Ecole Doctorale GAIA - Université de Montpellier

Habilitation à Diriger des Recherches

L'écologie chimique et fonctionnelle au service de la transition agroécologique

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Equipe : Durabilité des systèmes de production agricole et Sécurité alimentaire

Remerciements

Mes remerciements vont d’abord à mes mentors qui ont toujours répondu présents lorsque j’avais besoin d’eux : Thibaud Martin, Denis Bourguet, Baldwyn Torto.

Je remercie également mes collègues d’unité et plus largement du Cirad ainsi que mes collègues des instituts dans lequel j’ai été accueillie.

Mes remerciements vont également aux étudiants que j’ai encadrés et sans qui, tout ce travail n’aurait pas pu se faire.

Merci aux membres du jury qui prendront de leur temps pour évaluer mon travail.

Et je n’oublie pas ma famille qui est toujours là, à mes côtés.

DECLARATION

Je déclare avoir respecté, dans la conception et la rédaction de ce mémoire d'HDR, les valeurs et principes d'intégrité scientifique destinés à garantir le caractère honnête et scientifiquement rigoureux de tout travail de recherche, visés à l'article L.211-2 du Code de la recherche et énoncés par la Charte nationale de déontologie des métiers de la recherche et la Charte d'intégrité scientifique de l'Université de Montpellier. Je m'engage à les promouvoir dans le cadre de mes activités futures d'encadrement de recherche.

SOMMAIRE

1. Curriculum Vitae

1.1 Parcours professionnel

1.1.1 Etat civil

Emilie DELETRE
Née le 3 août 1987 à Roubaix (59)
Nationalité française
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Pentagone Valley, Kayahwe road, Kilimani, Nairobi, Kenya

1.1.2 Adresse

Centre Suisse de Recherche Scientifique (CSRS), 17 Rte de Dabou, Abidjan, Côte d'Ivoire
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1.1.3 Formation

2005 : Baccalauréat Série S, Lycée Camille Sée, Paris, Mention Bien
2008 : Concours d'entrée aux écoles nationales d'agronomie après classe préparatoire BCSPT, Lycée Lakanal, Bourg la Reine
2011 : Ingénieure agronome d'AgroCampus-ouest, spécialisée en protection des cultures, Rennes
2014 : Doctorat en écologie chimique et sciences agronomiques à Montpellier Supagro : Recherche sur la répulsion chez les insectes et ses applications en IPM.

1.1.4 Cursus professionnel

2009 : Stage à l'ONG Baobab Trust, Mombasa, Kenya (Etude comparative de purification de l'eau avec du *Moringa oleifera* et la méthode SODIS)
2010 : Stage à Sakata Vegetabe Europe, Nîmes, France (Développement d'une nouvelle méthode de conservation de l'oïdium et amélioration de son test de résistance)
2011 : Stage de fin d'études à l'unité Hortsys (Fonctionnement agroécologique et performances des systèmes de cultures horticoles) du Cirad, Montpellier, France (Développement d'une nouvelle méthode de lutte contre *Tetranychus urticae* en production de roses avec des matériaux imprégnés)
2011-2014 : Doctorante à l'unité Hortsys du Cirad, Montpellier, France et l'UMR Mivegec de l'IRD, France en collaboration avec la faculté de pharmacie de Montpellier
Depuis 2015- : Chercheuse Agroécologue, spécialiste des interactions chimiques plantes-insectes recrutée à l'unité Hortsys du Cirad, Montpellier, France
2015-2022: chercheuse invitée à l'icipe (international centre of insect physiology and ecology) à Nairobi, Kenya dans le département santé des plantes

Depuis 2022- : chercheuse invitée au Centre Suisse de Recherche Scientifique (CSRS), Abidjan, Côte d'Ivoire dans l'équipe : Durabilité des systèmes de production agricole et Sécurité alimentaire

1.1.5 Compétences

Ecologie et gestion des ravageurs des cultures horticoles
Outils de l'écologique chimique (Olfactomètre, GC-MS, EAG, SPME…)

1.1.6 Initiatives scientifiques

2012 -2015 : Membre fondateur et actif de l'association française des jeunes scientifiques en écologie chimique et organisateur des premières rencontres nationales d'écologie chimique, juin 2013, Montpellier, France
2016-2017 : Co-organisateur de l'atelier sur la Durabilité de la lutte agro-écologique contre les ravageurs : relever les défis écologiques, évolutifs et sociologiques avec la recherche sur les mouches des fruits, septembre 2016, Montpellier, France

1.1.7 Expertise

2018 : Rédaction de fiches techniques sur *Tetranychus urticae* et *Bemisia tabaci* pour la FAO

1.2 Contrats de recherche obtenus

Tous les contrats de recherche ci-dessous ont été contracté par le cirad :

1. **Marigo** Transition Agroécologique des Maraichers en Côte d'Ivoire (coordinateur de WP, 4 partenaires dans 1 pays, European Union: 2M€, 2021-2024)
2. **Pest-Free Fruit**: Sustainable intensification of fruit production systems through innovative pest biocontrol technologies (coordinateur de WP, 7 partenaires dans 5 pays, Leapagri: 665k€, 2018-2021)
3. **Eco+** : Exploiting semiochemical compounds combined with physical control for the design of an ecologically intensive farming system. (coordinateur du projet, 1 partenaire dans 4 pays, ANR: 225k€, 2017-2020)
4. **BionetAgro**: Netting Technology for small-scale vegetable growers in Africa (Participant, 6 partenaires dans 4 pays, USAID 200k$, 2016-2017)
5. **HortiNet-CI**: Adaptation et développement de l'agriculture protégée aux conditions climatiques et parasitaires de la Côte d'Ivoire (Participant, 3 partenaires dans 2 pays, Presed/CI :160k€, 2018-2020)
6. **KairOlive2**: Identification of the attractant kairomone from olive tree to Bactrocera oleae for the design of a mass trapping system (coordinateur du projet, 1 partenaire dans 1 pays, Région Occitanie, 65k€, 2020-2022)
7. **Biological control of aphids on Kalanchoe crops**: Identification and assessmentof the efficiency of local natural enemies (Participant, 4 partenaires dans 3 pays, MultiFlower company: 80k€, 2018-2019)
8. **Biophora**: Biocontrol Phoretic Agents (Participant, 5 partenaires dans 1 pays, CIRAD: 47.5k€, 2017)

9. **GRABT**: Evaluation du potentiel répulsif du Gros-thym contre l'aleurode *Bemisia tabaci* (Participant, 1 partenaire dans 1 pays, EcoPhyto: 46k€, 2018-2019)
10. **HortImpact**: Stimulating production of green leafy vegetables and improving food safety (Participant, 2 partenaires dans 2 pays, SNV: 29k€, 2018)
11. **RESCALE**: Répartition Spatiale des Maladies & Ravageurs et des Communautés associées à large échelle (co-coordinateur du projet, 2 partenaires dans 1 pays, CIRAD, 20k€, 2021)
12. **PAIV**: Pest of African Indigenous Vegetables (coordinateur du projet, 3 partenaires dans 3 pays, USAID: 20k€, 2018-2019)
13. **IMPRESS**: Impact assessment of netting technology in Kenya (coordinateur du projet, 2 partenaires dans 1 pays, CIRAD, 20k€, 2021)
14. **Kair-Olive**: Identification of the attractant kairomone from olive tree to *Bactrocera oleae* for the design of a mass trapping system (coordinateur du projet, 3 partenaires dans 1 pays, FranceOlive: 12k€, 2018-2019).
15. **IPPO**: Impact of predator on pollination (coordinateur du projet, 2 partenaires dans 1 pays, CIRAD, 14k€, 2020)

1.3 Liste de publications

1.3.1 Articles

Depuis 2013, j'ai publié 35 articles dans des journaux à comité de lecture dont 9 comme premier auteur et 14 en dernier auteur. Mon h-index est de 12 avec 580 citations depuis 2013, année de ma première publication (Figure 1). Les noms soulignés dans les listes d'auteurs sont les étudiants de Master ou les doctorants que j'ai encadrés.

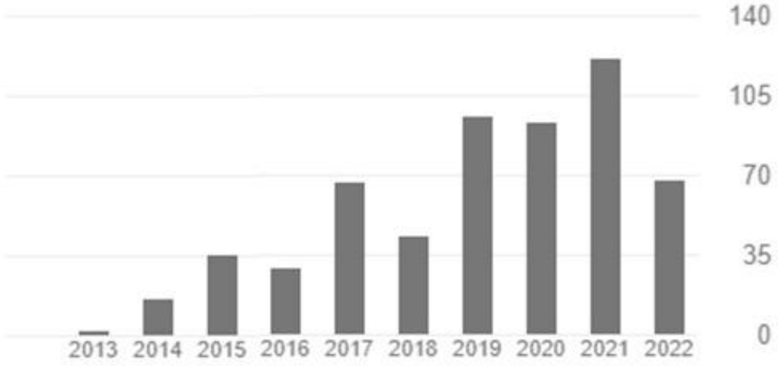


Figure 1. Nombre de citation par an depuis 2013 (source scholar.google.com)

1. P Ayelo, A Yusuf, A Chailleux, S Mohamed, C Pirk, **E Deletre**. 2022. Chemical cues from honeydew and cuticular extracts of *Trialeurodes vaporariorum* serve as kairomones for the parasitoid *Encarsia Formosa. Journal of Chemical Ecology*, 1-14 (IF=2.63)
2. **E Deletre**, F Matu, L Kananu, S Mohamed. 2022. Repellency potential of tomato herbivore induced volatiles against the greenhouse whitefly (*Trialeurodes vaporariorum*) (Homoptera: Aleyrodidae). *Journal of Economonic Entomology*, 15(2), 565-572 (IF=2.38)
3. A Chailleux, J Chepkemoi, J M Haran, L Benoit, R Copeland, **E Deletre**. 2022. Impact of net houses on the natural regulation of the populations of *Trialeurodes vaporariorum* (Homoptera:

Aleyrodidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae), two major tomato pests in Kenya. *International Journal of Tropical Insect Science,* 1-10 (IF=0.82)

4. C Pouët, **E Deletre**, B Rhino. 2022. Repellency of wild oregano plant volatiles, *Plectranthus amboinicus*, and their essential oils to the silverleaf whitefly, *Bemisia tabaci*, on tomato. *Neotropical Entomology*, 51(1), 133-142 (IF=1.33)
5. P Ayelo, S Mohamed, A Chailleux, A Yusuf, C Pirk, **E Deletre**. 2021. The parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato plants. *Journal of Pest Science*. 95, 633–652 (IF=5.15)
6. P Ayelo, A Yusuf, C Pirk, A Chailleux, S Mohamed, **E Deletre**. 2021. Terpenes from herbivore-induced tomato plant volatiles attract *Nesidiocoris tenuis* (Hemiptera: Miridae), a predator of major tomato pests. *Pest Management Science*. 77 (11), 5255-5267 (IF=4.38)
7. T Nordey, S Boni, M Agbodzavu, R Mwashimaha, N Mlowe, S Ramasamy, **E Deletre.** 2021. Comparison of biological methods to control *Aphis fabae* Scopoli (Hemiptera: Aphididae) on kalanchoe crops in East Africa*.* *Crop Protection*, 142, 105520. (IF=2.57)
8. S Diabate, T Martin, L Murungi, K Fiaboe, J Wesonga, J Kimani, **E Deletre**. 2021. Push-pull strategy combined with net houses for controlling cowpea insect pests and enhancing crop yields. *Crop Protection*, 141, 105480. (IF=2.57)
9. B Mekonnen, A Yusuf, C Pirk, S Ekesi, **E Deletre**. 2021. Oviposition responses of *Bactrocera dorsalis* and *Ceratitis cosyra* to Dufour’s and poison gland extracts of *Oecophylla longinoda* (Hymenoptera: Formicidae). *International Journal of Tropical Insect Science*, 1-9. (IF=0.82)
10. P Ayelo, A Yusuf, C Pirk, S Mohamed, A Chailleux, **E Deletre**. 2021. The role of *trialeurodes vaporariorum*-infested tomato plant volatiles in the attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Journal of Chemical Ecology*, 47(2), 192-203. (IF=2.63)
11. F Matu, L Murungi, S Mohamed, **E Deletre**. 2021. Behavioral response of the greenhouse whitefly (*Trialeurodes vaporariorum*) to plant volatiles of *Ocimum basilicum* and *Tagetes minuta*. *Chemoecology*, 31(1), 47-62. (IF=1.40)
12. P Ayelo, C Pirk, A Yusuf, A Chailleux, S Mohamed, **E Deletre**. 2021. Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: A Review. *Frontiers in Ecology and Evolution*, 9, 143. (IF=3.26)
13. B Mekonnen, X Cheseto, C Pirk, A Yusuf, S Ekesi, **E Deletre**, B Torto. 2021. Re-Analysis of Abdominal Gland Volatilome Secretions of the African Weaver Ant, *Oecophylla longinoda* (Hymenoptera: Formicidae). *Molecules*, 26(4), 871. (IF=4.41)
14. T Nordey, **E Deletre**, N Mlowe, T Martin. 2020. Nethouses protect cucumber plants from insect pests and increase yields in eastern Africa, *Journal of Horticultural Science & Biotechnology,* 95(5), 673-678. (IF=1.48)
15. B Mekonnen, J Haran, R Copeland, C Pirk, A Yusuf, **E Deletre**, 2020. First report of a gall midge as a parasitoid of weaver ants, *Entomologia Generalis* 40(4): 437-441. (IF=5.63)
16. T Nordey, N Mlowe, T Martin, **E Deletre**. 2020. Small mesh nets protect tomatoes from insect pests and increase yields in eastern Africa. *Journal of Horticultural Science & Biotechnology,* 95 (2), 222-228. (IF=1.48)
17. A Antwi-Agyakwa, A Fombong, **E Deletre,** A. Yusuf, C Pirk, B Torto. 2019. Lemon terpenes elicit arrestment behavior in the African citrus triozid *Trioza erytreae* (Hemiptera: Triozidae). *Journal of Chemical Ecology*, 45 (11), 934-945 (IF=2.63)

18. S. Diabate, T Martin, LK Murungi, KK Fiaboe, S Subramanian, J Wesonga, **E Deletre**. 2019. Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*. *Journal of Applied Entomology,* 143(8), 855-866. (IF=2.24)
19. S Diabate, **E Deletre**, LK Murungi, KK Fiaboe, S Subramanian, J Wesonga, T Martin. 2019. Behavioural responses of bean flower thrips (*Megalurothrips sjostedti*) to vegetative and floral volatiles from different cowpea cultivars. *Chemoecology*, *29*(2) :73-88. (IF=1.40)
20. S Diabate, **E Deletre**, L Kananu Murungi, K Fiaboe, J Wesonga T Martin. 2019. Behavioural response of alate *Aphis craccivora* Koch (Homoptera: Aphididae) to volatiles from different cowpea cultivars. *Journal of Applied Entomology.* (IF=2.24)
21. A Chailleux, A Stirnemann, J Leyes, **E Deletre**. 2019. Manipulating natural enemy behavior to improve biological control: attract and repulse. *Entomologia Generalis* 38(3):191-210 (IF=5.63)
22. **E Deletre,** F Chandre, C Duménil, T Martin. 2019. Insecticide resistance modifies mosquito behavior to DEET and natural repellents. *Parasites & vectors*. 12(1):89. (IF=3.88)
23. M Kungu, **E Deletre**, S Subramanian, K Fiaboe, T. Martin. 2019. A new mite IPM strategy: predator avoidance behaviour resulting from the synergetic effects of predator release and acaricide-treated nets. *Pest management science*. *75*(4) :979-985 (IF=4.38)
24. G Azandémè Hounmalona, N Maniania, N Saliou, S Fellous, S Kreiter, **E Deletre**, K Fiaboe, T Martin. 2018. Performance of *Metarhizium anisopliae*-treated foam in combination with *Phytoseiulus longipes* Evans on *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). *Pest management science*. *74*(12):2835-2841. (IF=4.38)
25. T Nordey, C Basset-Mens; H De Bon; T Martin; **E Deletre**; S Simon; L Parrot; H Despretz; Y Biard; J Huat; T Dubois; E Malezieux. 2017. Protected cultivation of vegetable crops in sub-Saharan Africa: limits and prospects for smallholders. A review. *Agronomy for Sustainable Development*. 37(6):53. (IF=4.10)
26. Martin T., M. Saidi, S. Niassy, S. Simon, F. Vidogbena, L. Parrot, S. Ekesi, **E. Deletre**, S. Subramanian, F. Assogba-Komlan, V. Baird, K. Fiaboe, M. Ngouajio, J.E. Simon, A. Ratnadass. 2016. Insect net: A Novel Technology to Promote Integrated Pest Management on Horticultural Crops in Africa. *III All Africa Horticultural Congress.* 1225 :43-52. (IF=0.26)
27. **E Deletre**, B Schatz, L Williams, D Bourguet, A Ratnadass, F Chandre, T Martin. Prospects for repellency in pest control – current developments and future challenges. 2016. C*hemoecology.* 26(4):127-142 (IF=1.40)
28. **E Deletre**, F Chandre, L Williams, C Duménil, C Menut, T Martin. 2016. Electrophysiological and behavioral characterization of bioactive compounds of four essential oils on *Anopheles gambiae* and prospects for use in treated bed nets. *Parasites & Vectors.* 14(1):1-5 (IF=3.88)
29. **E Deletre**, F Chandre, B Barkman, C Menut, T Martin. 2016. Naturally occurring bioactive compounds from four repellent essential oils against the whitefly *Bemisia tabaci. Pest Management Science. 72*(1), 179-189. (IF=4.38)
30. **E Deletre**, M Mallent, F Chandre, C Menut, T Martin. 2015. Behavioral response of *Bemisia tabaci* to 20 plant extracts. *Journal of Economonic Entomology* Tov118 (IF=2.38)
31. T Martin, A Kamal, E Gogo, M Saidi, **E Deletre**, R Bonafos, S Simon, M Ngouajio. 2014. Repellent effect of alphacypermethrin-treated netting against *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Economonic Entomology* 107(2):684-90. (IF=2.38)
32. **E. Deletre**, R. Bonafos, T. Martin. 2014.Evaluation of acaricide-treated string curtains for control of two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse roses and impact of the string curtain on the predatory mite *Phytoseiulus persimilis* Athias-

Henriot (Acari: Phytoseiidae). *Crop Protection*, 60:34–43. (IF=2.57)

33. **E Deletre**, T Martin, P Campagne, D Bourguet, A Cadin, C Menut, R Bonafos, F Chandre. 2013. Repellent, Irritant and Toxic Effects of 20 Plant Extracts on Adults of the Malaria Vector *Anopheles gambiae* Mosquito. *PLoS ONE,* 8(12):e82103. (IF=3.24)
34. T Martin, R Palix, A Kamal, **E Deletre**, R Bonafos, S Simon, M Ngouajio. 2013.A repellent net as a new technology to protect cabbage crops. *Journal of Economonic Entomology* 106(4):1699-706. (IF=2.38)
35. F Baldacchino, C Tramut, A Salem, E Liénard, **E Deletre**, M Franc, T Martin, G Duvallet, P Jay-Robert. 2013. The repellency of lemongrass oil against stable flies, tested using video tracking. *Parasite*, 20:21. (IF=3.01)

Articles en préparation ou soumis:

- Chailleux A, Ndjiliw , Diakhaté M, Akodjetin GF, Correa P, **Deletre E,** Brévault T (soumis) First steps toward conservation biological control programs using *Nesidiocoris tenuis* in Senegal. *Biological control.*
- **Deletre E,** Wangari M, Musila R, Rhino B (soumis) Use of natural enemies attractant to attract and retain parasitoids to control whitefly, *Trialeurodes vaporariorum* in openfield and nethouse.
- Mujukaa E, Parrot L, Baird W, **Deletre E**, Simone J, Mburuf J, Martin T (soumis) A Cost: Benefit analysis of netting technology in real farming conditions among smallholder farmers in Kenya, *Agronomy for Sustainable Development*
- **Deletre E**, Aika C, Mohamed S (In prep) Effects of African weaver ant semiochemicals from Dufour and poison gland on fruit fly parasitoids.
- **Deletre E**, Guthiri J, Mohamed S (In prep) Role of herbivore induced plant volatiles in tomato defense against the invasive pest *Tuta absoluta*
- **Deletre E,** Mekonnen B, Aika C, Yusuf A, Pirk C, Ekesi S (In prep) Effect of synthetic compounds from the Dufour's gland of the African weaver ant, *Oecophylla longinoda* (Hymenoptera: Formicidae) as oviposition deterrent for *Bactrocera dorsalis* and *Ceratitis cosyra*
- **Deletre E,** Aika C, Shemweno S, Brévault T (In prep) Impact of rearing, sterility and entomovectoring of male fruit flies on mating success compared to wild male fruit flies
- **Deletre E**, Mohamed S, Kimani J, Kambo C, Martin T (In prep) Netting technology: an alternative to chemical spray for protecting tomato crop in Africa.

1.3.2 Ouvrages

36. Atta-Krah K., Chotte J. L., Gascuel, C., Gitz, V., Hainzelin, E., Hubert, B., ..., **Deletre E**., … & Sinclair, F. (2021). Agroecological transformation for sustainable food systems: insight on France-CGIAR research.
37. M Kungu, S Subramanian, D Salifu, K Fiaboe, G Azandémè-Hounmalon, Gitonga L, GlOnyambu, **E Deletre**, Martin T. (2020). Influence of Predatory Mites, *Phytoseiulus longipes* Evans. on the Within-Plant Diurnal Migration and Distribution of the Red Spider Mite, *Tetranychus evansi*, Baker and Pritchard on African Nightshade, *Solanum scabrum*. In *Sustainable Management of Invasive Pests in Africa* (pp. 267-282). Springer, Cham.
38. Martin T., Parrot L., Belmin R., Nordey T., Basset-Mens C., Biard Y., **Deletre E.**, Simon S., Le Bellec **F.**. 2019. Anti-insect nets to facilitate the agroecological transition in Africa. In : The agroecological transition of agricultural systems in the Global South. Côte François-Xavier (ed.), Poirier-Magona

Emmanuelle (ed.), Perret Sylvain (ed.), Roudier Philippe (ed.), Bruno Rapidel (ed.), Thirion Marie-Cécile (ed.). Versailles : Ed Quae, pp. 75-87. (Agricultures et défis du monde) ISBN 978-2-7592-3056-3

39. Silvie P., Vayssières J-F, Babin R., Bagny-Beilhe L., Brévault T., Chailleux A., Delatte H., **Deletre E.**, Dufour B., Martin T., Michel B., Pagès C., Pinard F., Ratnadass A.. 2018. Directives pour la gestion d'arthropodes ravageurs transfrontières des plantes autres que les acridiens. Volume I. Montpellier : FAO-CIRAD, 70 p.

1.3.3 Communications dans des congrès

Communications orales:

40. **E Deletre** & G. Desurmond, Identification of olive tree kairomones attractant to *Bactrocera oleae* for mass trapping, TEAM, 2020, La Grande Motte, France
41. **E Deletre**, B Mekonnen, X Cheseto, C Pirk, A Yusuf, S Ekesi, B Torto, Trait-mediated avoidance behavior of fruit flies to semiochemicals of *Oecophylla longinoda,* ISCE meeting, 2019, Atlanta, USA
42. **E Deletre**, B Mekonnen, X Cheseto, C Pirk, A Yusuf, S Ekesi, B Torto .Trait-mediated avoidance behavior of fruit flies to semiochemicals of *Oecophylla longinoda* L. (Hymenoptera: Formicidae) AAIS meeting, 2017, Wad Medani, Sudan
43. Martin T., Parrot L., Belmin R., Nordey T., Basset-Mens C., Biard Y., **Deletre E.**, Simon S., Le Bellec **F.**. 2019. Anti-insect nets to facilitate the agroecological transition in Africa. III All Africa Horticultural Congress, 2016, Ibadan, Nigeria
44. **E. Deletre** & A. Chailleux, Improving biocontrol and adoption by farmers of an aggressive generalist predator, *Oecophylla longinoda* , XXV International Congress of Entomology, 2016, Orlando, USA
45. **E. Deletre,** Naturally occuring bioactive compounds against *Bemisia tabaci,* II International whitefly symposium, 2016, Arusha, Tanzania
46. **E. Deletre,** F. Chandre, T. Martin Mise au point d'une méthode de lutte contre les insectes vecteurs d'agents pathogènes : combinaison entre une barrière physique et olfactive, Mediatec conference (french researchers group in chemical ecology), 2014, Paris, France
47. **E. Deletre,** F. Chandre, T. Martin, Naturally occuring bioactive compounds against *Bemisia tabaci*, CIRA 2014 international congress on pests in agriculture, 2014, Montpellier, France
48. **E. Deletre,** F. Chandre, T. Martin, Naturally occuring bioactive compounds against *Bemisia tabaci*, Congress on natural products and biocontrol, 2014, Perpignan, France
49. **E. Deletre,** F. Chandre, T. Martin, Naturally occuring bioactive compounds against *Bemisia tabaci*, ISCE meeting, 2014, Urbana-Champaign, USA
50. **E. Deletre,** T. Martin, F. Chandre, Natural products as alternative to pyrethroids for malaria control, ISCE meeting, 2013, Melbourne, Australia (Student travel award)

Session poster:

51. African Indigenous Vegetables: From Income Generation to Health and Nutrition, 2019, Nairobi, Kenya
52. ISCE workshop, 2019, Nairobi, Kenya
53. Workshop of Horticultural Association of Kenya, 2018, Taita, Kenya (best poster award)
54. ISCE workshop, 2018, Alnarp, Sweden

55. First international conference on pesticidal plants, 2013, Nairobi, Kenya
56. Congress on natural products and biocontrol, 2102, Perpignan, France
57. Congress of the European society of vector ecology, 2012, Montpellier, France (best poster award)
58. International symposium on essential oils, 2012, Lisbon, Portugal

1.4 Liste des encadrements

1.4.1 Encadrement de doctorants

En tant qu'encadrant principal:
1) **Seydou Diabaté**: Combination of 'push-pull' strategy and netting technology for cowpea management (Jomo Kenyatta University of Agriculture and Technology, 2015-2019). Seydou est ajourd'hui en post doc sous ma direction à Abidjan depuis le 1 février 2022. Il a entre temps travaillé au Centre National de Recherche Agronomique de Côte d'ivoire à Bouaké comme enseignant et ingénieur de recherche.
2) **Bethelihem Mekonnen**: Improving biological control of fruit flies using *Oecophylla longinoda* (University of Pretoria, South Africa, 2016-2021). Betty est actuellement en post doc à l'université de Pretoria.
3) **Pascal Ayelo**: Recruitment of natural enemies through semiochemicals for tomato crop protection (University of Pretoria, South Africa, 2017-2021). Pascal est à la recherche d'un post doc, il attend une réponse de l'université de Birminghmam.
➔ Exploiting semiochemicals to develop crop protection innovations against fruit flies on cucurbitaceae (University of Montpellier, ED Gaia, 2022-2025). Cette thèse débutera en octobre 2022.

En tant que co-encadrant :
4) **Lionel Attoumou:** Utilisation de Trichoderma comme biofertilisant et biostimulant pour la culture de la tomate (*Solanum lycopersicum* L.) en Côte d'Ivoire (University Nangui Abrogoua, 2022-2025)
5) **Elodie Yeo:** Mise au point de solutions innovantes à base de crotalaire (*Crotalaria retusa)* pour la gestion du flétrissement bactérien (*Ralstonia solanacearum*) et élucidation du mécanisme d'action (University Felix Houphouet Boigny, 2022-2025)

1.4.2 Encadrement de stagiaires

J'ai encadré 20 étudiants (1-21), co-encadré 15 étudiants (21-36) et dirigé 3 techniciens (*) :

1. Maelle Mallent (2013): Repellent effect of essential oils for the management of *B. tabaci* (Faculté des sciences Montpellier, France)
2. Andy Cadin (2013): Repellent effect of essential oils for the management of *A. gambiae* (Faculté des sciences Montpellier, France)
3. Barbara Barkman (2014): repellent effect of natural compounds for the management of *B. tabaci* (Amsterdam University, Netherland)
4. Claire Duménil (2014): repellent effect of natural compounds for the management of *A. gambiae* (Faculty of sciences: Amsterdam, Netherland)

5. Aurélien Puisieux (2014): Study of coloured netting for the management of *Bemisia tabaci* (Ecole National Suprèrieur de Paris, France)
6. Beth Kerwitha (2017): Effect of tomato trichomes on the oviposition of *Tuta absoluta* (Meru University of Science and Technology, Kenya)
7. Judy Guthiri (2017): Effect of tomato volatiles on attractiveness/repellence of *Tuta absoluta* (Jomo Kenyatta University of Agriculture and Technology, Kenya)
8. Junitor Chepkemoi (2017): Survey of local natural enemies of *Bemisia tabaci* and *Tuta absoluta* in Kenya (Jomo Kenyatta University of Agriculture and Technology, Kenya)
9. Frank Matu (2018): Study of trap crop plants for the management of *Trialeurodes vaporariorum* (Jomo Kenyatta University of Agriculture and Technology, Kenya)
10. Marion Canale (2019): Study of olive tree volatiles on attractiveness/repellence of *Bactrocera oleae* (Sup'Biotech, France)
11. Sharon Chemweno (2019): Study of lekking and mating behaviour of *Bactrocera dorsalis* (Jomo Kenyatta University of Agriculture and Technology, Kenya)
12. Maureen Kimani* (2019): Lethal effect of different strains of *Metharizium anisopliae* on *Bactrocera dorsalis*
13. Cornelius Aika (2020): Study of methyl eugenol on lekking and mating behaviour of *Bactrocera dorsalis* (University of Nairobi, Kenya)
14. Thomas Bourge (2020): Identification of kairomone from olive tree to attract *Bactrocera oleae* (IRBI, université de Tours, France)
15. Maureen Wangari (2020): Study of attractant semiochemical to enhance biological control of whiteflies (Kenya Methodist University, Kenya)
16. Isaac Mbeche* (2020): Survey of impact assessment of netting technology in Kenya following the IMPRESS methodology
17. Ephantus Kimani* (2021): Study of spatial repartition of Coffee Leaf Rust and Coffee Berry Borer and associated parasitoids
18. Emma Giordanengo (2021): Identification of visual stimuli to attract *Bactrocera oleae* (Aix Marseille Université, France)
19. Béatrice Fedit (2021): Field trial of attractant semiochemical to enhance *Bactrocera oleae* trapping (Agrocampus Ouest, France)
20. Chloé Mouillac (2021): Field trial of attractant visual stimuli to enhance *Bactrocera oleae* trapping ((Agrocampus Ouest, France)
21. Aurélien Stirnemann (2015): Study of attractant plant for the management of *Oecophylla longinoda* (Université de Rennes1, France)
22. Jimmy Leyes (2015): Study of repellent natural compounds for the management of *Oecophylla longinoda* (Université de Rennes1, France)
23. Reagan Okoth (2018): Socio-technical analysis of the tomato value chain in Kenya (Kenyatta University, Kenya)
24. Cyrane Pouet (2019): Study of repellent effect of different gros thym chemotype on *Bemisia tabaci* (Institut Polytechnique Lasalle, France)
25. Aude Sany (2019): Study of political/economic aspects of the tomato value chain in Kenya (SciencesPo, France)
26. Virgile Verdi (2019): Study of constraints in tomato value chain for netting technology transfer in Kenya (ISTOM, France)

27. Samba Sanogo (2021) : Etude des ravageurs et ennemis naturels de 10 spéculations dans la région d'Abidjan (Université Felix Houphouet Boigny, Côte d'ivoire)
28. Lionel Attoumou (2021) : Etude des maladies de 10 spéculations dans la région d'Abidjan (Université de Nagui Abrogoua, Côte d'ivoire)
29. Samuel Skreudado (2021) : Etude des ravageurs et ennemis naturels de 10 spéculations dans la région de Yamoussoukro (Ecole Supérieure d'Agronomie, Côte d'Ivoire)
30. Esdras Akaffou (2021) : Etude des maladies de 10 spéculations dans la région de Yamoussoukro (ISARA Lyon, France)
31. Fokin Soro (2021) : Etude des ravageurs et ennemis naturels de 10 spéculations dans la région de Korhogo (Université Gon Koulibaly, Côte d'Ivoire)
32. Vanessa Yao (2021) : Etude des maladies de 10 spéculations dans la région de Korogho (Université Gon Koulibaly, Côte d'Ivoire)
33. Glwadys Akissi (2022) : Etude des ravageurs et ennemis naturels de 10 spéculations dans la région d'Abidjan (Université de Nagui Abrogoua, Côte d'ivoire)
34. Alidou Soumahoro (2022) : Etude des maladies de 10 spéculations dans la région d'Abidjan (Université Felix Houphouet Boigny, Côte d'ivoire)
35. Appia Kouame (2022) : Etude des ravageurs et ennemis naturels de 10 spéculations dans la région de Bouake (Université Felix Houphouet Boigny, Côte d'ivoire)
36. Chloé Latapie (2022) : Cartographie et abondance des acariens et de leurs ennemis naturels en Côte d'Ivoire (ISTOM, France)

2. Bilan des activités de Recherche

2.1 Contexte

En 2050 avec une population plus riche et plus urbaine de 9,1 milliards, la production agricole devra augmenter de 70 pourcent, ce qui causera de sérieux problèmes de sécurité alimentaire (FAO 2009a). D'après la FAO, l'agriculture africaine a le plus grand potentiel de croissance (FAO 2009b). La production de légumes y est en pleine croissance pour nourrir les populations urbaines grandissantes des régions sub-sahariennes. C'est aussi un moteur prometteur de croissance économique et une importante source de revenus pour les petits exploitants et d'emplois dans les zones rurales et périurbaines. Le maraichage contribue également à la sécurité alimentaire et à l'équilibre nutritionnel des populations les plus vulnérables. Les fruits et légumes sont des éléments importants pour une alimentation saine et la FAO et l'OMS en recommandent une consommation d'au moins 500 g/jour. L'augmentation de la production agricole viendra de l'augmentation des rendements et de l'expansion des terres agricoles. Nous devons donc produire plus mais également produire mieux. Aujourd'hui pour protéger leurs cultures, les producteurs utilisent surtout les insecticides de synthèse (De Bon et al. 2014)**.** Cependant, cette protection des cultures basée sur les insecticides de synthèse et cette dépendance excessive aux intrants chimiques n'est pas durable compte tenu de la résistance des ravageurs, de leur impact négatif sur les pollinisateurs et les ennemis naturels, de la pollution de l'environnement et de la menace pour la santé publique. Le contexte mondial actuel - ressources naturelles et biodiversité réduites, changement climatique, mobilité humaine et commerce transfrontalier - aggrave également les problèmes de sécurité alimentaire et augmente le risque d'invasion de ravageurs. Les innovations reposant sur un contrôle écologique sont cruciales pour répondre efficacement au besoin d'une production horticole durable, ainsi qu'à l'appel sociétal pour des régimes alimentaires plus sûrs et équilibrés sur le plan nutritionnel, une meilleure santé et une meilleure protection de l'environnement. **Ainsi, mes projets de recherche visent à concevoir des systèmes de productions agroécologiques et intensifs, qui permettront de produire plus, tout en respectant l'environnement.**

2.1.1 L'agroécologie

La FAO définit l'agroécologie comme « une approche holistique et intégrée qui applique simultanément des concepts et des principes écologiques et sociaux à la conception et à la gestion de systèmes agricoles et alimentaires durables. Elle cherche à optimiser les interactions entre les plantes, les animaux, les hommes et l'environnement tout en répondant à la nécessité de systèmes alimentaires socialement équitables au sein desquels les gens peuvent choisir ce qu'ils mangent et comment et où il est produit ». Le ministère de l'agriculture français, quant à lui, définit l'agroécologie comme « une façon de concevoir des systèmes de production qui s'appuient sur les fonctionnalités offertes par les écosystèmes. Elle les amplifie tout en visant à diminuer les pressions sur l'environnement (…) et à préserver les ressources naturelles. Il s'agit d'utiliser au maximum la nature comme facteur de production en maintenant ses capacités de renouvellement ». Le cirad s'intéresse particulièrement aux problématiques de transition agroécologique comme alternative économique et sociale, viable et durable, par rapport aux modèles conventionnels d'intensification. Avec l'identification des leviers à ces transitions agroécologiques, son objectif est de proposer une méthode globale adaptée aux différents types d'agriculture. L'agroécologie est une approche multidisciplinaire qui s'applique à des échelles multiples (Wezel et al. 2020). La transition agroécologique commence souvent à l'échelle de la parcelle avec la réduction des intrants nocifs pour l'environnement (niveau 1) puis avec leurs remplacements par des innovations agroécologiques (niveau 2) (figure 2). Suite à l'introduction d'innovations agroécologiques (diversification végétale, fertilisation organique, rotation, agroforesterie…), le système de production doit alors être reconçu à l'échelle de l'exploitation et du territoire (niveau 3). Enfin, des liens directs entre producteurs et consommateurs doivent être établis à l'échelle locale et nationale pour créer de nouvelles chaines de valeurs et des nouveaux marchés (niveau 4) mais un nouveau système alimentaire mondiale doit également être reconstruit, qui soit équitable et durable (niveau 5). Parmi ces différents niveaux de transition, je m'intéresse principalement au niveau 1 et 2. **Mes questions de recherches s'articulent autour de la réduction des pesticides et de la conception d'innovations agroécologiques, afin de tester et d'implémenter de nouvelles façons de produire. Ces innovations s'appuient sur les processus de régulations naturelles et de diversification végétale et font appels à différentes disciplines comme l'agronomie, l'écologie fonctionnelle ou encore l'écologie chimique.**

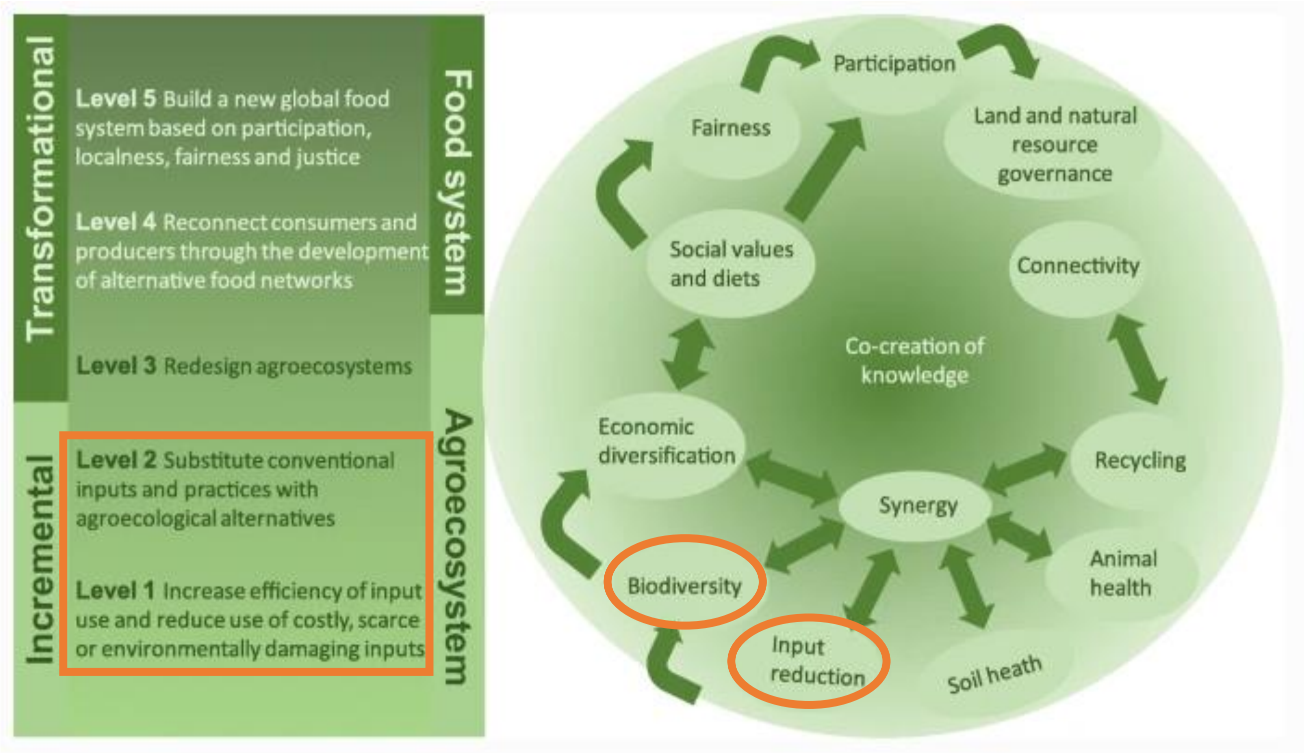


Figure 2. Les 5 différents niveaux de transition agroécologique vers des systèmes alimentaires durables associés aux principes agroécologiques (ovales). Les parties oranges sont celles qui concernent mes questions de recherche. Adapté de Wezel et al. 2020.

2.1.2 L'écologie fonctionnelle

L'écologie fonctionnelle analyse la dynamique de la biodiversité et ses conséquences sur le fonctionnement des écosystèmes. Elle s'intéresse aux traits fonctionnels des espèces, i.e. les caractéristiques physiologiques, morphologiques, reproductives ou comportementales des espèces, et aux interactions trophiques entre les espèces (Figure 3). **Ainsi, un de mes objectifs de recherche**

est de comprendre la structure et le fonctionnement de l’agroécosystème afin, par exemple, d’optimiser les services écosystémiques. En effet, l'intensification agroécologique a pour objectifs d'augmenter la productivité et de remplacer l'utilisation d'intrants chimiques par l'optimisation des services écosystémiques. Parmi les services écosystémiques, deux services régulateurs : la pollinisation et la suppression des ravageurs par les ennemis naturels peuvent particulièrement limiter la production, même lorsque tous les autres services sont optimisés (Bommarco et al. 2013). La contribution de la biodiversité à la pollinisation et à la lutte contre les ravageurs a été au centre des préoccupations de la communauté scientifique au cours des dernières décennies (Duru et al. 2015). Cependant, parmi les manques importants de connaissances, il y a la quantification des liens entre les services écosystémiques et la composition de la communauté (i.e. biodiversité). Or ces connaissances sont nécessaires pour une intensification écologique réussie, il faut « la bonne biodiversité ». En effet, l’augmentation de la biodiversité n’est pas toujours bénéfique, elle peut avoir un effet négatif en raison de l’augmentation des interactions intraguildes d’ennemis naturels (e.g. compétition) à mesure que de nouvelles espèces sont ajoutées au réseau trophique (Wilby & Thomas 2002, Finke & Snyder 2010, Chailleux et al. 2014, Dainese et al. 2017). Cependant, lorsque des espèces d’ennemis naturels se divisent les ressources en attaquant différents sous-ensembles de la population de ravageurs (i.e. complémentarité de niche), la biodiversité peut être considérée comme un facteur positif améliorant l'efficacité de la suppression des ravageurs (Straub & Snnyder 2008). En résumé, la compétition et le mutualisme sont des interactions indirectes bidirectionnelles symétriques négatives et positives respectivement qui peuvent se produire par exemple entre des proies/hôtes partageant un ennemi naturel commun ou des ennemis naturels partageant la même proie/hôte; à l'inverse, l'amensalisme et le commensalisme sont des interactions indirectes négatifves et positives respectivement à sens unique asymétrique qui se produisent par exemple lorsqu'une seule proie affecte indirectement l'autre ou qu’un seul ennemi naturel affecte l’autre. La plupart des études examinent la présence et/ou l'abondance des ennemis naturels (composition de la communauté), certaines examinent également l'impact direct (effet de la consommation ou du parasitisme) des ennemis naturels, voire la combinaison d’ennemis naturels (effet indirect) en termes de réduction des ravageurs (services écosystémiques) mais très peu y associent les signaux chimiques qui régissent ces interactions.

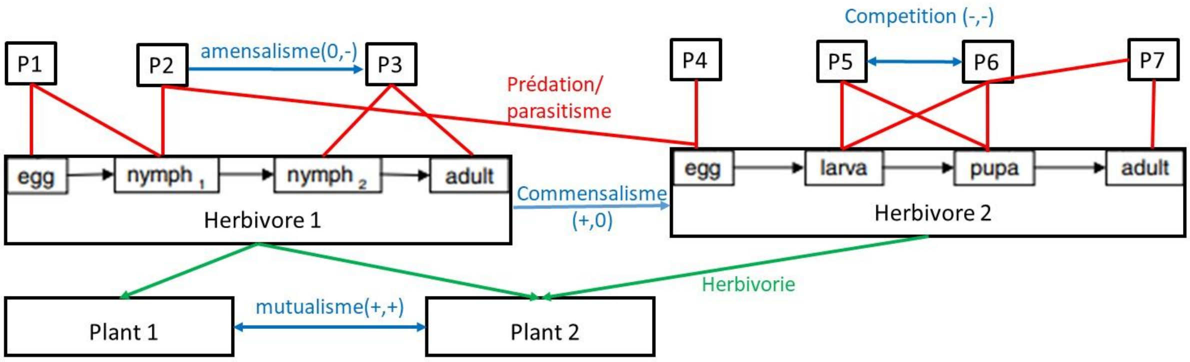


Figure 3. Schéma simplifier des interactions directes et indirectes d’un réseau trophique (P= prédateur/parasitoïde)

2.1.3 L’écologie chimique

L’écologie chimique s’intéresse aux interactions entre les organismes et leur environnement par l’intermédiaire de molécules. Ces molécules sont appelées les sémiochimiques (Figure 4). Il y a les phéromones qui sont intraspécifiques et les kairomones, allomones, synomones qui sont interspécifiques. Une kairomone affecte positivement le comportement du récepteur du signal chimique mais pas du producteur alors qu'une allomone affecte positivement le comportement du producteur mais pas du récepteur. Une synomone affecte positivement le producteur et le récepteur. Ces molécules chimiques sont impliquées dans divers comportements, comme par exemple (i) le comportement de marquage (territorial, oviposition, …), (ii) la recherche d'un partenaire sexuel, (iii) la reconnaissance intra-espèce pour les insectes sociaux, (iv) la détection d'un hôte par un parasitoïde, (v) le choix d'une source de nourriture… (Mbaluto et al. 2020). Ainsi l'écologie chimique est un domaine de recherches pluridisciplinaires qui implique : des chimistes qui identifient et synthétisent les sémiochimiques, des physiologistes qui étudient la biosynthèse ou la perception de ces molécules chimiques chez les plantes et les insectes, et des écologistes qui examinent les effets des interactions chimique au niveau des individus et des écosystèmes.

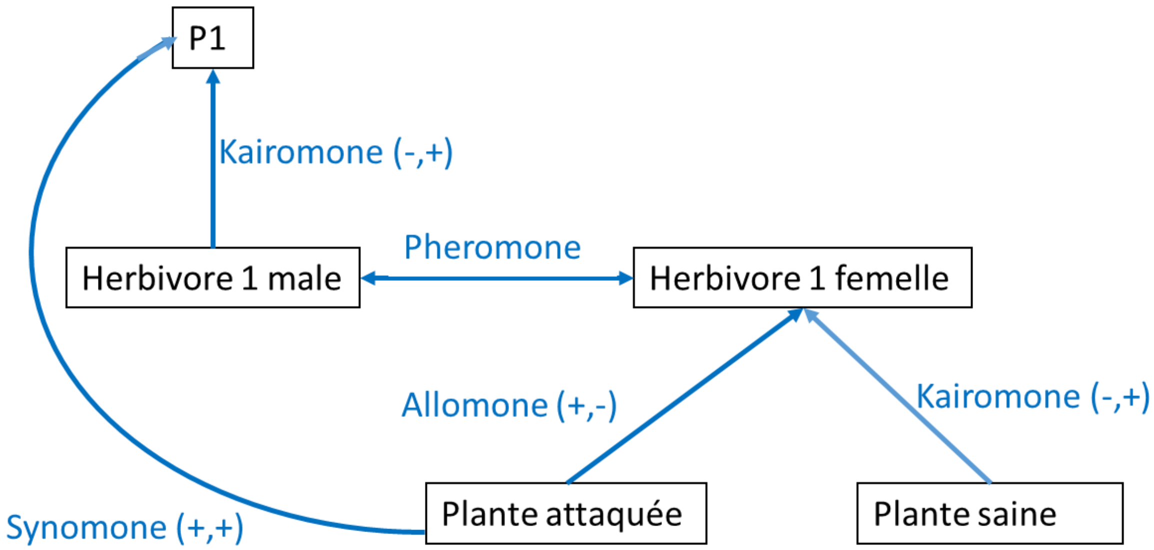


Figure 4. Schéma simplifié des différents types de sémiochimiques impliqués dans les interactions chimiques d'un réseau trophique (P= prédateur/parasitoïde).

Parmi les grands domaines de recherches de l'écologie chimique, je m'intéresse : au niveau des interactions chimiques plantes-insectes, à (i) la compréhension de la sélection de la plante hôte par un ravageur, que ce soit une source de nourriture ou un site d'oviposition (kairomone) ; (ii) la réponse défensive de la plante face aux herbivores (allomone) ; (iii) l'attraction des ennemis naturels par la plante attaquée (synomone) ; au niveau des interactions chimiques insectes-insectes, à (i) l'identification des phéromones sexuelles qui régit le choix du partenaire sexuel (phéromones) ; (ii) les stratégies de détection d'un hôte/proie par un parasitoïde/ prédateur et inversement (kairomones) ; (iii) la communication entre les insectes sociaux (phéromones).

La compréhension de ces interactions chimiques au sein du réseau trophique de l'agroécosystème a permis (i) de développer des systèmes push-pull (i.e. « stratégie de manipulation comportementale des insectes ravageurs et de leurs ennemis naturels via l'intégration de stimuli répulsifs (push) qui agissent pour rendre la ressource à protéger peu attrayante ou inadaptée aux ravageurs tout en les attirant vers une source attractive (pull) d'où les ravageurs sont ensuite éliminés » Cook et al. 2007), (ii) d'améliorer les systèmes de lutte biologique par conservation (i.e. pratique de modification de l'environnement ou des pratiques existantes pour protéger et favoriser les populations d'ennemis naturels afin de réduire l'impact des ravageurs sur les cultures (Shields et al. 2019)), (iii) de favoriser et d'augmenter les services écosystémiques mais également (iv) de développer des pièges ou la lutte par la confusion sexuelle. **L'écologie chimique est un formidable outil qui me permet de concevoir et de développer des innovations pour la protection des cultures.**

2.2 Volet 1 : Fonctionnement de l'agroécosystème de la tomate au Kenya

2.2.1 Niveau 1 de la transition agroécologique : diminution de l'utilisation des pesticides

Articles associés à ce volet :

- Mujukaa E, Parrot L, Baird W, **Deletre E**, Simone J, Mburuf J, Martin T (soumis) A Cost: Benefit analysis of netting technology in real farming conditions among smallholder farmers in Kenya, *Agronomy for Sustainable Development*
- T Nordey, C Basset-Mens; H De Bon; T Martin; **E Deletre**; S Simon; L Parrot; H Despretz; Y Biard; J Huat; T Dubois; E Malezieux. 2017. Protected cultivation of vegetable crops in sub-Saharan Africa: limits and prospects for smallholders. A review. *Agronomy for Sustainable Development*. 37(6):53.
- Martin T., M. Saidi, S. Niassy, S. Simon, F. Vidogbena, L. Parrot, S. Ekesi, **E. Deletre**, S. Subramanian, F. Assogba-Komlan, V. Baird, K. Fiaboe, M. Ngouajio, J.E. Simon, A. Ratnadass. 2016. Insect net: A Novel Technology to Promote Integrated Pest Management on Horticultural Crops in Africa. *III All Africa Horticultural Congress.* 1225 :43-52.
- T Martin, A Kamal, E Gogo, M Saidi, **E Deletre**, R Bonafos, S Simon, M Ngouajio. 2014. Repellent effect of alphacypermethrin-treated netting against *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J. Econ. Entom*. 107(2):684-90.

Comment augmenter les rendements des cultures, sans dépendre des intrants chimiques, tout en favorisant l'agroécologie ? En Afrique, le transfert et l'adoption de techniques *low-tech* abordables pourraient relever ce défi. Pour réduire l'utilisation des pesticides et augmenter les rendements, mon unité de recherche travaille depuis 15 ans sur l'utilisation et le développement des filets anti-insectes pour la protection des cultures (Martin et al. 2006, Martin et al. 2014, Martin et al. 2016) Nos recherches au Bénin, en Tanzanie et au Kenya ont montré que les filets sont faciles à utiliser et augmentent le rendement de 30% en moyenne des plantes maraichères telles que les tomates, haricots, choux, poivrons, etc… En effet, la production de légumes sous ces filets permet de réduire les attaques des ravageurs de grande taille, en particulier ceux responsables des dommages directs causés aux fruits ou aux feuilles, comme les oiseaux, les escargots, les criquets, les chenilles et les mouches. Mais ces filets ne protègent pas complètement les cultures contre les ravageurs de petite taille, tels que les pucerons, les aleurodes, les thrips et les acariens, mais ils retardent leur infestation (Deletre et al., in prep). Ces ravageurs se nourrissent principalement du phloème ou du contenu cellulaire de l'épiderme des feuilles et certains peuvent transmettre des virus. L'environnement confiné sous filets facilite néanmoins la lutte biologique contre ces ravageurs. La pollinisation par les abeilles peut également être contrôlée avec l'installation de ruches à deux ouvertures, qui leur permettent d'accéder à l'intérieur et à l'extérieur du filet. Cette technologie permet ainsi aux agriculteurs de réduire drastiquement l'utilisation des pesticides, tout en atténuant les effets des conditions climatiques extrêmes telles que le rayonnement solaire élevé, les fortes pluies et les vents secs (Nordey et al. 2017). Les filets anti-insectes peuvent être combinés avec des filets d'ombrage qui diminuent le stress thermique pendant la saison sèche et un toit en plastique qui peut réduire davantage le risque de maladies fongiques pendant la saison des pluies. Ainsi, les filets anti insectes permettent de prolonger la période de production, d'augmenter les rendements des cultures et d'améliorer la qualité en termes de caractéristiques organoleptiques et de réduction de résidus de pesticides. Les techniques liées à la culture protégée sont souvent décriées à cause de l'utilisation du plastique. Cependant, le filet peut être recyclé et la réduction conséquente de l'utilisation des intrants agricoles compenserait les impacts négatifs, comme le suggèrent les analyses du cycle de vie. Mais la faible capacité d'investissement des agriculteurs freine l’adoption de cette technologie. Pourtant les filets permettent de limiter les variations de rendement des cultures et donc d’assurer un revenu plus stable aux agriculteurs, en protégeant les cultures contre les intempéries et les attaques des ravageurs. Des analyses coût-bénéfices ont montré que l'utilisation de filets aide à stabiliser les flux de trésorerie et à réduire la volatilité de la production et les variations de qualité (Mujukaa et al. Soumis). Elle améliore ainsi la vision à long terme des agriculteurs en réduisant les risques et en leur permettant de faire des investissements à moyen terme à moindre risque.

2.2.2 La biodiversité de l’agroécosystème de la tomate au Kenya

Etudiant encadré associé à ce volet :

J. Chepkemoi (2017): Survey of local natural enemies of *B. tabaci* and *T. absoluta* in Kenya (JKUAT, Kenya)

Articles publiés associés à ce volet :

- A Chailleux, J Chepkemoi, J M Haran, L Benoit, R Copeland, **E Deletre**. 2022. Impact of net houses on the natural regulation of the populations of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae), two major tomato pests in Kenya. *International Journal of Tropical Insect Science,* 1-10
- **Deletre E**, Mohamed S, Kimani J, Kambo C, Martin T (In prep) Netting technology: an alternative to chemical spray for protecting tomato crop in Africa.

Des recherches en station (Deletre et al. In prep) et chez des producteurs (Chailleux et al. 2022) nous ont permis d’établir en partie la biodiversité de l’agroécosystème de la tomate au Kenya (Figure 5).

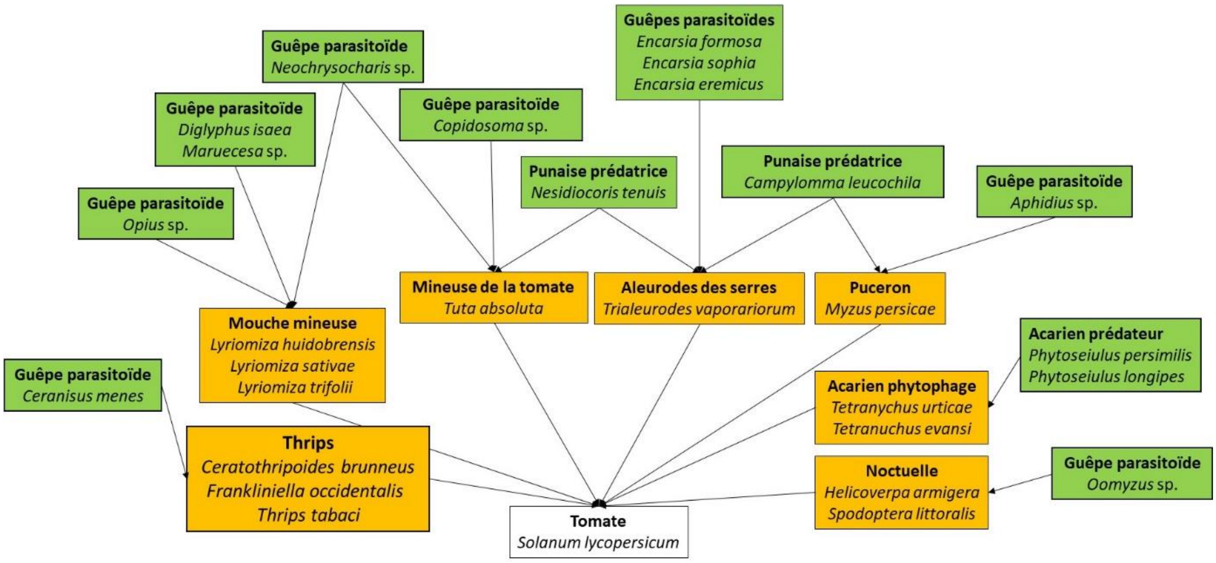


Figure 5. Biodiversité de l’agroécosystème de la tomate au Kenya. (Orange = ravageurs, vert = ennemis naturels)

Parmi les espèces identifiées, *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) est une espèce invasive venant d'Amérique du sud. Elle a été détectée pour la première fois en Espagne en 2006 (Urbaneja et al. 2007). Peu de temps après, le ravageur s'est propagé et s'est établi dans plusieurs pays d'Europe, d'Asie, d'Haïti et de presque tout le continent africain avec un impact dévastateur sur la production de tomates (Desneux et al. 2010 ; Mansour et al. 2018 ; Verheggen & Fontus 2019 ; Han et al. 2019). Les dégâts causés aux cultures par *T. absoluta* sont principalement causés par les larves qui se nourrissent des feuilles, des tiges et des fruits des plants de tomates, causant entre 80 et 100 % de pertes de rendement lorsqu'aucune mesure de contrôle n'est utilisée (Desneux et al. 2010 ; Mansour et al. 2018). *T. absoluta* est un spécialiste, il préfère la tomate comme plante hôte, mais il est également connu pour attaquer d'autres cultures de solanacées comme la pomme de terre, l'aubergine ou la morelle. Actuellement, l'utilisation des insecticides chimiques synthétiques à large spectre reste la principale stratégie de lutte dans le monde entier (Desneux et al. 2010 ; Biondi et al. 2018). Les applications aveugles et fréquentes de ces pesticides associées à la nature multivoltine de *T. absoluta* ont conduit au développement de résistances à différentes classes d'insecticides chimiques synthétiques, rendant leur utilisation obsolète (Guedes et al. 2019). Il existe une demande croissante pour l'utilisation de stratégies de contrôle alternatives présentant des avantages écologiques et environnementaux : comme par exemple l'utilisation de phéromones sexuelles pour le piégeage de masse et la confusion sexuelle, l'utilisation des organismes entomopathogènes comme *Bacillus thuringiensis* et l'utilisation d'ennemis naturels comme le prédateur généraliste, *Macrolophus pygmaeus* ou le parasitoïde, *Trichogramma achaeae* (Chailleux et al. 2013). Mais, mise à part la phéromone, ces solutions n'existent pas localement.

Contrairement à notre hypothèse de départ, nous n'avons pas trouvé de *Bemisia tabaci* sur les plants de tomate mais du *Trialeurodes vaporariorum* (Westwood) (Hemiptera : Aleyrodidae). Cet aleurode, très polyphage, est une espèce invasive en Europe et en Afrique subsaharienne où il menace la production de tomates (Hanssen et Lapidot 2012 ; Gamarra et al. 2016). Ce ravageur cause des pertes de rendement par des dégâts directs en prélevant de la sève du phloème ainsi qu'indirectement en excrétant du miellat sur le feuillage, entraînant le développement de fumagines qui réduisent la photosynthèse des feuilles, et par la transmission de virus, comme le *tomato chlorosis crinivirus* ou *le tomato curly stunt begomovirus* (Moodley et al. 2019). Les dommages causés à la production de tomates par cet aleurode provoqueraient des pertes de 5 à 30 % dans la région subsaharienne (Johnson et al. 1992). Les insecticides synthétiques à large spectre sont principalement utilisés pour lutter contre *T. vaporariorum* mais avec de nombreux problèmes de résistance (Lapidot et al. 2014).

Ces deux ravageurs sont les espèces pour lesquels les producteurs kenyans utilisaient le plus d'insecticides. Nous avons donc focalisé nos recherches sur ces 2 ravageurs afin de trouver des alternatives à l'utilisation des insecticides. Une des alternatives était de favoriser la lutte biologique par conservation. Dans un premier temps, nous avons donc cherché à identifier les ennemis naturels présents localement. Nous avons identifié un prédateur généraliste zoophytophage : *Nesidiocoris tenuis* (Hemiptera : miridae), de *Tuta absoluta* et de *Trialeurodes vaporariorum.* Il représente plus de 96% des prédateurs que nous avons trouvé. Ce prédateur se nourrit des œufs et des larves de premier stade de *T. absoluta*, et des nymphes de troisième et quatrième stades de l'aleurode (Perez-Hedo et al. 2020). En absence de proies, ce prédateur peut se nourrir de la sève de tomate lui permettant ainsi de survivre (Perez-Hedo & Urbaneja 2016). C'est pourquoi l'utilisation de prédateurs hémiptères zoophytophages pour la lutte contre les ravageurs a fait l'objet d'une attention considérable ces dernières années (Dumont et al. 2018). Nous avons également identifié des parasitoïdes de *T. vaporariorum* : *Encarsia formosa* Gahan (Hymenoptera : Aphelinidae)*, Encarsia sophia, Encarsia* *eremicus*. Le plus abondant était *Encarsia formosa*, nous avons émis l'hypothèse que ce parasitoïde était plus résistant aux insecticides que les deux autres car *E. sophia* et *E. eremicus* n'ont été identifié qu'en station expérimentale où nous utilisions très peu d'insecticides. *E. formosa* est l'un des parasitoïdes les plus efficaces pour lutter contre les aleurodes (Liu et al. 2015). Ce parasitoïde parasite de préférence les nymphes de troisième et quatrième stades de *T. vaporariorum*. Nous avons également identifié un parasitoïde de *T. absoluta* : *Copidosoma* sp. (Hymenoptera : Encyrtidae) mais en très faible quantité. Cela peut s'expliquer par le fait qu'étant un ravageur invasif, *T. absoluta* n'a pas de parasitoïde spécialiste présent localement. Au moment de ces recherches, l'icipe avait un projet sur l'introduction d'un parasitoïde venant du Pérou (zone d'origine du ravageur) pour lutter contre *T. absoluta* : *Dolichogenidea gelechiidivoris* March (Hymenoptera : Braconidae). Il parasite préférentiellement les larves de premier et deuxième stades de *T. absoluta* (Aigbedion-Atalor et al. 2020), et il est connu pour être efficace contre *T. absoluta* (Salas et al. 2019). Au pérou, il est le parasitoïdes le plus répandu de *T. absoluta*, atteignant environ 26 à 41 % et 57 % de parasitisme avec et sans l'utilisation de pesticides chimiques, respectivement (Palacios et Cisneros, 1995).

Suite à ces travaux, nous avons donc sélectionnés les modèles biologiques sur lesquels nous voulions travailler : *T. absoluta, T. vaporariorum, N. tenuis, E. formosa* et *D. gelechiidivoris.*

2.2.3 Manipulation du comportement des ravageurs de la tomate

Etudiants encadrés associés à ce volet :

M. Mallent (2013): Repellent effect of essential oils for the *B. tabaci* control (Fac sciences Montpellier, France)

B. Barkman (2014): Repellent effect of compounds for the control of *B. tabaci* (Amsterdam Univ, Netherland)

J. Guthiri (2017): Effect of tomato volatiles on attractiveness/repellence of *Tuta absoluta* (JKUAT, Kenya)

F. Matu (2018): Study of trap crop plants for the management of *T. vaporariorum* (JKUAT, Kenya)

C. Pouet (2019): Study of gros thym repellent effect on *B. tabaci* (Institut Polytechnique Lasalle, France)

S. Diabaté: Combination of netting and 'push-pull' strategy for cowpea management (JKUAT, 2015-19)

Articles publiés associés à ce volet :

- **Deletre E**, Guthiri J, Mohamed S (In prep) Role of herbivore induced plant volatiles in tomato defense against the invasive pest *Tuta absoluta*
- **E Deletre**, F Matu, L Kananu, S Mohamed. 2022. Repellency potential of tomato herbivore induced volatiles against the greenhouse whitefly (*Trialeurodes vaporariorum*) (Homoptera: Aleyrodidae). *Journal of Economonic Entomology,* 15(2), 565-572
- F Matu, L Murungi, S Mohamed, **E Deletre**. 2021. Behavioral response of the greenhouse whitefly (*Trialeurodes vaporariorum*) to plant volatiles of *Ocimum basilicum* and *Tagetes minuta*. *Chemoecology*, 31(1), 47-62.
- S Diabate, T Martin, L Murungi, K Fiaboe, J Wesonga, J Kimani, E Deletre. 2021. Push-pull strategy combined with net houses for controlling cowpea insect pests and enhancing crop yields. Crop Protection, 141, 105480.
- C Pouët, **E Deletre**, B Rhino. 2022. Repellency of wild oregano plant volatiles, *Plectranthus amboinicus*, and their essential oils to the silverleaf whitefly, *Bemisia tabaci*, on tomato. *Neotropical Entomology*, 51(1), 133-142
- S. Diabate, T Martin, LK Murungi, KK Fiaboe, S Subramanian, J Wesonga, E Deletre. 2019. Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*. *Journal of Applied Entomology*, 143(8), 855-866.
- **E Deletre**, F Chandre, B Barkman, C Menut, T Martin. 2016b. Naturally occurring bioactive compounds from four repellent essential oils against the whitefly *Bemisia tabaci. Pest Manag Sci. 72*(1), 179-189.

- **E Deletre**, M Mallent, F Chandre, C Menut, T Martin. 2015. Behavioral response of *Bemisia tabaci* to 20 plant extracts, *J. Econ. Entom.* Tov118
- **E Deletre**, B Schatz, L Williams, D Bourguet, A Ratnadass, F Chandre, T Martin. 2016a. Prospects for repellency in pest control – current developments and future challenges. *Chemoecology.* 26(4):127-142

Le comportement d'un ravageur peut être manipulé de deux manières : soit on l'attire avec un stimulus attractif, soit on le repousse avec un stimulus répulsif. Dans les stratégies push pull, qui utilisent les deux types de stimuli en même temps, le principe est de repousser le ravageur de la ressource à protéger, tout en l'attirant vers une ressource attractive d'où le ravageur est éliminé (Cook et al. 2007). Avant de concevoir des systèmes push-pull pour la protection des plantes maraichères, j'ai commencé ma carrière en étudiant les comportements de répulsion pendant ma thèse. Au sens large, un comportement de répulsion est un comportement d'éloignement par rapport à la source d'odeur ou l'incapacité à trouver une source d'odeur (Deletre et al. 2016a). Il existe plusieurs types de répulsifs associés à plusieurs types de mécanismes d'action (Figure 6). Trois types de répulsifs nous intéressent particulièrement en protection des cultures : (1) le répulsif spatial qui provoque un mouvement de fuite de l'insecte par rapport à la source d'odeur sans contact direct, (2) le répulsif masquant qui correspond à une réduction de l'attractivité d'une source d'odeur ou à une perturbation de la localisation de la source d'odeur, et (3) l'anti-appétant qui correspond à une perturbation de l'activité alimentaire par contact ou ingestion du répulsif.

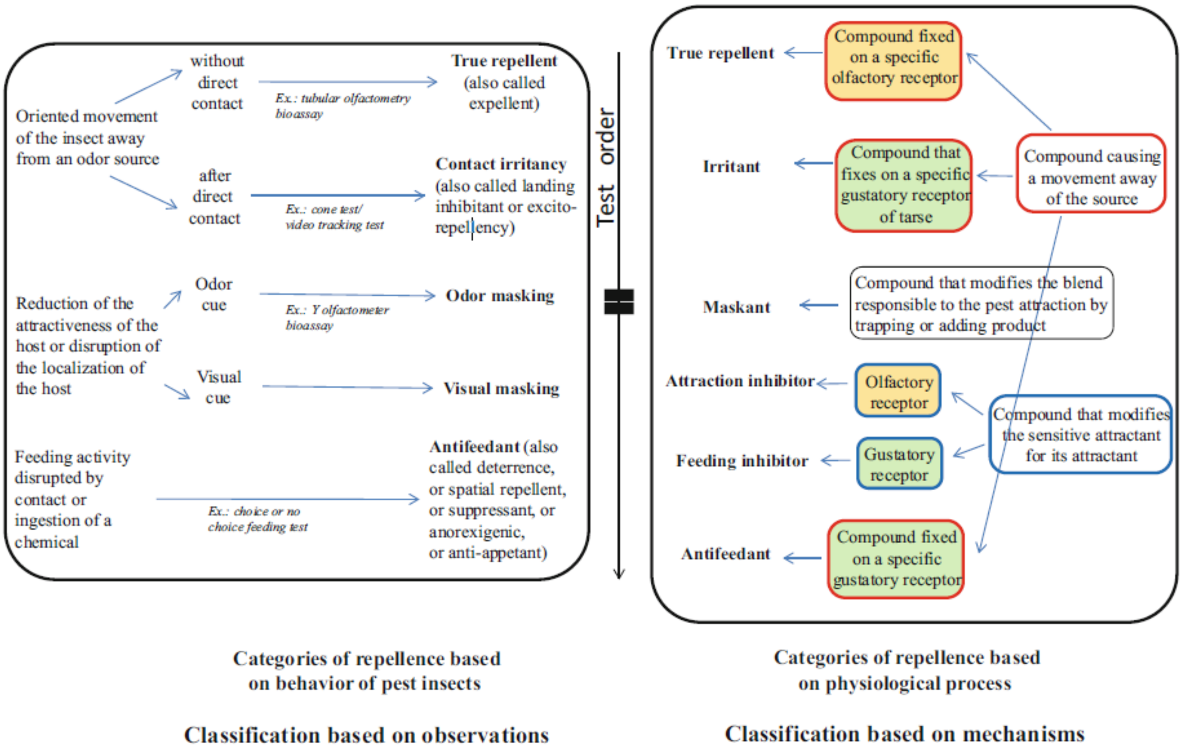


Figure 6 : Définitions des différents types de répulsifs associés à leurs mécanismes d'action

Pendant ma thèse, j'ai ainsi montré que les huiles essentielles de lemongrass (*Cymbopogon citratus*), de cannelle (*Cinnamomum zeylanicum*), de cumin (*Cuminum cyminum*) et de citronelle (*Cymbopogon winternarius*) étaient des répulsifs spatiaux de *Bemisia tabaci* (Deletre et al. 2015). Parmi les composés de ces huiles essentielles, le cinnamaldéhyde, le cuminaldéhyde, le geraniol, le citronellol et l'acétate de géranyl étaient les composés bioactifs des répulsifs spatiaux de *B. tabaci* (Deletre et al. 2016b). Mais le plus important, c'est que nous avons noté que le phénomène de répulsion pouvait être dû à un ou plusieurs composés, qu'il s'agissait aussi bien du composé majoritaire que d'un composé minoritaire et que l'effet dépendait également de la concentration. Plus récemment, nous avons montré que l'huile essentielle de gros thym, *Plectranthus amboinicus* (Lour.) Spreng, avait un effet répulsif spatial et masquant contre *Bemisia tabaci* (Pouet et al. 2022). Cette plante est donc prometteuse pour être utilisée comme plante compagne afin de retarder l'infestation des plants de tomate par *B. tabaci.*

Après mes travaux de thèse sur la compréhension des comportements de répulsion, j'ai été recrutée par l'unité HortSys afin de mettre au point une stratégie push-pull contre les principaux ravageurs de la tomate, identifiés suite à nos travaux comme *T. absoluta* et *T. vaporariorum.* Pour ce qui est des stimuli répulsifs (push), je me suis intéressée aux composés induits par l'herbivorie. En effet, les plantes émettent des composés organiques volatils mais elles libèrent des mélanges de composés organiques volatils différents lorsqu'elles sont attaquées par des insectes herbivores, appelés composés organiques volatils induites par l'herbivorie (HIPVs) (Turlings et Erb, 2018). Ces composés peuvent être différenciés quantitativement et qualitativement des composés organiques volatils émis par les plantes saines. Pour la lutte contre les ravageurs, ces composés offrent deux mécanismes directs de défense. Ils agissent comme moyen de dissuasion pour la ponte et l'alimentation des ravageurs. Par exemple, les plantes de tabac infestées par *Heliothis virescens* émettent des HIPVs qui sont répulsifs pour les femelles, diminuant ainsi son activité d'oviposition (De Morales et al. 2001). Mais ces composés sont également impliqués dans la communication de plante à plante (Turlings et Erb, 2018). Par exemple, le (Z)-3-hexénol émis par des plants de tomates infestés de *Spodoptera litura* a été absorbé et transformé en (Z)-3-hexényl-vicianoside par des plants de tomates voisins, ce qui réduit l'endurance et le développement des nouvelles chenilles (Sugimoto et al. 2014).

Dans le cas de *T. absoluta,* nous avons montré que même si cet insecte était un spécialiste de la tomate, les mâles et les femelles accouplées étaient plus attirés par les plants de tomates sains qu'infestés de certaines variétés (Deletre et al. In prep). Parmi les composés volatils induit par *T. absoluta*, nous avons identifié l'α-pinene, le trans-isolimonene, le sabinene, le β-myrcene, le 2-carene, l'α-phellandrene, le 3-carene, l'α-terpinène, le β-phellandrene, le (Z)-β-ocimène, l'(E)-β-ocimène, l'allo-ocimène, le δ-élemène, le terpinolene, l'(E)-β-caryophyllene et le salicylate de méthyle. Parmi ces composés, l'α-phellandrène, le γ-terpinène, le terpinolène et l'ocimène ont diminué l'attractivité de la tomate (répulsif masquant) pour *T. absoluta*. Ces composés pourraient être utilisés comme des répulsifs masquants (simuli push) en combinaison avec l'utilisation de la phéromone sexuelle (stimuli pull) de *T. absoluta* dans une stratégie push pull. Cette phéromone est déjà utilisée pour de la surveillance, du piégeage de masse ou de la confusion sexuelle mais l'efficacité est encore faible surtout en plein champs (Caparros Megido et al. 2013). La combinaison des deux techniques pourrait avoir un effet synergique.

Dans le cas de *T. vaporariorum,* nous avons montré que cet insecte était plus attiré par les plants de tomates sains qu'infestés de certaines variétés (Deletre et al. 2022). Parmi les composés induits par l'herbivorie de *T. vaporariorum,* nous avons identifié l'o-xylène, le sabinene, le β-pinène, le β-ocimène, le β-myrcène, l'α-phellandrène, le 3-carène, le p-cymène, le β-phellandrène, l'α-humulène, le γ-terpinène, le (E)-β-caryophyllene, l'allo-ocimène, le β-elemène et le terpinolène. Parmi les composés émis par la tomate, le limonène, le p-cymène et le 1,8 cinéole ont diminué l'attractivité de la tomate (répulsif masquant) et seul le p-cymène s'est avéré être un HIPV. Mais parmi les HIPVs, le

(E)-β-ocimène, le p-cymène et l'α-pinène ont été identifiés comme des répulsifs spatiaux pour *T. vaporariorum* ainsi que le limonène, tandis que le 1,8-cinéole s'est avéré attractif lorsqu'il était testé contre l'air pur. Les résultats sur la répulsion des HIPVs vis-à-vis des ravageurs ont ainsi été moins probants pour *T. vaporariorum* que pour *T. absoluta*. Cela pourrait s'expliquer par le fait que *T. vaporariorum* est un insecte polyphage alors que *T. absoluta* est un insecte spécialiste de la tomate, même s'il peut s'attaquer à d'autres solanaceae. *T. absoluta* et *T. vaporariorum* ont également différents modes d'alimentation (broyage/extraction de feuilles versus succion de sève de phloème), ainsi la variation de composés organiques volatils induits par ces herbivores est par conséquent attribuée à l'activation des différentes voies de défense (Silva et al. 2017, Danner et al. 2018). Les larves de *T. absoluta* activent la voie de biosynthèse de l'acide jasmonique et déclenchent l'émission de grandes quantités de composés organiques volatils par rapport aux aleurodes qui sont connus pour activer la voie de l'acide salicylique (Danner et al. 2018, Yang et al. 2021).

Avec l'identification de composés répulsifs contre *T. vaporariorum*, nous avions identifié les stimuli push de notre stratégie push-pull. Pour cette stratégie, les stimuli pull sont souvent des attractifs visuels, des composés volatils de plantes hôtes, des phéromones d'agrégation ou sexuelles, des attractifs d'oviposition ou des attractifs gustatifs (Cook et al. 2007). Contrairement à *T. absoluta,* aucune phéromone d'agrégation ou sexuelle n'a été identifié pour *T. vaporariorum.* Nous aurions pu utiliser des attractifs visuels puisqu'aujourd'hui les pièges englués jaunes sont utilisés pour la surveillance de *T. vaporariorum* mais l'efficacité nous semblait trop faible. Contrairement à *T. absoluta, T. vaporariorum* est un ravageur polyphage, l'identification d'une plante piège, i.e. une plante plus attractive que la tomate, nous semblait être une bonne piste de recherche. En 2014, nous avions observé sur le terrain des *T. vaporariorum* sur l'œillet d'inde géant ou tagete des décombres (*Tagetes minuta*) et le basilic (*Ocimum basilicum*). Lors de deux expériences en station expérimentale au Kenya, nous avons observé que l'œillet d'inde géant réduisait significativement la population d'aleurodes des serres dans les cultures de niébé, tandis que la culture intercalaire de basilic et de tomates avait entraîné une réduction de 68,7 % dans la population d'aleurodes (Diabate et al. 2019 ; Mutisya et al. 2016 ; Diabate et al. 2021). Au brésil, il avait également été montré que la tomate cultivée en intercalaire avec le basilic avait de meilleur rendement (Carvalho et al. 2009). De plus, l'utilisation d'huile essentielle de basilic avec des pièges jaunes englués augmente l'attractivité et donc le piégeage de *T. vaporariorum* de 4,8 fois (Górski 2004). Par la suite au laboratoire, nous avons montré que le basilic en fleurs et l'œillet d'inde au stage végétatif ou en floraison ont été plus attractif que la tomate pour *T. vaporariorum* (Matu et al. 2021). Les composés majeurs de l'œillet d'inde étaient le limonène, la dihydrotagétone, le (Z)-β-ocimène, l'α-pinène, et l'acétate de (Z)-3-hexényle, et ceux du basilic étaient le linalool, le 1,8-cinéole, l'eugénol et le β-élémène. Parmi ces composés, le 1,8-cinéole et l'acétate de (Z)-3-hexényle ont provoqué une forte attraction chez l'aleurode des serres, tandis que le (Z)-β-ocimène et le linalool ont provoqué une forte répulsion. Le 1,8 cinéole en fonction de sa concentration et des odeurs en arrière plant peut avoir des effets très différents sur le comportement de *T. vaporariorum.* L'œillet d'inde géant, le basilic ou l'acétate de (Z)-3-hexényle pourraient donc être utilisés comme des stimuli pull dans notre stratégie push pull.

2.2.4 Manipulation du comportement des ennemis naturels

Etudiants associés à ce volet :
P. Ayelo: Recruitment of natural enemies through kairomones for crop protection (Univ Pretoria, 2017-21)
M. Wangari (2020): Study of kairomones to enhance biological control of whiteflies (Methodist Univ, Kenya)

Articles associés à ce volet :

- Chailleux A, Ndjiliw , Diakhaté M, Akodjetin GF, Correa P, **Deletre E,** Brévault T (soumis) First steps toward conservation biological control programs using *Nesidiocoris tenuis* in Senegal. *Biological control.*
- **Deletre E,** Wangari M, Musila R, Rhino B (soumis) Use of natural enemies attractant to attract and retain parasitoids to control whitefly, *Trialeurodes vaporariorum* in openfield and nethouse.
- P Ayelo, S Mohamed, A Chailleux, A Yusuf, C Pirk, **E Deletre**. 2022b. The parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato plants. *Journal of Pest Science*. 95, 633–652
- P. Ayelo, A. Yusuf, A. Chailleux, S. Mohamed, C. Pirk, E. Deletre. 2022a. Chemical cues from honeydew and cuticular extracts of *Trialeurodes vaporariorum* serve as kairomones for the parasitoid *Encarsia Formosa. Journal of Chemical Ecology*, 1-14
- P Ayelo, A Yusuf, C Pirk, A Chailleux, S Mohamed, **E Deletre**. 2021c. Terpenes from herbivore-induced tomato plant volatiles attract *Nesidiocoris tenuis* (Hemiptera: Miridae), a predator of major tomato pests. *Pest Management Science*. 77 (11), 5255-5267
- P Ayelo, A Yusuf, C Pirk, S Mohamed, A Chailleux, **E Deletre**. 2021b. The role of *trialeurodes vaporariorum*-infested tomato plant volatiles in the attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Journal of Chemical Ecology*, 47(2), 192-203.
- P Ayelo, C Pirk, A Yusuf, A Chailleux, S Mohamed, **E Deletre**. 2021a. Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: A Review. *Frontiers in Ecology and Evolution*, 9, 143.

La lutte biologique classique et l'industrie associée à cette stratégie de lutte sont peu développées en Afrique pour le moment, même si le kenya fait exception à la règle à cause de l'importance de son industrie de production de fleurs coupées. Cependant, les petits producteurs n'ont pas recours à la lutte biologique classique, de par leur manque de connaissances et de par le manque d'accessiblité aux produits : ennemis naturels, entomopathogènes, … La lutte biologique par conservation semble donc être la plus indiquée pour les petits producteurs. L'idée est de manipuler l'habitat des ennemis naturels afin d'améliorer leur survie, leurs performances comportementales et/ou physiologique ce qui se traduit par une efficacité accrue de la gestion des ravageurs (Rusch et al. 2017). Ce type de biocontrôle est une alternative prometteuse à l'utilisation des pesticides et une composante importante de l'agroécologie. La manipulation de l'habitat consiste principalement à réduire les perturbations de l'agroécosystème, tout en augmentant la biodiversité végétale. La biodiversité végétale peut conduire à l'augmentation de la biodiversité des ennemis naturels et de leur abondance mais c'est rarement suffisant pour atteindre un niveau de gestion des ravageurs satisfaisant. Par conséquent, le principal défi de cette stratégie de lutte est de savoir comment attirer et concentrer suffisamment d'ennemis naturels au sein de la culture pour atteindre un niveau satisfaisant de gestion des ravageurs. Ce défi se retrouve également en lutte biologique classique. En effet, lorsque les ennemis naturels sont relâchés, ils sont susceptibles d'émigrer ou de se disperser en dehors de la culture à protéger, réduisant ainsi leur efficacité dans la lutte contre les insectes ravageurs de la culture cible (Heimpel et Asplen 2011). Or l'écologie chimique s'intéresse aux interactions plantes-ennemis naturels, ravageurs-ennemis naturels et entre ennemis naturels, nous permettant ainsi d'identifier des sémiochimiques attractifs d'ennemis naturels. Les applications de leurres à base de sémiochimiques se sont avérés être une solution judicieuse pour recruter les ennemis naturels, les retenir à proximité des cultures cibles et limiter leur dispersion, améliorant ainsi les stratégies de lutte biologique (Ayelo et al. 2021a). Par exemple, Kelly et al. (2014) ont montré que l'utilisation de salicylate de méthyle (HIPV) ou de phéromone d'agrégation de *Podisus maculiventris* avait permis de retenir les punaises prédatrices relâchés sur la parcelle mais également d'attirer des punaises sauvages. Dans les parcelles avec les leurres, la consommation des chenilles de *Manduca sexta,* sa proie, avait augmenté. Nous avons ainsi émis l'hypothèse que l'utilisation de composés sémiochimiques et de plantes compagnes pouvaient augmenter l'efficacité de la lutte biologique ou des services écosystémiques de suppression des ravageurs.

Parmi les composés sémiochimiques impliqués dans la localisation et la reconnaissance des proies ou hôtes par les prédateurs ou parasitoides, il y a (i) les composés organiques volatiles induits par l'herbivorie ou l'oviposition, (ii) les phéromones des ravageurs et (iii) des signaux chimiques associés aux ravageurs provenant de leur fèces, du miellat, de leur cuticule, … (Figure 7) (Ayelo et al. 2021). Ces composés sont impliqués dans les différentes séquences comportementales de recherche de l'hôtes/proies. Les composés organiques volatiles induits par l'herbivorie ou l'oviposition interviendraient lors de la localisation de l'habitat, les phéromones lors de la localisation du ravageur, et les signaux chimiques associés aux ravageurs lors de sa sélection (Figure 8). Nous pourrions utiliser les composés organiques volatiles pour attirer plus d'ennemis naturels sur les parcelles et utiliser les phéromones pour limiter l'émigration des ennemis naturels vers d'autres parcelles.

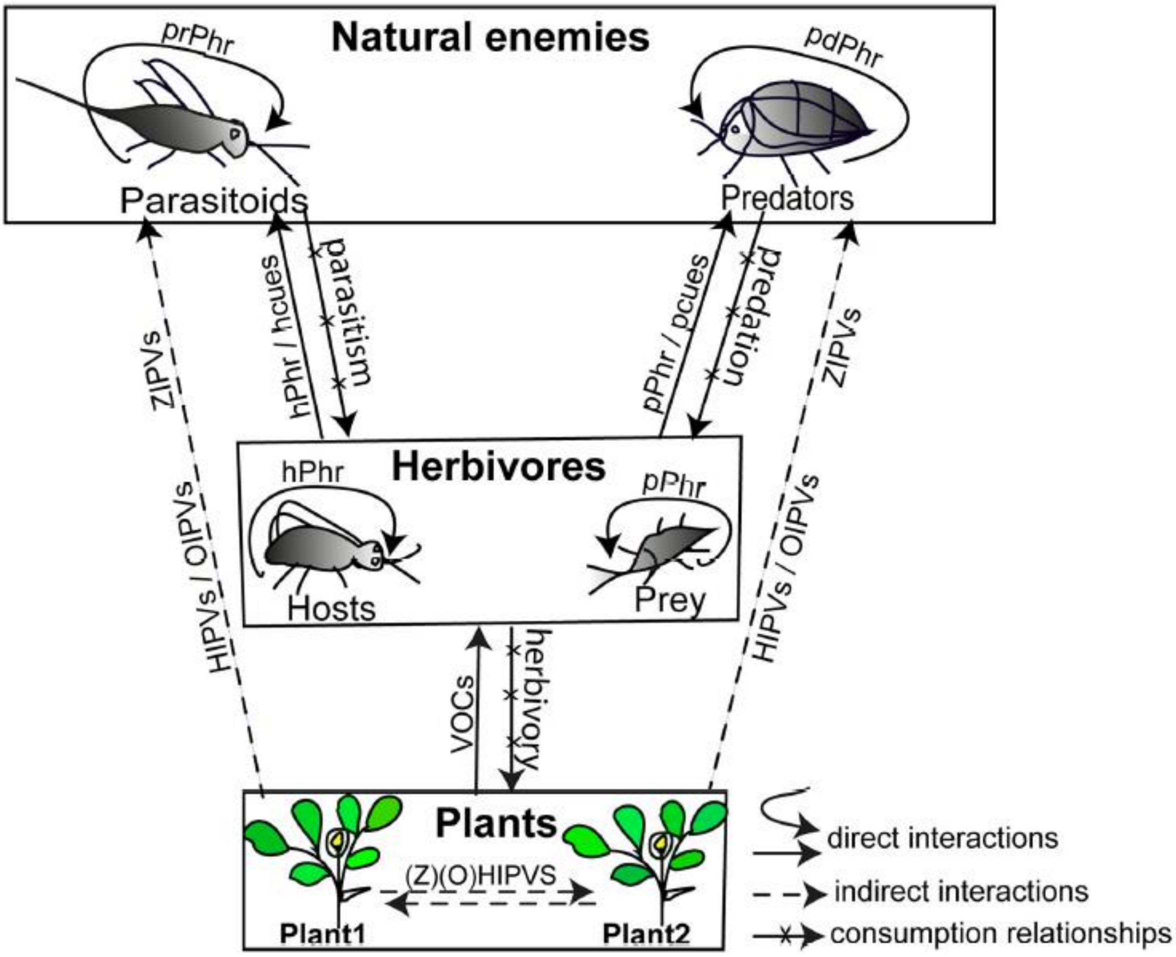


Figure 7. Les interactions chimiques au sein d'un système tritrophique. (HIPVs, composés organiques volatils induits par l'herbivorie ; OIPVs, composés organiques volatils induits par l'oviposition; ZIPV, composés organiques volatils induits par les zoophytophages ; COV, composés organiques volatils ; pPhr, phéromone de proie ; hPhr, phéromone d'hôte ; pdPhr, phéromone de prédateur ; prPhr, phéromone de parasitoïde ; pcues, signaux chimiques associés aux proies ; hcues, signaux chimiques associés à l'hôte) (Ayelo et al. 2021).

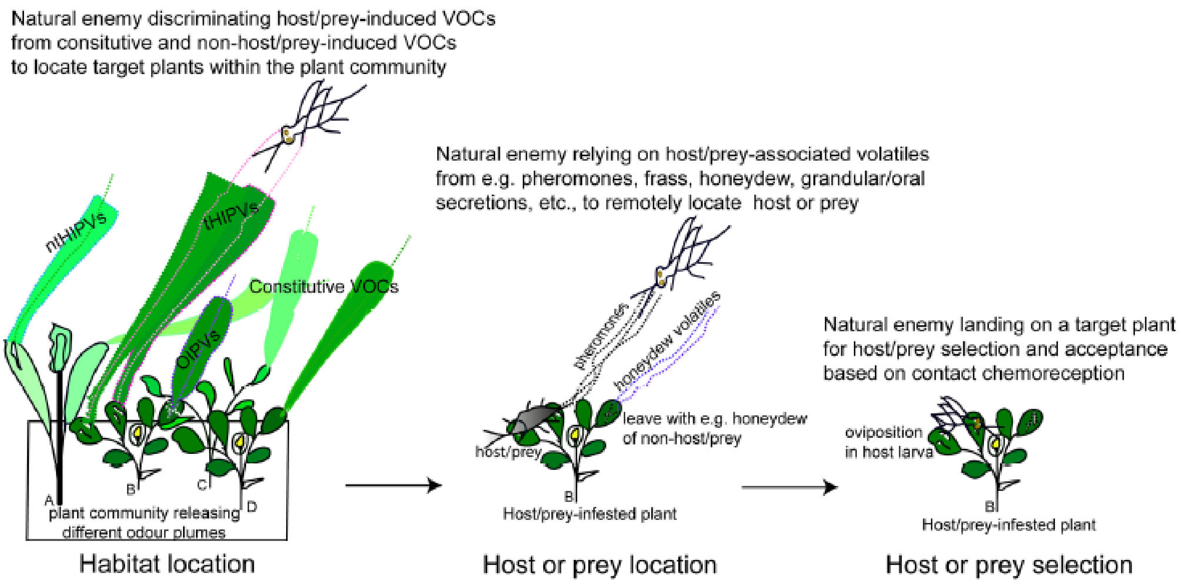


Figure 8. Utilisation des kairomones par les parasitoïdes/prédateurs lors des différentes séquences comportementales impliquées dans la recherche d'hôtes/proies (Communauté végétale composée de (A) plante non hôte infestée, (B) plante hôte infestée, (C) plante non hôte non infestée, et (D) plante hôte non infestée. VOC: composé organique volatil, tHIPV: composé organique volatil de plante hôte induite par l'herbivorie, ntHIPV: composé organique volatil de plante non hôte induite par l'herbivorie, OIPV: composé organique volatil de plante hôte induit par l'oviposition) (Ayelo et al. 2021).

Dans le cas d'*Encarsia formosa,* parasitoïde de *Trialeurodes vaporariorum,* nous avions émis l'hypothèse qu'il serait attiré par (i) les HIPVs de plants de tomate infestés par des aleurodes, (ii) le miellat des aleurodes et/ou (iii) les composés de la cuticule des nymphes. Nous avons montré qu'*E. formosa* était plus attiré par des plants de tomate infestés que par des plants sain (Ayelo et al. 2021b). Ce parasitoïde a donc été attiré par les HIPVs de plants de tomates infestés par *T. vaporariorum*, mais sa réponse comportementale a varié en fonction de la densité d'infestation. En effet, la plante n'émet pas la même quantité ou les mêmes composés organiques volatils en fonction de la densité d'infestation (Shiojiri et al. 2010). Le parasitoïde *E. formosa* a été attiré par les composés organiques volatils émis par des plants de tomates infestés de 100 *T. vaporariorum* adultes mais pas par des plants de tomates infestés de 50 ou 200 adultes. De même, Lou et al. (2005) ont montré que le parasitoïde *Anagrus nilaparvatae* a été attiré par les composés organiques volatils de riz infestés de 10 ou 20 cicadelles adultes de *Nilaparvata lugens*, mais qu'il n'était pas attiré par des plants de riz avec un niveau d'infestation inférieure (1 ou 5) ou supérieure (40 ou 80). Lorsque nous analysons les composés organiques volatiles émis par les plants de tomate faiblement (50) et fortement (200) infestés, nous observons que les HIPVs sont émis en faible quantité. Il faudrait donc un niveau d'infestation minimum pour activer les défenses de la plante. Par conséquent, les parasitoïdes ne sont attirés par des plantes infestées qu'à partir d'un seuil minimal d'infestation (Lou et al. 2005). De plus, les herbivores se nourrissant de phloème tels que les aleurodes qui sont des inducteurs de la voie de l'acide salicylique peuvent activer mais aussi supprimer les mécanismes de défense de la plante, et donc l'émission de HIPVs (Walling 2018). Par conséquent, au-delà d'un certain seuil d'infestation, ici 200 adultes, les défenses de la plante sont inhibées par l'herbivore et le parasitoïde n'est plus attiré. A une infestation de 100 *T. vaporariorum* adultes, la qualité et la quantité des composés organiques volatils émis par les plants de tomates ont varié par rapport aux plants sains, mettant ainsi en évidence une activation des défenses de la plante. Parmi les HIPVs de plants de tomate infestés par 100 *T. vaporariorum* adultes, nous avons identifié le 3-carene, l'allo-ocimene, le β-elemene, le p-cymene, l'α-phellandrene, le β-phellandrene, l'(E)-β-ocimene, l'α-pinene, le β-myrcene, le terpinolene et le δ-elemene. Nous avons aussi noté que l'émission d'α-phellandrène, de p-cymène, d'(E)-β-caryophyllène et d'α-humulène avait diminué pour les plants de tomates infestés par 200 adultes par rapport aux plants infestés par 100 adultes. Parmi les HIPVs*,* nous avons trouvé que le 3-carène, le β-myrcène, le β-ocimène et l'α-phellandrène attiraient *E. formosa*. Le mélange de ces quatre composés était également très attractif pour *E. formosa.* Ces quatre composés ont été rapportés comme des composés attractifs chez d'autres espèces de parasitoïdes. Par exemple, l'α-phellandrène et l'(E)-β-ocimène induits par une infestation de pucerons *Acyrthosiphon pisum* étaient attractifs pour le parasitoïde *Aphidius ervi* (Takemoto & Takabayashi 2015). Le mélange de ces quatres composés a ensuite été testé sur le terrain afin de tester notre hypothèse sur l'augmentation de l'efficacité des services écosystémiques (i.e. suppression des ravageurs) grâce à l'utilisation des composés sémiochimiques (Deletre et al. In prep). Ces composés ont été testé avec ou sans l'utilisation de filet anti-insectes. Pour cet essai, le nombre d'aleurodes sur les pièges jaunes englués, qui ont contribué au suivi des populations d'adultes, était plus important en plein champ que sous les filets (Figure 9a). Mais les aleurodes, qui ont émergé des feuilles échantillonnées, étaient plus nombreux sous les filets qu'en plein champs (Figure 9b). En effet, les plants de tomates en plein champs ont des feuilles qui sèchent plus rapidement que sous les filets, ce qui empêchent le bon développement des nymphes. Nordey et al. (2017) ont montré que les besoins en rayonnement de la tomate sont inférieurs au rayonnement du Kenya ce qui provoque une senescence précoce des feuilles. Contrairement au plein champ, l'environnement climatique sous les filets était propice à la croissance de la tomate et au développement des nymphes d'aleurodes. En effet, les plants de tomates cultivés sous filet ont une conductance stomatique plus élevée et des feuilles riches en chlorophylle (Saidi et al., 2013). En peu de temps, ils produisent plus de feuilles que les plants de tomates de plein champs. Kaur et al. (2010) ont également rapporté que la température et l'humidité élevées sous les filets fournissent un environnement optimal pour une multiplication rapide des ravageurs comme les aleurodes. Le mélange des quatres kairomones attractives d'*Encarsia formosa* ont attiré plus de parasitoïdes (Figure 9c). En effet, plus de parasitoïdes ont émergé des feuilles collectées dans les parcelles avec attractifs que sans attractifs. Le taux de parasitisme était de 11,60% et 42,08% avec attractifs en plein champ et sous filet respectivement, contre 7,8% et 20,33% sans attractif. Le taux plus important de nymphes sous filet a pu également joué un rôle dans l'attraction des parasitoïdes et l'environnement climatique sous les filets était également plus propice au développement des parasitoïdes. Mais l'attractif n'a pas eu d'impact proprement dit sur la population d'aleurodes. L'arrivée des parasitoïdes pourrait être trop tardive pour contrôler efficacement la population d'aleurodes. Même si les HIPVs ont montré des résultats prometteurs, nous nous sommes également intéressés au potentiel attractif du miellat et des composés de la cuticule de nymphes (Ayelo et al. 2022a). Nous avons montré que le parasitoïde *E. formosa* passait plus de temps sur les zones traitées avec des extraits de miellat et de nymphes que sur des zones non traitées. Par contre, l'odeur du miellat de *T. vaporariorum* attirait *E. formosa* mais pas l'odeur émise par les nymphes. Ceci suggère que le miellat sert à la localisation de l'hôte et la cuticule à la sélection de l'hôte. Les volatils du miellat étaient des composés tels que le 3-carène, l'α-phellandrène, le salicylate de méthyle, le β-ocimène, le β-myrcène et le (E)-β-caryophyllène qui sont connus pour être attractifs pour *E. formosa*. Les extraits cuticulaires des nymphes contenaient principalement des alcanes, des alcènes et des esters. Parmi ces composés, le parasitoïde a passé plus de temps sur les zones traitées avec du nonacosane que sur les zones non traitées suggérant que le nonacosane était impliqué dans la sélection des nymphes de *T. vaporariorum* par *E. formosa*.

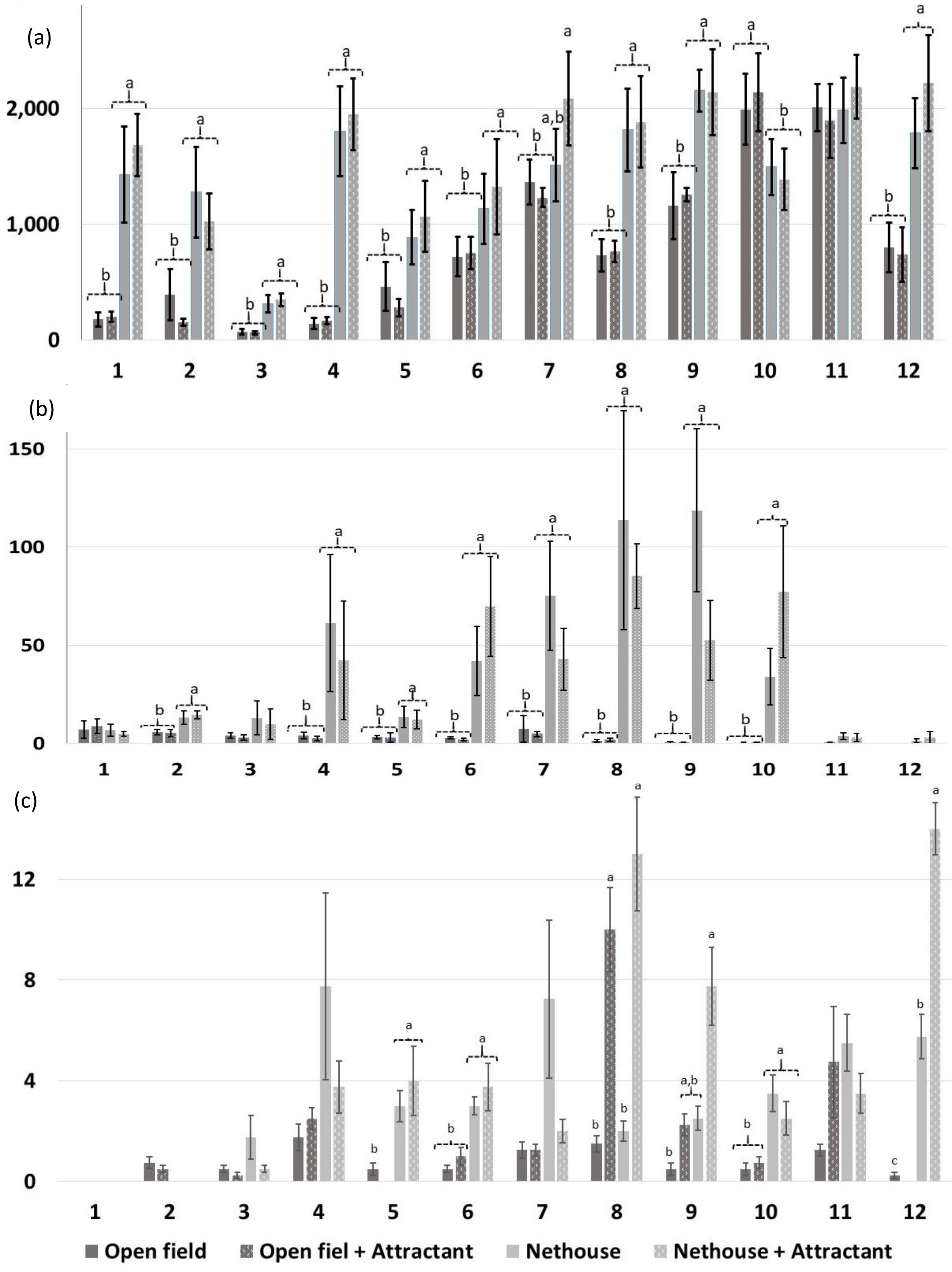


Figure 9. Population moyenne (±ES) par semaine de *T. vaporariorum* (a) sur les pièges jaunes englués, (b) émergeant des feuilles échantillonnées et de parasitoïdes (c) émergeant des feuilles échantillonnées. (Analyse statistique par GLMMs suivis d'une Anova de la déviance puis des comparaisons de moyennes. Selon les données, les lois de poisson, négative binomiale ou des modèles avec inflation de zéros ont été utilisées.)

Dans le cas du parasitoïde larvaire *D. gelechiidivoris*, nous avons émis l'hypothèse qu'il utilisait (i) les HIPVs de plants de tomate infestés par *T. absoluta*, (ii) la phéromone sexuelle de *T. absoluta*, (iii) les fécès de *T. absoluta* et/ou (iv) les composés de la cuticule des larves de *T. absoluta* pour détecter son hôte. Nous avons montré que les HIPVs d'un plant de tomate infesté par *T. absoluta* était attractif pour le parasitoïde *D. gelechiidivoris.* De plus, il était plus attiré par les odeurs de plants de tomates infestés que de plants sains, distinguant ainsi les différences quantitatives et qualitatives de la composition des composés organiques volatils émis par les plants de tomates infestés par rapport aux plants sains. De même, Suckling et al. (2012) ont également rapporté que le parasitoïde, *Dolichogenidea tasmanica* était plus attiré par les odeurs émises par les plantules de pommier (*Malus domestica*) infestées par la teigne brune de la pomme, *Epiphyas postvittana* que les odeurs émises par les plantules saines. Contrairement à *E. formosa,* plus le niveau d'infestation de la tomate par *T. absoluta* était élevé, plus le parasitoïde *D. gelechiidivoris* était attiré, c'est-à-dire que les plants infestés de 20 *T. absoluta* était plus attractif que ceux infestés par 5 ou 10 hôtes. De telles réponses comportementales dépendantes de la densité d'hôtes ont également été observées dans autres interactions tritrophiques plante-hôte-parasitoïde, en particulier pour les parasitoïdes dont l'hôte est un herbivore de type broyeur (Shiojiri et al. 2010). Par exemple, le parasitoïde *Cotesia vestalis* est attiré par les odeurs de choux infestés avec une forte densité (15–30 larves) de son hôte, *Plutella xylostella* par rapport à ceux avec une faible (5) densité d'infestation. En effet, les larves mineuses de feuilles sont connues pour activer les voies de biosynthèse de l'acide jasmonique (JA) et de l'acide salicylique qui peuvent agir en synergie pour induire l'émission de volatiles spécifiques (Turlings et al. 2004, Chen et al. 2006, Degenhardt et al. 2010, McCormick et al. 2012). L'activation de la voie JA induit la production des métabolites secondaires et des composés volatils induits par les herbivores qui augmentent avec la densité d'infestation et la durée d'alimentation. Dans notre étude, l'augmentation du niveau d'infestation par *T. absoluta* était positivement corrélée avec l'augmentation de l'émission d'α-pinène, de β-myrcène, d'α-phellandrène, d'α-terpinène, d'(E)-β-ocimène et d'(E)-β-caryophyllène. A forte densité d'infestation, les plants de tomate ont également émis de nouveaux composés par rapport aux plants de tomates sains : le (Z)-3-hexénol, l'ester (Z)-3-hexényl butanoate, le salicylate de méthyle, l'ester benzénoïde, le sabinène, le linalol, l'allo-ocimène et le β-élémène. Parmi ces composés, le parasitoïde *D. gelechiidivoris* a été attiré par l'α-pinène, le β-myrcène, l'α-phellandrène, l'α-terpinène, le β-ocimène, l'(E)-β-caryophyllène et le salicylate de méthyle. Ces attractifs sont également connus comme des kairomones pour d'autres espèces d'ennemis naturels. Le mélange de ces 7 attractifs s'est révélé être aussi attractif que les composés organiques volatils de tomates infestées par 20 larves de *T. absoluta.* Sur le terrain, ce mélange pourrait servir à attirer plus de parasitoïdes et/ou limiter leur émigration. Dans notre étude, nous avons également montré que le parasitoïde *D. gelechiidivoris* était attiré par l'odeur des fécès de larves de *T. absoluta.* A l'exception de l'oxyde de caryophyllène, qui auraient pu résulter d'un processus d'oxydation, tous les composés volatils émis par ces fécès de *T. absoluta*, que nous avons identifiés, sont aussi des composés émis par les plants de tomates dont le β-myrcène, l'α-terpinène, l'(E)-β-caryophyllène et le salicylate de méthyle, qui sont des attractifs du parasitoïde. Nous avons également montré que le parasitoïde *D. gelechiidivoris* était attiré par la phéromone sexuelle de *T. absoluta*. Cette phéromone qui est aujourd'hui utilisée pour le piégeage de masse et la confusion sexuelle pourrait donc avoir un effet bénéfique sur la lutte biologique en attirant plus de parasitoïdes mais aussi un effet néfaste avec le risque potentiel de les piéger. La détection de ce type d'odeur (fécès, phéromone) aide le parasitoïde à localiser précisément son hôte dans la parcelle de tomate. En effet, il a déjà été montré que les parasitoïdes localisent leurs hôtes en utilisant les kairomones émanant d'eux, comme les fécès ou les phéromones. Par exemple, le parasitoïde *Telenomus euproctidis* est attiré par la phéromone sexuelle de son hôte, *Orgyia postica* (Arakaki et al. 2011) ou le parasitoïde *Dibrachys cavus* est attiré par l'odeur des fécès des larves de son hôte, *Lobesia botrana* (Chuche et al. 2006). Par contre, lors de nos essais, l'odeur des larves de *T. absoluta* n'a pas attiré le parasitoïde, *D. gelechiidivoris*.

Dans le cas du prédateur généraliste *Nesidiocoris tenuis*, nous avons émis l'hypothèse qu'il utilisait: (i) les HIPVs de plants de tomate infestés par *T. absoluta* ou par *T. vaporariorum*, (ii) la phéromone sexuelle de *T. absoluta*, (iv) les fécès de *T. absoluta,* (v) le miellat de *T. vaporariorum* et/ou (vi) les composés de la cuticule des larves de *T. absoluta* ou des nymphes *T. vaporariorum* pour détecter ses proies. Nos résultats ont révélé que les kairomones associées aux proies jouent un rôle mineur dans l'attraction du prédateur généraliste *N. tenuis*. Même si l'odeur des fèces de *T. absoluta* a attiré le prédateur ; le miellat de *T. vaporariorum,* la phéromone sexuelle de *T. absoluta,* et l'odeur des larves de *T. absoluta* ou des nymphes *T. vaporariorum* n'ont pas attiré le prédateur. Comme indiqué précédemment, les composés volatils émis par les fécès de *T. absoluta*, que nous avons identifié, sont aussi des composés émis par les plants de tomates (e.g. l'α-pinène, le 3-carène, l'α-phellandrène, le β-phellandrène et le β-ocimène) ce qui pourrait expliquer l'attraction de *N. tenuis* par les fécès. L'absence d'attraction de *N. tenuis* aux odeurs de miellat peut s'expliquer par le fait qu'il s'agit d'un prédateur zoophytophage généraliste qui préféreraient se nourrir des plantes plutôt que de miellat, contrairement au parasitoïde *E. formosa* qui s'en nourrit. De plus, *N. tenuis* est un prédateur généraliste et qui n'a pas co-évolué avec *T. absoluta*, il n'a donc pas développé d'attraction spécifique pour la phéromone sexuelle de *T. absoluta.* L'utilisation de cette phéromone pour la gestion de ce ravageur n'a donc pas d'influence sur ce prédateur. Par contre, nous avons montré que *N. tenuis* était attiré par les HIPVs de plants de tomate infestés par *T. absoluta* ou par *T. vaporariorum* mais qu'il était plus attiré par les plants infestés de *T. absoluta* que de *T. vaporariorum.* Comme indiqué précédemment les HIPVs de ces 2 ravageurs ne sont pas les mêmes car ils n'activent pas les mêmes voies de biosynthèse. Ce prédateur arrive donc à discriminer la présence des ravageurs et le type de ravageurs. De plus, *N. tenuis* se nourrit davantage de *T. absoluta* que d'aleurodes (Calvo et al. 2012), il est donc possible que le prédateur ait une préférence innée pour les odeurs de plantes induite par son espèce de proie préférée. Nos résultats ont montré que parmi les HIPVs, l'α-pinène, le 3-carène, l'α-phellandrène, le β-phellandrène, l'(E)-β-caryophyllène et le β-ocimène étaient les composés attractifs de *N. tenuis*. Ces composés étaient d'ailleurs plus fortement émis par les plants infestés par *T. absoluta* que par *T. vaporariorum.* Nous pouvons de nouveau noter l'importance de la quantité des composés organiques volatils dans la réponse comportementale des insectes. Le mélange de ces quatre attractifs l'α-pinène, le 3-carène, l'α-phellandrène, et le β-ocimène s'est montré extrêmement attractif. Lors de notre essai terrain sur l'utilisation des attractifs d'*E. formosa*, nous avons utilisé un mélange de quatre composées dont trois sont aussi des attractifs de *N. tenuis* : le 3-carène, le β-ocimène et l'α-phellandrène. Nous avons également mesuré l'impact de ce mélange sur la population de *N. tenuis.* Le mélange de ces attractifs ne semblent pas avoir eu d'effet sur la population de prédateur et comme attendu, très peu de prédateurs ont été observé sous les filets (Figure 10). Afin d'introduire des prédateurs sous les filets sans avoir à passer par des entreprises de biocontrole, nous avons eu l'idée de rechercher des plantes insectarium afin d'attirer des prédateurs et pouvoir les relâcher par la suite (Chailleux et al. Soumis). Parmi les plantes compagnes, les plantes insectarium sont des éléments clés de la lutte biologique par conservation. Elles sont définies comme une plante qui attire et entretient une population d'ennemis naturels, généralement grâce aux nectars ou aux pollen et qui contribuent à la lutte biologique contre les ravageurs des cultures (Parolin et al. 2012). Nous avons identifié une plante insectarium prometteuse, *Sesamum radiatum* (faux sésame ou sésame noir) qui pourrait également servir de légume feuilles et de plantes médicinales. Cette plante permet le bon développement de *N. tenuis* même sans source de nourriture alternative. Si cette plante est utilisée en interculture pour de la lutte biologique par conservation, nous avons montré que *N. tenuis* était plus attiré par les plants de tomate que de sésame, il n'y a donc pas de risque qu'il quitte la culture de tomate. Cette plante pourrait également être utilisée entre 2 saisons de culture de tomates afin de maintenir une population constante de *N. tenuis.* Elle peut aussi être utilisée comme plante piège pour relâcher des *N. tenuis* sous les filets.

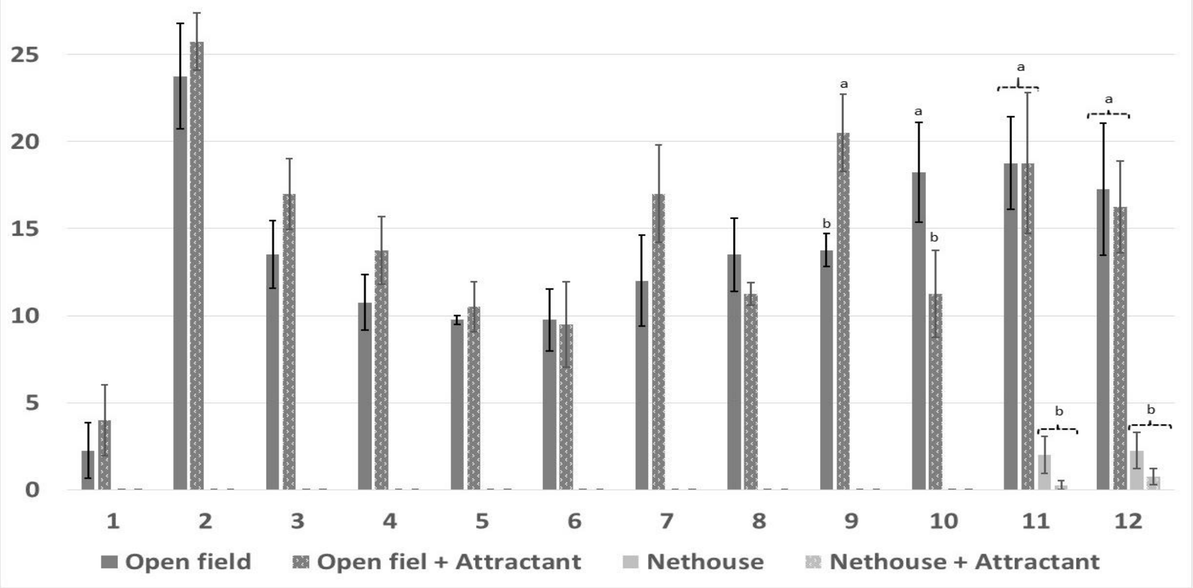


Figure 10. Population moyenne (±ES) par semaine de *N. tenuis* par plant de tomate. (Analyse statistique par GLMMs suivis d'une Anova de la déviance puis des comparaisons de moyennes. Selon les données, les lois de poisson, négative binomiale ou des modèles avec inflation de zéros ont été utilisées.)

2.2.5 Conclusion

L'étude de la biodiversité et des différentes interactions trophiques nous a permis d'identifier les ravageurs et les ennemis naturels qui jouent un rôle majeur dans la production de tomates et son rendement. Ainsi nous avons pu montrer par exemple qu'il n'y avait pas de parasitoïdes locales abondants contre *T. absoluta* d'où l'introduction d'un parasitoïde exotique *D. gelechiidivoris*. Nous avons pu également montré que l'aleurode local est *T. vaporariorum* et non *B. tabaci,* qui est la cause d'un nombre important de traitements chimiques par les producteurs, et qui est pourtant un ravageur moins problématique que *B. tabaci.*

Grâce à l'identification de composés sémiochimiques, qui sont impliqués dans les différentes interactions du réseau trophiques de la tomate (figure 11), nous avons pu développer différentes innovations, comme par exemple:

- un attractif pour chacun des ennemis naturels sélectionnés à base d'HIPVs,
- une stratégie push-pull pour lutter contre *Tuta absoluta* à base de phéromone sexuelle et d'HIPVs,
- une stratégie push-pull pour lutter contre *Trialeurodes vaporariorum* à base de plantes pièges et d'HIPVs.

Nous pouvons remarquer qu'une même molécule peut jouer plusieurs rôles dans le réseau trophique. Par exemple, une phéromone sexuelle peut aussi bien attirer un congénère qu'un ennemi naturel et un HIPV peut avoir un effet répulsif sur un ravageur ou un effet attractif sur un ennemi naturel.

En conclusion, l'utilisation des filets anti-insectes nous permet de diminuer l'usage des pesticides et les innovations conçues grâce à l'écologie chimique nous permettent, entre autres, d'augmenter les services écosystémiques de suppression des ravageurs. Cette combinaison de technique nous permet d'avoir un système de production plus agroécologique et plus durable.

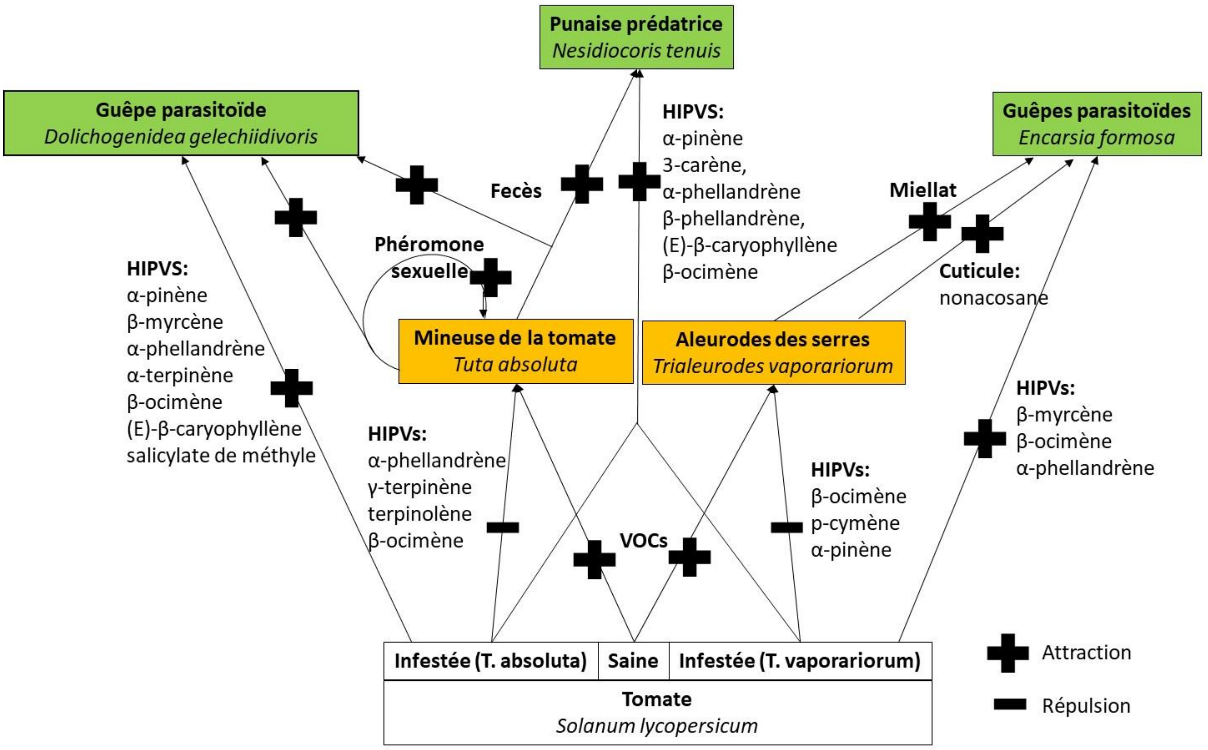


Figure 11. Schéma récapitulatif des interactions chimique de notre agroécosystème de la tomate

2.3 Volet 2 : Fonctionnement de l'agroécosystème de la mangue mouche des fruits

2.3.1 La biodiversité de l'agroécosystème de la mangue

Etudiants encadrés associés à ce volet :
B. Mekonnen: Improving biological control of fruit flies using *O. longinoda* (Univ Pretoria, 2016-21)
A Stirnemann (2015): Study of attractant plant for the management of *O. longinoda* (Univ Rennes, France)
J Leyes (2015): Study of repellent natural compounds for the control of *O. longinoda* (Univ Rennes, France)

Articles associés à ce volet :

- B Mekonnen, J Haran, R Copeland, C Pirk, A Yusuf, **E Deletre**, 2020. First report of a gall midge as a parasitoid of weaver ants, *Entomologia Generalis* 40(4): 437-441
- A Chailleux, A Stirnemann, J Leyes, **E Deletre**. 2019. Manipulating natural enemy behavior to improve biological control: attract and repulse. *Entomologia Generalis* 38(3):191-210

En afrique, les ravageurs de la mangue sont les cochenilles farineuses (Rastrococcus sp.), les cochenilles (Coccus spp.), les aleurodes, etc mais principalement les mouches des fruits. Les espèces de *Ceratitis* sp. qui affectent la production de mangues sont *Ceratitis quinaria*, *C. fasciventris*, *C. rosa*, *C. anonae*, *C. capitata*, et *C. cosyra,* cette dernière est celle qui cause le plus de dégâts (Figure 12) (Ekesi et al. 2006). L'autre ravageur qui cause le plus de dégâts à la mangue en Afrique est également une mouche des fruits : *Bactrocera dorsalis* qui est aussi une espèce invasive. Dans les zones propices à *B. dorsalis, C. cosyra* serait quasiment remplacée par *B. dorsalis* qui est plus agressive (Ekesi et al. 2009). Ces 2 espèces sont polyphages et attaquent un large éventail de cultures fruitières en plus de la mangue comme la goyave, les agrumes et l'avocat. Les mouches des fruits pondent leurs œufs dans les fruits où se développent ensuite les larves causant ainsi de 20 à 80% de perte selon l'espèce et les pays. En plus des pertes directes, une perte indirecte importante se produit également lorsque les opportunités d'accès au marché sont perdues en raison des restrictions européennes de quarantaine de *B. dorsalis.*

Parmi les mesures mise place pour lutter contre ces ravageurs, il y a l'utilisation d'une large gamme d'insecticides mais aussi des biopesticides comme l'utilisation du spinosad. La technique de piégeage de masse est largement utilisée pour les mouches des fruits. Le methyle eugenol est une kairomone efficace pour piéger les mâles de *Bactrocera dorsalis* et l'acétate de terpinyle est utilisé pour piéger *Ceratitis cosyra.* Le piégeage peut être combiné avec un agent biocide comme le champignon entomopathogène *Metharizium anisopliea,* qui est aussi utilisé en pulvérisation au niveau du sol pour tuer les pupes. Le piégeage alimentaire est également utilisé avec des attractifs à base de levures ou dérivés et un biocide. Cette technique a l'avantage de piéger les mâles et les femelles mais le désavantage d'être très peu sélectif. L'assainissement des vergers avec l'utilisation d'un augmentorium, qui favorise également l'accroissement des populations de parasitoïdes, fait partie des techniques simples à mettre en place mais avec un fort impact sur les populations de mouches des fruits. Les producteurs récoltent également leur mangue plus tôt dans la saison avant que les mouches des fruits piquent les mangues. Enfin la lutte biologique est également utilisée pour réduire la population de mouches des fruits et ses dégâts.

Au Kenya, trois agents de lutte biologique sont présents abondamment, deux parasitoïdes : *Fopius arisanus* (Sonan) et *Diachasmimorpha longicaudata* (Ashmead), qui ont été introduits par l'icipe en 2006 en provenance d'Hawaï pour lutter principalement contre *B. dorsalis,* et un prédateur natif : *Oecophylla longinoda* (Latreille). *Fopius arisanus* parasite les œufs des mouches des fruits et l'adulte émerge au stage nymphal. *Diachasmimorpha longicaudata* parasite les larves des mouches des fruits et l'adulte émerge également au stage nymphal. *Oecophylla longinoda* est une fourmi prédatrice généraliste, elle se nourrit principalement des larves et des pupes de mouches des fruits. Malgré son efficacité comme agent de lutte, les nids de fourmis d'*O. longinoda* sont parfois détruits dans les vergers car elles sont très agressives et deviennent gênante au moment de la récolte. Nous avons cherché à pouvoir manipuler leur comportement pour faciliter la récolte. Notre objectif était de trouver un répulsif afin de protéger les producteurs tout en proposant des moyens d'augmenter les populations de fourmis oecophylles, en trouvant des sources de glucides à partir de plantes cultivées locales (attractif). La diversification végétale est, en effet, un outil souvent utilisé en lutte biologique par conservation pour augmenter les populations d'ennemis naturels et ainsi les services écosystémiques qu'ils apportent. Nous avons identifié des répulsifs huileux (huile de paraffine ou huile de coco) ou poudreux (cendres, farine, talc) qui pourraient aider les producteurs à se protéger des morsures. De plus, le niébé en interculture avec les manguiers pourrait servir de sources de glucides grâce à la production de nectar extra-florale, ce qui favoriserait la croissance de la population de fourmis. En effet, nous avons clairement observé un comportement de recrutement vers le nectar extra-floral avec des comportements de frottements de l'abdomen pour libérer les phéromones de marquage, de mouvements antennaires et d'ouverture de la bouche comme précédemment décrits par Hölldobler & Wilson (1978).

Lors de nos différents travaux de recherche, nous avons également eu la surprise de découvrir un parasitoïde d'*O. longinoda* qui s'est avéré être une nouvelle espèce : une cécidomyie (Diptera: Cecidomyiidae) (Figure 13). Malheureusement nous n'avons jamais pu observer le stade adulte, seul le stade larvaire a pu être observé. Nous avons émis les hypothèses que (i) lorsque les fourmis parasitées sont tenues à l'écart de la colonie, leur fitness diminue à cause du manque de comportements sociaux et les larves de parasitoïdes quittent alors leur hôte en raison de sa mauvaise qualité avant d'avoir complété leur cycle; ou (ii) lorsqu'elles sont gardées dans la colonie, les larves de parasitoïdes sont consommées par les fourmis en quittant l'hôte. Quoiqu'il en soit, la séquence (MN603974) a été enregistrée dans les bases de données (GenBank), d'autres chercheurs pourraient trouver la forme adulte de cet insecte et nous pourrions ainsi décrire cette espèce.

Nous nous sommes également intéressés aux pollinisateurs du manguier au Kenya. Deux pollinisateurs ont été identifié : *Hypotrigona gribodoi* (Magretti), une espèce de Meliponini et un Apidae. Nous les avons aussi bien observés dans les vergers avec que sans des fourmis oecophylles, suggérant que les fourmis oecophylles n'avaient pas d'impact néfastes sur la pollinisation.

Ainsi, nos recherches au laboratoire, en station et chez des producteurs nous ont permis d'établir en partie la biodiversité de l'agroécosystème de la mangue au Kenya (Figure 12).

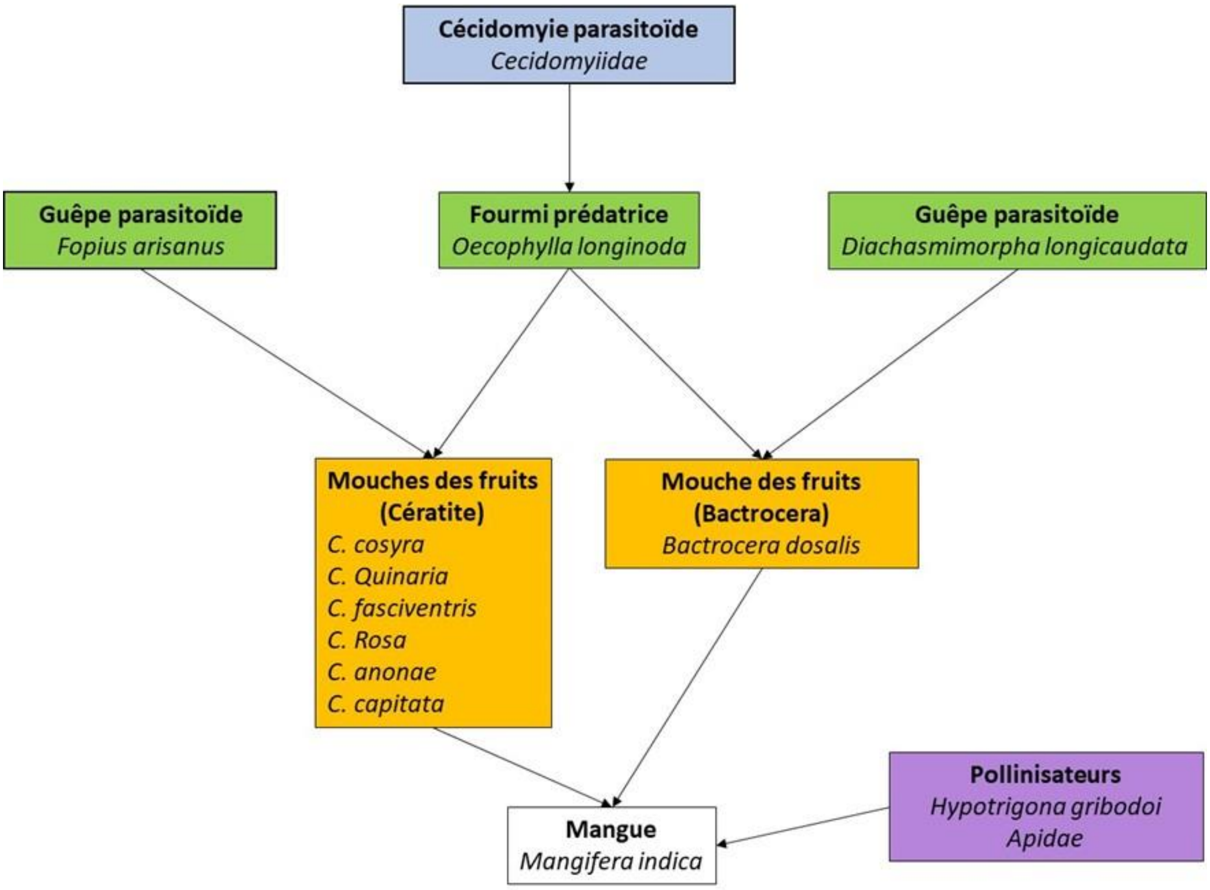


Figure 12. Biodiversité de l'agroécosystème de la mangue au Kenya. (orange = ravageurs, vert = ennemis naturels, violet = pollinsateurs, bleu = hyperparasitoïde)

Figure 13. Fourmi ouvrière *Oecophylla longinoda* parasitée avec la sortie de la larve de cécidomyie (Photo Dr S. Sevgan) et larve de la cécidomyie (Photo Dr R.S. Copeland).

2.3.2 Compréhension des interactions chimiques des fourmis oecophylles au sein de l'agroecosystème

Etudiants encadrés associés à ce volet :

B. Mekonnen: Improving biological control of fruit flies using *O. longinoda* (Univ Pretoria, 2016-21)

C. Aika (2020): Study of methyl eugenol on lekking and mating behaviour of *B. dorsalis* (Univ Nairobi, Kenya)

Articles associés à ce volet :

- **E. Deletre**, C. Aika, S. Mohamed (In prep) Effects of African weaver ant semiochemicals from Dufour and poison gland on fruit fly parasitoids.
- **Deletre E,** Mekonnen B, Aika C, Yusuf A, Pirk C, Ekesi S (In prep) Effect of synthetic compounds from the Dufour's gland of the African weaver ant, *Oecophylla longinoda* (Hymenoptera: Formicidae) as oviposition deterrent for *Bactrocera dorsalis* and *Ceratitis cosyra*
- B Mekonnen, A Yusuf, C Pirk, S Ekesi, **E Deletre**. 2021b. Oviposition responses of *Bactrocera dorsalis* and *Ceratitis cosyra* to Dufour's and poison gland extracts of *Oecophylla longinoda* (Hymenoptera: Formicidae). *International Journal of Tropical Insect Science*, 1-9.
- B Mekonnen, X Cheseto, C Pirk, A Yusuf, S Ekesi, **E Deletre**, B Torto. 2021a. Re-Analysis of Abdominal Gland Volatilome Secretions of the African Weaver Ant, *Oecophylla longinoda* (Hymenoptera: Formicidae). *Molecules*, 26(4), 871.

Des études récentes menées par des collègues de l'unité HortSys et leurs partenaires ont montré que le contrôle biologique des mouches des fruits par *O. longinoda* est dû à leur activité de prédation mais aussi à l'effet répulsif de composés chimiques déposés par ces fourmis. Van Mele et al. (2007) ont montré que le pourcentage de mangues infestées par *B. dorsalis* et *Ceratitis spp*. diminue avec l'abondance de nids de fourmis dans l'arbre, passant de 24 % de mangues infestées dans un arbre sans fourmis à 1 % de mangues infestées dans des arbres ayant plus de 8 nids par arbre. Au laboratoire, Adandonon et al. (2009) ont montré grâce à des bioessais avec choix et sans choix que *B. dorsalis* et *C. capitata* pondaient moins d'œufs dans des mangues collectées à 1 et 3 m de distance aux nids de fourmis que dans des mangues provenant d'arbres exempts de fourmis. Le taux de ponte des mouches était corrélé à la distance au nid, donc probablement plus ou moins affecté selon la densité de composés déposés par les fourmis. Van Mele et al. (2009) ont également montré que les deux espèces de mouches des fruits atterrissaient moins et pondaient moins d'œufs sur des fruits préalablement exposés aux fourmis que sur des fruits non exposés (i.e. exempt de composés de fourmis). Le nombre de pupes collectées sur les mangues non exposées était environ huit fois plus élevé que sur celles exposées aux fourmis. Les mouches des fruits adultes seraient donc plus affectées, en particulier le comportement d'oviposition, par les signaux chimiques déposés par les fourmis que par la prédation directe.

La sélection du site de ponte est, en effet, cruciale pour le succès reproducteur des insectes herbivores. Ce comportement inné implique des signaux olfactifs et visuels (Renwick et Chew, 1994). Les composés organiques volatils de la plante hôte jouent un rôle majeur dans la recherche du site d'oviposition par les femelles gravides. Après la localisation de la plante hôte, la combinaison de signaux visuels, physiques et chimiques conduit à la sélection ou le rejet du site de ponte. Nous pouvons nous attendre à ce que la sélection du site de ponte soit soumise à une forte pression évolutive, car la fitness des descendants dépendra de la bonne sélection du site. Ceci est d'autant plus important que les larves sont souvent limitées dans leurs capacités de dispersion. L'hypothèse de préférence-performance est que la *mère* sélectionne le meilleur site de ponte en termes de quantité et de qualité de nourriture pour une meilleure fitness des larves (Thompson & Pellmyr, 1991). En effet, de nombreuses études illustrent le fait que la sélection du site de ponte est influencée par la qualité et la quantité de la plante hôte, qui sont positivement corrélées aux performances des descendants. Cependant, cette hypothèse n'est pas toujours correcte et plusieurs exemples peuvent être trouvés où le comportement de ponte de la mère améliore ses propres performances. En effet, lorsque la nourriture des larves est différente de celle des adultes, le choix d'une durée de vie plus longue pour les adultes suffit à compenser le coût d'une diminution du taux de survie des larves. Certains insectes ont également un stock d'œufs important ou un temps de reproduction limité et pondent donc leurs œufs sur une grande variété de plantes (Moreau et al. 2017). Outre ces hypothèses, la sélection du site de ponte peut également être influencée par les niveaux trophiques supérieurs. En effet, une pression évolutive critique est l'impact des ennemis naturels, qui conduit à la sélection d'un site de ponte sans ennemi pour optimiser la survie de la progéniture. Mais l'effet de la présence d'ennemis naturels sur la sélection des sites de ponte n'est pas bien documenté et les mécanismes impliqués mal compris. Et plus largement, contrairement à l'effet de prédation, les réponses comportementales au risque de prédation ont été peu étudiées à ce jour. En effet, la perception du risque de prédation par les proies a le potentiel de modifier le choix de la plante hôte, la survie, la reproduction et la croissance de la population, ainsi que des effets indirects sur les membres et la dynamique de la communauté (Hermann & Thaler, 2014). Mais l'identité chimique des kairomones libérées par le prédateur et reconnu par la proie est souvent inconnue ; or ces composés pourraient être utilisés en protection des plantes comme bio-répulsifs.

Dans le cas de notre fourmi prédatrice *Oecophylla longinoda,* Offenberg et al. (2004) ont suggéré les marques anales déposées par les fourmis étaient les signaux des prédateurs reconnus par les mouches des fruits, alors que Van Mele et al. (2009) ont suggéré qu'il s'agissait de la phéromone de marquage du territoire. *O. longinoda* a plusieurs glandes exocrines, qui produisent différents types de phéromones, telles que les glandes mandibulaires situées dans la région céphalique, et les glandes de poison, de Dufour, rectal et sternal situées dans l'abdomen (Figure 14). N'ayant pas une idée précise de l'origine et de l'identité chimique de la kairomone impliquée dans la détection de la fourmi par les mouches des fruits, nous avons commencé par refaire le test avec des mangues exposés et non exposés au fourmi (Mekonnen et al. 2021a). Nous avons montré le nombre d'œufs pondu par *B. dorsalis* et *C. cosyra* était respectivement 2 fois et 4 fois plus élevé sur les mangues non-exposées que sur les mangues exposées aux fourmis oecophylles, montrant de nouveau que les fourmis déposent bien un composé chimique qui affecte l'oviposition des mouches des fruits. Nous avons ensuite préparé et testé des extraits de fourmis : du corps entier puis de tête/thorax/abdomen séparés et enfin des différentes glandes abdominales (Dufour/poison/sternal/rectal) dans différents solvants (hexane, methanol, acétate d'éthyle et eau) comme nous ne connaissions pas la famille chimique de la kairomone impliquée. Les extraits de corps entier ont affecté le comportement d'oviposition (i.e. diminution du nombre d'œufs pondus) de *C. cosyra* quelque soit le solvant et les extraits de l'abdomen dans l'hexane ont affecté le comportement d'oviposition de *C. cosyra* et de *B. dorsalis.* Les extraits de glandes de Dufour et de Poison dans l'hexane ont également affecté le comportement d'oviposition de *C. cosyra* et de *B. dorsalis.* Ces résultats suggèrent que la kairomone impliquée dans la détection de la fourmi oecophylle par les mouches des fruits est synthétisé dans ces deux glandes. Nous avons observé que le comportement d'oviposition des femelles de *C. cosyra* était plus affecté que celui des femelles de *B. dorsalis*. Cette différence peut probablement s'expliquer par le fait que l'espèce indigène de mouches des fruits, *C. cosyra,* aurait peut-être appris à éviter *O. longinoda* au cours de l'évolution, contrairement à *B. dorsalis,* qui est une espèce invasive. Mais une espèce proche d'*O. longinoda, O. smaragdina* existe en asie, région d'origine de *B. dorsalis*, ce qui explique que nous ayons quand même observé une réponse comportementale.

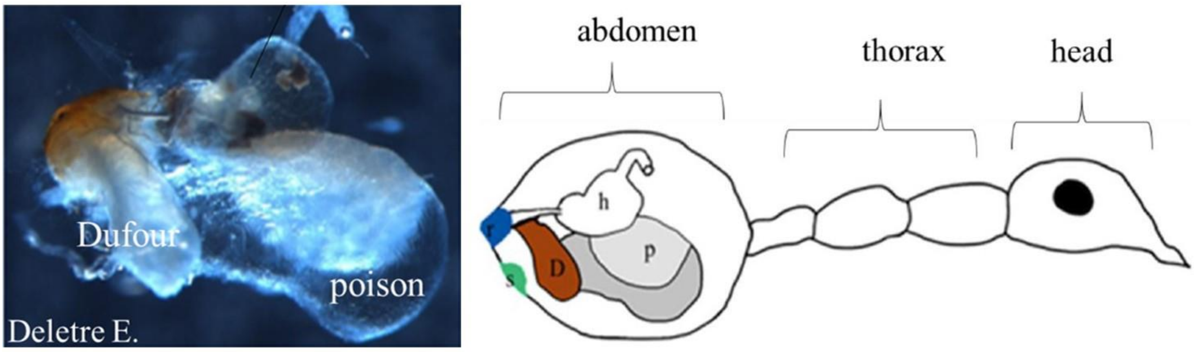


Figure 14. Photo et schéma des glandes exocrines de la fourmi oecophylle, *Oecophylla longinoda* ((p) poison, (D) Dufour, (s) sternale, (r) rectale et (h) intestin postérieur)

Nous avons ensuite analysé la composition chimique des glandes de Dufour et de poison (Mekonnen et al. 2021b). L'undécane, l'hénéicosane et le tricosane ont constitué 70% de la composition de la glande de Dufour, qui agissent comme des phéromones d'alarme pour la fourmi. Bradshaw et al. (1979) avait identifié ces mêmes composés. Plus surprenant, nous avons identifié sept classes de produits chimiques (hydrocarbures, acides carboxyliques, terpènes, aldéhydes, alcools, cétones et benzénoïde), alors que ces auteurs ont identifié seulement deux classes de produits chimiques (hydrocarbures et ester). Le composé le plus abondant de la glande de poison était l'acide formique suivi de l'undécane, l'hénéicosane et le tricosane. Une fois ces composés identifiés, nous les avons testés sur le comportement d'oviposition des mouches des fruits (Deletre et al. In prep). Nous avons commencé par tester les cinq alcanes majoritaires : l'undécane, l'hénéicosane, le tricosane, le tridecane et le pentadecane. Mais aucun d'eux n'a affecté le comportement d'oviposition des mouches des fruits sauf l'undécane à une dose équivalente à 1 glande qui a affecté *C. cosyra* (Figure 15). Le mélange des cinq alcanes n'a pas affecté le comportement d'oviposition des mouches des fruits. Nous avons ensuite testé les acides carboxyliques dont l'acide formique. L'acide acétique à une dose équivalente à 1 glande a affecté le comportement d'oviposition de *C. cosyra* et l'acide octadecanoïque à une dose équivalente à 100 glandes a affecté le comportement d'oviposition de *B.*

dorsalis. Nous avons également testé deux alcènes (le nom des alcènes n'est pas communiqué car un potentiel brevet pourrait être déposé). L'alcène 2 à une dose équivalente à 1 glande a affecté le comportement d'oviposition de *C. cosyra.* L'alcène 1 à une dose équivalente à 10 et 100 glandes a affecté le comportement d'oviposition de *B. dorsalis* et de *C. cosyra.* Même si nous avons identifié certains composés affectant le comportement des mouches des fruits, nous n'obtenons pas un effet aussi important qu'avec les extraits des glandes de Dufour et de poison. Une étude réalisée (pas encore publiée) par l'équipe de Kempraj de l'université de Macquarie, Sydney, Australie a montré que le 1-octanol, un composé volatil émis par *O. smaragdina,* diminuait fortement le nombre d'oeufs pondu par *Bactrocera tryoni*. Nous n'avons pas identifié ce composé chez *O. longinoda,* d'autres alcools ont été identifiés pour lesquels des tests au labo seront effectués. Un test sur le terrain est tout de même en cours de réalisation pour tester l'effet de l'undécane et tridecane et de l'alcène 1 sur le comportement d'oviposition des mouches des fruits. Nous espérons pouvoir développer un biorépulsif qui pourrait diminuer le niveau d'infestation des mouches des fruits.

		Bactrocera dorsalis			***Cosyra cosyra***		
Traitement	**dose**	**Moyenne (±ES) nb d'oeuf sur C[1]**	**Moyenne (±ES) nb d'oeuf sur T[2]**	***P value*[3]**	**Moyenne (±ES) nb d'oeuf sur C**	**Moyenne (±ES) nb d'oeuf sur T**	***P value***
Undecane	1	9.9 ± 1.7	7.8 ± 1.9	0.293	**48.8 ± 5.0**	**36.0 ± 5.1**	**0.045**
Undecane	10	29.0 ± 3.1	30.2 ± 3.7	0.810	13.8 ± 3.0	16.6 ± 3.0	0.430
Undecane	100	9.9 ± 1.3	8.9 ± 2.0	0.648	51.0 ± 5.2	43.3 ± 4.6	0.232
Ac. acetique	1	28.9 ± 3.7	30.9 ± 3.6	0.584	**10.3 ± 2.0**	**5.6 ± 1.6**	**0.025**
Ac. acetique	10	24.3 ± 3.8	28.5 ± 4.0	0.330	18.1 ± 3.0	16.1 ± 3.1	0.492
Ac. acetique	100	21.3 ± 2.9	19.5 ± 3.2	0.683	27.4 ± 3.6	19.7 ± 2.7	0.137
Ac. octadecanoique	1	11.4 ± 1.9	11.0 ± 2.2	0.839	16.3 ± 3.0	18.8 ± 3.9	0.574
Ac. octadecanoique	10	9.3 ± 1.3	9.0 ± 1.9	0.900	22.2 ± 3.8	23.4 ± 3.6	0.812
Ac. octadecanoique	100	**32.2 ± 3.9**	**22.4 ± 2.4**	**0.009**	12.4 ± 2.4	8.1 ± 1.6	0.162
Alcene1	1	22.9 ± 3.3	18.0 ± 2.5	0.140	8.9 ± 1.6	8.7 ± 1.3	0.926
Alcene1	10	**13.6 ± 1.8**	**10.8 ± 1.8**	**0.026**	12.2 ± 2.4	9 ± 1.7	0.103
Alcene1	100	20.2 ± 2.6	16.8 ± 2.2	0.264	**22.5 ± 2.1**	**14.5 ± 2.1**	**0.003**
Alcene2	1	8.3 ± 1.3	6.4 ± 1.2	0.242	**16.7 ± 2.4**	**11.6 ± 1.9**	**0.041**
Alcene2	10	15.4 ± 2.6	14.5 ± 2.3	0.783	14.0 ± 2.4	16.1 ± 2.6	0.488
Alcene2	100	16.8 ± 2.2	13.1 ± 2.5	0.291	11.3 ± 2.4	10.6 ± 2.1	0.818

Figure 15. Nombre moyen (±ES) d'œufs pondus par *B. dorsalis* et *C. cosyra* sur des mangues non traitées (C) ou des mangues traitées (T) à des doses de 1, 10 et 100 glandes équivalents. (P-value, venant d'un test de student apparié)

Comme indiqué précédemment, des interactions directes et indirectes peuvent se produire, comme dans les écosystèmes agricoles où trois espèces ou plus interagissent. Lors de la création de programme de lutte biologique, toutes ces interactions doivent être prise en compte. Dans les cas où un prédateur comme *O. longinoda* se nourrit d'une proie, comme *B. dorsalis,* qui est aussi l'hôte d'un parasitoïde, comme *F. arisanus* ou *D. longicaudata,* nous pouvons observer des interactions indirectes comme de la compétition pour la même ressource (Werner & Peacor 2003). Mais les prédateurs impactent le comportement, la physiologie, le développement et les traits morphologiques des proies, il pourrait également avoir un impact direct sur ses concurrents intraguildes, en particulier si le prédateur est généraliste. Migani et al. (2017) ont montré que le succès reproducteur des parasitoïdes

F. arisanus et *D. longicaudata* était affecté négativement par la présence de fourmis et que le parasitisme était réduit d'environ 50 % (Migani et al., 2017). Mais ces effets négatifs ne semblaient pas liés directement à des effets de prédation. En effet, Appiah et al. (2014) ont montré que le comportement d'oviposition de *F. arisanus* était affecté même lorsque les fruits étaient préalablement exposés aux fourmis avec une diminution du temps de recherche du site d'oviposition et du taux de parasitisme. Nous avons alors émis l'hypothèse que le comportement d'oviposition des parasitoïdes était affecté par des kairomones provenant des glandes de Dufour et de Poison comme pour *B. dorsalis* et *C. cosyra.* Nous avons donc testé les extraits des glandes de Dufour et de Poison dans de l'hexane et l'alcène 1 mais aucun des traitements n'a affecté le taux de parasitisme de *F. arisanus* et de *D. longicaudata.* Contrairement aux mouches qui se base sur la phéromone d'alarme venant des glandes de Dufour et de poison, ces parasitoïdes détecteraient plutôt les marques anales ou la phéromone de marquage provenant de la glande rectal ou sternal respectivement comme le suggérait Offenberg et al. (2004) et Van Mele et al. (2009) dans le cas des mouches des fruits. Les parasitoïdes pourraient également détecter les phéromones provenant des glandes mandibulaires des fourmis oecophylles. Des essais sont actuellement en cours pour compléter cette étude. Quoiqu'il en soit, si l'alcène 1 se révèle efficace sur le terrain pour lutter contre les mouches des fruits, il n'aura pas d'impact néfaste sur la lutte biologique par *F. arisanus* et de *D. longicaudata.*

2.3.3 Le comportement sexuel des mouches des fruits

Etudiants encadrés associés à ce volet:
S. Chemweno (2019): Study of lekking and mating behaviour of *B. dorsalis* (JKUAT, Kenya)
C. Aika (2020): Study of methyl eugenol on lekking and mating behaviour of *B. dorsalis* (Univ Nairobi, Kenya)
Articles associés à ce volet :

- **Deletre E,** Aika C, Shemweno S, Brévault T (In prep) Impact of rearing, sterility and entomovectoring of male fruit flies on mating success compared to wild male fruit flies

Aujourd'hui, malgré la diversité des moyens de lutte contre *Bactrocera dorsalis,* l'utilisation excessive d'insecticides à large spectre reste l'outil principal, avec une surveillance des populations grâce au piégeage. En effet, atteindre un niveau satisfaisant de contrôle des populations en utilisant des méthodes sans pesticide reste un défi majeur pour l'agriculture moderne. Les stratégies de lutte biologique sont étudiées depuis des années mais les cas où elles ont démontré une efficacité satisfaisante sont rares, surtout dans le cas de la lutte biologique par conservation. Concernant les mouches des fruits, d'autres méthodes sans pesticide sont aujourd'hui explorées comme l'utilisation de la technique de l'insecte stérile (SIT). Cette technique consiste à élever en masse l'insecte cible, à le stériliser par irradiation avant de le relâcher. Les mâles stériles s'accouplent avec les femelles et empêchent ainsi une reproduction efficace, par conséquent les populations d'insectes cibles diminuent. Récemment, des collègues en entomologie médicale du cirad ont proposé « *de considérer le mâle stérile non seulement comme un compétiteur sexuel, mais surtout comme un transporteur spécifique permettant de transmettre des biocides aux femelles, voire aux gîtes larvaires dans le cas des moustiques* » (Pleydell & Bouyer 2019). Ces chercheurs ont démontré par modélisation que cette technique pourrait réduire de plus de 95 % le nombre total de moustiques mâles stériles nécessaires pour contrôler les épidémies de dengue. Cette avancée engendrerait une réduction drastique du coût qui est une des limites de cette technique, en particulier dans le contexte africain.

Nous avons émis l'hypothèse que cette technique pourrait également être boostée dans le cadre de la lutte contre *B. dorsalis.* L'entomopathogène, le plus approprié pour cette idée d'entomovectoring, était le champignon *Metharizium anisopliae,* de par son efficacité et de la grande banque de souches qu'avait l'icipe. La première étape était d'identifier les facteurs pouvant influencer le succès reproducteur des mouches mâles puis d'identifier la meilleure souche de biopesticide. La plupart des Tephritidae ont des comportements de parade (lekking), c'est une agrégation d'au moins trois mâles émettant des phéromones d'agrégations ou sexuelles sur une plante hôte ou non hôte. Au sein de ces agrégations, les mâles se battent pour défendre un petit territoire puis initient un comportement sexuel en libérant des phéromones à longue portée qui attirent les femelles (Benelli, 2014). Ce comportement est généralement caractérisé par (i) un mouvement des ailes (l'attaquant fait face à l'adversaire et amène les deux ailes vers l'avant perpendiculairement à l'axe longitudinal de son corps, tandis que la surface ventrale des ailes est tournée vers l'avant), (ii) suivie d'une poursuite et des coups de tête (l'attaquant se précipite sur l'adversaire et lui donne un coup de tête) et/ou (iii) des coups avec les pattes avant (l'attaquant lève une patte avant et frappe l'adversaire à la tête ou au thorax), mais d'autres séquences agressives peuvent survenir. Suite à ces combats, le mâle vainqueur s'est approprié un territoire. Parmi le regroupement de mâles, les femelles choisissent et copulent ensuite avec les mâles qui ont exécuté la meilleure séquence de comportement de parade nuptiale. Les vibrations des ailes combinées à des signaux olfactifs et tactiles jouent également un rôle dans leur choix. Pour *Bactrocera dorsalis*, des comportements agressifs entre mâles ont déjà été observés (Ekesi et al. 2009). Pendant la parade nuptiale, les mâles de *B. dorsalis* présentent également un comportement d'éventail rigoureux, vraisemblablement pour augmenter la dispersion de la phéromone sexuelle (Shelly, 2000). Chez les Tephritidae, les comportements sexuels, comprenant la parade nuptiale et l'accouplement, impliquent donc un contact physique entre les mâles et entre mâles-femelles. Nous avons donc émis l'hypothèse qu'un mâle stérile vecteur de champignons entomopathogènes pourrait le transmettre aussi bien à un mâle qu'à une femelle. Cependant, les effets du transport de biopesticides par un insecte n'ont pas encore été clairement étudiés sur le comportement de parade et le succès reproducteur. D'un point de vu évolutif, il serait avantageux que les champignons entomopathogènes augmentent les rencontres conspécifiques afin de faciliter la transmission des spores. Pour y parvenir, certains champignons modifieraient la communication chimique et la signalisation du comportement sexuel des insectes, mais il existe très peu d'exemples documentés (Hansen & Henrik 2019). Nous avions émis l'hypothèse que *M. anisopliae* ne diminuerait pas le succès d'accouplement des mâles *B. dorsalis* jusqu'à la pénétration de l'appressorium dans le corps de la mouche. Dans les facteurs qui influencent le comportement de parade et le succès reproducteur, nous savons que l'élevage de masse des Tephritidae change leur comportement sexuel. Par exemple, la production de phéromones joue un rôle limité dans les élevages et les mâles s'accouplent tout au long de la journée. En milieu naturel, *B. dorsalis* s'accouple normalement au crépucule. Le comportement de parade nuptiale n'est pas possible avec les conditions de surpeuplement des élevages et le temps de parade est également réduit pour éviter l'interruption par d'autres mâles. Le choix des femelles est limité en raison du même âge et de la même taille que les mâles et la copulation peut être forcée et rapide. Par conséquent, les mâles d'élevage ont perdu certains comportements naturels et leur compétitivité à l'accouplement pourrait être réduite sur le terrain. Notre deuxième hypothèse était donc que les mouches des fruits mâles élevées en masse auraient un plus faible succès d'accouplement. Pour contrer cet effet néfaste, nous avions émis l'hypothèse que les mâles nourris avec du méthyleugénol auraient un meilleur succès d'accouplement. En effet, les mâles de *B. dorsalis* sont fortement attirés par le méthyleugénol et s'en nourrissent car il est un précurseur de leur phéromone sexuelle. Ce composé est transformé par la mouche en deux composants: l'alcool E-coniférylique (ECF) et le 2-allyl-4,5-diméthoxyphénol (DMP) et stocké dans les glandes rectales (Tan et al. 2011). Il a été démontré que ces deux composés agissent comme des phéromones sexuelles attirant les mâles et les femelles de *B. dorsalis* (Nishida et al. 2000). Dans la nature, les mâles recherchent et ingèrent le méthyleugénol provenant des plantes. La consommation de méthyleugénol améliore les performances d'accouplement et la compétitivité des mâles de *B. dorsalis* (Shelly, 2001). Pereira et al. (2013) précisent également que le régime alimentaire (sucres et protéines) avant les lâchers affecte le succès d'accouplement des mâles par la suite. Enfin Rull et al. (2005) ont montré sur *Anastrepha lundes* que la stérilité et la dose d'irradiation ont un effet négatif négligeable sur le succès reproducteur comparé à d'autres facteurs (Rull et al. 2007). Ainsi, nous avions émis l'hypothèse que la stérilisation n'aurait aucun effet sur le succès reproducteur des mâles.

Nous avons montré que le succès reproducteur était d'environ 65% pour les mâles d'élevage et était supérieur aux mâles sauvages, ce qui n'est pas surprenant car les tests ont été effectués au laboratoire (Deletre et al In prep). L'ingestion de méthyleugénol a augmenté le succès reproducteur des mâles après 3 et 6 jours et la stérilité n'a pas influencé le succès reproducteur des mâles, confirmant nos hypothèses. Par contre, l'infestation par *M. anisopliae* a diminué le succès reproducteur des mâles. Lorsque l'ingestion de méthyleugénol était combinée aux autres facteurs (stérilité, infestation, …), le succès reproducteur des mâles était également plus important, limitant ainsi l'impact négatif de l'infestation par *M. anisopliae* sur le succès reproducteur et favorisant la transmision de *M. anisopliae* des mâles aux femelles.

Au moment de la parade nuptiale, le nombre de contacts entres mâles infestés par *M. anisopliae,* entre eux-mêmes et avec les mâles non contaminés, a augmenté. La stérilité et l'ingestion de méthyleugénol ont également augmenté le nombre de contacts entre mâles, favorisant ainsi la transmission de *M. anisopliae* entre mâles. Mais seul les contacts entre mâles d'élevage et sauvages ont augmenté, pas les contacts entre eux-mêmes.

Nous avons également montré que les femelles *B. dorsalis* étaient attirées par l'odeur des mâles 3 jours après l'ingestion de méthyleugénol et les mâles ont été attirés par l'odeur des mâles 3 jours et 12 jours après l'ingestion de méthyleugénol. Après l'analyse des glandes rectales des mouches mâles, nous observons que la production de ECF et DMP augmente jusqu'à 6 jours après ingestion pour ensuite diminué, expliquant l'attraction des mouches 3 jours après l'ingestion.

Pour tester, l'efficacité de notre stratégie d'insecte stérile boosté avec l'ingestion de méthyleugénol et le transport de *M. anisopliae*, nous avons fait des tests en mésocosme. Nous avons observé qu'après 3 semaines d'essai, le taux de survie dans les témoins (20 femelles sauvages + 20 mâles sauvages) était de plus de 95%, le taux de survie pour la modalité de SIT classique (20 femelles sauvages + 20 mâles sauvages + 20 mâles stériles) était de plus de 80% pour les populations sauvages et de 25% pour les mâles steriles, et le taux de survie pour la modalité de SIT boostée (20 femelles sauvages + 20 mâles sauvages + 20 mâles stériles, infestés par *M. anisopliae* et nourris avec du méthyleugénol) était de moins de 50% pour les femelles sauvages, de moins de 40% pour les mâles sauvages et de 0% pour les mâles stériles boostés après seulement 9 jours pour ces derniers (figure 16). La mortalité observée dans cette dernière modalité était due à plus de 90% par l'infestation des mouches par *M. anisopliae.* L'un des objectifs de la SIT boostée était de diminuer les populations sauvages, cette objectif est donc atteint. Le deuxième objectif était de diminuer la taille des prochaines générations de population sauvage*,* i.e. diminuer le nombre d'œufs pondus, et donc des dégâts sur les mangues. Pour ce deuxième objectif, les observations sur ce court pas de temps ont permis de ne montrer que l'intérêt de la technique SIT mais pas d'intérêt supplémentaires de la technique de SIT boostée (figure 17). En effet, le nombres d'œufs pondus par les femelles pour la SIT et la SIT boostée était inférieur au témoin, mais le nombre pour la SIT boostée n'était pas inférieur à la SIT classique, même si le nombre de femelles avait diminué pour la SIT boostée. Des observations a plus long terme auraient peut-être permis d'observer l'impact sur le nombre d'œufs pondus. Sur le terrain, un essai de la SIT classique et de la SIT boostée est actuellement en cours au Sénégal.

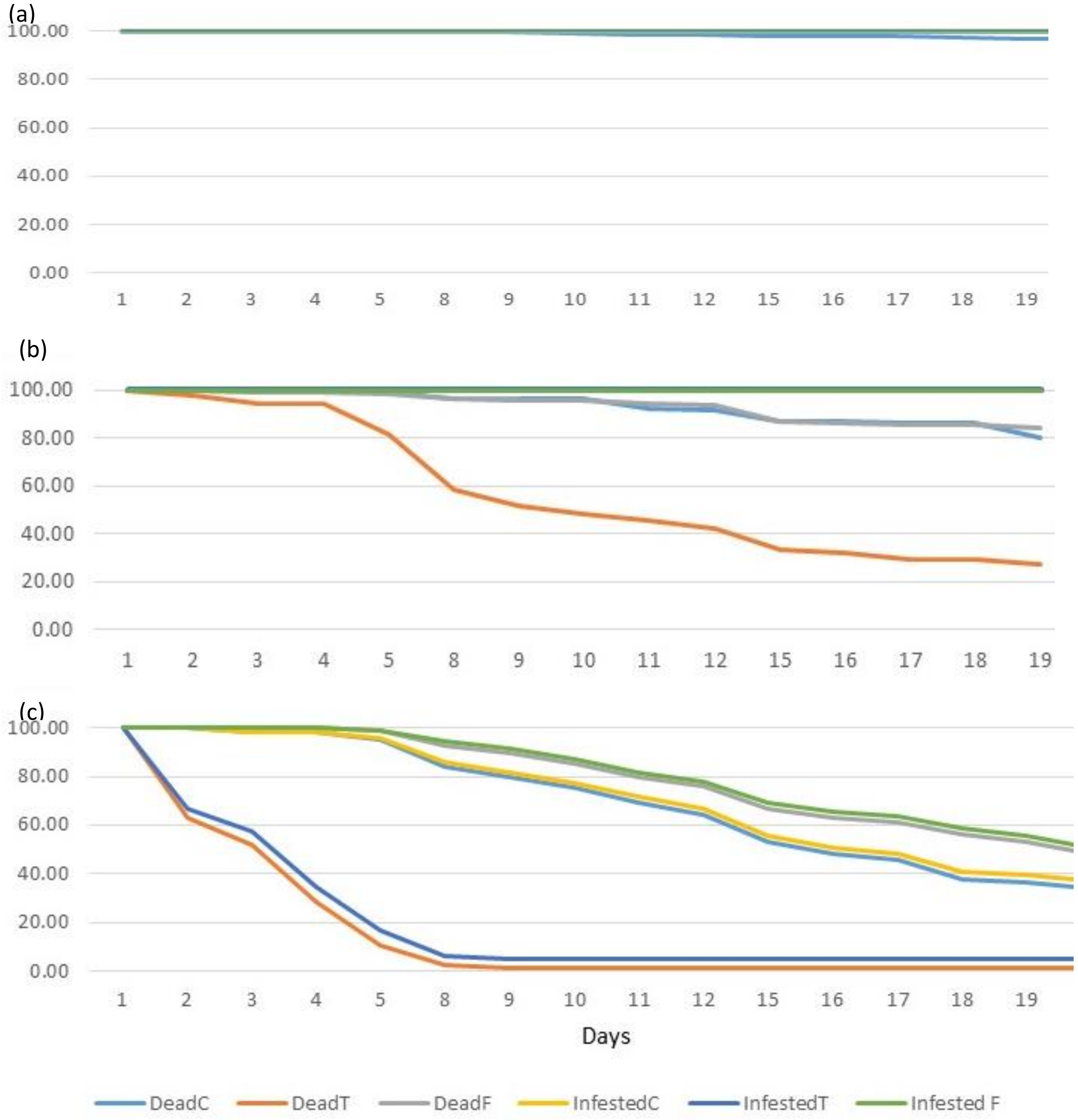


Figure 16. Taux de survie et taux sain (non infesté) de femelles *Bactrocera dorsalis* sauvages (DeadF, InfestedF), de mâles sauvages (DeadC, InfestedC) et de mâles stériles ou stériles, infestés par *M. anisopliae* et nourris avec du méthyleugénol (DeadT, Infested T).

(a)20 femelles + 20 mâles sauvages qui est le témoin, (b) 20 femelles + 20 mâles sauvages + 20 mâles stériles ce qui correspond à la technique de l'insecte stérile classique, (c) 20 femelles + 20 mâles sauvages + 20 mâles stériles, infestés par *M. anisopliae* et nourris avec du méthyleugénol ce qui correspond à la technique de l'insecte stérile boostée

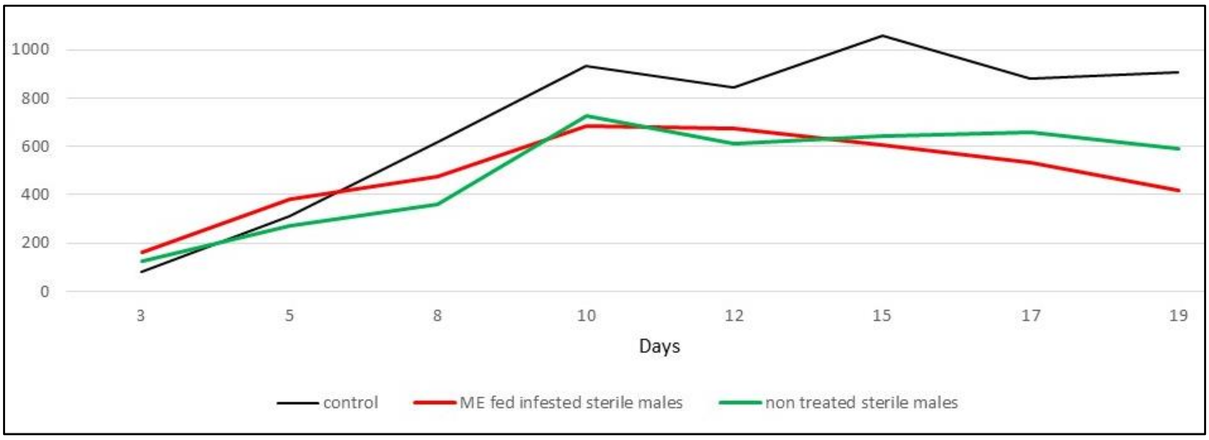


Figure 17. Nombre d'œufs moyen pondus par 20 femelles *B. dorsalis* sauvages avec 20 mâles sauvages et aucun autre mâle (témoin en noir) ou 20 mâles stériles (SIT classique en vert) ou 20 mâles stériles, infestés par *M. anisopliae* et nourris avec du méthyleugénol (SIT boostée en rouge).

2.3.4 Conclusion

L'étude de la biodiversité et des différentes interactions trophiques nous ont permis de montrer que la présence du prédateur généraliste, *O. longinoda,* ne perturbait pas la biodiversité des pollinisateurs et la pollinisation mais qu'il avait un impact sur le comportement des mouches des fruits et leurs parasitoïdes.

Grâce à l'écologie chimique (Figure 18), nous avons pu développer des innovations pour la lutte contre les mouches des fruits :

- un bio-répulsif à base de phéromone de fourmis contre les mouches des fruits et qui n'a pas d'effet néfastes sur les parasitoïdes des mouches des fruits.
- une stratégie SIT boosté avec un précurseur de phéromone sexuelle pour augmenter le succès reproducteur des mâles stériles et avec le transport d'un champignon entomopathogène pour diminuer les populations de mouches des fruits sauvages.

Au cours des ces travaux, nous avons pu mettre en évidence que la détection du prédateur par une proie n'implique pas les mêmes sémiochimiques selon l'espèce et que la compréhension du comportement reproducteur est crucial pour optimiser des techniques comme celle de l'insecte stérile.

La combinaison d'un bio-répulsif, de la lutte biologique et de la SIT boostée pourrait être alors un système de production de la mangue plus agroécologique et plus durable.

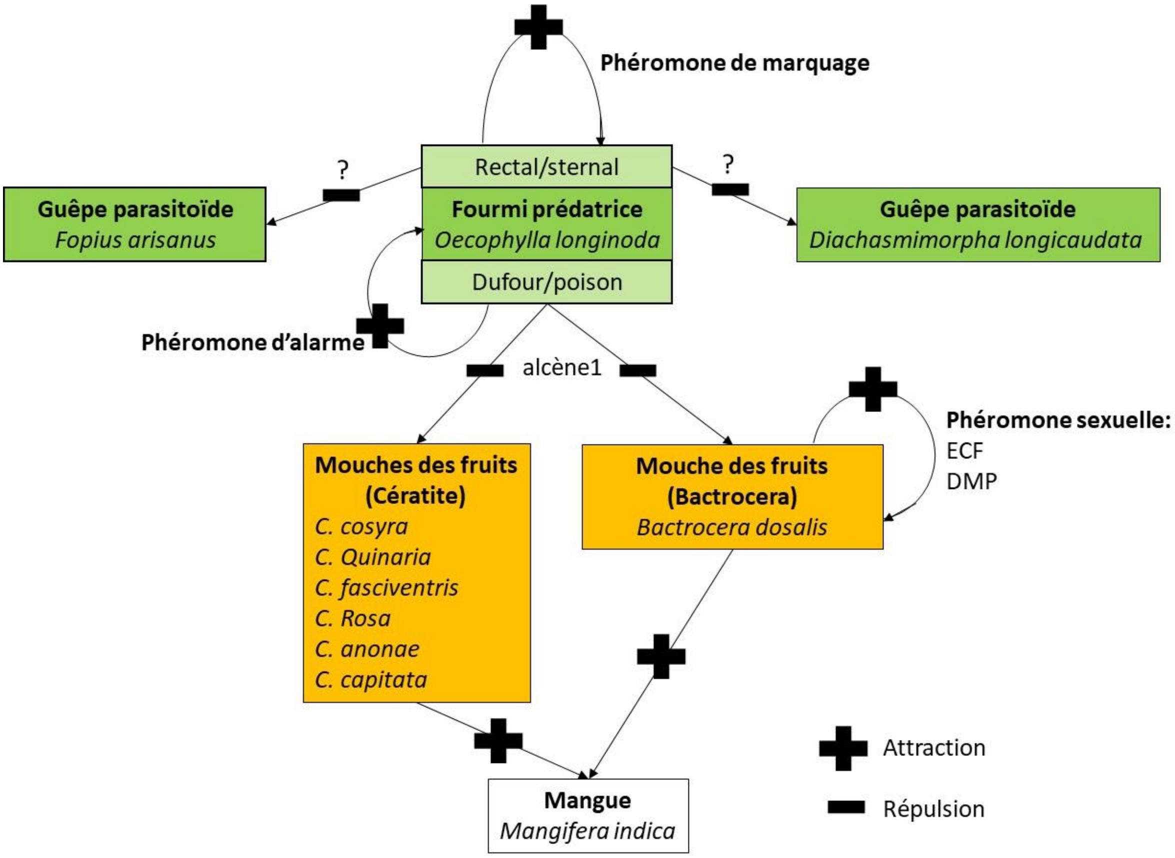


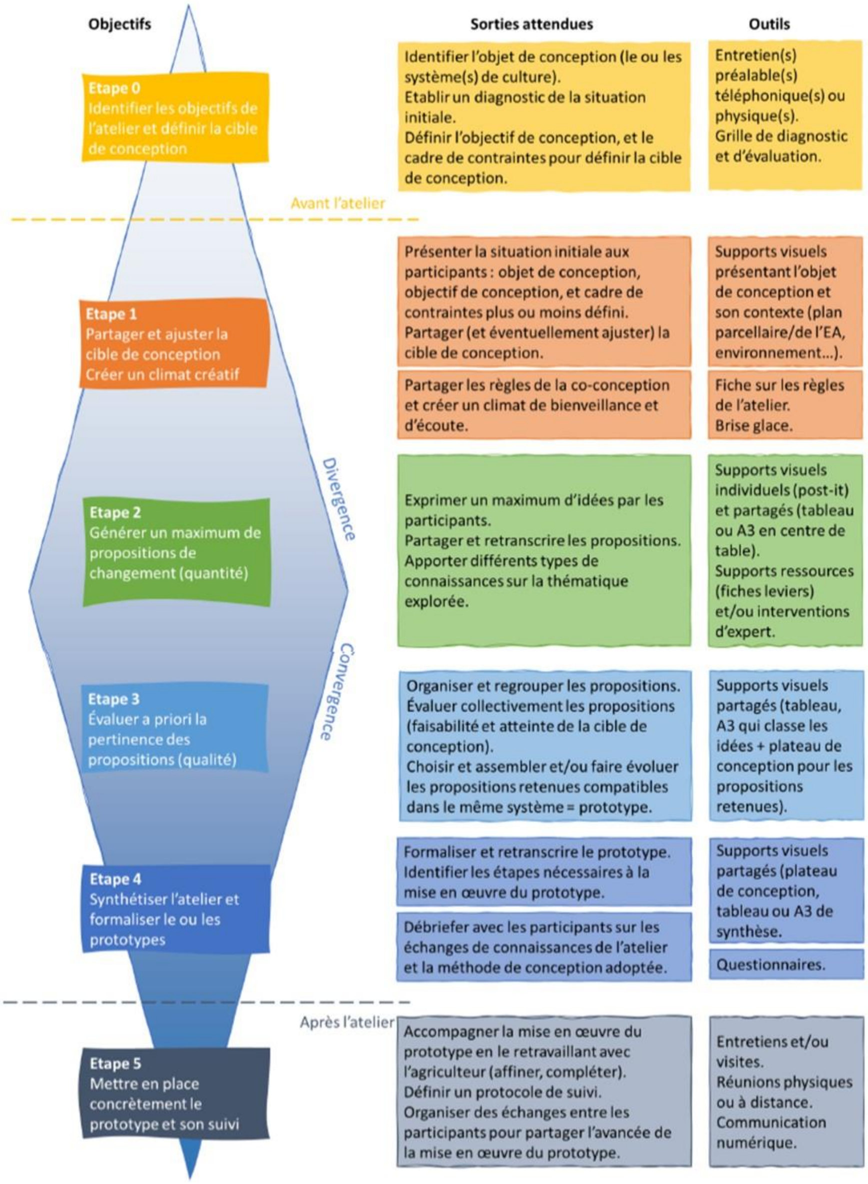
Figure 18. Schéma récapitulatif des interactions chimique de notre agroécosystème de la mangue

3 Projet de recherche

3.1 Introduction

Au cours de ces 10 dernières années, je me suis intéressée aux 2 premiers niveaux de la transition agroécologique : la réduction des intrants chimiques (niveau 1) et leurs remplacements par des innovations agroécologique (niveau 2) (Figure 2). J'ai travaillé sur l'utilisation des filets anti insectes afin de réduire l'usage des pesticides et j'ai développé des innovations agroécologiques, comme le développement d'attractifs ou de répulsifs pour manipuler le comportement des ravageurs ou des ennemis naturels afin de remplacer l'usage des pesticides. Mais j'ai effectué ce travail seulement à l'échelle de la parcelle, or lorsque l'on veut faire adopter ces innovations par les producteurs, il faut repenser le système de production à l'échelle de l'exploitation et du territoire (niveau 3 de la transition). **Dans mes futurs projets de recherche, je souhaite intégrer l'échelle de l'exploitation voire du territoire pour aller plus loin que la simple conception d'innovations agroécologiques et aller jusqu'à leur adoption.** Je devrais donc faire appel à de nouvelles méthodologies de travail comme la co-conception. La co-conception ou conception participative est une démarche consistant à impliquer différents acteurs (producteurs, consommateurs, …) dans le processus de développement du nouveau système de production (Chieze et al. 2021). Elle repose sur le principe d'un travail partagé et collectif lors d'ateliers, qui va de l'idée jusqu'à la création de prototypes réalistes. L'intérêt de ce travail est de combiner les savoirs empiriques des producteurs avec les connaissances scientifiques, ainsi que les contraintes politiques, économiques et logistiques. L'un des avantages de cette méthode est que le producteur est un acteur du changement. Il est alors plus enclin à initier les changements nécessaires à la transition agroécologique. La conception d'un nouveau système de production peut se faire pas à pas avec un processus progressif en boucles d'apprentissage ou de novo à partir d'une page blanche avec la mise en place d'un système complet puis son évaluation. Quoiqu'il en soit, ces ateliers font appel à des outils d'animation et des supports de discussion bien spécifiques aux objectifs prédéfinis, mais dont le déroulé et l'enchaînement des étapes restent identiques (figure 19).

Aujourd'hui, les expériences professionnelles les plus proches de la co-conception que j'ai eu sont (i) la mise en place d'un piège de masse pour la mouche de l'olive avec les oléiculteurs et (ii) le transfert de la technologie des filets anti-insectes auprès d'agriculteurs kényans. Dans le cas des projets sur la mouche de l'olive, j'avais été contacté par la coopérative d'oléiculteurs d'Occitanie, FranceOlive, pour rechercher une solution innovante contre *Bactrocera oleae.* Nous avions organisé une réunion avec les ingénieurs de FranceOlive et des oléiculteurs afin de comprendre les problématiques de la gestion de la mouche de l'olive (interdiction par l'UE d'un grand nombre de pesticides, prix très élevés des traitements biologiques comme la kaolinite, lutte biologique non efficace, SIT impossible dû aux problèmes d'élevage, …). Suite aux discussions, la solution la plus prometteuse semblait le piégeage de masse. Il existait un piège spécifique à base de phéromone mais efficace uniquement contre les mâles et un piège alimentaire mais très peu spécifique. Il a donc été convenu que je travaille à l'élaboration d'un piège spécifique aux femelles à base de kairomones. Les oléiculteurs avaient noté que certaines variétés étaient plus attaquées que d'autres, nous sommes donc partis de l'hypothèse que certaines variétés émettaient des composés organiques volatiles plus attractifs que d'autres. 4 ans après ces discussions, nous allons tester en été 2022 chez des oléiculteurs, un prototype (piège + diffuseur de kairomones) spécifique aux femelles *B. oleae,* fait par une entreprise privée. Pour ce qui est du transfert de la technologie des filets anti-insectes, nous avions travaillé de concert avec 30 producteurs kenyans, une entreprise locale de fabrication de filet anti-insectes et une entreprise locale de structure de serres (Mujuka et al. soumis). L'objectif était d'évaluer le retour sur investissement et l'adoption de cette technologie. Dans de précédents travaux, nous avions hiérarchisé, par ordre de priorité, les technologies à transférer aux producteurs afin d'améliorer leur production (figure 20), le filet anti-insectes étant la dernière technologie à transférer. Pour cette étude, nous avions sélectionné des producteurs plus ou moins avancés en terme de technologie pour avoir une idée de la potentielle adoption de cette technologie par tous types d'agriculteurs. Nous leur avions fourni une serre en filet anti-insecte de 8*20*2m d'un coût avoisinant les 1000$ pour produire de la tomate, du chou ou du haricot vert, des cultures à haute valeur ajoutée. Nous avons suivi ces producteurs de manière hebdomadaires et nous avons fait plusieurs ateliers de restitution. Avec ces cultures, le retour sur investissement était de 15 mois en moyenne. Quant à l'adoption, tous les producteurs ont vu l'intérêt de cette technologie e.g. réduction de l'utilisation des pesticides, augmentation du rendement de 30% en moyenne mais malgré une amélioration de la qualité des produits, leur prix de vente est resté le même et l'investissement initial reste un frein à l'adoption.



Objectifs	Sorties attendues	Outils
Etape 0 Identifier les objectifs de l'atelier et définir la cible de conception	Identifier l'objet de conception (le ou les système(s) de culture). Etablir un diagnostic de la situation initiale. Définir l'objectif de conception, et le cadre de contraintes pour définir la cible de conception.	Entretien(s) préalable(s) téléphonique(s) ou physique(s). Grille de diagnostic et d'évaluation.
Etape 1 Partager et ajuster la cible de conception Créer un climat créatif	Présenter la situation initiale aux participants : objet de conception, objectif de conception, et cadre de contraintes plus ou moins défini. Partager (et éventuellement ajuster) la cible de conception.	Supports visuels présentant l'objet de conception et son contexte (plan parcellaire/de l'EA, environnement...).
	Partager les règles de la co-conception et créer un climat de bienveillance et d'écoute.	Fiche sur les règles de l'atelier. Brise glace.
Etape 2 Générer un maximum de propositions de changement (quantité)	Exprimer un maximum d'idées par les participants. Partager et retranscrire les propositions. Apporter différents types de connaissances sur la thématique explorée.	Supports visuels individuels (post-it) et partagés (tableau ou A3 en centre de table). Supports ressources (fiches leviers) et/ou interventions d'expert.
Etape 3 Évaluer a priori la pertinence des propositions (qualité)	Organiser et regrouper les propositions. Évaluer collectivement les propositions (faisabilité et atteinte de la cible de conception). Choisir et assembler et/ou faire évoluer les propositions retenues compatibles dans le même système = prototype.	Supports visuels partagés (tableau, A3 qui classe les idées + plateau de conception pour les propositions retenues).
Etape 4 Synthétiser l'atelier et formaliser le ou les prototypes	Formaliser et retranscrire le prototype. Identifier les étapes nécessaires à la mise en œuvre du prototype.	Supports visuels partagés (plateau de conception, tableau ou A3 de synthèse.
	Débriefer avec les participants sur les échanges de connaissances de l'atelier et la méthode de conception adoptée.	Questionnaires.
Etape 5 Mettre en place concrètement le prototype et son suivi	Accompagner la mise en œuvre du prototype en le retravaillant avec l'agriculteur (affiner, compléter). Définir un protocole de suivi. Organiser des échanges entre les participants pour partager l'avancée de la mise en œuvre du prototype.	Entretiens et/ou visites. Réunions physiques ou à distance. Communication numérique.

Figure 19. Déroulé et enchaînement des étapes d'un atelier de co-conception avec les objectifs et les outils d'animation associés (Chieze et al. 2021)

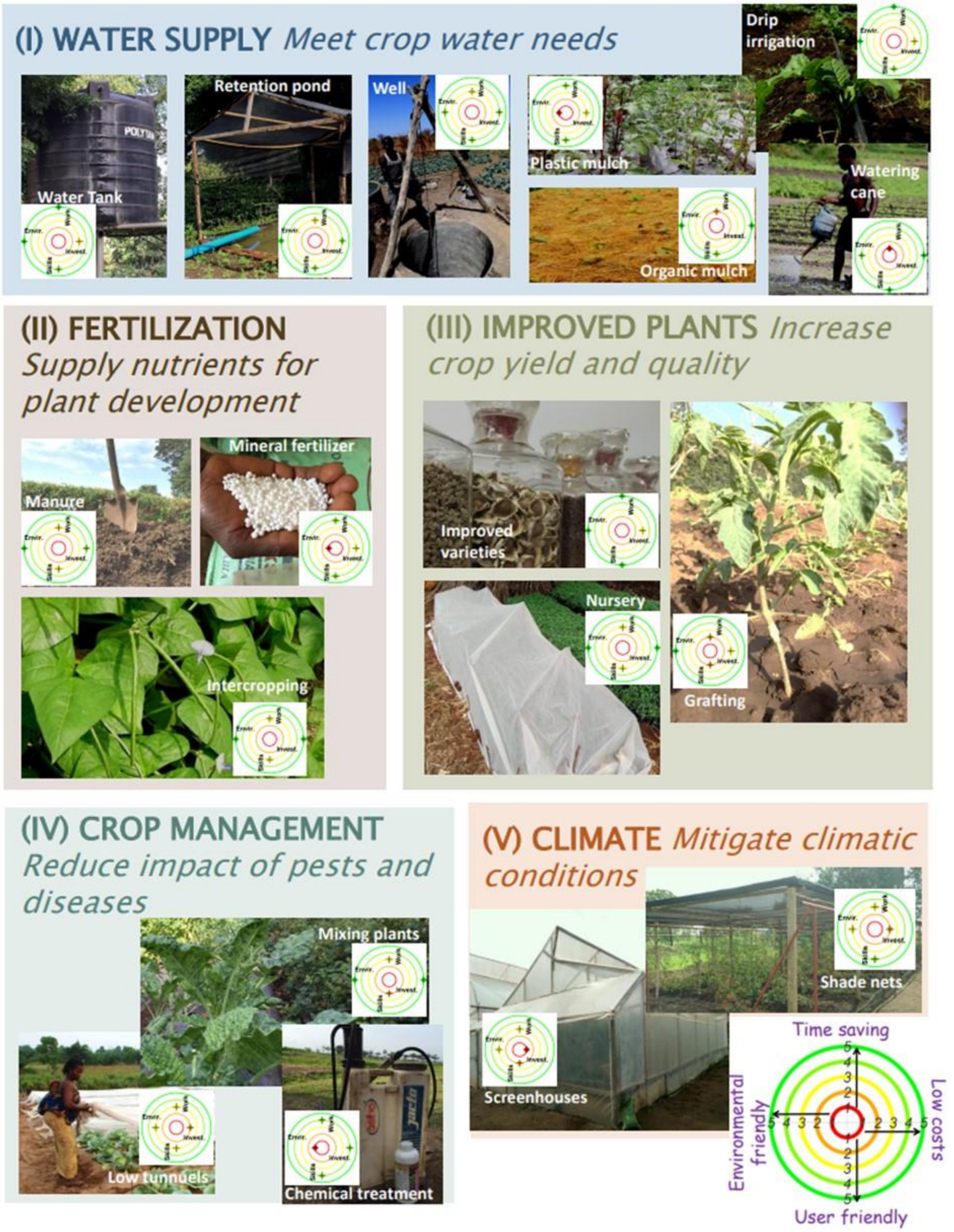


Figure 20. Priorisation des technologies (avantages/inconvénients) à transférer pour améliorer le rendement et la qualité de la production (Nordey et al. 2017)

Mon nouveau projet de recherche, qui inclut une nouvelle méthodologie et de nouvelles échelles d'étude, s'inscrit dans les missions de ma nouvelle affectation à Abidjan en Côte d'ivoire au Centre Suisse de Recherche Scientifique (CSRS) depuis février 2022. Il s'appuie entre autres sur l'obtention d'un financement de l'union européenne de 2M€ pour 4 ans dont j'assure la co-coordination de la tâche 4 « Santé des plantes ». Ce projet s'intitule *Développement d'un maraichage péri-urbain agroécologique encourageant le mutualisme entre les acteurs des territoires* (projet MARIGO : Maraichage Agroécologique Peri-urbain). L'objectif de ce projet est de contribuer à une transformation durable et productive de l'agriculture et des systèmes alimentaire de la Côte d'Ivoire.

3.1.1 Logique d'intervention

Le cœur du projet MARIGO est la création de 4 plateformes multi-acteurs dans les 4 grandes zones péri-urbaine : Abidjan, Yamoussoukro, Bouaké et Korhogo, qui sont réparties sur un axe Sud-Nord et bénéficient de conditions économiques, sociales et climatiques contrastées (Figure 21). Ces plateformes seront constituées d'agriculteurs, de formateurs, de chercheurs, d'acteurs de la filière maraichère, de décideurs de politiques publiques sectorielles, d'ONGs et de membres de la société civile. L'objectif de ces plateformes est d'élaborer ensemble des solutions durables aux enjeux de la filière maraichère ivoirienne : économie locale favorisant le recyclage, réduction des coûts et des risques de pertes, changement climatique… (WP1). Les solutions passeront (i) par l'élaboration et la mise en œuvre de politiques et de programmes de sécurité alimentaire et nutritionnelle, (ii) par la transition agroécologique des systèmes de productions et (iii) par le développement d'une économie circulaire. Nous pouvons noter que la mise en place des liens directs entre producteurs et consommateurs à l'échelle locale et nationale, correspond au niveau 4 de la transition agroécologique (figure 2).

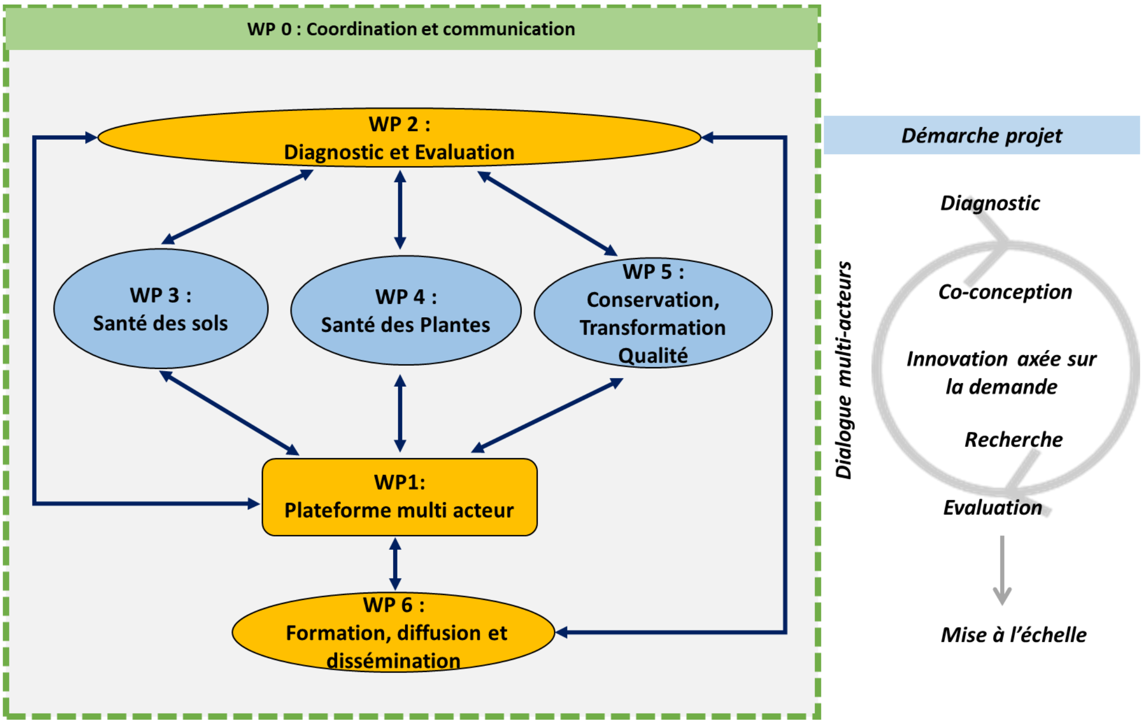


Figure 21. Organisation et démarche du projet Marigo

La première étape du projet sera de faire un diagnostic des pratiques agricoles utilisées dans les 4 zones périurbaines, d'identifier les verrous sociotechniques qui freinent la transition agroécologique et d'identifier des agriculteurs pilotes (WP2). Suite à cette première étape, des ateliers de co-conception seront réalisés avec les plateformes multi-acteurs afin d'identifier des solutions possibles à ces verrous sociotechniques répartis dans 3 domaines : la santé des sols (WP3), la santé des plantes (WP4) et le post-récolte (WP5). Pour les verrous sociotechniques sans solutions agroécologiques, des recherches seront entreprises afin de développer des innovations agroécologiques dans ces 3 domaines (WP3, WP4, WP5). Les solutions existantes et les innovations agroécologiques seront transférées aux producteurs grâce à plusieurs ateliers avec les plateformes multi-acteurs et grâce à des fiches techniques ou clips vidéos pédagogiques (WP6). Pour finir, les impacts sociétaux, économiques et environnementaux des nouveaux systèmes agroécologiques seront évalués (WP2).

Pour ce projet, je commencerai par faire un diagnostic des maladies, des ravageurs, des ennemis naturels et des pollinisateurs des 10 spéculations les plus cultivées en Côte d'Ivoire : tomate, aubergine africaine, piment, haricot vert, concombre/courgette, oignon, chou, salade, gombo et amarante dans les 4 grandes zones péri-urbaines : Abidjan, Yamoussoukro, Bouake et Korhogo en saison sèche et en saison des pluies. Les 30 producteurs pilotes de chaque zone seront également interviewés afin de relever leurs pratiques agricoles et les facteurs pouvant influer la biodiversité (e.g. paysage). Ces études chez les producteurs seront complétées par des études en station expérimentales dans les 4 zones, afin de disposer de parcelles non traitées pour suivre plus facilement l'entomofaune (nuisible et utile) et les maladies ainsi que leur dynamique en saison sèche et en saison des pluies. Ce diagnostic sera suivi par des ateliers afin de restituer les résultats et d'identifier et hiérarchiser les problèmes de protection des cultures en terme de transition agroécologique. Des ateliers de co-conception seront ensuite réalisés afin de trouver des solutions agro-écologiques pour remplacer l'usage des pesticides. Pendant ces ateliers, des innovations agroécologiques permettant d'améliorer la santé des plantes seront alors proposées aux producteurs et évaluées dans une approche de concertation et d'adaptation. Il y aura 3 catégories d'innovations : soit l'innovation existera déjà au niveau local (e.g. filets anti insectes, plantes de service, mesures sanitaires, piégeage, …) et des ateliers de formation seront organisés pour transférer la technologie, soit l'innovation sera en cours de développement (e.g. lutte biologique par conservation pour favoriser des prédateurs et parasitoïdes à fort potentiel de régulation des ravageurs, biocides à base d'extraits de plantes et d'enthomopathogènes, …) et des essais en station seront réalisés pour confirmer son efficacité avant de la transférer aux producteurs, soit l'innovation n'existera pas et des recherches seront lancées pour trouver développer des innovations. L'apiculture sera également développée chez les producteurs les plus avancés en terme de transition agroécologique. Ces connaissances permettront de produire in fine un guide sur les cultures maraichères en Côte d'Ivoire permettant aux producteurs d'identifier les maladies, les ravageurs, les ennemis naturels et les pollinisateurs et de mettre en place des innovations agroécologiques pour lutter contre les bioagresseurs. Les systèmes de cultures agroécologiques, reposant avant tout sur la diversité des espèces cultivées, leur association dans l'espace et leur rotation, seront mis en démonstration dans une ferme pédagogique et pourra servir lors des formations aux producteurs. En parallèle, des travaux de caractérisation, mise en collection, multiplication et diffusion de légumes indigènes non hybrides (aubergine africaine, gombo, amarante, célosie, oseille de Guinées, corète potagère, morelle noire, …) seront réalisés et une banque de semences sera disponible à la fin du projet. L'objectif avec cette banque de semence de légumes indigènes est de concevoir des systèmes de cultures plus diversifiés et résilients dans les différentes zones climatiques.

Lors de projets de recherche précédents en Côte d'Ivoire (Hortinet-CI, C2D,…), nous avons identifié plusieurs verrous technologiques à la transition agroécologique (e.g. problème de la mouche des cucurbitaceae et problème du flétrissement bactérient) et des innovations agroécologiques prometteuses (e.g. utilisation de *Trichoderma* spp. comme biostimulant). Ces 3 problématiques feront l'objet de trois thèses financées par le projet MARIGO, que je co-dirigirerai, et qui débuteront en 2022.

3.1.2 Résultats attendus

Parmi les résultats attendus du projet MARIGO, ceux qui touchent à la transition agroécologique de la filière maraichère en Côte d'Ivoire, sont les suivants :

- Une contribution à l'organisation d'un dialogue politique autour de l'agroécologie
- Une pérennisation de plateformes multi-acteurs sur l'agriculture maraichère
- La création d'un module de formation en agroécologie destiné aux agriculteurs leur permettant d'accéder à différents niveaux de compétence et selon le niveau acquis de bénéficier du label de production biologique ou de former d'autres agriculteurs.
- La création d'un module de formation en agroécologie pour les écoles et les universités.
- La formation à l'agroécologie d'au moins 30 maraichers pilotes dans chacun des 4 sites d'étude, capable de pratiquer une agriculture diversifiée, durable et résiliente face aux changements climatiques et d'approvisionner les villes en produits frais ou transformés répondant en qualité et quantité à la demande sociétale.
- Une contribution à la conception d'un label de production biologique.
- La création d'un comité national représentatif des acteurs de la filière maraichère pour la définition, l'attribution et le respect d'un label biologique ivoirien.
- La création d'un réseau d'agriculteurs labélisés qui s'engagent à respecter une charte locale de bonnes pratiques agroécologiques voire biologiques.
- La création de réseaux locaux d'acteurs autour de la production maraichères pratiquant une économie circulaire où les services comme les produits et les sous-produits des uns sont utilisés par d'autres.
- Une disponibilité d'équipements pilotes de conservation des denrées.
- La production de fiches techniques, de vidéos et d'outils numériques pour faciliter la diffusion et la mise en pratique de systèmes de cultures agroécologiques mais aussi pour faciliter l'accès aux intrants biologiques, au matériel et aux services locaux.
- La participation à la création de microentreprises répondant aux nouveaux métiers de l'agroécologie: production d'intrants biologiques (biofertilisant, biopesticide), production de plants et de semences (pépinière, greffage, …), transformateurs, transporteurs, coopératives.
- La production d'un catalogue variétal des espèces locales et exotiques produites et adaptées aux conditions environnementales de la Côte d'Ivoire.
- La valorisation des savoirs locaux sur la production des légumes traditionnels et leur transformation (recettes) et promouvoir leurs qualités sanitaires et nutritionnelles.

3.1.3 Stratégie de partenariat

Un tel projet nécessite le développement de collaborations étroites avec de nombreux partenaires locaux venant de la recherche et du développement dont les compétences seront complémentaires et les rôles bien définis.

Co-Partenaires impliqués de la recherche :

- Cirad : diagnostic, approche agrotechnique et socio-économique des systèmes alimentaires, santé des plantes, technologies post récolte, ACV

- IRD : protection des sols et fertilisation biologique
- Centre Scientifique de la Recherche Suisse (CSRS) : Evaluation de la qualité nutritionnelle, sanitaire et fonctionnelle
- Institut National Polytechnique FH Boigny - Ecole Nationale Supérieure d'Agriculture : formation académique et technologies numériques et analyses socio-économiques

Partenaires associés de la recherche :

- Université Felix Houphouet Boigny, Centre d'excellence CCBAD/WASCAL: biocontrôle, biodiversité et changement climatique
- Université Nangui Abrogoua : biofertilisant, conservation, transformation, évaluation de la qualité, recyclage
- Université Gon Koulibaly : production de biocides à base d'extraits de plantes
- CNRA : Evaluation de l'efficacité des produits et techniques et analyse des sols

Partenaires associés du développement :

- ANADER : formation et réseaux de producteurs
- ONG IECD : transformation, commercialisation, micro-entreprenariat et systèmes de culture agroécologiques, Yamoussoukro
- ONG NITIDAE : analyse de la demande, communication et label qualité
- ONG FERT : systèmes de culture agroécologiques, Agboville, Bouaké, Korhogo

3.2 Un projet en continuité : utilisation de composés sémio-chimiques pour lutter contre les mouches des cucurbitaceae

3.2.1 Contexte

Les politiques de développement agricoles de la Côte d'Ivoire ont surtout mis l'accent sur les cultures vivrières de base comme le riz et le manioc ou les cultures d'exportations comme le café ou le cacao. Or, la production maraîchère diversifiée du pays est très intéressante sur le plan nutritionnel. Elle est la principale source de vitamines et d'oligoéléments pour les populations rurales les plus vulnérables, soumises à des carences qui peuvent induire des problèmes de santé infantile et aussi pour la population en générale. Les cucurbitacées sont des productions prisées dans la cuisine locale. Mais cette production maraîchère est soumise à de fortes pressions de ravageurs, et principalement les mouches des légumes (Kone et al. 2018 ; N'Dépo & My 2019 ; données personnelles). Pour protéger leurs cultures contre ces ravageurs, les producteurs ivoiriens ont recours à la lutte chimique à forte dose pour ne pas prendre le risque de perdre leur production. De plus, peu ou pas de pratiques agroécologiques sont à ce jour efficaces et/ou présentes localement contre les mouches des légumes. Sans innovations agroécologiques locales et efficaces, les producteurs pilotes du projet MARIGO n'auront pas de solutions pour transformer leur système actuel en système agroécologique. Ce projet de thèse vise à concevoir et évaluer la pertinence d'innovations agroécologiques utilisant les effets de biocontrôle des composés sémiochimiques impliqués dans le système tritrophique mouches-cucurbites-ennemis naturel. Ce projet de thèse est très similaire en termes de méthodologie, que les travaux que j'ai effectués au Kenya, mais sur un nouvel agroécosystème. Les composés sémiochimiques seraient utilisés à la fois pour faire un système « push-pull » qui modifieraient la distribution et l'abondance des mouches de cucurbitacées (Cook et al, 2007) mais aussi pour attirer leurs ennemis naturels (Ayelo et al, 2021a). Aujourd'hui, il existe des pièges attractifs pour les mâles Bactrocera, Dacus, et Zeugodacus (e.g. Anisyl Acetone, Cue-Lure, Raspberry Ketone, and Zingerone) (Royer et al. 2020) mais peu de travaux scientifiques se sont intéressés au piégeage des femelles et il n'a pas été identifié de répulsifs efficaces. Des parasitoides de Bactrocera, Dacus et Zeugodacus ont été identifié en Afrique de l'est (Ekesi et al. 2016 ; Mohamed et al. 2016), mais leur diversité en Côte d'ivoire n'est pas connue et donc aucun attractif d'ennemis naturel n'a été identifié à ce jour.

3.2.1 Objectifs, questions de recherche, hypothèses

L'objectif de ce projet de thèse est de concevoir et d'évaluer la pertinence d'innovations agroécologiques utilisant les effets de biocontrôle des composés sémiochimiques impliqués dans le système tritrophique mouches-cucurbites-ennemis naturel. L'hypothèse principale du projet de thèse est qu'une combinaison d'innovations agroécologiques permettrait une gestion efficace des mouches des légumes. Les hypothèses testées durant ce travail de thèse viseront à vérifier que i) certains composés organiques volatils spécifiques des Cucurbitaceae sont attractifs pour les femelles des mouches des légumes afin de créer un piège et ii) certains composés volatils spécifiques de cucurbitaceae attaqués par les mouches des légumes attirent leurs ennemis naturels et sont répulsifs pour les mouches des légumes. Ainsi, les questions scientifiques suivantes sont posées :
(1) Quels sont les composés organiques volatils émis par la plante impliqués dans l'oviposition des femelles des mouches des légumes ? Peuvent ils être utilisés pour faire un piège efficace ?
(2) Quelles sont les composés induits par l'oviposition des mouches des légumes ? Peuvent ils être utilisés comme répulsif ?
(3) Quels sont les composés émis par les cucurbitaceae impliqués dans l'attraction des ennemis naturels ? Peuvent ils être utilisés pour augmenter le service écosystèmique de suppression des ravageurs ?

3.2.2 Méthodes

Les méthodes envisagées pour répondre à ces questions scientifiques seront divisées en trois tâches :

- La première tâche sera de cartographier et d'identifier les mouches des légumes et leurs ennemis naturels en culture de Cucurbitaceae sur un gradient nord-sud. Des fruits seront prélevés à Korhogo, Bouake, Yamoussoukro et Abidjan à différentes saisons (Appryiadi et al. 2021). Les fruits collectés seront conservés au laboratoire jusqu'à l'émergence des mouches ou des parasitoides. Des fruits pré-infestés au laboratoire pourront servir de fruits sentinelles pour augmenter les chances d'identifier des ennemis naturels. Un filet fauchoir sera également utilisé afin de collecter de potentiels prédateurs. Les itinéraires techniques et les données climatiques et de paysages seront également renseignés pour expliquer la diversité.
- La deuxième tâche sera consacrée à l'étude des interactions chimique mouches-Cucurbitaceae-ennemis naturels. Il s'agira d'analyser la réponse comportementale des mouches femelles aux odeurs de Cucurbitaceae afin d'identifier des attractifs (pull) et des répulsifs (push) (Deletre et al. 2022). La réponse comportementale d'un prédateur et/ou parasitoïde aux odeurs de Cucurbitaceae sera également analysée afin d'identifier des attractifs (Ayelo et al. 2021b ; Ayelo et al. 2021c). Pour permettre l'identification des attractifs et répulsifs, des tests de comportement en olfactomètre et des analyses chimiques par GC-MS seront indispensables. La réponse comportementale de la mouche

et de l'ennemi naturel a des plantes saines et des plantes infestées à différents niveaux d'infestions seront comparées. Les odeurs de ces plantes seront identifiées et quantifiées. Grâce à une méthode statistiques (RF+SPLSDA), les composés attractifs et répulsifs seront sélectionnés. Ces composés seront alors testés un à un et en mélange sur la ou les mouches des légumes et le ou les ennemis naturels.

- La troisième tâche se focalisera sur des tests de terrain pour évaluer les attractifs et répulsifs identifiés comme innovations agroécologique pour la gestion des mouches des légumes (Deletre et al. Soumis). Cette phase finale sera un test en station comparant : (i) témoin négatif (aucun traitement), (ii) témoin positif (traitement insecticide), et (iii) composés sémiochimiques.

3.2.3 Résultats attendus

R1 : cartographie de la biodiversité des mouches des cucurbitaceae et de leurs ennemis naturels en Côte d'Ivoire
R2 : liste de composés attractifs et répulsifs de mouches des cucurbitaceae
R3 : liste de composés attractifs de prédateurs/parasitoides de mouches des cucurbitaceae
R4 : système push-pull pour la lutte contre les mouches des cucurbitaceae
R5 : système agroécologique de production des cucurbitaceae avec attraction d'ennemis naturels
R6 : publications de 4 articles scientifiques et d'une thèse
R7 : communication orale à un congrès international

3.3 De nouveaux projets impliquant l'écologie chimique du sol

Après avoir travaillé de nombreuses années sur les ravageurs aériens de la tomate, je m'intéresserai au compartiment du sol sur des questions autour de biofertilisation, de biostimulation et de lutte biologique. Ces questions peuvent s'apparenter aux questions d'allelopathie soulevées en écologie chimique (Zheng 2014). L'allelopathie est une interaction chimique entre plantes (e.g. tomate et crotalaire) ou entre plantes et micro-organismes (e.g. tomate et trichoderma) dans laquel un organisme produit des sémiochimiques qui influencent la germination, la croissance, la survie et la reproduction d'autres organismes (Duke 2010). Aujourd'hui, il est admis que l'allélopathie joue un rôle prépondérant dans la structuration des communautés végétales, le maintien de la biodiversité et la détermination de la productivité agricole (Zheng 2014). Son application permet de trouver des alternatives écologiques aux problèmes de fertilité des sols, de désherbage et de protection des cultures.

3.3.1 Utilisation de Trichoderma comme biofertilisant et biostimulant pour la culture de la tomate (Solanum lycopersicum L.) en Côte d'Ivoire

Contexte. En Côte d'Ivoire, les besoins en tomate sont estimés à plus de 200 000 tonnes alors que la production nationale qui est de 40 000 tonnes/an reste largement inférieure pour couvrir cette demande (FAOstat 2020). Ces besoins sont satisfaits grâce aux importations massives du Burkina Faso et de l'Europe. De nombreux facteurs peuvent être évoqués pour expliquer la faiblesse de la production de cette culture en Côte d'Ivoire, comme l'impact des bioagresseurs et la baisse de fertilité des sols qui affecte la levée, la croissance et le rendement des cultures (Shankara et al. 2005). L'utilisation des intrants chimiques n'étant pas une solution durable, l'utilisation d'agents biologiques (i.e. bactéries ou champignons) est prometeuse pour ce qui concerne la biofertilisation, la biostimulation, ou la lutte biologique, sans perturber l'équilibre de l'environnement et de l'écosystème. Parmi ces agents biologiques, les *Trichoderma* spp. sont des champignons utilisés pour leur propriété en matière de lutte biologique, de biofertilisation et de biostimulation. En effet, ils sont connus pour attaquer d'autres champignons, pour améliorer la croissance des plantes et des racines et renforcer les mécanismes de défense de la plante (Waghunde et al. 2016). Le présent projet de thèse vise à la mise au point d'un biofertilisant/biostimulant pour l'amélioration de la production de tomate à partir de souches de Trichoderma spp. locales. En effet, *Trichoderma* spp. fabriquent des nutriments par différents processus biologiques qui favorisent la croissance des plantes et améliorent les propriétés du sol et les activités microbiennes pendant une période plus longue que les engrais chimiques (Bandhari et al. 2021). *Trichoderma* spp. agit également comme agent de lutte biologique par différents mécanismes: (i) l'antibiose qui résulte de la production de substances qui agissent comme des antibiotiques et qui inhibent la croissance de l'agent pathogène, (ii) la compétition qui se manifeste par l'aptitude de *Trichoderma* spp. à utiliser les mêmes ressources du milieu (aires d'alimentation, sites de développement) que les champignons pathogènes et qui est capable de se développer avec des concentrations faibles en éléments nutritifs par rapport à d'autres champignons, (iii) le mycoparasitisme qui se manifeste par la destruction de l'agent en l'étranglant, en pénétrant à l'intérieur et/ou en lui injectant des enzymes qui le détruisent, (iv) la résistance induite qui induit directement les défenses de la plante. Ce dernier mode d'action de résistance induite est ce qui nous intéresse ici pour l'étude de l'effet biostimulant.

Objectifs, questions de recherche, hypothèses. L'objectif de ce projet de thèse est de mettre au point un biofertilisant/biostimulant à partir de souches locales de Trichoderma spp. pour améliorer la production de tomates. L'hypothèse principale du projet de thèse est que les *Trichoderma* spp. auraient un effet biostimulant en activant les défenses des plants de tomates et un effet fertilisant en augmentant la croissance racinaire. Les hypothèses testées durant ce travail de thèse viseront à vérifier que (i) il éxiste des souches locales de *Trichoderma* spp. dans les parcelles de tomates, et (ii) des solutions de *Trichoderma* spp. augmentent la vigueur (i.e. croissance/résistance/rendement) de la tomate. Ainsi, les questions scientifiques suivantes sont posées :

(1) Quelle est la biodiversité des souches de *Trichoderma* spp. en Côte d'Ivoire ?
(2) Quel est l'effet biofertilisant/biostimulant de ces différentes souches sur la tomate?
(3) Est-ce que cette innovation pourrait être adoptée par les producteurs ?

Méthodes. Les méthodes envisagées pour répondre à ces questions scientifiques seront divisés en quatre tâches :

- *Inventaire et caractérisation macroscopique et microscopique des souches de Trichoderma sp.*

Des échantillons de sol seront prélevés à Korhogo, Bouake, Yamoussoukro et Abidjan dans des parcelles de cultures de tomate. Les différentes souches de *Trichoderma* spp. seront isolées en utilisant la méthode de piégeage avec pomme de terre, la méthode de dilution et la méthode de piégeage avec les grains de céréales. Ces souches seront purifiées sur des milieux PDA avant d'être identifiées au niveau macroscopique et microscopique. Les itinéraires techniques et les données climatiques et de paysages seront également renseignés pour expliquer la diversité des souches de Trichoderma spp.. Une banque des différentes souches sera réalisée.

- *Évaluation de l'efficacité des souches de Trichoderma sp. sur les paramètres agromorphologiques et le rendement de la tomate en station*

Cette tâche se fera en trois étapes : des parcelles de tomates seront mises en culture à la station expérimentale de l'université Nangui Abrogoua, puis les différentes parcelles seront traitées avec des suspensions sporales à la transplantation, 30 jours après la transplantation au moment de la floraison et 60 après la transplantation au moment de la fructification. L'évaluation de l'effet des souches de *Trichoderma* spp. sur les paramètres agromorphologiques (vitesse de croissance, surface foliaires, poids sec des parties aériennes et racinaires, …) et le rendement des plants de tomate seront comparés avec des parcelles utilisant les pratiques habituelles des agriculteurs.

- *Identification moléculaire des 6 souches de Trichoderma sp. les plus performantes*

Les 6 souches qui auront été les plus performantes en station expérimentale seront identifiées au niveau moléculaire par PCR.

- *Comparaison en milieu paysan de l'effet du biofertilisant/ biostimulant à base de Trichoderma sur les paramètres agromorphologiques et le rendement de la tomate par rapport aux pratiques habituelles des producteurs*

Un atelier de co-conception/sensibilisation auprès de 2 groupes de 10 producteurs sera réalisé afin d'expliquer l'innovation, de sélectionner la ou les modalités à tester et de sélectionner 5 agriculteurs par groupe qui testeront le biofertilisant/biostimulant à Abidjan, et Yamoussoukro ou Bouake. L'innovation sera ensuite transférée chez les agriculteurs et le même suivi que celui de la station sera effectué. A la fin du cycle de production, une enquête sera réalisée auprès des producteurs afin de recueillir leurs avis sur l'utilisation de ce nouveau produit à base de *Trichoderma* spp. Cette tâche nous permettra d'évaluer l'acceptabilité par les producteurs de ce nouveau produit et de tester l'effet biofertilisant/biostimulant des souches de *Trichoderma* spp. en milieu paysan par rapport à leurs pratiques habituelles.

Résultats attendus.

R1 : Bibliothèque des souches de *Trichoderma* spp.
R2: Caractérisation de l'activité biofertilisant/biostimulant des souches de *Trichoderma* spp.
R3 : Formulation et mise au point d'un biofertilisant/biostimulant à base de *Trichoderma* spp.
R4 : Publication de 3 articles scientifiques et d'une revue sur *Trichoderma* spp.
R5 : Mémoire et attestation de soutenance de la thèse
R6 : Communication orale à un congrès international

3.3.2 Evaluation de solutions innovantes à base de Crotalaria spp. pour lutter contre le flétrissement bactérien (R. solanacearum) des cultures maraîchères en Côte d'Ivoire

Contexte. Le flétrissement bactérien est une maladie causée par la bactérie tellurique *Ralstonia solanacearum*. Cette bactérie est répartie à travers le monde et est considérée comme l'un des agents pathogènes les plus importants sur cultures maraîchères de rente et vivrière (Lebeau et al, 2011). En Côte d'Ivoire, le flétrissement bactérien est la maladie tellurique majeure des cultures maraîchères, en particulier des cultures de tomate (N'guessan et al., 2012). En effet, *R. solanacearum* peut causer jusqu'à 100 % de pertes de rendement selon l'espèce et la variété, entraînant ainsi l'abandon de certaines parcelles par les producteurs (Fondio et al., 2008). Les facultés exceptionnelles d'adaptation de *R. solanacearum*, sa forte diversité génétique et phénotypique augmente considérablement les difficultés à trouver des méthodes de contrôle et de lutte durables (Poussier 2000). Les stratégies actuelles de lutte contre cette maladie reposent essentiellement sur la résistance génétique, mais cette résistance est partielle, fortement dépendante de l'environnement et s'avère aujourd'hui insuffisante (N'guessan et al. 2020). La stratégie de lutte intégrée combinant différentes pratiques culturales dont la résistance variétale, les rotations et les associations culturales avec des espèces assainissantes ainsi que les méthodes de biocontrôle à base de substances naturelles reste la voie privilégiée pour combattre cette maladie dévastatrice (N'guessan et al. 2020 ; Deberdt et al. 2018 ; Deberdt et al. 2012).

C'est dans ce contexte que s'inscrit ce projet de thèse qui vise à contribuer au développement de nouvelles solutions de biocontrôle à base d'extraits de crotalaires pour lutter contre le flétrissement bactérien des cultures maraîchères en Côte d'Ivoire, et plus particulièrement sur la tomate. Dans des études préliminaires réalisées par mon unité de recherche, nous avons montré que *Crotalaria spectabilis* pouvait être utilisée comme plante assainissante lorsqu'elle est plantée avant la culture de tomate (Deberdt et al. 2015). L'incidence du flétrissement bactérien sur la tomate est alors diminuée de 60% après l'utilisation de *C. spectabilis*, ainsi que la proportion de plantes infectées. *Crotalaria juncea* and *Crotalaria spectabilis* ont également montré des propriétés intéressantes au moment de leur décomposition dans le sol (Deberdt et al. 2018).

Objectifs, questions de recherche, hypothèses. L'objectif de ce projet de thèse est d'élaborer une nouvelle solution de biocontrôle à base de crotalaire pour lutter contre le flétrissement bactérien des cultures maraîchères en Côte d'Ivoire. L'hypothèse principale du projet de thèse est que la diversification végétale, comme l'utilisation des crotalaires, pourrait être utilisée pour lutter contre le flétrissement bacterien. Les hypothèses testées durant ce travail de thèse viseront à vérifier que i) les différentes espèces locales de crotalaires n'ont pas le même effet sur le flétrissement bactérien, ii) ces espèces doivent être utilisée différemment en fonction de leur matière active, et (iii) certains modes d'utilisation sont plus à même d'être adopté par les producteurs. Ainsi, les questions scientifiques suivantes sont posées :
(1) Existe-t-il une variété de crotalaire locale et efficace ?
(2) Quel est la meilleure utilisation des crotalaires : broyat sec, engrais vert, extrait aqueux, purin ?
(3) Quel sont les composés biocides ?
(4) Est ce que cette nouvelle pratique culturale pourrait être adoptée par les producteurs ?

Méthodes. Au préalable, les différents modèles biologiques seront identifiés et caractérisés : variété de la tomate, variété de crotalaires, souche de *Ralstonia solanacearum*. Les méthodes envisagées pour répondre à ces questions scientifiques seront divisés en six tâches :

➢ *Evaluation in vitro de l'efficacité de différents extraits de crotalaires :*

2 espèces de crotalaires seront étudiées : *Crotalaria retusa* et *Crotalaria juncea*. Le biopesticide NECO sera utilisé comme témoin positif et pour toute la durée de la thèse. 4 modes d'utilisation (extrait) de la crotalaire seront évalués :
- le broyat sec (gros morceaux de feuilles séchées et intégrées dans le sol)
- l'extrait aqueux (poudre de feuilles séchées dans l'eau pour aspersion au sol)
- l'engrais vert (gros morceaux de feuilles fraiches et intégrées dans le sol)
- le purin de crotalaire (fermentation de feuilles fraiches dans de l'eau pour aspersion au sol)

L'effet biocide (antibactérien) des solutions aqueuses de ces 4 modes d'utilisations sera testé sur la croissance in vitro de *Ralstonia solanacearum* en boite de pétri pour les 2 espèces de crotalaires, soient 8 modalités.

➢ *Evaluation in vivo de l'efficacité de différents extraits de crotalaires :*

Parmi les 8 modalités testées précédemment, les 4 plus efficaces (i.e. meilleur effet biocide) seront testées in vivo. Des plants de tomates seront produits en pots avec un sol infecté avec du *Ralstonia solanacearum* puis les solutions acqueuses de crotalaires seront ajoutées. Les symptômes de la maladie seront suivis ainsi que la dynamique de population de *R. solanacearum* dans le sol. A la fin du test, les infections latentes sur les plantes saines (indice de colonisation) seront enregistrées ainsi que le poids frais et sec des parties aériennes et des parties racinaires de la tomate.

- *Caractérisation de la composition chimique de différents extraits de crotalaires efficaces*

Parmi les 4 modalités testées précédemment, les 2 plus efficaces seront analysées en HPLC afin d'identifier les composés bio-actifs des extraits.

- *Démonstration de l'efficacité de ces extraits de crotalaires sur le contrôle du flétrissement en station expérimentale*

Un test en station sera réalisé afin d'évaluer l'effet biocide des 2 meilleurs extraits de crotalaires sur le flétrissement bactérien dans un sol naturellement infesté par R. solanacearum. 4 modalités seront testées avec 4 répétitions sur 2 saisons : (i) témoin négatif (pas de traitement), (ii) témoin positif (traitement NECO), (iii) extrait 1 de crotalaires (iv) extrait 2 de crotalaires. 2 fois par semaine, l'évolution de la maladie sera observée. Le rendement sera également enregistré. La bioactivité du sol et ses caractéristiques seront évaluées avant l'essai, après la saison 1 et après la saison 2.

- *Réalisation d'une étude d'acceptabilité de ce nouveau produit à base de crotalaires auprès des producteurs.*

Un atelier de co-conception/sensibilisation auprès de 2 groupes de 10 producteurs sera réalisé afin d'expliquer l'innovation, de sélectionner la ou les modalités à tester et de sélectionner 5 agriculteurs par groupe (Abidjan, et Yamoussoukro ou Bouake) qui testeront l'innovation. L'innovation sera ensuite transférée chez les agriculteurs et le même suivi que celui de la station sera effectué mais à raison d'une fois par semaine. A la fin du cycle de production, une enquête sera réalisée auprès des producteurs afin de recueillir leurs avis sur l'utilisation de ce nouveau produit à base de crotalaires. L'efficacité de ce nouveau produit sur le développement du flétrissement bactérien *Ralstonia solanacearum* de la tomate sera donc évaluée en conditions réelles ainsi que l'acceptabilité par les producteurs de ce nouveau produit.

Résultats attendus.

R1 : Connaissances sur l'efficacité des crotalaires sur le flétrissement bactérien
R2 : Formulation et mise au point d'un nouveau produit à base de crotalaires
R3 : Publication de 3 articles scientifiques et d'une revue littéraire
R4 : Mémoire et attestation de soutenance de la thèse
R5 : Communication orale à un congrès international

3.4 Un projet à plus long terme avec une démarche One Health

Je travaille avec mes collègues et partenaires de Côte d'Ivoire depuis un an sur le montage d'un projet one health qui incluera la santé des plantes, la santé des animaux sauvages et domestiques, la santé des hommes et la santé des écosystèmes (e.g. biodiversité, sol, eau, …). L'approche one health s'est considérablement développée ces dernières années mais la santé des plantes y est souvent sous-considérée. Notre hypothèse est qu'une approche socio-écologique de la santé couplée à la transition agroécologique pourrait améliorer la santé globale à l'échelle d'un territoire et contribuer à la mise en place d'agrosystèmes durables. L'objectif général du projet est d'accompagner la mise en place de changements de pratiques et d'interactions entre les acteurs d'un même territoire dans le cadre d'une transition agroécologique et la formulation d'indicateurs pour évaluer l'impact de ces changements sur la santé du territoire. Le projet a été construit en suivant les démarches de théorie du changement avec l'identification des *inputs* (ressources mobilisées par la recherche), des *outputs* (produits de la recherche), des *outcomes* (adoption des produits de la recherches) et des changements/impacts et la création d'un cadre logique.

La première tâche de ce projet consisterait à caractériser un territoire et faire le diagnostic de la santé de ce même territoire. 3 zones d'études ont été selectionnées : la zone de Korhogo au nord, la zone de Bouaflé au centre et la zone de Tiassalé au sud. Ces 3 zones seraient alors cartographiées, les systèmes de productions et la typologie des agriculteurs caractérisées et les principaux vecteurs, ravageurs et maladies identifiés. Des analyses de sol et d'eau seront également faites afin de quantifier les pollutions.

La deuxième tâche consisterait à mettre en évidence les interactions entre les différentes santés. Pour cela, une démarche participative semble la plus adaptée afin d'identifier les enjeux des socioécosystèmes qui ont du sens pour les différentes parties prenantes d'un territoire. Parmi ces interactions, il y aura les problèmatiques d'accès aux ressources du territoire (eau, sol, main d'œuvre, …) mais aussi les problématiques de prévention/gestion des bioagresseurs (maladies, vecteurs, …).

La troisième tâche consisterait à améliorer la santé globale du territoire par la transition agroécologique et l'appui technique et technologiques pour la résolution des interactions négatives. Pour favoriser ces changements, des ateliers de co-conception seront organisés afin de développer des actions concertées qui amélioreront de manière durable ces enjeux de santé du territoire comme (i) l'adoption de pratiques agro-écologiques, (ii) l'optimisation des flux de ressources entre les systèmes de production (problématiques liées à l'eau, flux de matière organique, …), (iii) la labellisation d'un territoire, (iv) la mise en place de filière locale, …

Enfin, la quatrième tâche serait l'analyse économique, environnemental et social de ce territoire avant et après le projet. Des indicateurs de la santé seront co-construits avec les différents acteurs afin de qualifier et de suivre l'état de santé du territoire mais aussi de suivre et d'évaluer les dynamiques des systèmes socioécologiques suite aux différents changement du système.

3.5 Conclusion générale

Depuis toujours, je vois le système de production comme un jeu de stratégie dans lequel il faudrait mettre en place des tactiques pour optimiser sa production et en particulier, pour lutter contre les bioagresseurs, c'est d'ailleurs cet aspect ludique, qui m'a fait choisir la spécialité « protection des plantes et environnement » à l'école d'agro. Au début, j'ai *joué* à l'échelle de la plante et de la parcelle et j'aimerais, dans le futur, *jouer* à l'échelle de l'exploitation et du territoire.

Mieux connaitre et comprendre les interactions trophiques et chimiques est un point commun de l'ensemble de mes activités de recherche. La description des agro-écosystèmes et de leurs interactions permet ainsi de développer des innovations agroécologiques et de concevoir des systèmes de production plus durable en identifiant les leviers de régulation à manier pour la lutte contre les ravageurs. Parmi ces leviers, la diversification végétale, la manipulation des comportements des ravageurs ou le développement de la lutte biologique ont été et resteront mes principaux thèmes de recherche.

Aujourd'hui, avec le projet européen Desira MARIGO, je souhaite poursuivre mes recherches sur la conception d'innovation agroécologique (e.g. innovations contre les mouches des légumes ou le flétrissement bactérien) mais aussi aller plus loin en travaillant sur leur adoption. Ce nouveau projet de recherche me permettra aussi de m'intéresser au compartiment du sol sur lequel j'ai peu travaillé jusqu'à maintenant (e.g. travaux sur Trichoderma spp.). Pour ce nouveau projet, j'essaierai d'intégrer des innovations agroécologiques dans un nouveau système de production à l'aide d'une démarche participative afin de les faire adopter plus facilement. La méthodologie de la co-conception sera l'outil qui me permettra de passer de l'échelle de la parcelle à l'échelle de l'exploitation et du territoire afin de transformer durablement les systèmes alimentaires par la transition agroécologique.

Ainsi après 10 ans de recherche à l'unité de recherche HortSys (Fonctionnement agroécologique et performances des systèmes de cultures horticoles) du cirad, la publication d'une trentaine d'articles et l'encadrement de trois étudiants en thèse, je présente avec ce mémoire ma candidature pour l'obtention du diplôme d'habilitation à diriger des recherches. Obtenir ce diplôme est une étape indispensable dans mon plan de carrière, puisqu'il me permettra d'atteindre mon objectif professionnel de diriger mes propres étudiants en thèse. En 2022, j'encadrerai un étudiant en thèse sur *l'utilisation de composés sémio-chimiques pour lutter contre les mouches des cucurbitaceae* qui sera inscrit l'ED GAIA. J'aimerais pouvoir assurer la direction de cette thèse suite à l'obtention de ce diplôme.

Travaillant en Afrique, j'espère pouvoir y former les chercheurs de demain qui deviendront mes futurs collègues. Je recrute mes futurs étudiants sur la base de leur motivation. J'estime que mon rôle est de les former quelques soient leurs cursus du fait des grandes inégalités d'accès à l'éducation en Afrique. J'identifie les forces et faiblesses du doctorant et j'adapte mon encadrement pour l'accompagner au mieux. De mon point de vue, l'encadrement de doctorants relèvent de deux principaux défis pour lesquels j'ai développé une méthodologie. Le premier est l'acquisition du raisonnement scientifique, i.e. savoir formuler une question de recherche et des hypothèses suite à une revue de la littérature, développer un protocole pour tester ses hypothèses puis analyser et discuter ses résultats. Mes doctorants commencent donc par rédiger leur projet de thèse en suivant ce raisonnement : questions, hypothèses, protocoles. Le second défi est, pour moi, la diffusion des connaissances scientifiques acquises, soit la publication d'articles. Pour relever ce défi, j'ai développé un guide pour aider le doctorant à écrire son premier article. Les doctorants que j'ai encadrés ont publié un minimum de 4 articles scientifiques. Aujourd'hui, je donne également des formations *introduction à l'écriture d'article*. En plus de développer un sens critique, mes doctorants doivent monter et gérer le budget et le planning de leur thèse afin de développer leur compétence de gestion de projet. Avec ces différentes méthodologies que j'ai développées, j'espère ainsi être une bonne future directrice de thèse.

4. References

1. Apriyadi, R., Saputra, H. M., Sintia, S., & Andini, D. E. (2021). The Diversity of Fruit Fly (Diptera: Tephritidae) on Combination of Attractant and Different Trap Height in Cucumber Field (Cucumis sativus L.). In IOP Conference Series: Earth and Environmental Science (Vol. 926, No. 1, p. 012059). IOP Publishing.
2. Adandonon A, Vayssieres JF, Sinzogan A, Van Mele P (2009) Density of pheromone sources of the weaver ant *Oecophylla longinoda* affects oviposition behaviour and damage by mango fruit flies (Diptera: Tephritidae). Int J Pest Manag 55:285–292
3. Aigbedion-Atalor PO, Mohamed SA, Hill MP, Zalucki MP, Azrag AGA, Srinivasan R, Ekesi S (2020) Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. Biol Control 144:104215.
4. Arakaki N, Yamazawa H, Wakamura S (2011) The egg parasitoid *Telenomus euproctidis* (Hymenoptera: Scelionidae) uses sex pheromone released by immobile female tussock moth *Orgyia postica* (Lepidoptera: Lymantriidae) as kairomone. Appl Entomol Zool 46:195–200.
5. Bhandari, S., Pandey, K. R., Joshi, Y. R., & Lamichhane, S. K. (2021). An overview of multifaceted role of Trichoderma spp. for sustainable agriculture. Archives of Agriculture and Environmental Science, 6(1), 72-79.
6. Benelli, G., Daane, K. M., Canale, A., Niu, C. Y., Messing, R. H., & Vargas, R. I. (2014). Sexual communication and related behaviours in Tephritidae: current knowledge and potential applications for Integrated Pest Management. Journal of Pest Science, 87(3), 385-405.
7. Biondi A, Guedes RNC, Wan F-H, Desneux N (2018) Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. Annu Rev Entomol 63:239–258.
8. Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: harnessing ecosystem services for food security. Trends in ecology & evolution, 28(4), 230-238.
9. Bradshaw, J.W.S.; Baker, R.; Howse, P.E. (1979) Chemical composition of the poison apparatus secretions of the African weaver ant, *Oecophylla longinoda*, and their role in behaviour. Physiol. Entomol., 4, 39–46.
10. Calvo FJ, Lorente MJ, Stansly PA and Belda JE (2012). Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisia tabaci* in greenhouse tomato. Entomol Exp Appl 143:111–119.
11. Caparros Megido, R., Haubruge, E., & Verheggen, F. (2013). Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review. Biotechnologie, Agronomie, Société et Environnement, 17(3), 475-482.
12. Cayol, J. P. (1999). Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes. In Fruit Flies (Tephritidae) (pp. 861-878). CRC press.
13. Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R., & Desneux, N. (2013). Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. Journal of Pest Science, 86(3), 533-541.
14. Chen H, Jones AD, Howe GA (2006) Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. FEBS Lett 580:2540–2546.
15. Chuche J, Xuéreb A, Thiéry D (2006) Attraction of *Dibrachys cavus* (Hymenoptera : Pteromalidae) to its host frass volatiles. J Chem Ecol 32:2721–2731
16. Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. Annu. Rev. Entomol., 52, 375-400.
17. Danner H, Desurmont GA, Cristescu SM and van Dam NM, (2018). Herbivore induced plant volatiles accurately predict history of coexistence, diet breadth, and feeding mode of herbivores. New Phytol 220:726–738
18. Dainese, M., Schneider, G., Krauss, J., & Steffan-Dewenter, I. (2017). Complementarity among natural enemies enhances pest suppression. Scientific reports, 7(1), 1-8.
19. De Bon, H., Huat, J., Parrot, L., Sinzogan, A., Martin, T., Malézieux, E., & Vayssières, J. F. (2014). Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. A review. *Agronomy for sustainable development*, *34*(4), 723-736.
20. De Carvalho LM, Nunes MUC, de Oliveira IR, de Leal M (2009) Yield of tomato in monocrop and intercropping with aromatics plants. Hort Brasil 27:458–464
21. De Moraes, C. M., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature, 410(6828), 577-580.
22. Deberdt P., Perrin B., Coranson-Beaudu R., Duyck P. & Wicker E. (2012). Effect of *Allium fistulosum* Extract on *Ralstonia solanacearum* Populations and Tomato Bacterial Wilt. Plant disease 96: 687-692.

23. Deberdt, P., Gozé, E., Coranson-Beaudu, R., Perrin, B., Fernandes, P., Lucas, P., & Ratnadass, A. (2015). *Crotalaria spectabilis* and *Raphanus sativus* as previous crops show promise for the control of bacterial wilt of tomato without reducing bacterial populations. *Journal of Phytopathology*, 163(5), 377-385.
24. Deberdt P., Fernandes P., Coranson-Beaudu R., Minatchi S., Ratnadass A. (2018). The use of biocontrol plants to manage bacterial wilt of tomato in the tropics. In: Proceedings of the V International Symposium on Tomato Diseases: Perspectives and Future Directions in Tomato Protection. Acta Horticulturae, 1207.
25. Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, González-Cabrera J et al (2010) Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J Pest Sci 83(3):197–215.
26. Duke, S. O. (2010). Allelopathy: current status of research and future of the discipline: a commentary.
27. Dumont F, Aubry O and Lucas E, (2018) From evolutionary aspects of zoophytophagy to biological control. Front Ecol Evol 6:221.
28. Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M. A., Justes, E., ... & Sarthou, J. P. (2015). How to implement biodiversity-based agriculture to enhance ecosystem services: a review. Agronomy for sustainable development, 35(4), 1259-1281.
29. Ekesi, S., Billah, M. K., Nderitu, P. W., Lux, S. A., & Rwomushana IV, I. (2009). Evidence for competitive displacement of *Ceratitis cosyra* by the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on mango and mechanisms contributing to the displacement. Journal of Economic Entomology, 102(3), 981-991
30. Ekesi, S., De Meyer, M., Mohamed, S. A., Virgilio, M., & Borgemeister, C. (2016). Taxonomy, ecology, and management of native and exotic fruit fly species in Africa. Annual review of entomology, 61, 219-238.
31. FAO (2009a) How to feed the world in 2050, Global agriculture toward 2050.
32. FAO (2009b) How to feed the world in 2050, The special challenge for sub-Saharan Africa.
33. Finke, D. L., & Snyder, W. E. (2010). Conserving the benefits of predator biodiversity. Biological Conservation, 143(10), 2260-2269.
34. Fondio L., N'Tamon L. N., Hala F. N., H. A. D. (2008). Evaluation agronomique de six cultivars d'aubergine africaine (Solanum spp.) de la nouvelle collection des plantes légumières du CNRA. Agronomie Africaine 20: 69-79.
35. Gamarra H, Carhuapoma P, Mujica M, Kreuze J, Kroschel J (2016) Greenhouse Whitefly, *Trialeurodes vaporariorum* (Westwood 1956). In: Kroschel J, Mujica N, Carhuapoma P, Sporleder M (eds.) Pest distribution and risk atlas for Africa. Potential, global and regional distribution and abundance of agricultural and horticultural pests and associated biocontrol agents under current and future climates, 154:154–168. Lima, Peru: International Potato Center (CIP).
36. Górski R (2004) Effectiveness of natural essential oils in the monitoring of greenhouse whitefly *(Trialeurodes vaporariorum* Westwood). Folia Hortic 16:183–187
37. Guedes RNC, Roditakis E, Campos MR, Haddi K, Bielza P, Siqueira HAA et al (2019) Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. J Pest Sci 92(4):1329–1342.
38. Han P, Bayram Y, Shaltiel-Harpaz L, Sohrabi F, Saji A, Esenali UT, Jalilov A et al (2019) *Tuta absoluta* continues to disperse in Asia: damage, ongoing management and future challenges. J Pest Sci 92(4):1317–1327.
39. Hansen, A. N., & Henrik, H. (2019). Why are there so few examples of entomopathogenic fungi that manipulate host sexual behaviors?. Fungal Ecology, 38, 21-27.
40. Hanssen IM, Lapidot M (2012) Major tomato viruses in the mediterranean basin. Adv Virus Res 84:31-66. Elsevier Inc.
41. Hermann, S. L., & Thaler, J. S. (2014). Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. Oecologia, 176(3), 669-676.
42. Kaur S, Kaur S, Srinivasan R, Cheema D, Lal T, Ghai T, Chadha M (2010) Monitoring of major pests on cucumber, sweet pepper and tomato under net-house conditions in Punjab, India. Pest Manag Hortic Ecosyst 16(2):148–155
43. Kelly, J. L., Hagler, J. R., & Kaplan, I. (2014). Semiochemical lures reduce emigration and enhance pest control services in open-field predator augmentation. Biological Control, 71, 70-77.
44. Kone, K., Tuo, Y., Yapo, M. L., & Kouakou, H. K. (2018). Entomofaune de la courgette (Cucurbita pepo L) en saison pluvieuse, à Korhogo, dans le nord de la Côte d'Ivoire. International Journal of Biological and Chemical Sciences, 12(3), 1286-1297.
45. Lapidot, M., J. P. Legg, W. M. Wintermantel, and J. E. Polston. 2014. Management of whitefly-transmitted viruses in open-field production systems. Adv. Virus Res. 90: 147–206.
46. Lebeau A., Daunay M. C., Frary A., Palloix A., Wang J. F., Dintinger J., Chiroleu F., Wicker E., Prior P. (20l1). Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. Phytopathology 101: 154-165.
47. Liu T-X, Stansly PA, Gerling D (2015) Whitefly parasitoids: distribution, life history, bionomics, and utilization. Annu Rev Entomol 60:1–15.

48. Mansour R, Brévault T, Chailleux A, Cherif A, Grissa-Lebdi K, Haddi K, Mohamed SA et al (2018) Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. Entomol Gen 38:38–112.
49. Martin, T., Assogba-Komlan, F., Houndete, T., Hougard, J. M., & Chandre, F. (2006). Efficacy of mosquito netting for sustainable small holders' cabbage production in Africa. Journal of economic entomology, 99(2), 450-454.
50. Mbaluto, C. M., Ayelo, P. M., Duffy, A. G., Erdei, A. L., Tallon, A. K., Xia, S., ... & Becher, P. G. (2020). Insect chemical ecology: chemically mediated interactions and novel applications in agriculture. Arthropod-plant interactions, 14(6), 671-684.
51. Mohamed, S. A., Ramadan, M. M., & Ekesi, S. (2016). In and out of Africa: Parasitoids used for biological control of fruit flies. In Fruit fly research and development in Africa-towards a sustainable management strategy to improve horticulture (pp. 325-368). Springer, Cham.
52. Moodley, V., A. Gubba, and P. L. Mafongoya. 2019. A survey of whitefly-transmitted viruses on tomato crops in South Africa. Crop. Prot. 123: 21–29.
53. Moreau, J., Desouhant, E., Louâpre, P., Goubault, M., Rajon, E., Jarrige, A., ... & Thiéry, D. (2017). How host plant and fluctuating environments affect insect reproductive strategies? In Advances in botanical research (Vol. 81, pp. 259-287). Academic Press.
54. Mutisya S, Opiyo A, Martin T, Ngouajio M, Saidi M (2016) Synergistic effects of agronet covers and companion cropping on reducing whitefly infestation and improving yield of open field-grown tomatoes. Agronomy 6:42.
55. N'Dépo, O. R., & My, M. (2019). Host plants associated with tephritidae in Côte d'Ivoire and discovery of a new fruit fly species: Dasus longistylus. Journal of Entomology and Zoology Studies, 7(3), 1301-1308.
56. N'guessan, A.C., 2012. Phylogénie, structure génétique et diversité de virulence de *Ralstonia solanacearum* Yabuuchi et al. (1995) [Burkhloderiacées], agent du flétrissement bactérien en Côte d'Ivoire. Thèse de Doctorat de l'Université de Cocody-Abidjan. C.I. 251 pp.
57. N'guessan A. C., Camara B., Amari L. N. D. G. E., Doumbouya M., Pakora G. A., Abo K., Koné D. (2020). Efficacy of the Biopesticide NECO in the control of Ralstonia solancearum, causal agent of tomato bacterial wilt in Côte d'Ivoire. Journal of Advances in Agriculture Vol 11. ISSN: 2349-0837.
58. Nishida R, Shelly TE, Kaneshiro KY, Tan KH (2000) Roles of semiochemicals in mating systems: a comparison between oriental fruit fly and medfly. In: Tan KH (ed) Area-wide control of fruit flies and other insect pests. Penerbit USM, Penang, pp 631–637
59. Palacios M., Cisneros F. (1995) Management of the Potato Tuber Moth. Program 4, Integrated pest management. International Potato Center Program, Report, pp. 87-91
60. Parolin, P., Bresch, C., Desneux, N., Brun, R., Bout, A., Boll, R., & Poncet, C. (2012). Secondary plants used in biological control: a review. International Journal of Pest Management, 58(2), 91-100.
61. Pereira, R., Yuval, B., Liedo, P., Teal, P. E. A., Shelly, T. E., McInnis, D. O., & Hendrichs, J. (2013). Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. Journal of Applied Entomology, 137, 178-190.
62. Pérez-Hedo M, Riahi C and Urbaneja A, (2020) Use of zoophytophagous mirid bugs in horticultural crops: current challenges and future perspectives. Pest Manag Sci 77:33–42.
63. Pérez-Hedo M and Urbaneja A, (2016) The zoophytophagous predator *Nesidiocoris tenuis*: a successful but controversal biological control agent in tomato crops, in Advances in Insect Control and Resistance Management, ed. by Horowitz AR and Ishaaya I. Springer, Cham, pp. 121–138.
64. Pleydell, D. R., & Bouyer, J. (2019). Biopesticides improve efficiency of the sterile insect technique for controlling mosquito-driven dengue epidemics. Communications biology, 2(1), 1-11.
65. Poussier S. (2000). Exploration de la diversité génétique de Ralstonia solanacearum, agent du flétrissement bactérien. Détection et dynamique des populations dans les réservoirs d'inoculum., Ecole Doctorale Vie-Agronomie-Santé, Rennes I, France. pp. 149.
66. Renwick JAA, Chew FS (1994) Oviposition behavior in Lepidoptera. Ann Rev Entomol 39:377–400
67. Royer, J. E., Tan, K. H., & Mayer, D. G. (2020). Comparative Trap Catches of Male Bactrocera, Dacus, and Zeugodacus Fruit Flies (Diptera: Tephritidae) With Four Floral Phenylbutanoid Lures (Anisyl Acetone, Cue-Lure, Raspberry Ketone, and Zingerone) in Queensland, Australia. Environmental entomology, 49(4), 815-822.
68. Rull, J., Diaz-Fleischer, F., & Arredondo, J. (2007). Irradiation of *Anastrepha ludens* (Diptera: Tephritidae) revisited: optimizing sterility induction. Journal of Economic Entomology, 100(4), 1153-1159.
69. Rusch, A., Bommarco, R., & Ekbom, B. (2017). Conservation biological control in agricultural landscapes. In Advances in botanical research (Vol. 81, pp. 333-360). Academic Press.

70. Saidi M, Gogo E, Itulya F, Martin T, Ngouajio M (2013 Microclimate modification using eco-friendly nets and floating row covers improves tomato (*Lycopersicon esculentum*) yield and quality for smallholder farmers in East Africa. Agric. Sci. 4, 577–584.
71. Salas Gervassio N.G., D. Aquino, C. Vallina, A. Biondi, M.G. Luna (2019) A re-examination of Tuta absoluta parasitoids in South America for optimized biological control J. Pest. Sci., 92 (4), pp. 1343-1357
72. Shankara N., Joep L J., Marja G., Martin H., Barbara D. (2005). La culture de la tomate : production, transformation et commercialisation. Agrodok, 17 : 1-105.
73. Shelly, T. E. (2000). Flower-feeding affects mating performance in male oriental fruit flies *Bactrocera dorsalis*. Ecological Entomology, 25(1), 109-114.
74. Shelly, Todd E. "Feeding on methyl eugenol and *Fagraea berteriana* flowers increases long-range female attraction by males of the oriental fruit fly (Diptera: Tephritidae)." Florida Entomologist (2001): 634-640.
75. Shields, M. W., Johnson, A. C., Pandey, S., Cullen, R., González-Chang, M., Wratten, S. D., & Gurr, G. M. (2019). History, current situation and challenges for conservation biological control. Biological control, 131, 25-35.
76. Shiojiri K, Ozawa R, Kugimiya S, Uefune M, Wijk MV, Sabelis MW, Takabayashi J (2010) Herbivore-specific, density-dependent induction of plant volatiles: honest or ‘cry wolf’ signals? PLoS One 5:1–11
77. Silva DB, Weldegergis BT, van Loon JJA, Bueno VHP (2017). Qualitative and quantitative differences in herbivore-induced plant volatile blends from tomato plants infested by either *Tuta absoluta* or *Bemisia tabaci*. J Chem Ecol 43:53–65
78. Straub, C. S., & Snyder, W. E. (2008). Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology, 89(6), 1605-1615.
79. Suckling DM, Twidle AM, Gibb AR, Manning LM, Mitchell VJ, Sullivan TES et al (2012) Volatiles from apple trees infested with light brown apple moth larvae attract the parasitoid *Dolichogenidea tasmanica*. J Agric Food Chem 60(38):9562–9566.
80. Sugimoto, K., Matsui, K., Iijima, Y., Akakabe, Y., Muramoto, S., Ozawa, R., ... & Takabayashi, J. (2014). Intake and transformation to a glycoside of (Z)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense. Proceedings of the National Academy of Sciences, 111(19), 7144-7149.
81. Takemoto H, Takabayashi J (2015) Parasitic wasps *Aphidius ervi* are more attracted to a blend of host-induced plant volatiles than to the independent compounds. J Chem Ecol 41:801–807
82. Tan, K. H., Tokushima, I., Ono, H., & Nishida, R. (2011). Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species: *Bactrocera invadens, B. dorsalis, B. correcta and B. zonata*. Chemoecology, 21(1), 25-33.
83. Thompson, J. N., & Pellmyr, O. (1991). Evolution of oviposition behavior and host preference in Lepidoptera. Annual review of entomology, 36(1), 65-89.
84. Turlings, T. C., & Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. Annual review of entomology, 63, 433-452.
85. Urbaneja A, Vercher R, Navarro V, Garcí-Marí F, Porcuna JL (2007) La Polilla Del Tomate, *Tuta absoluta*. Phytoma España 194:16–23
86. Van Mele P, Vayssières J-F, Van Tellingen E, Vrolijks J (2007) Effects of an African weaver ant, *Oecophylla longinoda*, in controlling mango fruit flies (Diptera: Tephritidae) in Benin. J Econ Entomol 100(3):695–701
87. Van Mele P, Vayssieres JF, Adandonon A, Sinzogan A (2009) Ant cues affect the oviposition behaviour of fruit flies (Diptera:Tephritidae) in Africa. Physiol Entomol 34:256–261
88. Verheggen F, Fontus RB (2019) First record of *Tuta absoluta* in Haiti. Entom Gen 38(4):349–353
89. Waghunde, R. R., Shelake, R. M., & Sabalpara, A. N. (2016). Trichoderma: A significant fungus for agriculture and environment. African journal of agricultural research, 11(22), 1952-1965.
90. Walling LL (2008) Avoiding effective defenses : strategies employed by phloem-feeding insects. Plant Physiol 146:859–866
91. Werner, E. E., & Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. Ecology, 84(5), 1083-1100.
92. Wezel, A., Herren, B. G., Kerr, R. B., Barrios, E., Gonçalves, A. L. R., & Sinclair, F. (2020). Agroecological principles and elements and their implications for transitioning to sustainable food systems. A review. *Agronomy for Sustainable Development*, *40*(6), 1-13.
93. Wilby, A., & Thomas, M. B. (2002). Natural enemy diversity and pest control: patterns of pest emergence with agricultural intensification. Ecology letters, 5(3), 353-360.
94. Yang J-N, Wei J-N and Kang L, (2021). Feeding of pea leafminer larvae simultaneously activates jasmonic and salicylic acid pathways in plants to release a terpenoid for indirect defense. Insect Sci. 28:811–824
95. Zeng, R. S. (2014). Allelopathy-the solution is indirect. Journal of Chemical Ecology, 40(6), 515-516.

5. Listes des tirés à part joint.

Pour les 5 tirés à part, j'ai sélectionné un article représentatif du travail en écologie que j'effectue au laboratoire qui fait partie de la thèse de Pascal Ayelo que j'ai co-encadré :
P Ayelo, A Yusuf, C Pirk, S Mohamed, A Chailleux, **E Deletre**. 2021. The role of *trialeurodes vaporariorum*-infested tomato plant volatiles in the attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Journal of Chemical Ecology*, 47(2), 192-203. (IF=2.63)
un article représentatif du travail en chimie que j'effectue au laboratoire qui fait partie de la thèse de Bethelihem Mekonnen que j'ai co-encadré :
B Mekonnen, X Cheseto, C Pirk, A Yusuf, S Ekesi, **E Deletre**, B Torto. 2021. Re-Analysis of Abdominal Gland Volatilome Secretions of the African Weaver Ant, *Oecophylla longinoda* (Hymenoptera: Formicidae). *Molecules*, 26(4), 871. (IF=4.41)
Un article représentatif du travail que j'effectue sur le terrain qui fait partie de la thèse de Seydou Diabaté que j'ai co-encadré :
S Diabate, T Martin, L Murungi, K Fiaboe, J Wesonga, J Kimani, **E Deletre**. 2021. Push-pull strategy combined with net houses for controlling cowpea insect pests and enhancing crop yields. *Crop Protection*, 141, 105480. (IF=2.57)
Et deux articles de review qui reprennent mes différents thèmes de recherche :
P Ayelo, C Pirk, A Yusuf, A Chailleux, S Mohamed, **E Deletre**. 2021. Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: A Review. *Frontiers in Ecology and Evolution*, 9, 143. (IF=3.26)
E Deletre, B Schatz, L Williams, D Bourguet, A Ratnadass, F Chandre, T Martin. Prospects for repellency in pest control – current developments and future challenges. 2016. C*hemoecology.* 26(4):127-142 (IF=1.40)

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The Role of *Trialeurodes vaporariorum*-Infested Tomato Plant Volatiles in the Attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae)

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Abstract

Natural enemies locate their herbivorous host and prey through kairomones emitted by host plants and herbivores. These kairomones could be exploited to attract and retain natural enemies in crop fields for insect pest control. The parasitoid *Encarsia formosa* preferentially parasitises its whitefly host, *Trialeurodes vaporariorum,* a major pest of tomato *Solanum lycopersicum*, thus offering an effective way to improve whitefly control. However, little is known about the chemical interactions that occur in *E. formosa-T. vaporariorum-S. lycopersicum* tritrophic system. Using behavioural assays and chemical analyses, we investigated the kairomones mediating attraction of the parasitoid to host-infested tomato plants. In Y-tube olfactometer bioassays, unlike volatiles of healthy tomato plants, those of *T. vaporariorum*-infested tomato plants attracted *E. formosa*, and this response varied with host infestation density. Coupled gas chromatography/mass spectrometric analyses revealed that host infestation densities induced varying qualitative and quantitative differences in volatile compositions between healthy and *T. vaporariorum* adult-infested tomato plants. Bioassays using synthetic chemicals revealed the attractiveness of 3-carene, β-ocimene, β-myrcene and α-phellandrene to the parasitoid, and the blend of the four compounds elicited the greatest attraction. Our results suggest that these terpenes could be used as an attractant lure to recruit the parasitoid *E. formosa* for the control of whiteflies in tomato crop fields.

Keywords HIPVs · Kairomone · Olfactometer · *Solanum lycopersicum* · Terpenes · Whitefly

Introduction

Natural enemies exploit kairomones from plants and herbivores to locate oviposition sites and feeding resources for their survival, growth and reproductive success (Afsheen et al. 2008; Colazza and Wanjberg 2013). Kairomones are chemical signals mediating interspecific interactions that are beneficial to organisms that detect them. Although healthy plants emit volatile organic compounds, herbivory results in the release of herbivore-induced plant volatiles (HIPVs), which serve as long-range kairomones for natural enemies seeking host and prey (Kessler and Baldwin 2001; Mumm and Dicke 2010; Takabayashi and Shiojiri 2019). Kairomones have been exploited to attract parasitoids for the biological control of insect pests (Kaplan 2012; Peri et al. 2018). For example, 4′-ethyl-acetophenone, a castor bean (*Ricinus communis* L. (Euphorbiaceae)) plant-emitted HIPV induced by *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae), has been applied to attract the parasitoid *Peristenus spretus* Chen & van Achterberg (Hymenoptera: Braconidae) for the control of the insect pest in the field (Xiu et al. 2019). However, HIPV blend compositions are influenced by biotic factors such as the herbivore-infesting species, its infestation density, infesting instar, and the host plant species, which in turn influence parasitoid responses (De Moraes et al. 1998; McCormick et al. 2012). Besides HIPVs, chemical cues from the host or prey, or its by-products such as feces and honeydew may also serve as kairomone signals that enable natural enemies to locate their host and prey (Afsheen et al. 2008). For example, the

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parasitoid *Psyllaephagus pistaciae* Ferrière (Hymenoptera: Encyrtidae) is attracted to volatiles from honeydew excreted by its host, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psylloidae) (Mehrnejad and Copland 2006). Kairomones identified from tritrophic interactions have been used for the recruitment of insect parasitoids to increase parasitism rates in field crops (Murali-Baskaran et al. 2018; Peñaflor and Bento 2013).

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is one the most efficient parasitoids used for controlling *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) whiteflies (De Vis and van Lenteren 2008; Hoddle et al. 1998; Liu et al. 2015). The parasitoid preferably parasitises third and fourth nymphal instars of both *T. vaporariorum* and *B. tabaci* (Antony et al. 2003; Hu et al. 2002). *Trialeurodes vaporariorum* and *B. tabaci* are devastating insect pests of tomato (*Solanum lycopersicum* L. (Solanaceae)) crops, responsible for 30 to 100% yield losses in both open fields and greenhouses worldwide (Gamarra et al. 2016; Hanssen and Lapidot 2012; Perring et al. 2018). Damage is mainly due to whitefly-vectored viruses which cause interveinal yellowing, irregular fruit ripening and poor plant growth (Hanssen and Lapidot 2012; Navas-Castillo et al. 2014). The phloem sap sucking feeding behaviour of whiteflies also causes plant wilting and defoliation, and their honeydew provides a medium which promotes sooty mold development on leaves, which in turn interferes with photosynthesis, and consequently limits plant growth (Gamarra et al. 2016; Palumbo et al. 2000). *Trialeurodes vaporariorum* is an invasive species in Europe and sub-Saharan Africa where it threatens tomato production (Hanssen and Lapidot 2012; Gamarra et al. 2016). *Trialeurodes vaporariorum* completes its life cycle (eggs, nymphs and adults) on tomato plants within 25 ± 2 days at 25 °C, with the completion of up to three to four generations before tomato harvest (Gamarra et al. 2016). Successful control of invasive insect pests is often challenging as their reproduction and population growth times are typically rapid, making them very destructive and more competitive than native pests (Wagh et al. 2014). However, the parasitoid *E. formosa* has been reported to successfully control *T. vaporariorum* on tomato plants grown in greenhouses (De Vis and van Lenteren 2008; Hoddle et al. 1998), and this performance could be achieved in the field with the application of kairomones to attract and retain the parasitoid in tomato crops.

Previous studies have demonstrated that kairomones attract *E. formosa* to various host plant species infested by whiteflies (Inbar and Gerling 2008; Zhang et al. 2013a). For example, *E. formosa* is attracted to volatiles from *Phaseolus vulgaris* L. (Fabaceae) infested by *T. vaporariorum*, and to volatiles of *Arabidopsis thaliana* (L.) (Brassicaceae) infested by *B. tabaci* (Birkett et al. 2003; Zhang et al. 2013a). Birkett et al. (2003) reported that the HIPVs (*Z*)-3-hexen-1-ol, 4,8-dimethyl-1,3,7-nonatriene, and 3-octanone released by *T. vaporariorum*-infested *P. vulgaris* attract *E. formosa*. However, *E. formosa* preferably parasitises its hosts, *T. vaporariorum* and *B. tabaci* on tomato (*S. lycopersicum*) plants compared to other host plants from Fabaceae, Brassicaceae and Solanaceae families (Kos et al. 2009; Zhang et al. 2005). These findings suggest that whitefly-infested *S. lycopersicum* plants release specific volatile compounds which attract *E. formosa*. Although, plant colour plays a role in host plant location by *E. formosa* (Romeis and Zebitz 1997), a field observation also reported that the density of the whitefly *B. tabaci* on plants influenced the abundance of *Encarsia* species (Kishinevsky et al. 2016). Hence, it would be interesting to investigate the kairomones allowing for a functional response to *T. vaporariorum* density on tomato plants. We thus assessed the attractiveness of *E. formosa* to tomato plant volatiles in relation to host infestation densities and identified the attractant compounds. We discuss our results in the light of the potential application of these attractants as kairomone-based lures to recruit the parasitoid for *T. vaporariorum* control in tomato crop fields.

Materials and Methods

Plant The Kilele F1 tomato hybrid (Syngenta, Nairobi, Kenya) was used in the experiments. Seeds were sown in nurseries on a 1:1 mixture of soil: manure placed in a seedling plastic tray. Three weeks after sowing, the seedlings were individually transplanted in plastic pots (10 × 15 cm) containing 3:1 soil: manure mixture and grown in a screen house at 30 ± 5 °C, 65 ± 5% RH at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. Plants were watered as needed, and water-dipped fertiliser containing 18% N, 20% P2O5, and 21% K2O (Easygrow, Osho Chemical Industries Ltd) was provided once a week. The plants were grown without insecticide application. Four-week-old plants with four developed leaves were used for behavioural assays and volatile chemical analyses.

Insects Colonies of the experimental insect species, *T. vaporariorum* and *E. formosa* were initiated with field collected nymphs and adults from tomato fields at the Kenyan Agricultural and Livestock Research Organization (KALRO) in Kimbimbi (0°37′11.3”S 37°22′08.0″E), Mwea county, Kirinyaga region. Insects were reared in the laboratory and maintained at 25 ± 2 °C and 65 ± 5% RH under a 12:12 L:D photoperiod regime.

Trialeurodes vaporariorum was reared on tomato plants in laboratory Plexiglass cages (40 × 40 × 50 cm). Six-week-old plants were exposed to whiteflies for oviposition for three days. Infested plants were thereafter transferred into a separate screen house where the eggs developed into nymphs. Three-week post-infestation leaves with mature nymphs (mostly fourth instars) were cut from plants and returned to the original infestation cage where the adults emerged.

Encarsia formosa was reared on *T. vaporariorum*-infested tomato plants. Four infested plants at 14–21 days post infestation were offered to parasitoids in a laboratory Plexiglass cage (40 × 40 × 50 cm) for parasitism. Every three days, the oldest parasitoid-exposed plant was removed and replaced with a plant 14 days post-infestation. Plants with parasitised nymphs were placed in another Plexiglass cage where the insects emerged. Newly emerged parasitoid adults were returned to the original cage. *Encarsia formosa* adults were provided 80% honey solution and water twice a week. Naive 3–5-day-old *E. formosa* females were used in the experiments as the parasitoid fecundity is known to be higher within this age interval (Qiu et al. 2004).

Y-Tube Olfactometer Bioassays The attractiveness of *E. formosa* to plant odours was assessed in dual choice assays using a vertically-oriented Y-tube olfactometer (0.5 cm internal diameter; 6 cm stem; two 6 cm side arms). A 10 L glass jar serving as an odour source container was connected to each side arm. A cardboard box (35 × 35 × 55 cm) served as observation chamber in which the Y-tube was placed, thus preventing insects from using plant visual cues. Uniform lighting was provided in the observation chamber using a 220–240 V cool white fluorescent light placed above the Y-tube. A unidirectional charcoal-filtered airflow was generated by an air pump (KNF lab LABOPORT N86KT.18, France) and passed through each odour source container toward the arms at a constant flow rate of 120 mL min^{-1} that was set using an AALBORG flow meter (Orangeburg, USA). Pots of growing test plants were wrapped in aluminium foil to avoid volatile contamination from the pot and soil. Experimental insects were individually introduced at the base of the Y-tube stem, and the insect was given 5 min during which its first choice was recorded. An insect was considered to have made a choice when it walked and reached the end of a given arm, and remained there for 30 s. Insects which did not make a choice within 5 min were considered as non-respondent and were subsequently not included in the data analysis. Ten insects were tested per plant per treatment per day, and the Y-tube was cleaned with dichloromethane after five insects and the volatile sources switched between the arms (left and right) to prevent contamination and positional bias. Overall, 80 insects were tested per choice test.

Encarsia formosa olfactory responses to volatiles from healthy and *T. vaporariorum* adult-infested tomato plants were investigated by performing the following dual choices (i) air vs. air (control); (ii) air vs. healthy plant; (iii) air vs. *T. vaporariorum*-infested plant; (iv) healthy plant vs. *T. vaporariorum*-infested plant. Infested plants were obtained by exposing a single 4-week-old plant to 50, 100 or 200 *T. vaporariorum* adults of mixed ages and sexes in a ventilated plastic box (35 × 25 × 35 cm) for four days which was enough to induce changes in volatile emission from tomato plants infested by *B. tabaci* adults (Su et al. 2018).

Collection of Volatiles Tomato headspace volatiles were collected using a dynamic push-pull system, and trapped onto prepacked 30 mg super-Q adsorbent (Analytical Research Systems, Gainesville, FL, USA). Volatiles were collected from healthy and *T. vaporariorum* adult-infested tomato plants, as well as from empty volatile collection chamber and pot without plant (controls). Healthy or infested plants, and the pots, were separately placed in a 10 L glass jar used in the behavioural response assays. A charcoal-filtered airflow was directed into the containers at a rate of 200 mL min^{-1} using an air pump (KNF lab LABOPORT N86KT.18, France). Collection of volatiles was done for 24 h with 4 replications. Volatiles were eluted with 150 μL of dichloromethane, then the solution was concentrated to 50 μL to which 5 μL of biphenyl (20 ng/μL) was added as internal standard, and then stored at −80 °C until analysis.

Analysis of Volatiles An aliquot (1 μL) of headspace tomato plant volatile extract was analysed by gas chromatography/mass spectrometry (GC/MS) on a Shimadzu QP2010 Ultra GC/MS (GL Sciences, Tokyo, Japan). The mass spectrometer was equipped with an Inert Cap 5MS/NP capillary column (5% diphenyl and 95% dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 μm film thickness). Analysis was performed in the splitless mode using helium as carrier gas at a constant flow rate of 1 mL min^{-1}. The oven temperature was set at 35 °C for 5 min and then programmed to increase at 10 °C min^{-1} until reaching a final temperature of 280 °C at which it was held for 10.5 min. The ion source temperature was set at 250 °C with an interface temperature of 270 °C, and spectra were recorded at 70 eV. Compound identification was done using retention time, library mass spectra (NIST11, Wiley9), and Kovats retention indices (RIs) determined using retention times of *n*-alkane (C_8-C_{23}) standards. All peaks detected in the control were considered as contaminants and therefore discarded in the volatile analysis. Compounds were quantified relative to peak area and concentration of the internal standard.

Response of *E. formosa* to Synthetic Plant Volatile Compounds A total of 11 compounds were found to be highly associated with 100-*T. vaporariorum* adult-infested tomato plants (see Results) which attracted the parasitoid. These VOCs included: β-myrcene, α-phellandrene, 3-carene, *p*-cymene, β-phellandrene, (*E*)-β-ocimene, terpinolene, allo-ocimene, β-elemene and (*E*)-β-caryophylene and α-humulene. They were therefore used in the bioassays with synthetic compounds, except β-phellandrene which was not commercially available. Based on the natural release rate (ng/

plant/h) of the compounds (Table 1), each compound was tested at doses corresponding to release rates of 10, 100 and 1000 equivalent plants in an hour. Based on the results obtained, four of these compounds (3-carene, β-ocimene, β-myrcene and α-phellandrene), found to be attractive to the parasitoid were formulated into a blend (B1) using their individual optimal attractant doses (100 ng 3-carene, 200 ng α-phellandrene, 600 ng β-ocimene and 800 ng β-myrcene). Two other blends B2 (100 ng 3-carene, 200 ng α-phellandrene, 60 ng β-ocimene and 80 ng β-myrcene) and B3 (1 ng 3-carene, 20 ng α-phellandrene, 6 ng β-ocimene and 8 ng β-myrcene) were tested. The test compounds were diluted in dichloromethane and a 10 μL aliquot of the test solution was applied on a filter paper (2 × 2 cm) (Whatman, UK) and tested against the control (i.e. filter paper loaded with 10 μL dichloromethane). The solvent was allowed to evaporate for 30 s. Thereafter, the impregnated filter papers were placed in Pasteur pipettes which were directly connected to the olfactometer arms. The test aliquot was used for a single insect with a 5 min observation period, and 80 insects were tested per choice test.

Chemicals The β-myrcene, α-phellandrene, 3-carene, *p*-cymene, β-ocimene, terpinolene, allo-ocimene, β-elemene, (*E*)-β-caryophylene and α-humulene synthetic standards used in the bioassays were all purchased from Sigma-Aldrich (France). All the chemicals were at 90–99% purity, except β-elemene (80% purity) and α-phellandrene (85% purity). Dichloromethane (99.9% purity) was purchased from Merck, Germany.

Statistical Analyses *Encarsia formosa* preference for odours in the Y-tube olfactometer was determined by comparing the recorded frequencies of choice of either of the olfactometer arms using a chi-squared test. Analysis of concentrations of volatile compounds between healthy and whitefly-infested plants was performed using a non-parametric Kruskal-Wallis test to account for distribution normality and variance homogeneity, and when a significant difference was noted a post-hoc Dunn's test associated with Bonferroni's adjustment was applied for mean separation (Dinno 2015). The Random Forest (RF) analysis (Breiman 2001) was used to select the most discriminant volatiles between healthy and *T. vaporariorum* adult-infested tomato plants, as used in previous studies (Ranganathan and Borges 2010; McCormick et al. 2014, 2016). The most predictive variables (VOCs in our case) were defined using the RF "importance" function which provides the mean decrease in accuracy (MDA), where a higher MDA indicates higher importance in the classification (Liaw and Wiener 2002; Ranganathan and Borges 2010). Using concentrations of the most discriminant volatile compounds, a Sparse Partial Least Square Discriminant Analysis (sPLS-DA) biplot was performed to highlight variations in the emission of compounds between healthy and herbivore-infested plants, and to identify VOCs highly associated with the attractant plants (i.e. 100-*T. vaporariorum* adult-infested plants) using the mixOmics package (Hervé et al. 2018; Lê Cao et al. 2011). The sPLS-DA model was validated using the "perf" function and the leave-one-group-out cross-validation method in the mixOmics package (Rohart et al. 2017). All statistical analyses were performed using R statistical software, version 4.0.2 (R Core Team 2020).

Results

Olfactory Response of *E. formosa* to Plant Volatiles The whitefly parasitoid *E. formosa* was attracted to volatiles from tomato plants infested with 100 *T. vaporariorum* adults compared to clean air (control) ($\chi^2_=$ 9.59, $df = 1$, $P = 0.002$) and healthy tomato plant volatiles ($\chi^2_=$ 4.63, $df = 1$, $P = 0.031$) (Fig. 1). However, volatiles from tomato plants infested with either 50 ($\chi^2_=$ 0.63, $df = 1$, $P = 0.428$; $\chi^2_=$ 0.01, $df = 1$, $P = 0.91$) or 200 *T. vaporariorum* adults ($\chi^2_=$ 0, $df = 1$, $P = 1$; $\chi^2_=$ 0.11, $df = 1$, $P = 0.737$) did not attract the parasitoid when compared to clean air or healthy tomato plant volatiles, respectively. Similarly, the parasitoid was not attracted to volatiles of healthy tomato plants compared to clean air ($\chi^2_=$ 1.04, $df = 1$, $P = 0.308$).

Analysis of Tomato Volatiles A total of 25 volatile organic compounds (VOCs) belonging to six chemical classes: monoterpene (14), sesquiterpene (6), ketone (2), homoterpene (1), aldehyde (1) and benzenoid (1) were detected in the volatile profiles of both healthy and whitefly-infested tomato plants (Table 1; Fig. 2). Quantitative and qualitative differences were observed in volatile emission between healthy and *T. vaporariorum* adult-infested tomato volatiles (Table 1; Fig. 2). The monoterpenes 2-carene and β-phellandrene were the most abundant compounds and accounted for about 80% of the total volatiles released from healthy and whitefly-infested tomato plants (Table 1; Fig. 2). Six VOCs, i.e. benzaldehyde (aldehyde); β-pinene, 6-methyl-5-hepten-2-one, 3-carene, allo-ocimene (monoterpenes) and β-elemene (sesquiterpene) were specific to *T. vaporariorum*-infested tomato plants. Among these compounds, 3-carene, allo-ocimene and β-elemene were found only in volatiles of plants infested with 100 *T. vaporariorum* adults.

Emission rates of some VOCs such as *p*-cymene, α-phellandrene, β-phellandrene, (*E*)-β-ocimene, α-pinene, β-myrcene, terpinolene and δ-elemene increased by ≥2 fold in plants infested with 100 *T. vaporariorum* adults than in healthy plants (Table 1). Differences in some VOCs such as the monoterpenes α-phellandrene and (*E*)-β-ocimene, and the sesquiterpenes (*E*)-β-caryophyllene and α-humulene were also observed between the different infestation densities, with a

Table 1 Mean amount (ng/plant/h) of volatile compounds identified in the headspace of healthy and *Trialeurodes vaporariorum* adult-infested tomato plants ($n = 4$)

Peak No.[1]	RT (min)	RI_{alk}[2]	RI_{lit}[3]	Compound[4]	Chemical class	Healthy tomato	*T. vaporariorum* (Tv) infestation densities			*P*-value[5]
							50	100	200	
1	8.34	865	865	*p*-xylene*	benzenoid	0.15±0.06	0.23±0.09	0.26 ± 0.12	0.22±0.02	0.675
2	9.83	936	934	α-pinene*	monoterpene	1.02±0.23	1.53±0.41	2.46 ± 0.74	1.00±0.18	0.248
3	10.42	964	963	benzaldehyde	aldehyde	nd	0.17±0.11	0.08 ± 0.05	0.29±0.17	0.366
4	10.62	973	972	3,7,7-trimethyl-1,3,5-cycloheptatriene	Homoterpene	2.66±1.33	1.67±0.59	3.20 ± 0.55	1.21±0.38	0.192
5	10.72	978	981	β-pinene*	monoterpene	nd	0.01±0.01	0.09 ± 0.06	0.03±0.03	0.422
6	10.99	989	987	6-methyl-5-hepten-2-one*	ketone	nd	0.10±0.06	0.14 ± 0.08	0.11±0.06	0.353
7	11.03	993	992	β-myrcene*	monoterpene	0.30±0.03	0.49±0.18	0.78 ± 0.16	0.43±0.21	0.334
8	11.21	1002	1001	2-carene *	monoterpene	11.11±2.18	15.26±3.97	22.38 ± 4.31	9.42±2.51	0.168
9	11.30	1006	1005	α-phellandrene*	monoterpene	$0.95{\pm}0.22^{a}$	$1.30{\pm}0.36^{a}$	2.05 ± 0.20^{b}	$0.69{\pm}0.19^{a}$	**0.031**
10	11.40	1011	1011	3-carene*	monoterpene	nd	nd	0.10 ± 0.04	nd	–
11	11.51	1016	1018	α-terpinene*	monoterpene	0.83±0.15	0.95±0.27	1.48 ± 0.30	0.62±0.21	0.216
12	11.67	1028	1026	*p*-cymene*	monoterpene	$0.14{\pm}0.05^{a}$	$0.47{\pm}0.09^{ab}$	0.61 ± 0.17^{b}	$0.47{\pm}0.11^{ab}$	**0.044**
13	11.78	1034	1032	β-phellandrene	monoterpene	32.56±5.78	43.36±11.37	63.73 ± 12.30	27.22±6.85	0.134
14	11.92	1042	1039	(*Z*)-β-ocimene*	monoterpene	0.16±0.03	0.16±0.05	0.30 ± 0.03	0.32±0.02	0.244
15	12.09	1051	1050	(*E*)-β-ocimene*	monoterpene	$0.26{\pm}0.04^{a}$	$0.24{\pm}0.08^{a}$	0.57 ± 0.09^{b}	$0.39{\pm}0.10^{a}$	**0.044**
16	12.81	1092	1090	terpinolene*	monoterpene	0.22±0.03	0.34±0.12	0.56 ± 0.17	0.15±0.09	0.134
17	13.23	1115	–	unidentified	monoterpene	0.71±0.13	1.08±0.32	1.33 ± 0.15	1.59±0.69	0.446
18	13.33	1121	1128	allo-ocimene*	monoterpene	nd	nd	0.10 ± 0.03	nd	–
19	16.65	1345	1342	δ-elemene	sesquiterpene	0.53±0.12	0.60±0.13	1.09 ± 0.30	0.58±0.14	0.334
20	17.40	1388	1385	β-elemene*	sesquiterpene	nd	nd	0.17 ± 0.05	nd	–
21	17.71	1424	1422	α-cedrene*	sesquiterpene	0.08±0.04	0.09±0.04	0.10 ± 0.02	0.07±0.01	0.581
22	17.80	1430	1427	(*E*)-β-caryophyllene*	sesquiterpene	$0.93{\pm}0.11^{b}$	$0.82{\pm}0.14^{ab}$	1.70 ± 0.13^{c}	$0.55{\pm}0.07^{a}$	**0.009**
23	17.93	1441	1440	γ-elemene*	sesquiterpene	0.05±0.03	0.05±0.03	0.08 ± 0.03	0.05±0.03	0.851
24	18.09	1453	1454	geranyl acetone*	ketone	0.34±0.12	0.62±0.15	0.61 ± 0.07	0.42±0.18	0.384
25	18.25	1465	1462	α-humulene*	sesquiterpene	$0.47{\pm}0.04^{ab}$	$0.30{\pm}0.04^{a}$	0.55 ± 0.06^{b}	$0.34{\pm}0.08^{a}$	**0.035**
–	–	–	–	Average total amount	–	$53.50{\pm}8.18^{a}$	$69.83{\pm}17.92^{ab}$	104.57 ± 18.76^{b}	$46.17{\pm}10.86^{a}$	**0.045**

nd not detected

[1] Peak numbers correspond to peaks indicated in Fig. 3

[2] Retention index relative to C8-C23 n-alkanes of an Inert Cap 5MS/NP capillary column

[3] Retention index obtained from the literature (Khan et al. 2012)

[4] Identification of compounds based on the retention time (RT) and comparison of mass spectra with published mass spectral library data from NIST11 and Wiley9. * indicates compounds confirmed with authentic standards

[5] *P*-value of the non-parametric Kruskal Wallis test for comparison of volatile compounds between healthy and *Trialeurodes vaporariorum* adult-infested tomato plants. Significant values are highlighted in bold

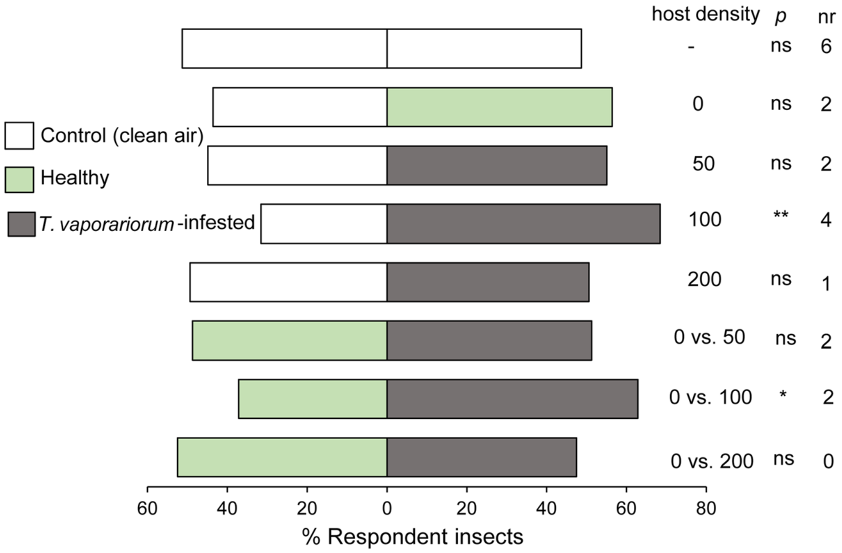

Fig. 1 Responses of *Encarsia formosa* to volatiles from healthy tomato plants and others infested with different densities of *Trialeurodes vaporariorum* adults in a Y-tube olfactometer choice tests. 80 insects were tested per choice. nr = number of non-respondent insects (i.e. no choice). P = statistical significance level with ns = no significant difference ($P > 0.05$); *, ** = significant differences at $P < 0.05$ or $P < 0.01$ from χ^2 test at $\alpha = 0.05$

decline in the emission at the highest whitefly infestation density (Table 1). However, the emission rates of 3,7,7-trimethyl-1,3,5-cycloheptatriene, α-cedrene and γ-elemene did not change between healthy plants and *T. vaporariorum* adult-infested plants.

Determination of Discriminant Volatiles for Bioassays with Synthetic Compounds The RF analysis and the sPLS-DA biplot highlighted the VOCs that mostly characterised the attractant 100-*T. vaporariorum* adult-infested plants compared to the unattractant plants (combination of healthy, 50 and 200-*T. vaporariorum* adult-infested plants). The results of the RF analysis revealed 13 VOCs discriminating *T. vaporariorum* adult-infested tomato plants and healthy plants (Fig. 3a). Using these VOCs, the sPLS-DA classified healthy and whitefly-infested plants into two clusters: one group composed of 100-*T. vaporariorum* adult-infested plants, which was separated from the other group composed of healthy, and 50 or 200-*T. vaporariorum* adult-infested plants (Fig. 3b). The sPLS-DA biplot displayed the correlation between the discriminant VOCs and the plants (Fig. 3c). The first two dimensions of the sPLS-DA explained 62% of the total variation (Fig. 3c). Dimension 1 explained 47% of the total variation and was highly associated mainly with (*E*)-β-caryophyllene, β-elemene, allo-ocimene, 3-carene and α-phellandrene. Whereas dimension 2 accounted for 15% of the total variation, and was closely correlated mainly with *p*-cymene, (*Z*)-β-ocimene and 6-methyl-5-hepten-2-one. Heatmap clustering was performed to illustrate variations in VOCs across replicates of healthy and infested tomato plants, and the findings showed that most of the discriminant VOCs were abundant in tomato plants infested with

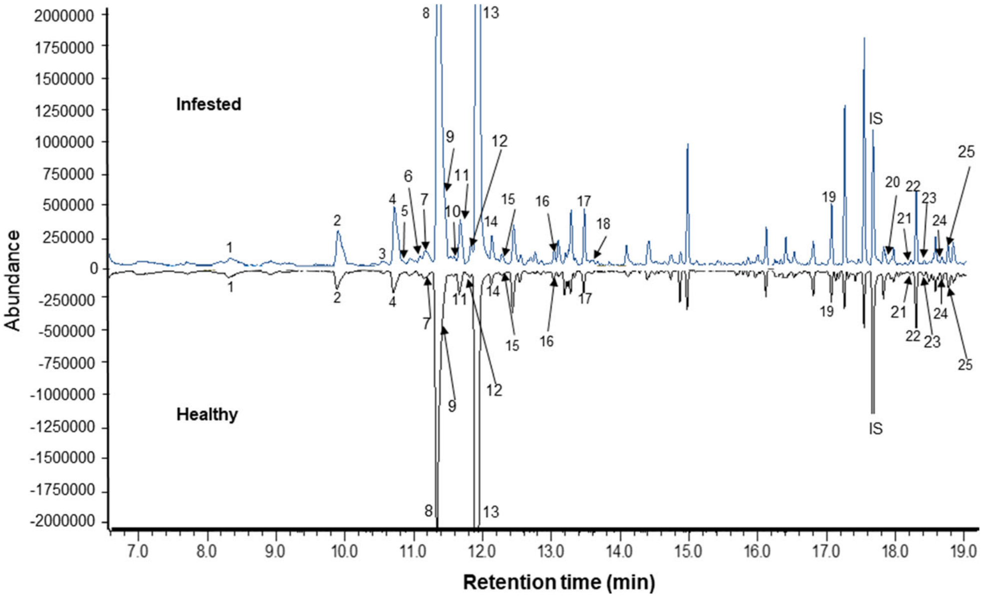

Fig. 2 GC/MS profiles of headspace volatiles from healthy tomato plants and 100 *Trialeurodes vaporariorum* adult-infested tomato plants. Numbers correspond to volatile compounds indicated on Table 1. IS = internal standard (biphenyl)

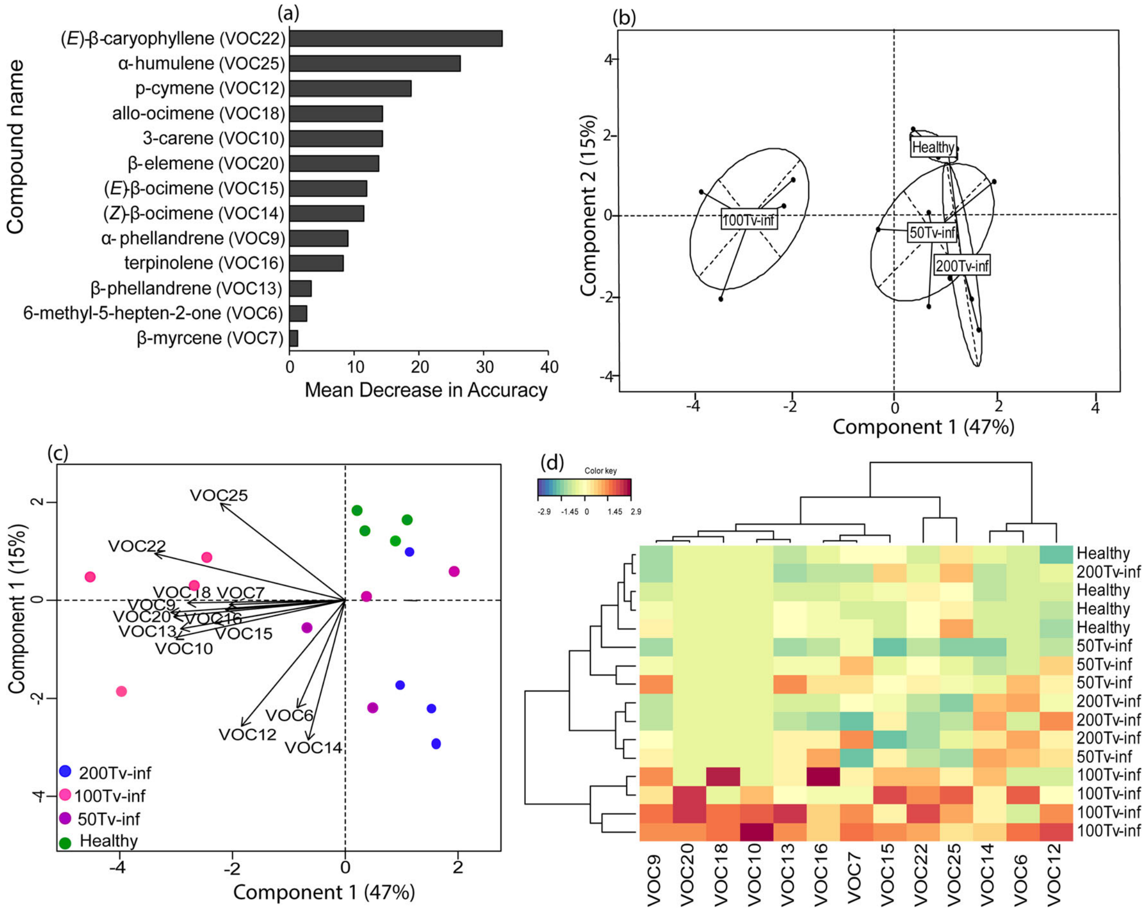

Fig. 3 Determination of the most discriminant volatiles and their correlation with healthy tomato plants and tomato plants infested by 50, 100 and 200 *Trialeurodes vaporariorum* adults (50Tv-inf, 100Tv-inf and 200Tv-inf, respectively). **a** The 13 most discriminant volatiles between healthy and infested tomato plants are listed in decreasing importance based on the mean decrease in accuracy (MDA > 1) in the random forest analysis. **b** A sPLS-DA plot displaying the distribution of healthy and infested tomato plants. **c** A sPLS-DA biplot showing the correlation of the most discriminant volatiles with healthy and infested tomato plants. **d** Heatmap clustering showing the abundance (in decreasing colour intensity) of the most discriminant VOCs across replicates of healthy and infested tomato plants

100 *T. vaporariorum* adults (Fig. 3d). According to the horizontal direction of the heatmap, all samples were classified in two main groups, i.e. one composed of plants infested with 100 *T. vaporariorum* adults, and another group composed of the rest of the samples (i.e. combination of healthy plants and others infested with 50 and 200 *T. vaporariorum* adults). The results of the sPLS-DA biplot specifically showed that (*E*)-β-caryophyllene, β-elemene, allo-ocimene, α-phellandrene, (*E*)-β-ocimene, β-phellandrene, 3-carene, terpinolene, *p*-cymene, β-myrcene and α-humulene were highly associated with plants infested with 100 *T. vaporariorum* adults, which attracted the parasitoid. Therefore, we focused on these VOCs for testing the attractiveness of the parasitoid *E. formosa* to synthetic compounds.

Bioassays with Synthetic Compounds *Encarsia formosa* responded differently to the various doses of compounds tested individually (Fig. 4). The parasitoid was attracted to β-ocimene at 60 ng ($\chi^2 = 5.06$, $df = 1$, $P = 0.024$) and 600 ng ($\chi^2 = 8.01$, $df = 1$, $P = 0.003$) doses compared to the control (dichloromethane). The parasitoid was also attracted to β-myrcene at 80 ng ($\chi^2 = 4.63$, $df = 1$, $P = 0.03$) and 800 ng ($\chi^2 = 6.13$, $df = 1$, $P = 0.013$) doses compared to the control. Moreover, 3-carene attracted *E. formosa* only at the 100 ng dose ($\chi^2 = 6.13$, $df = 1$, $P = 0.013$) and α-phellandrene at 200 ng dose ($\chi^2 = 7.81$, $df = 1$, $P = 0.005$) compared to the control. On the other hand, terpinolene, *p*-cymene, α-humulene and (*E*)-β-caryophyllene were not attractive to the parasitoid at any of the tested doses (Fig. 4).

The parasitoid *E. formosa* was attracted to the blend of the four attractants 3-carene, α-phellandrene, β-myrcene and β-ocimene mixed at their optimal attractant doses (B1) compared to the control (dichloromethane) ($\chi^2 = 6.28$, $df = 1$, $P = 0.012$) (Fig. 5). However, the parasitoid was highly attracted to the mixture of the four attractants when blended at the intermediate attractant doses (B2) compared to the control ($\chi^2 = 11.39$, $df = 1$, $P < 0.001$).

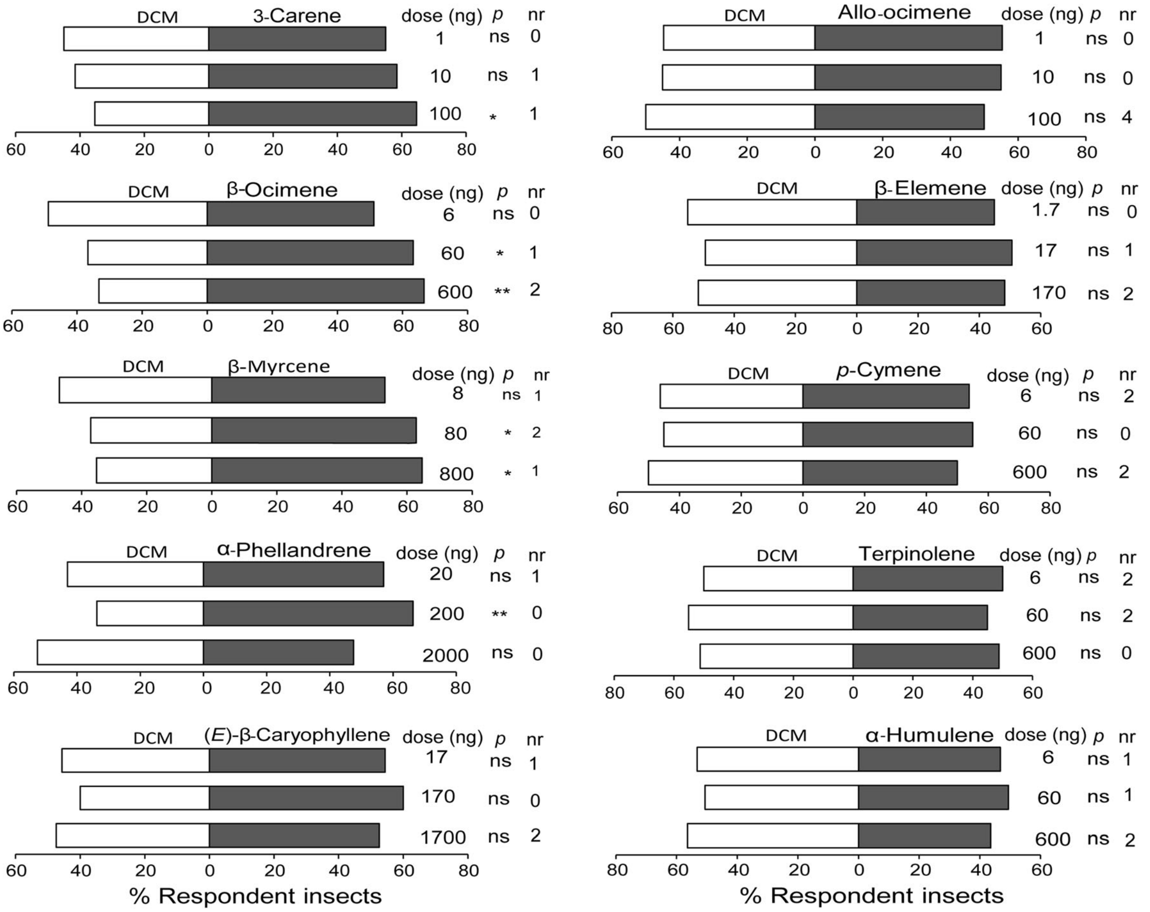

Fig. 4 Olfactory responses of *Encarsia formosa* to three doses of selected synthetic volatile compounds. 80 insects were tested per choice. DCM = dichloromethane. nr = number of non-respondent insects (i.e. no choice). P = statistical significance level with ns = no significant difference at $P > 0.05$; *, ** = significant differences at $P < 0.05$ and $P < 0.01$ from χ^2 test at $\alpha = 0.05$

On the other hand, *E. formosa* was not attracted to the blend when the attractant compounds were mixed at their lowest doses (B3) compared to the control ($\chi^2 = 3.24$, $df = 1$, $P = 0.072$) (Fig. 5).

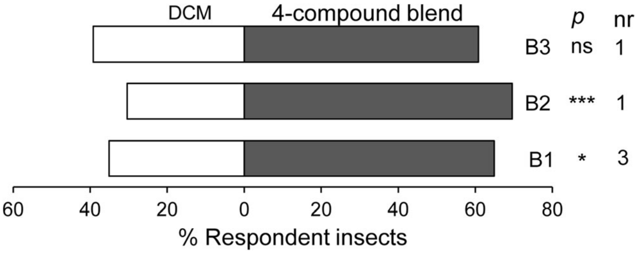

Fig. 5 *Encarsia formosa* attractiveness to mixtures of attractant compounds blended at the attractant doses of (B1): 100 ng 3-carene, 200 ng α-phellandrene, 600 ng β-ocimene and 800 ng β-myrcene and (B2): 100 ng 3-carene, 200 ng α-phellandrene, 60 ng β-ocimene and 80 ng β-myrcene, and at lowest tested doses of (B3): 1 ng 3-carene, 20 ng α-phellandrene, 6 ng β-ocimene and 8 ng β-myrcene. 80 insects were tested per choice test. DCM = dichloromethane. nr = number of non-respondent insects (i.e. no choice). P = statistical significance level with ns = no significant difference ($P > 0.05$); *, *** = significant differences at $P < 0.05$ and $P < 0.001$ from χ^2 test at $\alpha = 0.05$

Discussion

We have shown that the parasitoid *E. formosa* responded to volatiles of *T. vaporariorum* adult-infested tomato plants, and this response varied with the host infestation density. *Encarsia formosa* was attracted to volatiles of plants infested with 100 *T. vaporariorum* adults, and the parasitoid preferred 100-*T. vaporariorum* adult-infested tomato plant volatiles compared to healthy plant volatiles. Hence, plants infested with 100 *T. vaporariorum* adults produced specific induced plant volatiles which are detected by the parasitoid to discriminate between infested and healthy tomato plants. Our results support previous studies on other plant-host-parasitoid systems. For example, *E. formosa* was reported to be attracted to volatiles of *T. vaporariorum* adult-infested *P. vulgaris* plants and *B. tabaci* adult-infested *A. thaliana* plants compared to volatiles of healthy plants (Birkett et al. 2003; Zhang et al. 2013a). Similarly, *E. formosa* preferred *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) adult-infested tomato plant volatiles relative to volatiles of healthy plants (Tan and Liu 2014). The attraction of parasitoids to specific host infestation density is the result of qualitative and quantitative differences in volatile emission between healthy and herbivore-infested plants (McCormick et al. 2012; Shiojiri et al. 2010). We found that the infestation density of 100 *T. vaporariorum* adults changed the quality and quantity of released volatile compounds in tomato plants. Our tomato volatile analysis showed that volatile emission reached a maximum at an intermediate host density (100 *T. vaporariorum* adults). Similarly volatile emission by *Brasica nigra* L. (Brassicaceae) plants infested with *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) aphid increased at the infestation density of 50 aphid adults compared to infestation by lower (i.e. 25) and higher (i.e. 100) host densities (Ponzio et al. 2016). The VOCs β-pinene, 6-methyl-5-hepten-2-one, benzaldehyde, 3-carene, allo-ocimene and β-elemene were specific to 100-*T. vaporariorum* adult-infested tomato plants, and levels of *p*-cymene, α-phellandrene, (*E*)-β-ocimene and (*E*)-β-caryophyllene increased in the attractant 100 *T. vaporariorum* adult-infested tomato plants compared to healthy plants.

The parasitoid *E. formosa* was, however, not attracted to volatiles produced by tomato plants infested with 50 or 200 *T. vaporariorum* adults. Our findings on the host infestation density dependent-olfactory response in *E. formosa* corroborated the findings of previous studies on other parasitoid species of phloem-feeding herbivores, where an infestation threshold is generally required for the parasitoids for optimal response to herbivore-infested plants (Lou et al. 2005; Ponzio et al. 2016). Lou et al. (2005) reported that the parasitoid *Anagrus nilaparvatae* Pang and Wang (Hymenoptera: Mymaridae) was attracted to volatiles of *Oryza sativa* L. (Poaceae) plants infested with 10 or 20 *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) adults, but there was no attraction to plants with lower (1 or 5) or higher (40 or 80) infestation densities. This density-dependent olfactory response was also reported for the parasitoid *Dieaeretiella rapae* (McIntoch) (Hymenoptera: Braconidae) which preferred volatiles of *B. nigra* plants infested with 25 *M. persicae* aphid nymphs over those of healthy plants, but it showed no attraction to plants infested with higher host infestation density, i.e. 50 or 100 *M. persicae* nymphs (Ponzio et al. 2016).

In our study, the relatively low attraction of *E. formosa* to plants infested by either low or high *T. vaporariorum* adult infestation densities could be explained by the results obtained from our volatile analysis. Phloem-feeding herbivores such as whiteflies and aphids which are salicylic acid pathway inducers may activate or suppress the plant defence mechanisms, thus emission of volatiles, depending on their density and the infestation or feeding duration (Lou et al. 2005; Walling 2008; Zhang et al. 2013b). We found that 50 whitefly adults induced minor changes in the plant volatiles, where benzaldehyde, β-pinene, and 6-methyl-5-hepten-2-one were only released in 50 whitefly adult-infested tomato plants compared to healthy plants. On the other hand, we found that volatile emission declined in tomato plants infested with 200 *T. vaporariorum* adults compared to plants infested with 100 *T. vaporariorum* adults. Specifically, α-phellandrene, *p*-cymene, (*E*)-β-caryophyllene and α-humulene decreased in 200-*T. vaporariorum* adult-infested plants compared to 100-*T. vaporariorum* adult-infested plants, and also (*E*)-β-caryophyllene decreased in 200-*T. vaporariorum* adult-infested plants compared to healthy plants. Darshanee et al. (2017) reported that α-humulene and (*E*)-β-caryophyllene emission decreased in tomato plants infested with 200 *T. vaporariorum* adults compared to healthy plants. The decrease of volatile emission at the highest *T. vaporariorum* infestation density could be explained by an inhibitory effect on the jasmonic acid-regulated pathway, as observed for *B. tabaci* feeding on Arabidopsis and tomato plants (Su et al. 2018; Zarate et al. 2007; Zhang et al. 2013b, 2019). Estrada-Hernandez et al. (2009) also reported that a high *B. tabaci* feeding intensity on tomato plants led to downregulation of genes associated with photosynthesis and secondary metabolites. Therefore, we speculate that the high *T. vaporariorum* infestation rate could have induced a reduction in photosynthetic activity and secondary metabolite synthesis. Moreover, it is likely that the high *T. vaporariorum* density which correlated with a high feeding intensity could have weakened the plant quality and thus its ability to maintain normal physiological processes, which could potentially decrease whitefly survival and fitness, which in turn affects the fitness of the parasitoid. It has been observed that a high infestation density and longer feeding period of *B. tabaci* whiteflies on cucumber crops resulted in a reduction in plant photosynthetic activity (Shannag and Freihat 2009). Palumbo et al. (2000) also reported that heavy *Bemisia* whitefly density led to a reduction in the growth and vigor of alfalfa (*Medicago sativa* L. (Fabaceae)) plants through the reduction in the plant crude protein content.

Herbivore-induced plant volatiles serve as kairomones during host location by parasitoids. We found that synthetic 3-carene, β-myrcene, β-ocimene and α-phellandrene tested individually attracted *E. formosa.* These attractant compounds were either specific to or abundant in the attractant 100-*T. vaporariorum* adult-infested plants compared to unattractant plants, i.e. healthy plants and plants infested with 50 and 200 *T. vaporariorum* adults. Among these compounds, only β-myrcene has been previously identified as an attractant to *E. formosa*, from a study on HIPV of *B. tabaci* adult-infested *A. thaliana* plants (Zhang et al. 2013a). However, other *E. formosa* attractants: (*Z*)-3-hexen-1-ol, 4,8-dimethyl-1,3,7-nonatriene, and 3-octanone have been identified in *T. vaporariorum* adult-infested *P. vulgaris* plant volatiles (Birkett et al. 2003). The *E. formosa*-attracting compounds identified in our study have been reported to mediate olfactory responses in other parasitoid species. For instance, α-phellandrene and (*E*)-β-ocimene induced by phloem-feeding aphids were reported to be attractants for the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) (Takemoto and Takabayashi 2015). Moreover, 3-carene attracted *Chouioia cunea* (Yang) (Hymenoptera: Eulophidae), a pupal parasitoid of *Hypanthia cunea* (Drury) (Lepidoptera: Arctiidae) (Pan et al. 2020). The dose-response assays in our study revealed that, except for α-phellandrene, the proportion of attracted parasitoids slightly increased as the test dose increased. Doses of compounds attractive to *E. formosa* in previous studies were: e.g. 360 ng β-myrcene in olfactometer (Zhang et al. 2013a), and 10 μg (*Z*)-3-hexen-1-ol in wind tunnels (Birkett et al. 2003), suggesting that the doses tested in our study were in relevant range to elicit attraction in the parasitoid. Natural enemies are likely to use blend of odours when foraging for host and prey (Conchou et al. 2019; Thomas-Danguin et al. 2014). Our results showed that the parasitoid *E. formosa* was highly attracted to the blend of the four attractants, i.e. 3-carene, β-myrcene, β-ocimene and α-phellandrene when tested against clean air. However, the parasitoid attractiveness was reduced when concentrations of two compounds, β-myrcene and β-ocimene, were increased by 10-fold, indicating that the parasitoid could be sensitive to the relative concentration and ratio of these compounds in the blend. A previous study reported that the mixture of (*Z*)-3-hexen-1-ol and 3-octanone elicited the greatest attraction by *E. formosa* in wind tunnel assays (Birkett et al. 2003).

In summary, our findings show that the density of the whitefly *T. vaporariorum* adults on tomato plants influences both the olfactory response of *E. formosa* and the volatile composition of infested tomato plants. We also demonstrate that terpenes are responsible for *E. formosa* attraction to *T. vaporariorum* adult-infested tomato plants. The 4-component terpene blend of 3-carene, β-myrcene, β-ocimene and α-phellandrene, could be used as a kairomone-based attractant lure to recruit and retain the parasitoid for the control of *T. vaporariorum* in tomato crop fields.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval Not applicable.

References

Afsheen S, Wang X, Li R, Zhu C-S, Lou Y-G (2008) Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. Insect Sci 15:381–397. https://doi.org/10.1111/j.1744-7917.2008.00225.x

Antony B, Palaniswami MS, Henneberry TJ (2003) *Encarsia transvena* (Hymenoptera: Aphelinidae) development on different *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) instars. Environ Entomol 32:584–591. https://doi.org/10.1603/0046-225x-32.3.584

Birkett MA, Chamberlaln K, Guerrieri E, Pickett JA, Wadhams LJ, Yasuda T (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. J Chem Ecol 29:1589–1600. https://doi.org/10.1023/A:1024218729423

Breiman L (2001) Random forests. Mach Learn 45:5–32. https://doi.org/10.1023/A:1010933404324

Colazza S, Wanjberg E (2013) Chemical ecology of insect parasitoids: Towards a new area. In : Wanjberg E, Colazza S (eds) Chemical Ecology of Insect Parasitoids. First edit. Wiley-Blackwell, Chichester, UK: John Wiley & Sons, Ltd.

Conchou L, Lucas P, Meslin C, Proffit M, Staudt M, Renou M (2019) Insect odorscapes : from plant volatiles to natural olfactory scenes. Front Physiol 10:1–20. https://doi.org/10.3389/fphys.2019.00972

Darshanee HLC, Ren H, Ahmed N, Zhang Z-F, Liu Y-H, Liu T-X (2017) Volatile-mediated attraction of greenhouse whitefly *Trialeurodes vaporariorum* to tomato and eggplant. Front Plant Sci 8:1–13. https://doi.org/10.3389/fpls.2017.01285

De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573. https://doi.org/10.1038/31219

De Vis RMJ, Van Lenteren JC (2008) Biological control of *Trialeurodes vaporariorum* by *Encarsia formosa* on tomato in unheated greenhouses in the high altitude tropics. Bull Insectology 61:43–57

Dinno A (2015) Nonparametric pairwise multiple comparisons in independent groups using Dunn's test. Stata J 15:292–300. https://doi.org/10.1177/1536867X1501500117

Estrada-Hernandez MG, Valenzuela-Soto JH, Ibarra-Laclette E, John Paul Delano-Frier JP (2009) Differential gene expression in whitefly bemisia tabaci-infested tomato (*Solanum lycopersicum*) plants at progressing developmental stages of the insect's life cycle. Physiol Plant 137:44–60. https://doi.org/10.1111/j.1399-3054.2009.01260.x

Gamarra H, Carhuapoma P, Mujica M, Kreuze J, Kroschel J (2016) Greenhouse Whitefly, *Trialeurodes vaporariorum* (Westwood 1956). In: Kroschel J, Mujica N, Carhuapoma P, Sporleder M (eds.) Pest distribution and risk Atlas for Africa. Potential, Global and Regional Distribution and Abundance of Agricultural and Horticultural Pests and Associated Biocontrol Agents under Current and Future Climates, 154:154–168. Lima, Peru: International Potato Center (CIP). ISBN 978-92-9060-476-1. https://doi.org/10.4160/9789290604761

Hanssen IM, Lapidot M (2012) Major tomato viruses in the mediterranean basin. Adv Virus Res 84:31-66. Elsevier Inc. https://doi.org/10.1016/B978-0-12-394314-9.00002-6

Hervé MR, Nicolè F, Lê Cao K-A (2018) Multivariate analysis of multiple datasets: a practical guide for chemical ecology. J Chem Ecol 44:215–234. https://doi.org/10.1007/s10886-018-0932-6

Hoddle MS, Van Driesche RG, Sanderson JP (1998) Biology and use of the whitefly parasitoid *Encarsia formosa*. Annu Rev Entomol 43:645–669. https://doi.org/10.1146/annurev.ento.43.1.645

Hu JS, Gelman DB, Blackburn MB (2002) Growth and development of *Encarsia formosa* (Hymenoptera: Aphelinidae) in the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae): effect of host age. Arch Insect Biochem Physiol 49:125–136. https://doi.org/10.1002/arch.10015

Inbar M, Gerling D (2008) Plant-mediated interactions between whiteflies, herbivores, and natural enemies. Annu Rev Entomol 53:431–448. https://doi.org/10.1146/annurev.ento.53.032107.122456

Kaplan I (2012) Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? Biol. Control. 60:77–89. https://doi.org/10.1016/j.biocontrol.2011.10.017

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291(5511):2141–2144. https://doi.org/10.1126/science.291.5511.2141

Khan M, Mousa AA, Syamasundar KV, Alkhathlan HZ (2012) Determination of chemical constituents of leaf and stem essential oils of *Artemisia monosperma* from Central Saudi Arabia. Nat Prod Commun 7:1079–1082. https://doi.org/10.1177/1934578x1200700829

Kishinevsky M, Keasar T, Bar-Massada A (2016) Parasitoid *Encarsia* spp abundance on plants: effects of host abundance, plant species, and plant fLowering state. Arthropod Plant Interact 11:1–7. https://doi.org/10.1007/s11829-016-9476-2

Kos K, Tomanović Ž, Rojht H, Vidrih M, Trdan S (2009) First massive occurrence of greenhouse whitefly parasitoid, *Encarsia formosa* Gahan (Hymenoptera : Aphelinidae) on greenhouse whitefly, *Trialeurodes vaporariorum* [Westwood] (Homoptera : Aleyrodidae) in Slovenia. Acta Agric Slov 93:285–291. https://doi.org/10.2478/v10014-009-0017-x

Lê Cao K-A, Boitard S, Besse P (2011) Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinform 12:1–17. https://doi.org/10.1186/1471-2105-12-253

Liaw A, Wiener M (2002) Classification and regression by random Forest. R News 2:18–22

Liu T-X, Stansly PA, Gerling D (2015) Whitefly parasitoids: distribution, life history, bionomics, and utilization. Annu Rev Entomol 60:1–15. https://doi.org/10.1146/annurev-ento-010814-021101

Lou Y-G, Ma B, Cheng J-A (2005) Attraction of the parasitoid *Anagrus nilaparvatae* to rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*. J Chem Ecol 31:2357–2372. https://doi.org/10.1007/s10886-005-7106-z

McCormick AC, Unsicker SB, Gershenzon J (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends Plant Sci 17:303–310. https://doi.org/10.1016/j.tplants.2012.03.012

Mccormick AC, Gershenzon J, Unsicker SB (2014) Little peaks with big effects: establishing the role of minor plant volatiles in plant–insect interactions. Plant Cell Environ 37(8):1836–1844

McCormick AC, Reinecke A, Gershenzon J, Unsicker SB (2016) Feeding experience affects the behavioral response of polyphagous gypsy moth caterpillars to herbivore-induced poplar volatiles. J Chem Ecol 42:382–393. https://doi.org/10.1007/s10886-016-0698-7

Mehrnejad MR, Copland MJW (2006) Behavioral responses of the parasitoid *Psyllaephagus pistaciae* (Hymenoptera : Encyrtidae) to host plant volatiles and honeydew. Entomol Sci 9:31–37. https://doi.org/10.1111/j.1479-8298.2006.00151.x

Mumm R, Dicke M (2010) Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Can J Zool 88:628–667. https://doi.org/10.1139/Z10-032

Murali-Baskaran KR, Chander K, Kaushal P, Kumar J, Parthiban P, Senthil-Nathan S, Mankin RW (2018) Role of kairomone in biological control of crop pests-a review. Physiol Mol Plant Pathol 101:3–15. https://doi.org/10.1016/j.pmpp.2017.07.004

Navas-Castillo J, López-Moya JJ, Aranda MA (2014) Whitefly-transmitted RNA viruses that affect intensive vegetable production. Ann Appl Biol 165:155–171

Palumbo JC, Toscano NC, Blua MJ, Yoshida HA (2000) Impact of *Bemisia* whiteflies (Homoptera: Aleyrodidae) on alfalfa growth, forage yield, and quality. J Econ Entomol 93:1688–1694. https://doi.org/10.1603/0022-0493-93.6.1688

Pan L, Xiang W, Sun Z, Yang Y, Han J, Wang Y, Yan C, Li M (2020) CcOBP2 plays a crucial role in 3-carene olfactory response of the parasitoid wasp *Chouioia cunea*. Insect Biochem Mol Biol 117:103286. https://doi.org/10.1016/j.ibmb.2019.103286

Peñaflor MFGV, Bento JMS (2013) Herbivore-induced plant volatiles to enhance biological control in agriculture. Neotrop Entomol 42:331–343. https://doi.org/10.1007/s13744-013-0147-z

Peri E, Moujahed R, Wajnberg E, Colazza S (2018) Applied chemical ecology to enhance insect parasitoid efficacy in the biological control of crop pests. In: Tabata J (ed) Chemical ecology of insects: applications and associations with plants and microbes. Taylor & Francis, New York, pp 234–267

Perring TM, Stansly PA, Liu TX, Smith HA, Andreason SA (2018) Whiteflies : biology, ecology, and management. In: Wakil W, Brust GE, and Perring TM (eds) Sustainable management of arthropod pests of tomato,73–110. Elsevier Inc. Academic Press. https://doi.org/10.1016/B978-0-12-802441-6.00004-8

Ponzio C, Cascone P, Cusumano A, Weldegergis BT, Fatouros NE, Guerrieri E, Dicke M, Gols R (2016) Volatile-mediated foraging behaviour of three parasitoid species under conditions of dual insect herbivore attack. Anim Behav 111:197–206. https://doi.org/10.1016/j.anbehav.2015.10.024

Qiu YT, Van Lenteren JC, Drost YC, Posthuma-Doodeman JAM (2004) Life-history parameters of *Encarsia formosa*, *Eretmocerus eremicus* and *E. mundus*, aphelinid parasitoids of *Bemisia argentifolii* (Hemiptera: Aleyrodidae). Eur J Entomol 101:83–94

R Core Team (2020) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna. URL https://www.R-project.org/. Accessed 11 Aug 2020

Ranganathan Y, Borges RM (2010) Reducing the babel in plant volatile communication: using the forest to see the trees. Plant Biol 12:735–742. https://doi.org/10.1111/j.1438-8677.2009.00278.x

Rohart F, Gautier B, Singh A, Lê Cao K-A (2017) MixOmics : an R package for 'Omics feature selection and multiple data integration. PLoS Comput Biol 13:e1005752. https://doi.org/10.1371/journal.pcbi.1005752

Romeis J, Zebitz CPW (1997) Searching behaviour of *Encarsia formosa* as mediated by colour and honeydew. Entomol Exp Appl 1990:299–309

Shannag HK, Freihat NM (2009) Gas exchange of cucumber impaired by tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera : Aleyrodidae). Jordan J Agric Sci 5:295–305

Shiojiri K, Ozawa R, Kugimiya S, Uefune M, Wijk MV, Sabelis MW, Takabayashi J (2010) Herbivore-specific, density-dependent induction of plant volatiles: honest or 'cry wolf' signals? PLoS One 5:1–11. https://doi.org/10.1371/journal.pone.0012161

Su Q, Chen G, Mescher MC, Peng Z, Xie W, Wang S, Wu Q, Liu J, Li C, Wang W, Zhang Y (2018) Whitefly aggregation on tomato is mediated by feeding-induced changes in plant metabolites that influence the behaviour and performance of conspecifics. Funct Ecol 32:1180–1193. https://doi.org/10.1111/1365-2435.13055

Takabayashi J, Shiojiri K (2019) Multifunctionality of herbivory-induced plant volatiles in chemical communication in tritrophic interactions. Curr Opin Insect Sci 32:110–117. https://doi.org/10.1016/j.cois.2019.01.003 2214-5745/ã

Takemoto H, Takabayashi J (2015) Parasitic wasps *Aphidius ervi* are more attracted to a blend of host-induced plant volatiles than to the independent compounds. J Chem Ecol 41:801–807. https://doi.org/10.1007/s10886-015-0615-5

Tan X-L, Liu T-X (2014) Aphid-induced plant volatiles affect the attractiveness of tomato plants to *Bemisia tabaci* and associated natural enemies. Entomol Exp Appl 151:259–269. https://doi.org/10.1111/eea.12190

Thomas-Danguin T, Sinding C, Romagny S, Mountassir FE, Atanasova B, Le Berre E, Anne-Marie L, Le Bon A-M, Coureaud G (2014) The perception of odor objects in everyday life : a review on the processing of odor mixtures. Front Physiol 5:1–18. https://doi.org/10.3389/fpsyg.2014.00504

Wagh VH, Dhote VW, Maimom S, Jha S (2014) Invasive insect pests: challenges of changing climate. J Interacademicia 18 : 695–700. https://www.cabdirect.org/cabdirect/abstract/20153061548

Walling LL (2008) Avoiding effective defenses : strategies employed by phloem-feeding insects. Plant Physiol 146:859–866. https://doi.org/10.1104/pp.107.113142

Xiu C, Dai W, Pan H, Zhang W, Luo S, Wyckhuys KAG, Yang Y, Lu Y (2019) Herbivore-induced plant volatiles enhance field-level parasitism of the mirid bug *Apolygus lucorum*. Biol Control 135:41–47. https://doi.org/10.1016/j.biocontrol.2019.05.004

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol 143:866–875. https://doi.org/10.1104/pp.106.090035

Zhang S-Z, Guo J-Y, Wan F-H, Zhang F (2005) Parasitic behavior and selectivity of *Encarsia formosa* (Hymenoptera: Aphelinidae) towards *Bemisia tabaci* (Homoptera: Aleyrodidae) on different host plants. Sheng Tai Xue Bao 25:2595–2600

Zhang P-J, Xu C-X, Zhang J-M, Lu Y-B, Wei J-N, Liu Y-Q, David A, Boland W, Turlings TCJ (2013a) Phloem-feeding whiteflies can fool their host plants, but not their parasitoids. Funct Ecol 27: 1304–1312. https://doi.org/10.1111/1365-2435.12132

Zhang P-J, Li W-D, Huang F, Zhang J-M, Xu F-C, Lu Y-B (2013b) Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. J Chem Ecol 39:612–619. https://doi.org/10.1007/s10886-013-0283-2

Zhang P-J, Wei J-N, Zhao C, Zhang Y-F, Li C-Y, Liu S-S, Dicke M, Yu X-P, Turlings TCJ (2019) Airborne host–plant manipulation by whiteflies via an inducible blend of plant volatiles. PNAS 116: 7387–7396. https://doi.org/10.1073/pnas.1818599116

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Re-Analysis of Abdominal Gland Volatilome Secretions of the African Weaver Ant, *Oecophylla longinoda* (Hymenoptera: Formicidae)

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Abstract: The African weaver ant, *Oecophylla longinoda*, is used as a biological control agent for the management of pests. The ant has several exocrine glands in the abdomen, including Dufour's, poison, rectal, and sternal glands, which are associated with pheromone secretions for intra-specific communication. Previous studies have analyzed the gland secretions of Dufour's and poison glands. The chemistry of the rectal and sternal glands is unknown. We re-analyzed the secretions from Dufour's and poison glands plus the rectal and sternal glands to compare their chemistries and identify additional components. We used the solid-phase microextraction (SPME) technique to collect gland headspace volatiles and solvent extraction for the secretions. Coupled gas chromatography–mass spectrometry (GC-MS) analysis detected a total of 78 components, of which 62 were being reported for the first time. These additional components included 32 hydrocarbons, 12 carboxylic acids, 5 aldehydes, 3 alcohols, 2 ketones, 4 terpenes, 3 sterols, and 1 benzenoid. The chemistry of Dufour's and poison glands showed a strong overlap and was distinct from that of the rectal and sternal glands. The different gland mixtures may contribute to the different physiological and behavioral functions in this ant species.

Keywords: weaver ant; biological control; hydrocarbons; solid-phase microextraction (SPME); mass spectrometry

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1. Introduction

Chemical communication is a well-documented phenomenon in ants [1], used within and outside the nest [2] for recruitment [3], defense [4], alarm [5], nestmate [6], and sexual recognition [7]. Ant semiochemicals are primarily released from different glands, for example, the Pavan gland located in the abdomen of ants in the families Dolichoderinae and Aneureinae [8], Dufour's gland in harvester ants, [3], the sternal gland in African stink ants (*Pachycondyla tarsata*) [9], and the hindgut of the jet ant (*Lasius fuliginosus*) [10].

Weaver ants of the genus *Oecophylla* consist of two extant species, *O. longinoda* and *O. smaragdina*, distributed in the old-world tropics and known for sophisticated nest-building behaviors [11]. These ants possess different glands located in the abdomen, which are used for various purposes [12,13]. The poison gland of *Oecophylla longinoda* contains a large quantity of formic acid, whereas in *O. smaragdina*, the key component in the gland secretion is undecane [13,14]. Dufour's gland, in both species, contains a mixture of hydrocarbons, including undecane and other n-alkanes (decane, dodecane, pentadecane, heptadecane, nonadecane, heneicosane, docosane, and tricosane) [13,14]. The two major compounds in the glands, formic acid and undecane, are used as an alarm/defense system in *O. longinoda* [13]. The functions of the gland secretions have not been well studied in *O. smaragdina.* Two more glands are reported in *O. longinoda*: the rectal gland releases pheromones that mediate recruitment to new food sources [12], whereas emigration to new sites and short-range recruitment to territorial intruders are facilitated by secretions from the sternal gland [12].

Previous studies have identified 23 compounds in Dufour's gland secretions, with two compounds identified in the poison gland [13]. This study, which was carried out over four decades ago, used less sensitive analytical chemical techniques based on mass spectrometry fitted with packed glass columns and degradative reactions of isolates obtained from preparative chromatographic analysis to identify compounds. A recent study using modern and more sensitive analytical chemical techniques provided a comprehensive comparison of the chemical profiles of the cuticle, Dufour's glands, poison glands, the head, headspace volatiles, and trails of a related species, *O. smaragdina* [14]. In this study, a total of 59 compounds were identified from these glands of worker ants, including aldehydes, alcohols, carboxylic acid, esters, fatty acids, terpenes, and hydrocarbons. Given these findings for *O. smaragdina*, we hypothesized that additional potential semiochemicals may be released in the abdominal gland secretions of *O. longinoda*.

Thus, in the present study, we re-analyzed the secretions of Dufour's and poison glands to identify additional components. Further, since little is known about the composition of the sternal and rectal glands, we explored the chemistry of their secretions.

2. Results

Our results demonstrate that the use of more than one technique to collect gland secretions, followed by gas chromatography–mass spectrometry (GC-MS) analysis, reveals a complex blend of compounds, including chemical signatures associated with the different glands. Interestingly, GC-MS analysis detected a total of 78 components from secretions of the four glands, of which 62 are reported for the first time (presented in bold font in Tables 1 and 2). Of these 62 additional components, the identities of 28 were confirmed with authentic samples. Quantitative variations in the detected components are shown in Tables 1 and 2. These additional components include a complex mixture of 32 hydrocarbons (51.6%), dominated by unsaturated alkanes, which ranged in chain length from 13 to 25 carbon atoms; saturated alkanes of chain length from 6 to 31 carbon atoms were also detected, as were 12 carboxylic acids (19.4%), dominated by short-chain fatty acids of chain length from two to seven carbon atoms; 5 aldehydes (8.1%); 3 alcohols (4.8%); 2 ketones (3.2%); 4 terpenes (6.5%); 3 sterols (4.8%) and 1 benzenoid (1.6%). Solvent-extracted glands and headspace volatiles gave similar profiles, dominated by hydrocarbons, which were also identified in the gland secretions of *O. smaragdina* [14]. However, we found that the headspace volatiles were richer in fatty acids than the solvent-extracted glands. These data suggest further investigation into the potential pheromonal roles of these fatty acids and possibly the terpene *p*-cymene [13] and caryophyllene detected in the headspace volatiles. The patterns of the composition of the hydrocarbons were similar for both Dufour's and poison glands. Likewise, the compositions of the rectal and sternal glands were similar, comprising saturated alkanes of chain length from 8 to 31 carbon atoms.

Table 1. Percentage compositions and amounts of compounds detected in Dufour's, poison, sternal, and rectal gland extracts of major workers of *Oecophylla longinoda*.

			Content (%) Mean ± SE				Absolute Amount (ng)			
Compounds	**RT**	**RI**	**Dufour's**	**Poison**	**Rectal**	**Sternal**	**Dufour's**	**Poison**	**Rectal**	**Sternal**
				Hydrocarbons						
Heptane [a]	3.82	700	-	-	-	1.65 ± 0.32	-	-	-	3.4
Octane [a]	6.51	800	-	0.08 ± 0.06	0.06 ± 0.15	0.09 ± 0.01	1.79	0.91	0.84	0.86
Unidentified branched alkane 1 [b]	7.85	842	-	-	-	0.19 ± 0.10	-	-	-	1.04
Nonane [a]	9.53	900	0.14 ± 0.02	1.14 ± 0.47	0.65 ± 0.21	-	0.94	3.42	1.89	
Decane [a]	11.88	1000	0.90 ± 0.08	-	0.37 ± 0.89	0.23 ± 0.10	2.16	-	1.39	1.09
Unidentified alkene 1 [b]	12.25	1041	0.16 ± 0.08	-	-	-	1.01	-	-	-
Undecane [a]	13.38	1100	39.64 ± 1.06	-	8.62 ± 3.27	7.33 ± 6.46	68.64	-	16.18	12.6
Dodecane [a]	14.84	1200	0.94 ± 0.05	0.28 ± 0.14	1.31 ± 1.15	0.80 ± 0.35	2.27	1.37	3.07	2.01
Unidentified branched alkane 2 [b]	16.07	1276	-	-	-	0.74 ± 0.27	-	-	-	1.92
Unidentified branched alkane 3 [b]	16.25	1288	-	-	-	0.85 ± 0.35	-	-	-	2.1
Unidentified alkene 2 [b]	16.29	1291	0.51 ± 0.07	-	-	-	1.54	-	-	-
Unidentified alkene 3 [b]	16.31	1292	1.61 ± 0.13	-	-	-	3.38	-	-	-
Tridecane [a]	16.61	1300	4.72 ± 0.24	0.77 ± 0.12	4.65 ± 3.72	3.12 ± 0.60	8.59	2.53	9.05	5.78
Tetradecane [a]	17.48	1400	0.37 ± 0.02	1.99 ± 0.85	1.35 ± 0.45	0.25 ± 0.03	1.3	5.42	3.15	1.13
Pentadecane [a]	18.69	1500	7.07 ± 0.09	1.66 ± 0.15	1.34 ± 0.59	4.18 ± 1.28	12.54	4.63	3.13	7.49
Unidentified alkene 4 [b]	18.99	1520	0.14 ± 0.02	-	-	-	0.96	-	-	-
Hexadecane [a]	20.48	1600	0.07 ± 0.01	1.13 ± 0.19	1.37 ± 0.32	0.50 ± 0.12	0.84	3.39	3.19	1.54
Unidentified alkene 5 [b]	21.05	1663	1.07 ± 0.12	-	-	-	2.48	-	-	-
Unidentified alkene 6 [b]	21.12	1669	0.86 ± 0.02	-	-	-	2.13	-	-	-
Heptadecane [a]	21.47	1700	0.11 ± 0.01	-	3.70 ± 1.03	3.44 ± 1.79	0.89	-	7.36	6.29
Octadecane [a]	22.46	1800	0.09 ± 0.02	3.84 ± 1.41	1.08 ± 0.32	0.56 ± 0.09	0.88	3.08	2.66	1.63
Nonadecane [a]	23.44	1900	1.16 ± 0.11	3.27 ± 0.86	1.81 ± 0.66	2.49 ± 0.37	2.74	8.45	3.97	4.75
Unidentified alkene 7 [b]	23.82	1949	0.24 ± 0.03	-	-	-	1.14	-	-	-
Eicosane [a]	24.31	2000	0.55 ± 0.06	4.84 ± 0.99	2.43 ± 1.03	1.77 ± 0.35	1.67	12.16	5.08	3.59
Unidentified alkene 8 [b]	24.95	2070	1.19 ± 0.07	-	-	-	2.67	-	-	-
Heneicosane [a]	25.01	2100	12.47 ± 0.42	5.15 ± 1.61	8.38 ± 1.75	16.5 ± 2.42	22.14	12.89	15.75	27.58
Docosane [a]	25.97	2200	1.77 ± 0.11	3.71 ± 0.72	3.66 ± 0.40	2.32 ± 0.15	3.73	9.49	7.29	4.48
Unidentified alkene 9 [b]	26.8	2239	2.57 ± 0.06	-	4.79 ± 2.38	-	4.82	-	9.31	-
Tricosane [a]	26.98	2300	16.84 ± 0.33	8.77 ± 2.18	12.78 ± 3.38	24.2 ± 2.17	28.99	21.45	23.64	40.07

Table 1. *Cont.*

			Content (%) Mean ± SE				Absolute Amount (ng)			
Compounds	**RT**	**RI**	**Dufour's**	**Poison**	**Rectal**	**Sternal**	**Dufour's**	**Poison**	**Rectal**	**Sternal**
Unidentified alkene 10 [b]	27.53	2364	0.35 ± 0.03	5.13 ± 1.45	4.07 ± 0.73	3.78 ± 0.53	1.33	12.89	8.02	6.85
Tetracosane [a]	27.61	2400	0.25 ± 0.03	-	-	-	1.11	-	-	
Pentacosane [a]	28.61	2500	1.10 ± 2.39	4.16 ± 1.78	4.58 ± 0.81	3.91 ± 0.54	2.54	10.56	8.94	7.06
Hexacosane [a]	29.87	2600	-	6.48 ± 1.16	3.04 ± 2.07	3.65 ± 0.47	-	16.05	6.16	6.63
Heptacosane [a]	30.43	2700	-	7.06 ± 1.99	4.95 ± 0.29	2.34 ± 0.35	-	17.42	9.6	4.52
Octacosane [a]	30.79	2800	-	4.80 ± 0.46	2.68 ± 0.61	3.56 ± 0.35	-	12.08	5.52	6.49
Nonacosane [a]	33.39	2900	-	4.83 ± 0.88	8.06 ± 1.82	5.30 ± 1.18	-	10.6	15.16	9.31
Squalene [a]	33.57	2934	-	4.18 ± 0.28	3.66 ± 0.53	2.42 ± 0.43	-	12.15	7.28	4.65
Triacontane [a]	34.1	3000	-	4.02 ± 1.25	3.91 ± 0.43	2.57 ± 0.47	-	10.23	7.73	4.89
Hentriacontane [a]	34.89	3100	-	6.20 ± 2.54	4.74 ± 1.97	-	-	15.38	9.22	-
Carboxylic acids										
Decanoic acid [a]	16.45	1301	0.77 ± 0.14	-	-	-	1.74	-	-	-
Hexadecanoic acid [a]	23.68	1933	0.40 ± 0.05	-	-	-	1.13	-	-	-
Unidentified carboxylic acid 1 [b]	25.75	2156	1.66 ± 0.19	-	-	-	2.43	-	-	-
Octadecanoic acid [a]	25.94	2177	0.30 ± 0.03	-	-	-	1.25	-	-	-
Terpene										
α-Cedrene[a]	18.11	1422	-	-	-	0.96 ± 0.46		-	-	-
Alcohol										
Unidentified alcohol 1 [b]	24.81	2055	-	3.84 ± 1.41	-	-		9.8	-	-
Sterols										
Cholesterol [a]	35.56	3074	-	6.10 ± 2.09	-	-		15.16	-	-
Unidentified sterol 1 [b]	36.14	3094	-	4.07 ± 1.95	-	-		10.33	-	-
Campesterol [a]	37.6		-	5.34 ± 1.00	-	-		13.34	-	-

RT (min): retention time in minutes; SE: standard error; [a] compounds identified by the injection of synthetic standards; [b] compounds tentatively identified by gas chromatography–mass spectrometry (GC-MS) library data only. RI: retention index relative to C$_6$–C$_{32}$ n-alkanes on an HP-5 MS column. Compound names in bold are additional components reported for the first time in gland secretions. RSD < 10, green; RSD < 50, yellow; RSD < 100, red; and RSD > 100, no color.

Table 2. Percentage compositions and amounts of headspace volatiles of Dufour's and poison glands of major workers of *O. longinoda*.

	Content (%) Mean ± SE				Absolute Amount (ng)	
Compounds	**RT**	**RI**	**Dufour's**	**Poison**	**Dufour's**	**Poison**
Hydrocarbons						
Nonane [a]	9.53	900	0.19 ± 0.12	0.02 ± 0.01	7.6	0.82
Decane [a]	11.88	1000	3.21 ± 0.85	-	117.19	-
Unidentified alkene 1 [b]	12.83	1077	0.13 ± 0.08	0.35 ± 0.24	5.47	2.46
Undecane [a]	13.38	1100	59.29 ± 4.40	32.52 ± 8.99	2149.44	164.64
Unidentified alkene 2 [b]	14.44	1167	0.14 ± 0.06	0.16 ± 0.10	5.89	1.51
Dodecane [a]	14.84	1200	3.22 ± 0.48	0.82 ± 0.20	117.52	4.85
Unidentified alkene 3 [b]	14.56	1245	0.47 ± 0.21	-	17.63	-
Unidentified alkene 4 [b]	15.7	1251	0.15 ± 0.11	-	6.02	-
Unidentified alkene 5 [b]	15.83	1260	0.85 ± 0.39	0.33 ± 0.08	31.67	2.37
Unidentified alkene 6 [b]	15.94	1265	3.89 ± 0.80	1.13 ± 0.36	141.75	6.39
Tridecane [a]	16.61	1300	11.29 ± 1.50	4.30 ± 1.37	109.73	22.4
Unidentified alkene 7 [b]	17.2	1352	0.25 ± 0.14	-	9.92	-
Tetradecane [a]	17.48	1400	1.14 ± 0.17	0.39 ± 0.23	42.21	2.68
Pentadecane [a]	18.69	1500	8.47 ± 3.20	6.08 ± 1.60	307.56	31.35
Unidentified alkene 8 [b]	19.62	1538	0.12 ± 0.05	-	4.97	-
Hexadecane [a]	19.84	1600	0.12 ± 0.06	0.20 ± 0.19	5.01	1.72
Unidentified alkene 9 [b]	21.12	1669	0.52 ± 0.06	0.37 ± 0.06	19.51	2.57
Heptadecane [a]	21.47	1700	0.12 ± 0.06	-	5.13	-
Octadecane [a]	22.46	1800	0.11 ± 0.08	0.12 ± 0.03	4.85	1.34
Eicosane [a]	24.31	2000	0.23 ± 0.11	-	9.02	-
Heneicosane [a]	25.01	2100	0.18 ± 0.11	0.08 ± 0.05	7.16	1.12
Docosane [a]	25.97	2200	0.36 ± 0.29	0.24 ± 0.23	13.75	1.93
Tricosane [a]	26.98	2300	0.13 ± 0.10	0.25 ± 0.21	5.49	1.95
Tetracosane [a]	27.61	2400	0.46 ± 0.32	0.35 ± 0.23	17.25	2.47
Carboxylic acids						
Formic acid [a]	1.9		-	38. 47 ± 8.85	-	194.59
Acetic acid [a]	2.37		0.68 ± 0.47	3.45 ± 0.32	25.39	18.11
Propanoic acid [a]	3.42		0.16 ± 0.10	0.43 ± 0.08	6.61	2.9
2-Propenoic acid [a]	3.63		0.01 ± 0.01	-	6.61	-
2-Methylpropanoic acid	5.22		0.11 ± 0.07	0.03 ± 0.02	1.1	0.88
Butanoic acid [a]	6.05		0.17 ± 0.15	0.10 ± 0.07	4.62	1.22

Table 2. *Cont.*

			Content (%) Mean ± SE		Absolute Amount (ng)	
Compounds	**RT**	**RI**	**Dufour's**	**Poison**	**Dufour's**	**Poison**
3-Methylbutanoic acid ^{a}	7.81		0.06 ± 0.05	0.09 ± 0.06	6.93	1.19
2-Methylhexanoic acid ^{a}	8.04		-	🟥 0.17 ± 0.07	-	1.58
Unidentified carboxylic acid 1 ^{b}	17.72		-	🟨 0.52 ± 0.27	-	3.31
			Aldehydes			
Acetaldehyde ^{a}	1.13		🟥 0.21 ± 0.16	🟥 2.68 ± 1.09	8.21	14.2
2-Methylbutanal ^{a}	2.84		-	0.08 ± 0.05	-	1.14
Hexanal ^{a}	6.34		🟨 0.03 ± 0.02	🟥 0.14 ± 0.05	1.91	1.44
Heptanal ^{a}	9.09		-	🟥 0.06 ± 0.01	-	1.04
Unidentified aldehyde 1 ^{b}	21.28		0.09 ± 0.06	-	4.12	
			Terpenes			
***p*-Cymene** ^{a}	11.6	1000	-	🟨 0.07 ± 0.04	-	1.06
Caryophyllene ^{a}	17.8	1400	0.13 ± 0.11	-	5.28	-
Unidentified sesquiterpene 1 ^{b}	19.08	1500	-	🟨 0.18 ± 0.11	-	1.64
			Alcohols			
Unidentified alcohol 1 ^{b}	17.87	1400	-	🟥 0.10 ± 0.17	-	2.24
1-Tridecanol ^{b}	18.43	1444	🟨 0.72 ± 0.16	-	27.31	-
			Ketones			
Acetone ^{a}	1.61		🟥 0.01 ± 0.00	-	0.89	-
Unidentified ketone 1 ^{b}	13.48			0.37 ± 0.26	-	2.1
			Benzenoid			
Styrene ^{a}	8.78	878	-	0.12 ± 0.12	-	1.3
			Miscellaneous			
Unknown 1	1.52		-	3.03 ± 2.06	-	-
Unknown 2	5.19		-	🟥 0.11 ± 0.10	-	-
Unknown 3	11.04		-	🟥 0.25 ± 0.06	-	-
Unknown 4	15.65		-	🟨 0.16 ± 0.13	-	-
Unknown 5	17.96		-	🟨 0.40 ± 0.15	-	-
Unknown 6	18.07		-	🟨 0.25 ± 0.13	-	-
Unknown 7	18.18		-	🟨 0.26 ± 0.25	-	-
Unknown 8	18.25		-	🟨 0.49 ± 0.29	-	-
Unknown 9	19.21		-	🟩 0.33 ± 0.23	-	-

RT (min): retention time in minutes; SE: standard error; ^{a} compounds identified by the injection of synthetic standards; ^{b} compounds tentatively identified by GC-MS library data only. RI: Retention index relative to C_6-C_{32} n-alkanes on an HP-5 MS column. Compound names in bold are additional components reported for the first time in gland secretions. RSD < 10, green; RSD < 50, yellow; RSD < 100, red; and RSD > 100, no color.

We found that undecane, heneicosane, and tricosane in Dufour's gland secretions formed ~70% of the composition. Our results agree with previous work that reported these three compounds as the major components of the secretions of Dufour's gland of *O. longinoda* [13], which act as alarm pheromones for the ant. It is possible that the low vapor pressures associated with some of these hydrocarbons, especially those ranging in chain length between 25 and 31 carbon atoms could help moderate the volatility of other components in Dufour's gland secretions. Comparing our results to those reported by Bradshaw et al. [13], we identified seven classes of chemicals (hydrocarbons, carboxylic acids, terpenes, aldehydes, alcohols, ketones, and benzenoid), whereas these authors identified only two classes of chemicals (hydrocarbons and ester), comprising 23 compounds in Dufour's gland of *O. longinoda*. This confirms the efficiency of our method (combining solid-phase microextraction (SPME) and solvent extraction) for both qualitative and quantitative analysis of gland secretions compared to the use of solvent extraction alone. Of the 23 compounds identified in [13], 22 were hydrocarbons (decane, undecane, dodecene, dodecane, 4-tridecene, tridecane, tetradecane, pentadecene, pentadecane, hexadecane, 8-heptadecene, heptadecane, octadecane, nonadecane, eicosane, heneicosene, heneicosane, docosane, tricosene, tricosane, pentacosane, and 4,7-heptadecadiene). The ester was partially identified as a hexadecyl ester. Notably, we did not identify any ester in Dufour's gland secretions, although we found several carboxylic acids and alcohols detected in low quantities. As such, it is possible that given the right enzymes, the population of *O. longinoda* used in the current study or another population can make esters in Dufour's gland secretions. Such differences in chemical signatures between populations may also be influenced by diet, geographic location, season, and environmental factors. Our results are in agreement with those of [13] and those found for the related species, the Asian weaver ant, *O. smaragdina* [14,15], which showed that the composition of Dufour's gland secretions is dominated by the hydrocarbons undecane, heneicosane, and pentadecane, constituting more than 60% of the gland components (Table 1). Thus, it appears that the presence of undecane, heneicosane, and pentadecane in a higher proportion in Dufour's gland secretions may serve as a generic chemical signature for these ant species, with the other constituents (ratio and concentration) providing the chemical profile for inter- and intra-species distinction, though further research is necessary to confirm this.

Interestingly, carboxylic acids have previously been detected in Dufour's gland of workers of the crazy ant, *Paratrechina longicornis* [16]. The role of these carboxylic acids in this ant species is unknown, although to the best of our knowledge, this is the first study to identify carboxylic acids in Dufour's gland of *O. longinoda.* As previously observed, the gland secretions of certain ant species also contain aldehydes and terpenes, for instance, the black garden ant, *Lasius niger* [17]; the black-headed bull ant, *Myrmecia nigriceps*; the giant bull ant, *M. gulosa* [18]; and the Sahara Desert ant, *Cataglyphis bicolor* [19]. Likewise, alcohols and ketones have been reported in the blood-red ant, *Formica sanguinea* [20]. Further studies are required to determine the role of these classes of compounds in the biology of ants. The specific chemical signature of the poison gland secretion dominated by the hydrocarbons undecane, tricosane, and heptacosane and the carboxylic acid formic acid (Tables 1 and 2), identified in the present study, seems to agree with the composition previously reported [13]. Formic acid is one of the components of the alarm pheromone in *O. longinoda* [13]. However, Bradshaw et al. [13] partially identified a compound described as a derivative of 1-hexadecanol in the poison gland. On the other hand, in the present study, we identified the carboxylic acid derivative hexadecanoic acid of this alcohol in the Dufour's gland but not in the poison gland secretion. This suggests that further studies to investigate the composition of *O. longinoda* gland secretions from different populations are necessary to enhance our understanding of gland chemistry. Nonetheless, our results agree with the composition of hydrocarbons recently reported for the poison gland secretions of the Asian weaver ant, *O. smaragdina*, which identified undecane, tricosane, and heneicosane as the most abundant components of the gland secretions [14], and formic acid previously identified as a major component of the poison gland secretions of carpenter ants

(*Camponotus* spp.) [21]; the lemon ant, *Myrmelachista schumanni* [22]; and the red wood ant, *Formica rufa* [23].

Chemical analysis of the rectal gland of *O. longinoda* located in the hindgut revealed a range of hydrocarbons (Table 1). The major compounds identified were tricosane, undecane, and heneicosane. The gland has no specialized glandular tissue but is comprised of glandular cells responsible for pheromone production [4]. However, in the present study, since the entire hindgut was examined to access the glandular cells, there was a possibility of extracting products associated with cuticular components and digested food. Nonetheless, similar straight-chain hydrocarbons had previously been reported from the anal gland secretions of the ant *Novomessor cockerelli* [24].

Likewise, the sternal gland of this ant species does not have a reservoir; instead, the gland is composed of a group of glandular cells whose ducts penetrate the sclerotized cuticle [13]. The compounds identified in the sternal gland secretions included mainly hydrocarbons (Table 1), in agreement with previous results reported for the sternal gland secretions from the European paper wasp, *Polistes dominulus* [25]. The sternal-specific components heptane, α-cedrene, and, especially, dimethyl-branched saturated components, which were not detected in the other gland secretions, could be associated with cuticular components. Such differences need further investigation. Consequently, because of the approach we used to obtain sternal and rectal gland secretions, and to rule out the possibility of the presence of additional artifacts in our analysis, we did not carry out headspace collections and analysis of secretions of these glands.

3. Materials and Methods

3.1. Chemicals and Standards

Synthetic standards of alkanes (analytical grade ≥98% purity) were purchased from EAD Milliore Corporation, Burlington, MA, USA. A mixture of the alkanes from C_6–C_{32} was prepared for the identification of the different alkanes. Formic acid (≥98%), acetic acid (≥99.8%), propanoic acid (≥99.5%), butanoic acid (≥99%), nonanoic acid (≥97%), decanoic acid (≥98%), hexadecanoic acid (≥99%), octadecanoic acid (≥98.5%), squalene (≥98%), cholesterol (≥99%), styrene (≥99%), acetaldehyde (≥99.5%), hexanal (≥97%), heptanal (≥95%), *p*-cymene (≥97%), and caryophyllene (≥97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Insects

Ant colonies were collected from mango trees at the Muhaka field station (−4°32′41″ S, 39°52′44″ E) of the International Centre of Insect Physiology and Ecology (*icipe*) in Kwale County, Kenya. Tree branches with nests were cut and placed in plastic containers ($45 \times 30 \times 15$ cm^3), with fine netting attached to the lid for ventilation. The nests were transferred to potted mango seedlings in a greenhouse at *icipe*'s main campus (−1°22′17″ S, 36°89′65″ E) in Nairobi, Kenya. Pots were thereafter placed in the center of a tray filled with soapy water to confine the ants to the host plant as well as to keep predatory insects from getting access to the ants. The plants were watered and branches pruned regularly to prevent the ants from escaping. The plants were maintained under natural lighting in the greenhouse (12 light:12 dark) at 29 ± 2 °C and 65 ± 5 RH%, and the ants were fed on a 10% sugar solution and freshly killed fruit flies (adult *Bactrocera dorsalis* and *Ceratitis cosyra*) twice a week. A total of seven colonies (four for solvent extraction and three for headspace sampling) on different seedlings were maintained and used for GC-MS analysis.

3.3. Extraction of Ant Gland Contents

To extract glandular contents, adult major worker ants were immobilized on ice and abdomens removed using dissection scissors. The four different abdominal glands (10 Dufour's glands and 30 sternal, rectal, and poison glands (Figure 1)) were extracted by carefully removing all the cuticles (dorsal and sternal) and the remnant tissues. To extract the rectal gland, the whole of the hindgut was removed, and for the sternal gland, the last sternite was included to ensure the removal of all the contents. To avoid contamination among gland components, the water in the petri dish was replaced after each dissection and the forceps cleaned upon removal of each gland, using cotton soaked in distilled water followed by washing with acetone. The dissections were carried out in a petri dish containing distilled water under a simple microscope with a magnification power ranging from 10× to 30× using fine forceps (5SF, 11250-00 Inox-Biology CE). The glands were placed in vials containing 2000 µL of hexane and vortexed for 10 s, followed by 30 min of sonication (ultrasonic bath at room temperature) to agitate particles in the sample. After this, the suspension was centrifuged at 15,000 rpm for 30 min at 4 °C and the supernatant was filtered through glass wool. Sodium sulfate ($NaSO_4$) was added to remove water in the samples, vortexed, and centrifuged at 15,000 rpm for 10 min at 4 °C. The solutions were evaporated to dryness and reconstituted in 100 µL of hexane.

Figure 1. Schematic diagram of worker African weaver ant's exocrine glands in the abdomen; (P) poison, (D) Dufour's, (S) sternal, (R) rectal, and (H) hindgut.

Each extraction was repeated four times using different glands obtained from different ant populations for each extraction, and samples were stored at −80 °C until use.

3.4. Headspace Sampling by SPME

Collection of headspace volatiles was performed using a manual solid-phase microextraction (SPME) fiber, with a layer of polydimethylsiloxane (PDMS) obtained from Supelco Co (Bellefonte, PA, USA; Taufkirchen, Germany). Volatile contents of Dufour's and poison glands were collected from 10 glands each, combined and obtained from different ant populations. The gland reservoir was punctured and introduced into 2 mL vials (Supelco) sealed with a Teflon/silicon septum (Supelco) cap containing an insert. Each fiber was conditioned at 250 °C for 15 min before use by putting it into the injector port of a GC instrument operated in split mode with septum purge and purge flow set at 3 mL/min. Volatiles were collected from the glands by piercing the vial cap with the sample using a needle. The fiber was then exposed to the headspace, 2 mm above the sample, for 1 h. The fiber was drawn into the protecting needle before retracting the SPME fiber holder, and volatile collection was repeated three times using a different fiber. For all samples, a blank collection was made using the 2 mL vial and repeated three times.

3.5. Coupled Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Compounds in the glands were identified by coupled gas chromatography–mass spectrometry (GC-MS) on an HP 7890A series gas chromatograph (Agilent Technologies, Wilmington, NC, USA) linked to an HP 5975C mass spectrometer (Agilent Technologies, Wilmington, NC, USA) operated in electron ionization mode (70 eV). The instrument was equipped with a non-polar HP-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness; J &W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at 1.2 mL min^{-1}. One microliter of each sample was injected in splitless mode at 35 °C for 5 min, increasing the temperature to 280 °C at 10 °C min^{-1}. The solvent hexane used for extraction was analyzed similarly. The injector and the detector were held isothermal at 280 °C for 35 min. The ion source temperature was 230 °C. Electron ionization mass spectra were acquired at 70 eV within a mass range of 38–550 Daltons (Da) during a scan time of 0.73 scans s^{-1}. Volatile compounds were identified using their retention times and mass fragmentation spectra against authentic standards (those available). Others were tentatively identified using matches of three mass spectral libraries: Adams, Chemoecol, and National Institute of Standards and Technology (NIST) (MSD Chemstation E.02.00.493, MS HP, USA). A blend of alkanes (C_6–C_{32}) was injected to calculate the retention index (RI) = $[TR(X) - TR(n)]/[TR(n+1) - TR(n)]*100 + (100*n)$, where TR(X) is the retention time of the studied product, TR(n) is the retention time of the alkane with n carbons that eluted before X, and TR(n + 1) is the retention time of the alkane of n + 1 carbons that eluted after X. Components identified in the blank volatile collections were excluded from the analysis. Serial dilutions of the authentic standard undecane (0.1–100 ng/µL) were analyzed by GC-MS to generate a linear calibration curve (peak area vs. concentration), which gave the coefficient of determination (R^2 = 0.9937). This regression equation was used for the external quantification of the different volatile organic compounds (VOCs).

3.6. Statistical Analysis

The relative amounts of compounds were computed by dividing the peak area of the compound by the sum of the peak areas of all compounds. The data were presented as the average percentage of replicates. Relative standard deviations (RSDs) were determined from (1) four different colonies for Table 1 and (2) three colonies for Table 2, using the following formula RSD = $(S*100)/X$, where S is the standard deviation and X the mean of peak areas of compounds.

4. Conclusions

Our results demonstrate that Dufour's and poison glands of *O. longinoda* secrete a wider range of compounds than previously reported. Moreover, we found some compounds specific to the secretions of these two glands plus the sternal and rectal glands. As such, our results suggest that using a combination of analytical chemistry techniques, SPME and solvent extraction, could be a more effective method to unravel the composition of ant gland secretions. Our findings of the compositions of the gland secretions are similar and different in some cases between *O. longinoda* and other ant species, which may be important for chemotaxonomic studies and phylogenetic relationships. Additionally, future research should investigate the roles of these additional components identified in the gland secretions in the biology and physiology of *O. longinoda*.

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References

1. Morgan, E.D. Chemical sorcery for sociality: Exocrine secretions of ants (Hymenoptera: Formicidae). *Myrmecol News* **2008**, *11*, 79–90.
2. Sherman, G.; Visscher, P.K. Honeybee colonies achieve fitness through dancing. *Nature* **2002**, *419*, 920–922. [CrossRef]
3. Regnier, F.E.; Nieh, M.; Holldobler, B. The volatile Dufour's gland components of the harvester ants, *Pogonomyrmex rugosus* and *P. barbatus*. *J. Insect. Physiol.* **1973**, *19*, 981–992. [CrossRef]
4. Tappey, H.J.; Voegtle, H.L.; Miras, H.M.; Weatherford, R.G.; Spande, T.F.; Garraffo, H.M.; Snelling, R.R. Venom chemistry of the ant, *Myrmicaria melanogaster* from Brunei. *J. Nat. Prod.* **2007**, *70*, 160–168.
5. Vander Meer, R.K.; Preston, C.A.; Choi, M.-Y. Isolation of a Pyrazine alarm pheromone component from the fire Ant, *Solenopsis invicta*. *J. Chem. Ecol.* **2010**, *36*, 163–170. [CrossRef] [PubMed]
6. Lucas, C.; Pho, D.B.; Jallon, J.M.; Fresneau, D. Role of cuticular hydrocarbons in the chemical recognition between ant species in the *Pachycondyla villosa* species complex. *J. Insect Physiol.* **2005**, *51*, 1148–1157. [CrossRef] [PubMed]
7. Walter, F.; Fletcher, D.J.C.; Chautems, D.; Cherix, D.; Keller, L.; Francke, W.; Fortelius, W.; Rosengren, R.; Vargo, E.L. Identification of the sex pheromone of an ant, *Formica lugubris* (Hymenoptera, Formicidae). *Naturwissenschaften* **1993**, *80*, 30–34. [CrossRef]
8. Attygalle, A.B.; Mutti, A.; Rohe, W.; Maschwitz, U.; Garbe, W.; Bestmann,, H.J. Trail pheromone from the Pavan gland of the ant, *Dolichoderus thoracicus* (Smith). *Naturwissenschaften.* **1998**, *85*, 275–277. [CrossRef]
9. Janssen, E.; Hölldobler, B.; Bestmann, H.J. A trail pheromone component of the African stink ant, *Pachycondyla* (Paltothyreus) *tarsata* Fabricius (Hymenoptera: Formicidae: Ponerinae). *Chemoecology* **1999**, *9*, 9–11. [CrossRef]
10. Kern, F.; Klein, R.W.; Janssen, E.; Bestmann, H.J.; Attygalle, A.B.; Schäfer, D.; Maschwitz, U. Mellein, a trail pheromone component of the ant *Lasius fuliginosus*. *J. Chem. Ecol.* **1997**, *23*, 779–792. [CrossRef]
11. Crozier, R.H.; Newey, P.S.; Schlüns, E.A.; Robson, S.K.A. A masterpiece of evolution: *Oecophylla* weaver ants (Hymenoptera: Formicidae). *Myrmecol. News.* **2010**, *13*, 57–71.
12. Hölldobler, B.; Wilson, E.O. Multiple recruitment systems of African weaver ant *Oecophylla longinoda* (Latreille) (Hymenoptera Formicidae). *Behav. Ecol. Sociobiol.* **1978**, *3*, 19–60. [CrossRef]
13. Bradshaw, J.W.S.; Baker, R.; Howse, P.E. Chemical composition of the poison apparatus secretions of the African weaver ant, *Oecophylla longinoda*, and their role in behaviour. *Physiol. Entomol.* **1979**, *4*, 39–46. [CrossRef]
14. Kempraj, V.; Park, S.J.; DE Faveri, S.; Taylor, P.W. Overlooked Scents: Chemical profile of soma, volatile emissions and trails of the green tree ant, *Oecophylla smaragdina*. *Molecules* **2020**, *25*, 2112. [CrossRef] [PubMed]
15. Keegans, S.J.; Billen, J.; Morgan, E.D. Volatile secretions of the green tree ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae). *Comp. Biochem. Physiol. B.* **1991**, *100*, 681–685. [CrossRef]
16. Witte, V.; Abrell, L.; Attygalle, A.B.; Wu, X.; Meinwald, J. Structure and function of Dufour gland pheromones from the crazy ant, *Paratrechina longicornis*. *Chemoecology* **2007**, *17*, 63–69. [CrossRef]
17. Attygalle, A.B.; Vostrowsky, O.; Bestmann, H.J.; Morgan, E.D. New chemicals from the Dufour gland of the formicine ant *Lasius niger* (Hymenoptera: Formicidae). *Insect Biochem.* **1987**, *17*, 219–225. [CrossRef]
18. Jackson, B.D.; Billen, J.P.J.; Morgan, E.D. Dufour gland contents of three species of *Myrmecia* (Hymenoptera: Formicidae), primitive ants of Australia. *J. Chem. Ecol.* **1989**, *15*, 2191–2205. [CrossRef]
19. Gokcen, O.A.; Morgan, E.D.; Dani, F.; Agosti, D.; Wehner, R. Dufour gland contents of ants of the *Cataglyphis bicolor* group. *J. Chem. Ecol.* **2002**, *28*, 71–87. [CrossRef]
20. Bergstrom, G.; Lofqvist, J. Odour similarities between the slave-keeping ants, *Formica sanguinea* and *Polyergus rufescens* and their slaves *Formica fusca* and *Formica rufibarbis*. *J. Insect Physiol.* **1968**, *14*, 995–1011. [CrossRef]
21. Falótico, T.; Labruna, M.B.; Verderane, M.P.; De resende Briseida, D.; Izar, P.; Ottoni, E.B. Repellent Efficacy of Formic Acid and the Abdominal Secretion of Carpenter Ants (Hymenoptera: Formicidae) Against Amblyomma Ticks (Acari: Ixodidae). *J. Med. Entomol.* **2007**, *44*, 718–721. [CrossRef]
22. Frederickson, M.E.; Greene, M.J.; Gordon, D.M. 'Devil's gardens' bedevilled by ants. *Nature* **2005**, *437*, 495–496. [CrossRef] [PubMed]
23. Löfqvist, J. Formic acid and saturated hydrocarbons as alarm pheromones for the ant, *Formica rufa*. *J. Insect. Physiol.* **1976**, *22*, 1331–1346. [CrossRef]

24. Vick, K.; Drew, W.A.; McGurk, D.J.; Eisenbraun, E.J.; Waller, G.R. Identification of hydrocarbons from *Novomessor cockerelli*. *Ann. Entomol. Soc. Am.* **1969**, *62*, 723–725. [CrossRef]
25. Dani, F.R.; Jones, G.R.; Morgan, E.D.; Turillazzi, S. Reevaluation of the chemical secretion of the sternal glands of Polistes social wasps. *Ethol. Ecol. Evol.* **2003**, *15*, 73–82. [CrossRef]

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Push-pull strategy combined with net houses for controlling cowpea insect pests and enhancing crop yields

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ABSTRACT

Net houses can be used in tropical environments to protect crops such as cowpea against large insect pests, thereby avoiding pesticide treatments while sustainably mitigating the effects of climate change. We investigated a push-pull strategy to prevent small insect pest outbreaks in a net house. The push component consisted of two stimulus plants, i.e. *Cymbopogon citratus* and *Tagetes minuta*, and the pull stimuli consisted of visual cues from blue and yellow sticky traps. Field experiments were set up in central Kenya and conducted during a rainy and a dry season, involving an open field control treatment, and three management treatments consisting of (1) an open field push-pull treatment, (2) a net house treatment and (3) a combined net house + push-pull treatment. *Trialeurodes vaporariorum* infestations were lower in the net house and net house + push-pull treatments than in the two open field treatments during the dry period or in the control treatment during the rainy period. *Aphis craccivora* infestations were higher in the net house and net house + push-pull treatments than in the control and open field push-pull treatments during the dry period, while no differences were observed among treatments during the rainy period. *Megalurothrips sjostedti* infestations did not vary among treatments in both periods. Among the larger insect pests, *Clavigralla tomentosicollis* infestations were lower in the net house and net house + push-pull treatments than in the open field treatments during the dry period, while *Maruca vitrata* infestations were lower in the net house treatment than in the control. During the rainy period, *C. tomentosicollis* infestations were higher in the net house + push-pull treatment than in the net house treatment, whereas *M. vitrata* infestations did not vary among treatments. Compared to the control, *Empoasca* sp. infestations were lower in the net house and net house + push-pull treatments in both periods, and in the open field push-pull treatment in the rainy period. Cowpea pod and grain yield and quality were higher in the net house and net house + push-pull treatments than in the control irrespective of the period. Although the treatments 1 reduced some of the pests, the net house and net house + push-pull treatments were effective in protecting cowpeas against most of the pests while improving pod yields in both periods.

1. Introduction

Population growth in developing countries has increased the demand for safe nutritional food (Alexandratos and Bruinsma, 2012). Vegetable production plays an important role in feeding the growing urban population in sub-Saharan regions (OECD and FAO, 2016). Farmers have been resorting to intensive chemical pesticide treatments to reduce crop pest damage and thus help meet the rising food demand (de Bon et al., 2014). Chemical pesticides are effective for pest control but they often have concomitant negative impacts on beneficial arthropods, the environment and human health (de Bon et al., 2014). Netting technology provides an alternative for reducing chemical pesticide treatments (Martin et al., 2006, 2015) by protecting different vegetable crops against a wide range of pests, boosting soil moisture, stabilizing the air

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temperature and increasing crop yields and quality (Martin et al., 2006; Gogo et al., 2012, 2014b; Saidi et al., 2013; Simon et al., 2014; Nordey et al., 2017). Knitted nets with a 30–40 mesh size (0.9–0.4 mm diameter) have proven effective as a barrier against large size insect pests (body length > 2 mm) such as lepidopterans and dipterans, while providing enough ventilation to mitigate the adverse climate conditions that prevail particularly in the Kenyan highlands (Martin et al., 2015). However, net houses with such netting do not provide an effective barrier to small insects (body length < 2 mm) as they can pass through the mesh.

We investigated a push-pull crop protection strategy based on insect cues combined with the use of netting technology to curb outbreaks of small insect pests in net houses. In the push-pull strategy, attractant and repellent stimuli from different sources are simultaneously used to control the spatial distribution of insect pests and thus reduce their abundance on the target crop (Cook et al., 2006). Attractant stimuli divert insects from the target crop by means of taste, egg-laying stimulants, volatile plant attractants, sexual pheromones, and visual stimulants. Repellent stimuli prevent insect pests from finding or accepting its host via the emission of repellent volatiles, alarm pheromones, anti-feeding agents, visual distractions, egg-laying repellents and irritants (Cook et al., 2006; Eigenbrode et al., 2016).

Cowpea (*Vigna unguiculata* L. Walp), is an African indigenous vegetable (AIV) that can reduce food insecurity and malnutrition in Africa (Muniu, 2017) since about 200 million people consume cowpea grains or leaves (Popelka et al., 2006). For example, cowpea is the top ranking AIV cropped in Kenya, with a total of 65 million t produced over a 24, 431 ha area (HCDA, 2014). Netting technology and the push-pull strategy could be combined to provide an alternative solution for controlling large and small insect pests of cowpea, thus reducing the need for pesticide treatments.

The pod borer (*Maruca vitrata* Fabricius) (Lepidoptera: Crambidae), and the pod bug (*Clavigralla tomentosicollis* Stäl) (Hemiptera: Coreidae), are two large insect pests of cowpea that can cause 80% yield losses if no pesticides are applied (Ekesi et al., 2002; OECD, 2015). *C. tomentosicollis* feeds extensively on fresh pods (Koona et al., 2002), whereas *M. vitrata* feeds on both flowers and pods (Singh and Jackai, 1988). The bean flower thrips (*Megalurothrips sjostedti* Trybom) (Thysanoptera: Thripidae), and the black legume aphid (*Aphis craccivora* Koch) (Homoptera: Aphididae), are two small insect pests of cowpea that cause yield losses of 20–100%, depending on the outbreak severity (Ekesi et al., 1999; Obopile, 2006; Abtew et al., 2016; Mweke et al., 2018). The feeding activity of *M. sjostedti* larvae and adults can lead to flower abortion and shedding, thereby resulting in reduced crop yields, while heavy *A. craccivora* infestations may cause plant stunting and delayed flowering (Obopile and Ositile, 2010; Moritz et al., 2013). In addition, *A. craccivora* may transmit viruses to plants (Stoetzel and Miller, 2001; Borowiak-Sobkowiak et al., 2017). A previous study showed that *M. sjostedti* is attracted by blue colours (Muvea et al., 2014), while *A. craccivora* is attracted to yellow sticky traps (Webb et al., 1994)—we thus used sticky traps as a pull stimulus in our study. Our previous experiment using olfactory tests showed that Mexican marigold (*Tagetes minuta* L.), and lemongrass (*Cymbopogon citratus* DC. Stapf), plants in the vegetative stage were repellent to female *M. sjostedti* (Diabate et al., 2019). Freshly cut lemongrass leaves produce citral, an organic volatile which is repellent to *M. sjostedti*. Mexican marigold and lemongrass are either repellent or insecticidal to *A. craccivora* (Morallo-Rejesus and Decena, 1982; Ofuya and Okuku, 1994). In this study, we combined these repellent push plants with the use of a net house. Insect control using a push-pull strategy can avoid the need for chemical insecticides, thereby increasing the opportunity of natural enemies to reduce insect pest populations (Khan et al., 1997).

We hypothesised that nets could effectively protect cowpea crops against large insect pests but not small insect pests. The aim of the present study was to evaluate the effectiveness of three control strategies against cowpea pests, by combining (a) use of an insect net house to provide a physical barrier, (b) repellent companion plants to provide an olfactory barrier, and (c) a visual trap in the form of coloured sticky strips as a pest attractant to increase cowpea grain yields and quality.

2. Materials and methods

2.1. Site

The field trial was conducted at Kenya Agricultural and Livestock Research Organization (KALRO), Industrial Crops Research Centre (ICRC) Mwea (0°37′09.0"S 37°22′09.4"E) in Kirinyaga County, central Kenya. Mwea is one of the main areas in Kenya where vegetables, including cowpea, are grown (Musebe et al., 2005). The four seasons in Kenya include a short rainy season (October–November), a hot dry season (January–March), a long rainy season (March–June), and a cold dry season (July–August) (Hassan, 1998; Foba et al., 2015). The experiment was conducted during two periods: in the dry period from October 3, 2017 to January 16, 2018, and in the rainy period from January 30, 2018 to May 22, 2018. The dry period included one rainy month (10 October to November 7, 2017) and three dry months (14 November to January 9, 2018), with 358.8 mm total rainfall and 22.71 °C mean temperature. The rainy period included one dry month (January 30, 2018 to February 27, 2018) followed by three rainy months (27 February to May 15, 2018), with 679.5 mm total rainfall and 22.50 °C mean temperature. Rainfall and temperature data were collected at the KALRO ICRC Mwea Kirogo Research Farm.

2.2. Treatments and experimental design

The net house was a locally manufactured high iron framed tunnel covered with a transparent knitted polyethylene AgroNet 0.4 (A to Z Textile Mills, Arusha, Tanzania) with a 40 mesh size (0.4 mm diameter hole size). The high tunnel was 5 m wide, 10 m long and 2.5 m high (flat on top) in the middle and 2 m high at the sides with a double door. Rain drops seeped through the top of the net house. Coloured (blue, yellow) sticky traps were purchased from the RealIPM Company, Thika, Kenya. Cowpea, *V. unguiculata* var. Machakos 66 (M66), seeds were purchased from Dryland Seed Ltd., and lemongrass seedlings were from Simlaw Seeds Company Ltd., in Nairobi, Kenya. Mexican marigold seeds were field collected at KALRO-ICRC Mwea. About 40 kg of cow manure was spread per plot (10 m × 5 m) 2 weeks before sowing the cowpea seeds. Two cowpea seeds were sown in two hills on each side of the micro-irrigation emitters at a distance of 25 cm with 1 m inter-row spacing. Each experimental plot measured 5 m × 10 m and 60 cowpea plants were planted per row, for a total of 300 plants (60 plants × 5 rows) per plot.

The experiment involved a total of sixteen plots, with four replicate plots for each of the four treatments, including: an open field control treatment (open field), and three management treatments consisting of (1) an open field push-pull treatment, (2) a net house treatment and (3) a combined net house + push-pull strategy treatment. Plots were laid out in a Latin square design with four replicates (Fig. 1). The treatment 1 plots included two repellent plants, lemongrass and Mexican marigold as push components (Calumpang et al., 2013) and coloured sticky traps (blue and yellow) as pull components (Webb et al., 1994; Muvea et al., 2014). Mexican marigold was planted at 20 cm intervals and lemongrass at 2 m intervals around the plots. Mexican marigold served as a repellent plant via its continuous release of repellent volatiles. Moreover, on the basis of previous findings that volatiles emitted by freshly cut lemongrass leaves were effective in repelling *M. sjostedti* for 24 h (Diabate et al., 2019), freshly cut lemongrass leaf pieces (about 5 cm of each leaf cut with scissors once a week) were also applied between the rows as organic mulch to repel *M. sjostedti* and *A. craccivora*. In the treatment 3 plots, Mexican marigold was planted outside the net house to discourage insects from passing through the net and lemongrass was planted inside the net house along the sides to repel insects that managed to enter. The experimental plots were separated from each other by 2-m buffer strips

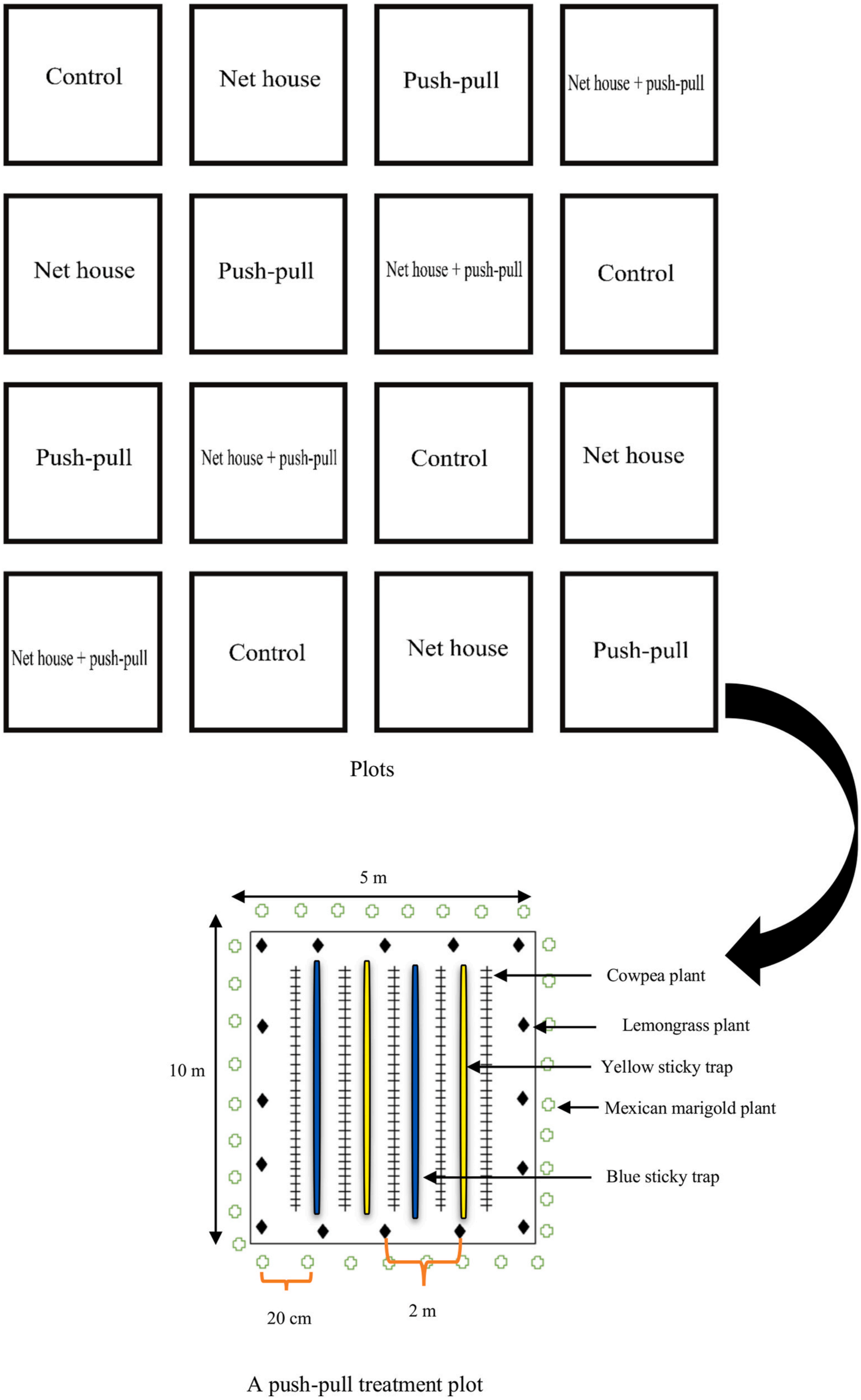

Fig. 1. Schematic representation of the experimental field set up in a 4 × 4 Latin square design with a total of 16 plots and randomized rows and columns. 300 plants were grown per plot and plots were separated by 2-m bare soil buffer strips. Four strips of sticky tape (15 cm × 10 m) per push-pull plot were placed at 1 m intervals between the rows.

of bare soil. Blue sticky traps were used to catch *M. sjostedti* and yellow sticky traps served to capture other insects including whiteflies and aphids. Four strips of sticky tape (15 cm × 10 m) per push-pull plot were placed at 1 m intervals between the rows, 1 m above the soil and attached to sticks fixed to the ground. The traps were removed and replaced with new ones monthly over the 4-month cowpea cropping period. Insects on 10 × 10 cm samples of each replaced trap were then counted in the laboratory. No chemical insecticide treatments were carried out during in the field experiment, but a biopesticide—a mixture of 5 ml of liquid soap and 5 g of pepper powder in 20 l of water—was applied when a threshold of 1000 aphids per plant was reached on three plants/plot in net house treatments. This biopesticide was sprayed twice in all treatments (on 17 and November 24, 2017) to slightly reduce the aphid populations, since high *A. craccivora* infestation of cowpea plants can cause high plant mortality (Annan et al., 1994). Biopesticide sprays were conducted during the dry period to avoid infested plant loss during the field trial, but this was not done during the rainy period. This soap/pepper solution provides a safe means to reduce aphid populations (Poswal and Akpa, 1991; Pahla et al., 2014), but it is less effective than synthetic insecticides in open field conditions (Ahmed et al., 2019; Smaili et al., 2014). To the best of our knowledge, there are no reports of this biopesticide impacting any of the other insects sampled in the study.

2.3. Sampling methods of insects and yield

The insect pests were counted 2 weeks after sowing and thereafter on a weekly basis for 14 and 15 weeks during the dry and rainy periods, respectively. Two plants per row were randomly selected and 10 individual plants were monitored in 5 rows per plot, for a total of 160 plants monitored per sampling date. The first and last plants of each row were not used to avoid border effects. The insects were counted between 9 a.m. and 3 p.m. in all plots. Adult and immature insects were visually counted on different parts of each cowpea plant—large insect pests such as leafhoppers, *Empoasca* sp., brown pod-sucking bugs, *C. tomentosicollis*, and larval bean pod borers, *M. vitrata*, were counted on whole plants. Small adult insects such as bean flower thrips, *M. sjostedti*, black legume aphids, *A. craccivora*, and greenhouse whiteflies, *Trialeurodes vaporariorum* Westwood, were counted on one old lower leaf and one young upper leaf per cowpea plant. *A. craccivora* and *M. sjostedti* were also counted on two picked open flowers, and on two pods per plant. *A. craccivora* was counted on a 5 cm portion of the plant stem (the number of insects counted on 1 cm were multiplied by 5 cm representing the portion of the plant stem).

The number of flowers and pods were counted on 10 plants randomly selected per plot. The harvested pods, undamaged pods or pods damaged by insects were counted and weighed to evaluate the quantity of marketable pods. Cowpea grains harvested on each plot were placed in bags and weighed on a mechanical kitchen scale.

2.4. Data analysis

The data were systematically log transformed (log (x+0.5) to ensure homogeneity of variance and conformation to normality before using a linear mixed model with random intercepts. Treatments were considered as fixed factors while plots were random factors. The model was fitted using the *lmer* function from the *lme4* package (Bates et al., 2015), and the fixed factor effects were tested using Type II Wald F tests. When the tests revealed a significant treatment effect, means were separated by least squares means (lsmeans) adjusted using the Tukey method. Between-period comparisons of insects counted on sticky traps and treatments were performed via Student's t tests after transformation. R version 3.3.2 software (R Core Team, 2016) was used for all analyses.

3. Results

3.1. Colonization of plants by small insect pests in both dry and rainy periods

In the dry period, cowpea colonization by insects with piercing-sucking mouthparts such as *T. vaporariorum* and *A. craccivora* started in the control and treatment 1 experiments (open field) 2 and 3 weeks after sowing, respectively (Fig. 2a and b). *A. craccivora* infestations were low in the open field treatments compared to the treatments 2 and 3. *T. vaporariorum* was observed on leaves while *A. craccivora* was noted on leaves and stems during cowpea seedling stage and on flowers and pods during the reproductive stage. Peak *T. vaporariorum* infestations were detected 6 weeks after sowing in the control plots, whereas *A. craccivora* outbreaks peaked 8 and 9 weeks after sowing in the treatment 3 and 2 during the flowering and podding stages, respectively. *M. sjostedti* infestations started during the cowpea flowering stage 8 weeks after sowing in both open field treatments (Fig. 2c).

In the rainy period, *T. vaporariorum, A. craccivora* and *M. sjostedti* infestations began in periods similar to those noted in the dry season treatments (Fig. 3a, b, c). *T. vaporariorum* infestations peaked 6 weeks after sowing in the control plots, while *A. craccivora* infestations peaked 7 weeks after sowing in the control plots. Three *M. sjostedti* infestation peaks occurred 13, 14 and 15 weeks after sowing in the treatments 2, 1 and control plots, respectively.

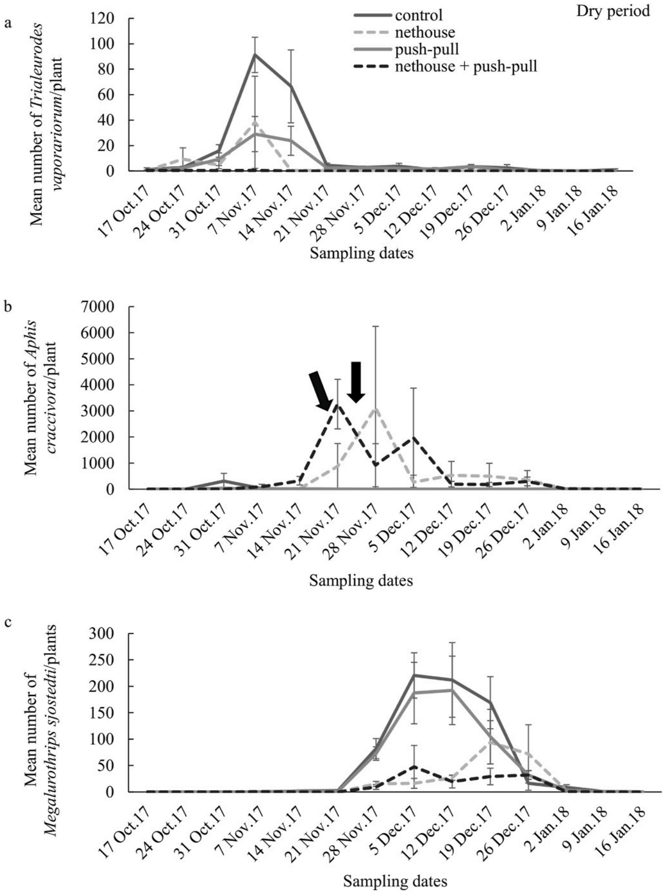

Fig. 2. Effects of treatments on adult *Trialeurodes vaporariorum (a)*, adult and larval *Aphis craccivora* (b), adult and larval *Megalurothrips sjostedti* (c) population fluctuations in the dry period at KALRO-Mwea (Kenya).
Two applications of a mixed pepper/soap solution in all treatments on 17 and November 24,
2017.

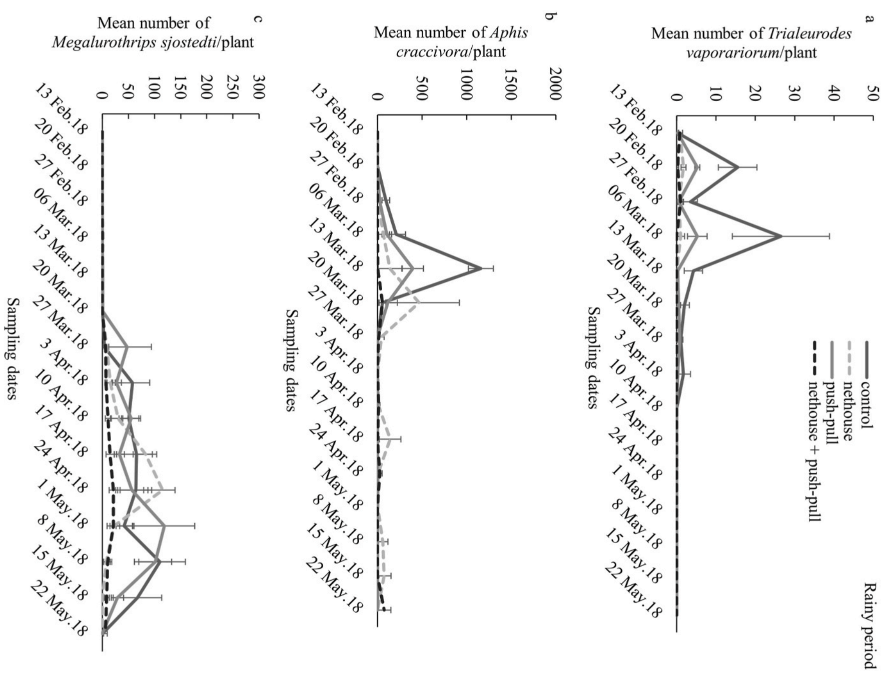

Fig. 3. Effects of treatments on adult *Trialeurodes vaporariorum*, adult and larval *Aphis craccivora,* adult and larval *Megalurothrips sjostedti* population fluctuations in the rainy period at KALRO-Mwea (Kenya).

3.2. Effects of dry and rainy periods on small insect pests

The between-period comparison showed that in the rainy period small insect pest populations were lower than or equal to those in the dry period (Table 1). In all treatments, the *M. sjostedti* population did not differ the dry and rainy periods. However, *A. craccivora* numbers were higher in the treatments 2 and 3 in the dry period than in the rainy period (treatment 2: $t = 2.186$, df = 114, p = 0.030; treatment 3: $t = 4.172$, df = 114, $p < 0.001$). Conversely, the *A. craccivora* population was higher in the control and treatments 1 in the rainy period than in the dry period (control $t = -1.899$, df = 114, p = 0.059; treatment 1: $t = -1.986$, df = 114, p = 0.049). In the control treatment ($t = 2.846$, df = 114, p = 0.005) and treatment 1 ($t = 4.010$, df = 114, $p < 0.001$), *T. vaporariorum* whitefly populations were higher in the dry period than in the rainy period.

3.3. Effects of each treatment on small insect pests

3.3.1. Megalurothrips sjostedti and Aphis craccivora

M. sjostedti populations did not vary among treatments in both periods (Table 1). *A. craccivora* infestations were higher in the treatments 2 and 3 than in control and treatment 1 during the dry period. Otherwise, *A. craccivora* infestations did not vary among treatments in the rainy period.

3.3.2. Trialeurodes vaporariorum

T. vaporariorum numbers were lower in the treatments 2 and 3 than in the open field treatments during the dry period. *T. vaporariorum* populations were higher in the control than in the treatments 2 and 3 in

Table 1
Mean (±SE) and abundance of insects observed per plant among the treatments in the dry period[a] and rainy period[2] at KALRO- Mwea (Kenya).

	Insect pests	Dry period						Rainy period					
		Control	Treatment 1	Treatment 2	Treatment 3	F	P-value	Control	Treatment 1	Treatment 2	Treatment 3	F	P-value
small pests	*Megalurothrips sjostedti*	51.0 ± 12.6 a	42.6 ± 11.2 a	16.3 ± 5.8 a	9.9 ± 3.7 a	2.54	0.10	31.4 ± 7.2 A	31.3 ± 7.1 A	18.8 ± 5.1 A	7.1 ± 1.5 A	1.67	0.22
	Aphis craccivora	24.6 ± 21.5 a[b]	5.10 ± 2.8 a[b]	406.5 ± 213.08 b[b]	518.8 ± 191.5 b[b]	17.33	P < 0.001	101.3 ± 39.0 A[b]	43.0 ± 16.5 A[b]	67.7 ± 32.9 A[b]	12.5 ± 6.0 A[b]	1.20	0.34
	Trialeurodes vaporariorum	14.0 ± 4.2 a[b]	5.6 ± 1.7 a[b]	3.8 ± 2.7 b	0.1 ± 0.1b	18.54	P < 0.001	3.7 ± 1.2 A[b]	1.0 ± 0.3 AB[b]	0.3 ± 0.1 B	0.1 ± 0.1 B	9.37	0.0018
large pests	*Maruca vitrata*	0.3 ± 0.1 a[b]	0.2 ± 0.1 ab	0 b	0.0 ± 0.0 ab	4.43	0.025	0 A[b]	0.0 ± 0.0 A	0A	0A	2.45	0.11
	Empoasca sp.	0.4 ± 0.1 a[b]	0.3 ± 0.1 a[b]	0 b	0.0 ± 0.0 b	8.77	0.002	8.0 ± 1.6 A[b]	3.1 ± 0.7 B[b]	0.1 ± 0.1 C	0 ± 0 C	35.62	P < 0.001
	Clavigralla tomentosicollis	7.5 ± 3.0 a	5.6 ± 2.5 a	0.2 ± 0.2 b[b]	0 b[b]	9.41	0.001	2.7 ± 0.8 AB	4.2 ± 1.2 AB	2.4 ± 1.3 A[b]	10.0 ± 2.5 B[b]	3.68	0.043

[a] From October 3, 2017 to January 9, 2018;[2] From January 30, 2018 to May 15, 2018, SE = Standard error, numerator and denominator degrees of freedom (df = 3, 12). Treatment 1 = Push-pull strategy; Treatment 2 = Net house; Treatment 3 = Combined net house + push-pull strategy. Small letters correspond to the comparison of treatments in the dry period and capital letters refer to the comparison of treatments in the rainy period using a linear mixed model (F, p-value). If significant, means were separated by least squares means (lsmeans) adjusted using the Tukey method.

[b] Indicates significant differences between the same treatment in the two periods according to the Student's *t*-test. The same letter in the same row means not different.

the rainy period.

3.4. *Effects of dry and rainy periods on large insect pests*

Infestations of the large insect pest *M. vitrata* were low infestations in all treatments, but they were significantly higher in the control during dry period than in the rainy period ($t = 2.810$, df = 114, $p = 0.005$). *Empoasca* sp. numbers were found to be highest during the rainy period. *Empoasca* sp. infestations were higher in the open field treatments in the rainy period than in the dry period (control: $t = -5.449$, df = 114, $p < 0.001$; treatment 1: $t = -4.273$, df = 114, $p < 0.001$). *C. tomentosicollis* infestations were higher in the treatment 2 and 3 during the rainy period than during dry period (treatment 2: $t = -2.457$, df = 114, $p = 0.015$; treatment 3: $t = -4.922$, df = 114, $p < 0.001$).

3.5. *Effects of each treatment on large insect pests*

3.5.1. *Maruca vitrata*

M. vitrata infestations were lower in the treatment 2 than in the control during the dry period, but no differences were observed among treatments during the rainy period.

3.5.2. *Empoasca sp.*

Empoasca sp. infestations were lower in the treatments 2 and 3 than in both open field treatments during the dry period. *Empoasca* sp. numbers were higher in the control than in all other treatments during the rainy period. In addition, *Empoasca* sp. numbers in the treatment 1 were higher than in the treatments 2 and 3 but lower than in the control.

3.5.3. *Clavigralla tomentosicollis*

C. tomentosicollis infestations were lower in the treatments 2 and 3 than in control and treatment 1 during the dry period (Table 1). However, *C. tomentosicollis* numbers were higher in the treatment 3 than in the treatment 2 in the rainy period. *C. tomentosicollis* infestations in control and treatment 1 were not significantly different.

3.6. *Pests caught by coloured sticky traps with the push-pull strategy*

The yellow sticky traps caught higher *T. vaporariorum* numbers in the treatment 1 than in the treatment 3, but no differences were observed between these two treatments with blue sticky traps (Table 2). The blue sticky traps caught more *M. sjostedti* in the treatment 1 than in the treatment 3. No differences were observed in *M. sjostedti* numbers caught on the yellow sticky traps when comparing the treatments 1 with treatments 3. *A. craccivora* numbers caught on yellow and blue sticky traps did not differ between the treatments 1 and 3. Only winged aphids were caught on the traps, whereas mostly nonwinged aphids were detected on cowpea plants.

Table 2

Mean (±SE) number of insects: *A. craccivora, M. sjostedti* and *T. vaporariorum* caught on the coloured sticky traps during the two periods. Dry period from October 3, 2017 to January 9, 2018. Rainy period from January 30, 2018 to May 15, 2018.

Insects		Blue trap	Yellow trap
T. vaporariorum	Treatment 1	10.6 ± 12.8a	2315.3 ± 1771.5a
	Treatment 3	1.0 ± 2.0a	91.2 ± 100.0b
	t-test	*1.438*	*2.583*
	P value	*0.172*	*0.021*
M. sjostedti	Treatment 1	265.4 ± 131.7a	4.4 ± 4.5a
	Treatment 3	27.0 ± 14.2b	2.1 ± 1.4a
	t-test	*3.487*	*0.241*
	P value	*0.003*	*0.812*
A. craccivora	Treatment 1	0a	64.6 ± 99.9a
	Treatment 3	0.25 ± 0.32a	93.9 ± 88.6a
	t-test	*−1.527*	*−0.993*
	P value	*0.148*	*0.337*

SE = Standard error, df = 14. Treatment 1 = Push-pull strategy; Treatment 3 = Combined net house + push-pull strategy. Small letters refer to the comparison of two treatments per insect in each column using the Student's *t*-test at $p < 0.05$. The same letter means not significantly different.

3.7. *Influence of the planting period on yield parameters*

There were no significant differences in the number of open flowers when comparing the same treatment in the two periods (Table 3). All yield parameters observed in the present study were lower in both of the open field treatments in the rainy period than in the dry period, including the total harvested pod number (control: $t = 4.486$, df = 114, $p < 0.001$; treatment 1: $t = 4.441$, df = 114, $p < 0.001$), the total harvested pod weight (control: $t = 4.469$, df = 114, $p < 0.001$; treatment 1: $t = 4.649$, df = 114, $p < 0.001$), marketable harvested pods (control: $t = 4.577$, df = 114, $p < 0.001$; treatment 1: $t = 4.518$, df = 114, $p < 0.001$) and the marketable harvested pod weight (control: $t = 4.528$, df = 114, $p < 0.001$; treatment 1: $t = 4.757$, df = 114, $p < 0.001$). On average, the harvested pod number in the open field treatments was 220-fold (control) and 55-fold (treatment 1) greater during the dry period compared to rainy period. No differences in yield parameters were observed between periods in both of the net house treatments.

3.8. *Effects of treatments on crop yield*

The open flower numbers did not differ among the treatments in both periods and nor any of the pod parameters differ among the treatments in the dry period (Table 3). However, the total harvested pods and their weight were 2-fold greater in the treatment 2 than in the control during the dry period. In addition, marketable harvested pods were 2-fold greater in the treatments 2 than in both of the open field treatments, while the marketable pod weights were 2-fold greater in the treatments 2 and 3 than in the control during the dry period. During the rainy period, the total harvested pods, harvested pod weight, marketable harvested pods and marketable pod and grain weights were greater in the treatments 2 and 3 than in the control and treatment 1. However, no differences were observed between the control and treatment 1 and between the treatment 2 and the treatment 3 during the rainy period. The grain quality in the plots was only assessed during the rainy period and the treatments 2 and 3 grain was better than that harvested in the open field treatments in the rainy period (Supplementary data).

4. Discussion

The present study showed that net house and net house + push-pull treatments were effective in protecting cowpea crops against most of the major large insect pests present. Previous studies revealed effective protection of vegetable crops grown in high or low tunnels under insect netting (Martin et al., 2015; Simon et al., 2014). For example, in Benin, netting reduced diamond back moth, *Plutella xylostella* L., large cabbage white, *Pieris brassicae* L., cabbage webworm, *Hellula undalis* Fabricius, and cotton bollworm *Helicoverpa armigera* Hübner, populations on cabbage crops (Simon et al., 2014).

Here we found that *T. vaporariorum* and *M. sjostedti* populations on cowpea crops in the net house and net house + push-pull treatments were always lower than in the control. The net house could work as a screen which disturbs the visual cues used by these insects to locate their host plants. Gogo et al. (2014b) suggested that the bright white colour of the nets could act as a visual barrier. Like many insects, whiteflies and thrips orient their movements visually (Teulon et al., 1999; Antignus et al., 2001). Net houses reduce UV light penetration, which may in turn interfere with insects' vision and dispersion under the net house (Raviv et al., 2004; Ben-Yakir et al., 2014). By contrast, we found that *A. craccivora* infestations were higher in the net house and net house + push-pull treatments than in open fields, particularly in the dry period. The aphid outbreaks that occurred in the net house in the dry period

Table 3

Mean (±SE) flowers per plant, the total and weight of harvested pods, marketable weight of harvested pods and number of grains per plot among the treatments in the dry period[a] and rainy period[2] at KALRO- Mwea (Kenya).

	Dry period						Rainy period					
	Control	Treatment 1	Treatment 2	Treatment 3	F	P-value	Control	Treatment 1	Treatment 2	Treatment 3	F	P-value
Flowers	2.9 ± 0.7 a	5.8 ± 2.0 a	3.7 ± 0.9 a	3.2 ± 0.9 a	0.13	0.93	1.7 ± 0.4 A	2.5 ± 0.5 A	3.3 ± 0.7 A	3.1 ± 0.5 A	1.02	0.41
Total harvested pods	110.3 ± 30.3 a^{b}	120.8 ± 31.7 a^{b}	181.60 ± 49.6 a	149.4 ± 39.1 a	0.18	0.90	0.5 ± 0.5 A^{b}	2.2 ± 1.7 A^{b}	105.3 ± 26.3 B	73.9 ± 17.8 B	11.63	P < 0.001
Total weight of pods (g)	282.4 ± 78.4 a^{b}	288.9 ± 73.7 a^{b}	478.2 ± 131.7 a	403.9 ± 101.6 a	0.16	0.91	1.3 ± 1.3 A^{b}	3.0 ± 2.7 A^{b}	276.6 ± 70.4 B	187.2 ± 44.7 B	11.84	P < 0.001
Marketable harvested pods	70.6 ± 19.1 a^{b}	75.4 ± 20.4 a^{b}	156.8 ± 43.4 a	124.3 ± 32.1 a	0.31	0.81	0 A^{b}	1.0 ± 0.7 A^{b}	104.9 ± 26.0 B	69.4 ± 16.4 B	13.32	P < 0.001
Marketable weight pod (g)	198.2 ± 53.6 a^{b}	201.1 ± 51.4 a^{b}	431.1 ± 119.9 a	348.9 ± 86.2 a	0.29	0.83	0 A^{b}	0.3 ± 0.3 A^{b}	272.3 ± 68.7 B	181.7 ± 43.5 B	13.62	P < 0.001
Grains							0.7 ± 0.7 A	0.8 ± 0.7 A	227.5 ± 57.3 B	136.2 ± 33.1 B	12.28	P < 0.001

[a] From October 3, 2017 to January 9, 2018; [2]From January 30, 2018 to May 15, 2018, SE = Standard error, numerator and denominator degrees of freedom (df = 3, 12). Treatment 1 = Push-pull strategy; Treatment 2 = Net house; Treatment 3 = Combined net house + push-pull strategy. Small letters refer to the comparison of treatments in the dry period and capital letters to comparison of treatments in the rainy period using a linear mixed model (F, p-value). If significant, means were separated by least squares means (lsmeans) adjusted using the Tukey method.

[b] Indicates significant differences between the same treatment in the two periods with the Student's *t*-test. The same letter in the same row means not different.

suggest that the temperature and humidity conditions under this shelter were suitable for aphids. Net houses are known to provide conditions conducive to aphid outbreaks, as reported with regard to *Lipaphis erysimi* (Kaltenbach) infestations on cabbage crops in Benin (Simon et al., 2014), *Myzus persica* (Sulzer) and *Aphis gossypii* (Glover) infestations on sweet pepper crops (Singh et al., 2004) grown under netting. Insect nets can also protect aphid populations from parasitoids and predators (Martin et al., 2013). The absence of natural enemies to regulate aphid populations in the net house and net house + push-pull treatments may explain the high *A. craccivora* numbers observed in our study. Conversely, the presence of natural enemies in open field treatments could explain the reduced *A. craccivora* numbers in the dry period—many adult and larval coccinellid beetles were actually observed on plants in open field treatments. Spraying a soap/pepper biopesticide solution on the cowpea crops reduced aphid populations in our study, in line with the findings of previous studies (Poswal and Akpa, 1991; Pahla et al., 2014). Releasing predators or parasitoids inside the net house is another way to reduce *A. craccivora* infestations. Yang et al. (2014) reported that the release of *Harmonia axyridis* (Pallas) natural enemies in the greenhouse was an effective way to reduce *Aphis gossypii* Glover populations on strawberry and cucumber. The severe aphid infestations we noted on cowpea flowers and pods could explain why cowpea yields were not as high as expected in the net house and net house + push-pull treatments. The best cowpea yields were nevertheless obtained in the net house treatment compared to other treatments despite the *A. craccivora* outbreak. The M66 cowpea cultivar used in this study may be tolerant to *A. craccivora*, since this cowpea genotype was an improved variety from the Kenya Agricultural Livestock Research Organization (KALRO) which is partly tolerant to aphid damage (Kenya Agricultural Research Institute (KARI), 2008; Kimutai, 2017).

We also hypothesised that the combined use of netting and the push-pull strategy (treatment 3) could help control small insect pests, particularly thrips on bean flowers. In our study, lemongrass (*C. citratus*) and Mexican marigold (*T. minuta*) were used to repel (push) insects from the cowpea crop and sticky traps were used as attractants (pull) to catch the flying insects. In laboratory assays, *M. sjostedti* was shown to be repelled by fresh cut lemongrass leaves but not by old cut leaves (Diabate et al., 2019), but *M. sjostedti* numbers did not vary among any of the treatments in our study. Lemongrass leaves were cut once a week and spread between the rows to repel thrips. In addition, *T. minuta* likely continuously released repellent volatiles that prevented *M. sjostedti* from locating the cowpea plants. The repellent effect of volatiles could have been of short duration or the cut lemongrass leaves were perhaps not sufficiently abundant to repel *M. sjostedti*. The fact that there was a greater abundance of cowpea plants relative to lemongrass and Mexican marigold plants in the field could mean that there was an overwhelming presence of attractive volatiles, thus decreasing repellent effect of the companion plants. The efficacy of repellent volatiles could be enhanced in the field by sowing companion plants that produce greater quantities of repellent volatiles, or otherwise repellent essential oils could be provided through dispensers or injected in the irrigation system. We previously identified repellent citral volatiles in lemongrass and repellent blends of dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene and *(Z)*-*β*-ocimene volatiles in marigold (Diabate et al., 2019). These compounds could be supplemented via dispensers to lengthen the repellence period in the field. Although the blue sticky traps were effective in catching *M. sjostedti* in the open field push-pull treatment, the number of thrips on cowpea plants in this treatment did not differ from that in the control. *M. sjostedti* was more attracted to cowpea flowers than to the blue traps. Frey et al. (1994) reported that the plant growth stage may have an impact on the efficacity of colour traps. For example, the number of *F. occidentalis* thrips caught on blue sticky traps in ornamental greenhouses was reported to be lower during flowering than during the vegetative stage (Frey et al., 1994).

By contrast, our findings showed that the *Empoasca* sp. population was lower in the open field push-pull treatment than in the control during the rainy period. Volatiles released by Mexican marigold and lemongrass could have been involved in the reduction of *Empoasca* sp. populations in the open field push-pull treatment. Calumpang et al. (2013) observed a reduction in green leafhopper, *Amrasca biguttula* (Ishida), populations when lemongrass was intercropped with eggplant. It was also reported that using Mexican marigold as an intercrop and maize as a border crop reduced *A. biguttula* leafhopper populations on eggplant crops in the field (Sujayanand et al., 2015). On the other hand, yellow sticky traps may contribute to reducing *Empoasca* sp. numbers. The attraction of sharp-nosed leafhoppers, *Scaphytopius magdalensis* (Provancher), to yellow traps was also demonstrated by Rodriguez-Saona et al. (2012). Combining visual and olfactory cues in a push-pull strategy could prove effective in controlling *Empoasca* sp. populations.

The open field push-pull treatment did not have an impact on insect populations except *Empoasca* sp. and therefore did not increase the pest control efficacy of the net house + push-pull treatment in the present study. Several authors have demonstrated the efficacy of non-host plant volatiles in reducing insect populations in the field (Parolin et al., 2012), whereas many other studies failed to reveal their efficacy in the same conditions (Held et al., 2003; Webster and Cardé, 2016). For example, intercropping French marigold, a non-host plant, with host potato plants did not reduce Colorado potato beetle, *Leptinotarsa decemlineata* (Say), populations but conversely it increased the incidence of *L. decemlineata* attacks (Moreau et al., 2006). In our study, we observed no such perverse effects of the push-pull strategy on any cowpea pests.

We observed that cowpea yields and insect populations varied with the period. Populations of large insect pests were higher in the rainy period than in the dry period. Conversely, small insect pests such as *M. sjostedti*, *T. vaporariorum* and *A. craccivora* were much lower in the rainy period than in the dry period. In Kenya, the heavy rainfall between March and June 2018 may have been responsible for the reduction in insect populations via egg destruction and partial larval mortality. Nyasani et al. (2013) reported that heavy rain destroyed western flower thrips, *F. occidentalis,* larvae in the field and hence reduced their population density. In our study, *A. craccivora* populations were reduced in the net house and net house + push-pull treatments in the rainy period compared to the dry period. The top of net house was flat and rain penetrated through the mesh. Rainfall is considered to regulate aphid population densities (Kaakeh and Dutcher, 1993). The lower aphid numbers in the net house and net house + push-pull treatments suggest that the microclimate was unsuitable for these pests during the rainy period. Rainfall increased the relative humidity under the net, which could have a negative impact on *A. craccivora* populations by enhancing the environmental conditions for entomopathogenic fungi. Kataria and Kumar (2017) reported that *A. craccivora* population patterns were negative correlated with minimum temperature, relative humidity and rainfall. In addition, *A. craccivora* propagate parthenogenetically in Africa (Irwin, 1980). This asexual reproduction enables aphids to multiply and spread rapidly under optimal conditions (Iluz, 2011). However, conditions might be different in the future and the lack of gene combinations could be a disadvantage to the offspring, thus curbing the growth populations of this pest. Cowpea pod yields in the open field treatments were very poor during the rainy period compared to the dry period. The reduction in pod yield may have been due to excessive rainfall in the rainy period, as already observed by Parwada (2016). In addition, cowpea plants in the open field treatments were heavily damaged by fungi and diseases in the rainy period. Edema et al. (1997) reported a higher incidence and severity of viral diseases, anthracnose and scab on cowpea crops in the rainy period than in the dry period. Fungi such as cercospora leaf spot may affect cowpea pod production and quality. Our study showed that net houses could help farmers to produce cowpea intensively. Despite the fact the rainy period was longer than usual during our study, cowpea production was always higher in the net house and net house + push-pull treatments than in the open field conditions. Net technology thus seems to be an efficient tool to help farmers grow vegetables in unpredictable weather conditions.

During both climate periods, all yield parameters evaluated were higher in the net house and net house + push-pull treatment than in the open field. The net house protected cowpeas against large insect pests or had no special effect on some of them, except in the rainy period when a *C. tomentosicollis* infestation was observed in the net house + push-pull treatment. Our findings did not enable us to explain why the *C. tomentosicollis* population was high in the net house + push-pull treatment in the rainy period, but the high density of Mexican marigold plants around the net house might have attracted or protected them. Further investigations are needed to elucidate this issue. Despite, the abundance of *Empoasca* sp. in the open fields during the rainy period, the net houses (used in treatments net house and net house + push-pull) prevented this pest from infesting the cowpea crops in the rainy period. The use of net houses in horticulture usually has a major impact on crop yields by reducing populations of large insect pests. *M. vitrata* and *C. tomentosicollis* can cause cowpea yield losses of up to 80% in Africa (Ekesi et al., 2002; OECD, 2015). The reduction of large insect pests could thus partially explain the increased crop yield in the net house and net house + push-pull treatments, whereas the modified microclimate in the net house could boost plant development. Saidi et al. (2013) reported that the constant high temperature and soil moisture in net houses improved plant growth and yield. Moreover, we noticed that the quality of the grains produced in the net house and net house + push-pull treatments were higher. In Kenya, Gogo et al. (2014) also reported faster development, higher pod yield, and better quality of green beans, another leguminous plant that was grown under low netting covered tunnels as compared to uncovered plants.

In our study, the cowpea pod numbers were higher in the dry period than in the rainy period. Conditions in the dry period were better for cowpea pod production compared to the rainy period. Cowpea is an herbaceous warm-season annual and many authors have reported its good adaptation to high temperatures and drought (Ehlers and Hall, 1997; Timko et al., 2007). Moreover, the reduction of large insect pests in the dry period consequently reduced cowpea flower and pod damage due to low feeding activity. Conversely, small insect pests were more abundant in the dry period. Populations of *M. sjostedti* thrips and *T. vaporariorum* whiteflies on cowpea plants in the net house and net house + push-pull treatments were always lower than the open field populations, which increased very rapidly, particularly in the dry period. *M. sjostedti* outbreaks were linked to the flower emergence. *M. sjostedti* preferentially feeds on flowers, causing them to fall (Moritz et al., 2013). However, there was no difference in the total number of pods and flowers among the different treatments in the dry period. The high *A. craccivora* infestation of cowpea vegetative parts, flowers and pods in the net house and net house + push-pull treatments did not decrease yields in the dry period. Small insect pests therefore seem to have a limited impact on cowpea production, contrary to large insect pests.

5. Conclusion

The findings of this study showed that net house and net house + push-pull treatments considerably reduced pest infestations on cowpeas and that the damage was mainly incurred by large insect pests such as *Empoasca* sp., *M. vitrata* and *C. tomentosicollis* along with smaller insect pests such as *M. sjostedti* and *T. vaporariorum*. We also noted that net house grown cowpeas could be subject to high *A. craccivora* infestation. Aphid outbreaks seemed to have a limited impact on cowpea yields, so populations of this pest could be reduced by biopesticide treatment. The net house + push-pull treatment did not improve *T. vaporariorum*, *A. craccivora* and *M. sjostedti* control. However, the open field push-pull treatment reduced *Empoasca* sp. infestations during the rainy period. The study also showed that cowpea production was much higher in the net house and net house + push-pull treatments than in the open field. In addition, cowpea pod yields were higher in all treatments during the dry period than in the rainy period. The large insect pest populations were low during the dry period and high in the rainy period, contrary to small insect pest populations. The study showed that cowpea production was impacted by the period and large insect pest infestations. This study showed that netting technology provides an effective alternative to chemical pesticide treatments for vegetable growing in the tropics regardless of the season. We showed that netting technology could be slightly improved to control sucking insect pests using the push-pull strategy. Cowpea yields were highly affected by heavy rains in both open field treatments but not in the net house and net house + push-pull treatments. Future research should be focused on investigating the effect of microclimate and insect pests on cowpea yield in net houses. In Mwea (Kenya), the rainy season is not the best period to grow cowpeas in the open fields despite the low insect pest pressure. But we found that the cowpea yield and quality in the net house treatment was still high, thus confirming the need for netting to produce off-season vegetable crops when prices may be higher due to the low market supply. This study confirmed that netting is an efficient tool for protecting vegetable crops against insect pests and producing higher yields of safe vegetables.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2020.105480.

References

Abtew, A., Niassy, S., Affognon, H., Subramanian, S., Kreiter, S., Garzia, G.T., Martin, T., 2016. Farmers' knowledge and perception of grain legume pests and their management in the Eastern province of Kenya. Crop Protect. 87, 90–97. https://doi.org/10.1016/j.cropro.2016.04.024.

Ahmed, M.T., Miah, M.R.U., Amin, M.R., Hossain, M.M., Suh, S.J., Kwon, Y.J., 2019. Plant material as an alternative tool for management of aphid in country bean field. Int. J. Pest Manag. 65, 171–176. https://doi.org/10.1080/09670874.2018.1494864.

Alexandratos, N., Bruinsma, J., 2012. World Agriculture towards 2030/2050: the 2012 Revision, ESA Working Paper No. 12-03. FAO, Rome. https://doi.org/10.1016/S0264-8377(03)00047-4, 2012.

Annan, I.B., Saxena, K.N., Schaefers, G.A., Tingey, W.M., 1994. Effects of infestation by cowpea aphid (Homoptera: Aphididae) on different growth stages of resistant and susceptible cowpea cultivars. Int. J. Trop. Insect Sci. 15, 401–410. https://doi.org/10.1017/s1742758400015733.

Antignus, Y., Nestel, D., Cohen, S., Lapidot, M., 2001. Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. Environ. Entomol. 30, 394–399. https://doi.org/10.1603/0046-225x-30.2.394.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. lme4: Linear Mixed-Effects Models Using Eigen and S4. R Package Version 1.1-8. Retrieved from. http://CRAN.R-project.org/package=lme4.

Ben-Yakir, D., Antignus, Y., Offir, Y., Shahak, Y., 2014. Photoselective nets and screens can reduce insect pests and diseases in agricultural crops. Acta Hortic. 1015, 95–102. https://doi.org/10.17660/ActaHortic.2014.1015.10.

Borowiak-Sobkowiak, B., Durak, R., Wilkaniec, B., 2017. Morphology, biology and behavioral aspects of *Aphis craccivora* (Hemiptera:Aphididae) on *Robinia pseudoacacia*. Acta Sci Pol-Hortoru. 16, 39–49.

Calumpang, S.M.F., Bayot, R.G., Vargas, D.G., Ebuenga, D.M., Gonzales, P.G., 2013. Impact of intercropping lemon grass (*Cymbopogon citratus* Stapf.) on infestation of eggplant fruit and Shoot borer (*Leucinodes orbonalis Guenee*) in eggplant (*Solanum melongena* L.). Siliman J 54, 114–130.

Cook, S.M., Khan, Z.R., Pickett, J.A., 2006. The use of push-pull strategies in integrated pest management. Annu. Rev. Entomol. 52, 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407.

de Bon, H., Huat, J., Parrot, L., Sinzogan, A., Martin, T., Malezieux, E., Vayssieres, J.F., 2014. Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. a review. Agron. Sustain. Dev. 34, 723–736. https://doi.org/10.1007/s13593-014-0216-7.

Diabate, S., Martin, T., Murungi, L.K., Fiaboe, K.K.M., Subramaniana, S., Wesonga, J., Deletre, E., 2019. Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*. J. Appl. Entomol. 1–12. https://doi.org/10.1111/jen.12651, 00.

Edema, R., Adipala, E., Florini, D.A., 1997. Influence of season and cropping system on occurrence of cowpea diseases in Uganda. Plant Dis. 81, 465–468. https://doi.org/10.1094/PDIS.1997.81.5.465.

Ehlers, J.D., Hall, A.E., 1997. Cowpea (*Vigna unguiculata* L. Walp.). Field Crop. Res. 53, 187–204. https://doi.org/10.1016/S0378-4290(97)00031-2.

Eigenbrode, S.D., Birch, A.N.E., Lindzey, S., Meadow, R., Snyder, W.E., 2016. A mechanistic framework to improve understanding and applications of push-pull systems in pest management. J. Appl. Ecol. 53, 202–212. https://doi.org/10.1111/1365-2664.12556.

Ekesi, S., Adamu, R.S., Maniania, N.K., 2002. Ovicidal activity of entomopathogenic hyphomycetes to the legume pod borer , *Maruca vitrata* and the pod sucking bug , *Clavigralla tomentosicollis*. Crop Protect. 21, 589–595.

Ekesi, S., Maniania, N.K., Onu, I., 1999. Effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti*. Entomol. Exp. Appl. 93, 149–155. https://doi.org/10.1046/j.1570-7458.1999.00573.x.

Foba, C.N., Salifu, D., Lagat, Z.O., Gitonga, L.M., Akutse, K.S., Fiaboe, K.K.M., 2015. Species composition, distribution, and seasonal abundance of *Liriomyza* leafminers (Diptera: Agromyzidae) under different vegetable production systems and agroecological zones in Kenya. Environ. Entomol. 44, 223–232. https://doi.org/10.1093/ee/nvu065.

Frey, J.E., Cortada, R.V., Helbling, H., 1994. The potential of flower odours for use in population monitoring of western flower thrips *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae). Biocontrol Sci. Technol. 4, 177–186.

Gogo, E.O., Saidi, M., Itulya, F.M., Martin, T., Ngouajio, M., 2012. Microclimate modification using eco-friendly nets for high-quality tomato transplant production by small-scale farmers in East Africa. HortTechnology 22, 292–298.

Gogo, E., Saidi, M., Itulya, F., Martin, T., Ngouajio, M., 2014a. Eco-friendly nets and floating row covers reduce pest infestation and improve tomato (*Solanum lycopersicon* L.) yields for smallholder farmers in Kenya. Agronomy 4, 1–12. https://doi.org/10.3390/agronomy4010001.

Gogo, E., Saidi, M., Ochieng, J.M., Martin, T., Baird, V., Ngouajio, M., 2014b. Microclimate modification and insect pest exclusion using agronet improve pod yield and quality of French bean. Hortscience 49, 1298–1304.

Hassan, R.M., 1998. Maize Technology Development and Transfer. A GIS Application for Research Planning in Kenya., CAB International. CIMMYT and Kenya Agricultural Research Institute, Walford, UK, p. 227.

HCDA, 2014. Horticulture Validated Report- Ministry of Agriculture & Horticultural Crops Development Authority.

Held, D.W., Gonsiska, P., Potter, D.A., 2003. Evaluating companion planting and non-host masking odors for protecting roses from the Japanese beetle (Coleoptera: Scarabaeidae). J. Econ. Entomol. 96, 81–87. https://doi.org/10.1093/jee/96.1.81.

Iluz, D., 2011. The plant–aphid universe. Z. In: Dubinsky, J.S. (Ed.), All Flesh Is Grass. Plant-Animal Interrelationships, pp. 91–118. https://doi.org/10.1093/aob/mcr214.

Irwin, M.E., 1980. Sampling methods in Soybean entomology. In: Kogan, M., Herzog, D. C. (Eds.), Sampling Methods in Soybean Entomology, Springer Series in Experimental Entomology. Springer New York, New York, NY, pp. 239–259. https://doi.org/10.1007/978-1-4612-9998-1.

Kaakeh, W., Dutcher, J., 1993. Effect of rainfall on population abundance of aphids (Homoptera : Aphididae) on Pecan. J. Entomol. Sci. 28, 283–286. https://doi.org/10.18474/0749-8004-28.3.283.

Kataria, R., Kumar, D., 2017. Population dynamics of *Aphis craccivora* (Koch) and its natural enemies on bean crop in relation to weather parameters in Vadodara, Gujarat, India. Legume Res. 40, 571–579. https://doi.org/10.18805/lr.v0iOF.10282.

Kenya Agricultural Research Institute (KARI), 2008. Growing Cow Peas in Dry Areas. http://www.kalro.org/fileadmin/publications/brochuresII/Growing_cow_peas_in_dry_areas.pdf. (Accessed 16 April 2018).

Khan, Z.R., Ampong-Nyarko, K., Chiliswa, P., Hassanali, A., Kimani, S., Lwande, W., Overholt, W.A., Pickett, J.A., Smart, L.E., Wadhams, L.J., Woodcock, C.M., 1997. Intercropping increases parasitism of pests. Nature 388, 631–632. https://doi.org/10.1038/41681.

Kimutai, W.J., 2017. Effect of Selected Bradyrhizobia and Nutrients on Cowpea Biomass, Biological Nitrogen and Yield in Kilifi and Mbeere Regions of Kenya. Masters Thesis. Kenyatta University, p. 69. Kenya.

Koona, P., Osisanya, E.O., Jackai, L., Tamo, M., Markham, R.H., 2002. Resistance in accessions of cowpea to the coreid pod-bug *Clavigralla tomentosicollis* (Hemiptera : Coreidae). J. Econ. Entomol. 95, 1281–1288.

Martin, T., Assogba-Komlan, F., Houndete, T., Hougard, J.M., Chandre, A.F., 2006. Efficacy of mosquito netting for sustainable small holders' cabbage production in Africa. J. Econ. Entomol. 99, 450–454. https://doi.org/10.1603/0022-0493-99.2.450.

Martin, T., Palix, R., Kamal, A., Delétré, E., Bonafos, R., Simon, S., Ngouajio, M., 2013. A repellent net as a new technology to protect cabbage crops. J. Econ. Entomol. 106, 1699–1706. https://doi.org/10.1603/EC13004.

Martin, T., Simon, S., Parrot, L., Komlan, F.A., Vidogbena, F., Adegbidi, A., Baird, V., Saidi, M., Kasina, M., Wasilwa, L.A., Subramanian, S., Ngouajio, M., 2015. Eco-friendly nets to improve vegetable production and quality in sub-Saharan Africa. Acta Hortic. 1105, 221–227. https://doi.org/10.17660/ActaHortic.2015.1105.31.

Morallo-Rejesus, B., Decena, A., 1982. The activity, isolation, purification and identification of the insecticidal principles from Tagetes. Philipp. J. Crop Sci. 7, 31–36.

Moreau, T.L., Warman, P.R., Hoyle, J., 2006. An evaluation of companion planting and botanical extracts as alternative pest controls for the Colorado potato beetle. Biol. Agric. Hortic. 23, 351–370. https://doi.org/10.1080/01448765.2006.9755336.

Moritz, G., Brandt, S., Triapitsyn, S., Subramanian, S., 2013. Pest thrips in east Africa: identification and information tools. QAAFI biological information technology (QBIT). The University of Queensland, Brisbane ht tps://doi.org/ISBN.978-1-74272-067-8.

Muniu, F.K., 2017. Characterization and Evaluation of Local Cowpea Accessions and Their Response to Organic and Inorganic Nitrogen Fertilizers in Coastal Kenya. B.S.c Thesis. University of Nairoby, p. 72.

Musebe, R., Dorward, P., Karanja, D., 2005. Socio-economic Report for Project (R8296/ZA0568), Promotion of Sustainable Approaches for the Management of Root-Knot Nematodes on Vegetables in Kenya.

Muvea, A.M., Waiganjo, M.M., Kutima, H.L., Osiemo, Z., Nyasani, J.O., Subramanian, S., 2014. Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. Int. J. Trop. Insect Sci. 34, 197–206. https://doi.org/10.1017/S174275841400040X.

Mweke, A., Ulrichs, C., Nana, P., Akutse, K.S., Fiaboe, K.K.M., Maniania, N.K., Ekesi, S., 2018. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae, Beauveria bassiana* and *isaria sp.* for the management of *Aphis craccivora* (Hemiptera: Aphididdae). J. Econ. Entomol. 111, 1587–1594. https://doi.org/10.1093/jee/toy135.

Nordey, T., Basset-Mens, C., De Bon, H., Martin, T., Déletré, E., Simon, S., Parrot, L., Despretz, H., Huat, J., Biard, Y., Dubois, T., Malézieux, E., 2017. Protected cultivation of vegetable crops in sub-Saharan Africa: limits and prospects for smallholders. a review. Agron. Sustain. Dev. 37, 53. https://doi.org/10.1007/s13593-017-0460-8.

Nyasani, J.O., Meyhöfer, R., Subramanian, S., Poehling, H.M., 2013. Seasonal abundance of western flower thrips and its natural enemies in different French bean agroecosystems in Kenya. J. Pest. Sci. 86, 515–523. https://doi.org/10.1007/s10340-013-0491-0, 2004.

Obopile, M., 2006. Economic threshold and injury levels for control of cowpea aphid, *Aphis crassivora* Linnaeus (Homoptera: Aphididae), on cowpea. Afr. Plant Protect. 12, 111–115. https://hdl.handle.net/10520/EJC87791.

Obopile, M., Ositile, B., 2010. Life table and population parameters of cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) on five cowpea *Vigna unguiculata* (L. Walp.) varieties. J. Pest. Sci. 83, 9–14. https://doi.org/10.1007/s10340-009-0262-0, 2004.

OECD, 2015. Consensus Document on the Biology of Cowpea (*Vigna Unguiculata* (L.) Walp.), Series on Harmonisation of Regulatory Oversight in Biotechnology No. 60 https://doi.org/ENV/JM/MONO(2015)48.

OECD, FAO, 2016. Agriculture in sub-Saharan Africa: prospects and challenges for the next decade. In: OECD-FAO Agricultural Outlook, pp. 2016–2025.

Ofuya, T.I., Okuku, I.E., 1994. Insecticidal effect of some plant extracts on the cowpea aphid *Aphis craccivora* Koch (Homoptera: Aphididae). Anzeiger für Schädlingskd. Pflanzenschutz Umweltschutz 67, 127–129. https://doi.org/10.1007/BF01909033.

Pahla, I., Moyo, M., Muzemu, S., Muziri, T., 2014. Evaluating the effectiveness of botanical sprays in controlling Aphids (*Brevicoryne brassicae*) on rape (*Brassica napus* L). Int. J. Agron. Agric. Res. 5, 1–6.

Parolin, P., Bresch, C., Desneux, N., Brun, R., Bout, A., Boll, R., Poncet, C., 2012. Secondary plants used in biological control: a review. Int. J. Pest Manag. 58, 91–100. https://doi.org/10.1080/09670874.2012.659229.

Parwada, C., 2016. Evaluation of seasonal rainfall changes on the growth performance of *Vigna unguiculta* (cowpeas) in Zimbabwe. Acad. J. Agric. Res. 4 https://doi.org/10.15413/ajar.2015.0188, 067–071.

Popelka, J.C., Gollasch, S., Moore, A., Molvig, L., Higgins, T.J.V., 2006. Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. Plant Cell Rep. 25, 304–312. https://doi.org/10.1007/s00299-005-0053-x.

Poswal, M.A.T., Akpa, A.D., 1991. Current trends in the use of traditional and organic methods for the control of crop pests and diseases in Nigeria. Trop. Pest Manag. 37, 329–333. https://doi.org/10.1080/09670879109371609.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Raviv, M., Antignus, Y., Yishay, R., 2004. Invited review UV radiation effects on pathogens and insect pests of greenhouse-grown crops. Photochem. Photobiol. 79, 219–226.

Rodriguez-Saona, C.R., Byers, J.A., Schiffhauer, D., 2012. Effect of trap color and height on captures of blunt-nosed and sharp-nosed leafhoppers (Hemiptera: Cicadellidae) and non-target arthropods in cranberry bogs. Crop Protect. 40, 132–144.

Saidi, M., Gogo, E.O., Itulya, F.M., Martin, T., Ngouajio, M., 2013. Microclimate modification using eco-friendly nets and floating row covers improves tomato (*Lycopersicon esculentum*) yield and quality for small holder farmers in East Africa. Agric. Sci. 4, 577–584.

Simon, S., Komlan, F.A., Adjaïto, L., Mensah, a., Coffi, H.K., Ngouajio, M., Martin, T., 2014. Efficacy of insect nets for cabbage production and pest management depending on the net removal frequency and microclimate. Int. J. Pest Manag. 60, 208–216. https://doi.org/10.1080/09670874.2014.956844.

Singh, D., Kaur, S., Dhillon, T.S., 2004. Protected cultivation of sweet pepper hybrids under net-house in indian conditions. Acta Hortic. 659, 515–521.

Singh, S.R., Jackai, L.E.N., 1988. The legume pod-borer, *Maruca Testulalis* (Geyer): past , present and future research. Insect Sci. its Appl. 9, 1–5.

Smaili, M.C., El Ghadraoui, L., Gaboun, F., Benkirane, R., Blenzar, A., 2014. Impact of some alternative methods to chemical control in controlling aphids (Hemiptera: Sternorrhyncha) and their side effects on natural enemies on young Moroccan citrus groves. Phytoparasitica 42, 421–436. https://doi.org/10.1007/s12600-013-0379-9.

Stoetzel, M.B., Miller Gary, L., 2001. Aerial feeding aphids of corn in the United States with reference to the root-feeding *Aphis maidiradicis* (Homoptera: Aphididae). Fla. Entomol. 84, 83–98. https://doi.org/10.2307/3496667.

Sujayanand, G.K., Sharma, R.K., Shankarganesh, K., Saha, S., Tomar, R.S., 2015. Crop diversification for sustainable insect pest management in Eggplant (Solanales: Solanaceae). Fla. Entomol. 98, 305–314. https://doi.org/10.1653/024.098.0149.

Teulon, D.A.J., Hollister, B., Butler, R.C., Cameron, E.A., 1999. Colour and odour responses of flying western flower thrips: wind tunnel and greenhouse experiments. Entomol. Exp. Appl. 93, 9–19. https://doi.org/10.1023/A:1003893905051.

Timko, M.P., Ehlers, J.D., Roberts, P.A., 2007. Cowpea. In: Genome Mapping and Molecular Breeding in Plants: Pulses, Sugar and Tuber Crops, pp. 49–67.

Webb, S.E., Kok-Yokomi, M.L., Voegtlin, D.J., 1994. Effect of trap color on species composition of alate aphids (Homoptera : Aphididae) caught over watermelon plants. Fla. Entomol. 77, 146–154. https://doi.org/10.2307/3495881.

Webster, B., Cardé, R.T., 2016. Use of habitat odour by host-seeking insects. Biol. Rev. 92, 1241–1249. https://doi.org/10.1111/brv.12281.

Yang, N.W., Zang, L.S., Wang, S., Guo, J.Y., Xu, H.X., Zhang, F., Wan, F.H., 2014. Biological pest management by predators and parasitoids in the greenhouse vegetables in China. Biol. Contr. 68, 92–102. https://doi.org/10.1016/j.biocontrol.2013.06.012.

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Exploring the Kairomone-Based Foraging Behaviour of Natural Enemies to Enhance Biological Control: A Review

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Kairomones are chemical signals that mediate interspecific interactions beneficial to organisms that detect the cues. These attractants can be individual compounds or mixtures of herbivore-induced plant volatiles (HIPVs) or herbivore chemicals such as pheromones, i.e., chemicals mediating intraspecific communication between herbivores. Natural enemies eavesdrop on kairomones during their foraging behaviour, i.e., location of oviposition sites and feeding resources in nature. Kairomone mixtures are likely to elicit stronger olfactory responses in natural enemies than single kairomones. Kairomone-based lures are used to enhance biological control strategies via the attraction and retention of natural enemies to reduce insect pest populations and crop damage in an environmentally friendly way. In this review, we focus on ways to improve the efficiency of kairomone use in crop fields. First, we highlight kairomone sources in tri-trophic systems and discuss how these attractants are used by natural enemies searching for hosts or prey. Then we summarise examples of field application of kairomones (pheromones vs. HIPVs) in recruiting natural enemies. We highlight the need for future field studies to focus on the application of kairomone blends rather than single kairomones which currently dominate the literature on field attractants for natural enemies. We further discuss ways for improving kairomone use through attract and reward technique, olfactory associative learning, and optimisation of kairomone lure formulations. Finally, we discuss why the effectiveness of kairomone use for enhancing biological control strategies should move from demonstration of increase in the number of attracted natural enemies, to reducing pest populations and crop damage below economic threshold levels and increasing crop yield.

Keywords: field application, attractant blend, HIPVs, pheromones, semiochemicals, crop yield, olfactory learning

INTRODUCTION

The foraging behaviour of a parasitoid or predator is a process by which it searches for oviposition sites and feeding resources for its survival, growth, and reproductive success [reviewed in Kramer (2001)]. Natural enemies (used hereafter in reference to both parasitoids and predators) play a key role in the biological control of chewing, sucking, and gall-feeding herbivores (Vidal and Murphy, 2018). Understanding the foraging behaviour of natural enemies is crucial for improving the biological control of herbivorous pests (e.g., Mills and Wajnberg, 2008; Gunton and Pöyry, 2016; Mills and Heimpel, 2018). Parasitoids have long served as an insect model in the study of foraging behaviour for oviposition sites, which has often been viewed as a three-sequence process: habitat location, host location, and host selection (Vinson, 1998; Fatouros et al., 2008). However, this behavioural process can also be applied to predatory insects searching for prey (Fellowes et al., 2007; Pervez and Yadav, 2018). During each of these foraging behavioural sequences, natural enemies rely on a combination of environmental stimuli to find their host and prey (hereafter jointly referred to as herbivores). Although kairomones and visual signals are exploited by natural enemies, the former is well-known to play an important role in their foraging behaviour (Colazza and Wanjberg, 2013; Lim and Ben-Yakir, 2020). Kairomones are individual semiochemical molecules or mixtures that mediate interspecific interactions between living organisms (Dicke and Sabelis, 1988; Kost, 2008), while serving as long-range, short-range, and contact cues during herbivore location by natural enemies (Afsheen et al., 2008; Heil and Ton, 2008). Kairomones exploited by natural enemies are either plant volatiles or herbivore-associated chemicals such as pheromones (Afsheen et al., 2008; Kaplan, 2012a; Kaiser et al., 2017; Peñaflor, 2019).

Herbivory-induced plant volatiles (HIPVs) and oviposition-induced plant volatiles (OIPVs) are the reliable plant volatile compounds that serve as long-range kairomones enabling natural enemies to locate herbivore-infested plants (e.g., Dicke and van Loon, 2000; Mumm and Dicke, 2010; Hilker and Fatouros, 2015; Turlings and Erb, 2018). The use of HIPVs and OIPVs to attract natural enemies has been pointed out as a novel avenue to achieve successful biological control (Kaplan, 2012a; Kelly et al., 2014; Murali-Baskaran et al., 2018; Peri et al., 2018). For example, (*E*)-4,8-dimethyl-1,3,7-non-atriene (DMNT), an elm plant (*Ulmus minor* Mill [Ulmaceae]) OIPV induced by *Xanthogaleruca luteola* (Müller) (Coleoptera: Chrysomelidae), attracted the parasitoid *Oomyzus gallerucae* (Fonscolombe) (Hymenoptera: Eulophidae) in the field (Büchel et al., 2011). In addition to HIPVs and OIPVs, kairomones include chemicals emitted from different herbivore stages (eggs, larvae/nymphs, pupae, adults), herbivore by-products (e.g., frass, honeydew, exuviae, mandibular gland secretions, defence secretions, etc.) which are exploited by natural enemies in their selection of oviposition and feeding sites [reviewed in Afsheen et al. (2008)]. For instance, application of hydrocarbons, e.g., tricosane identified in extracts of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) moth scales, increased the efficiency of host location by the parasitoids *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), thereby increasing the parasitisation rate in the field (Gross et al., 1975; Lewis et al., 1975).

Pheromones are highly species-specific in mediating intraspecific interactions between the emitter and its conspecifics (Dicke and Sabelis, 1988; Kost, 2008). They can be grouped into the following categories: (i) sex pheromones, (ii) aggregation pheromones, (iii) marking pheromones, and (iv) alarm pheromones (Kost, 2008; Ruther, 2013). Eavesdropping is a well-known phenomenon among natural enemies which use pheromones of herbivorous insects as kairomones to detect them (Aukema and Raffa, 2005). A typical example is reported by Kpongbe et al. (2019) who demonstrated that isopentyl-butanoate—the aggregation pheromone of *Clavigralla tomentosicollis* Stål (Hemiptera: Coreidae)—strongly attracts *Gryon* sp. (Hymenoptera: Scelionidae), the parasitoid of *Clavigralla* species. However, not only herbivore pheromones play a role in the attraction of herbivore enemies, i.e., pheromones released by natural enemies also attract conspecific individuals seeking for mates (Ruther, 2013), and may modulate their responses to HIPVs (Cabello et al., 2017). For example, pheromones from males of the predator *Nabis pseudoferus* Remane (Hemiptera: Nabidae) attracted conspecific females and enhanced their responses to HIPVs from *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)-infested wheat plants, whereas pheromones from males of the mirid predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) attracted conspecific females but were found to reduce their attraction to the HIPVs (Cabello et al., 2017).

Kairomone-based lures are deployed in crop plants of which the background odours shape the location of herbivores by natural enemies (e.g., Schröder and Hilker, 2008; Randlkofer et al., 2010). In this review, we focus on how to optimise field applications of kairomones for improving biological control of insect pests. First, we present sources of kairomones in tri-trophic systems and discuss how these kairomones are exploited by natural enemies foraging for hosts or prey. Then we provide examples of kairomone field applications, and we stress how the attraction of natural enemies into crop fields might be more efficient when using pheromones rather than HIPVs. Finally, we highlight potential ways to improve the use of kairomone-based lures for attracting enough natural enemies to reduce pest populations and crop damage below economic threshold levels while increasing crop yields in the field.

CHEMICAL INTERACTIONS IN TRI-TROPHIC SYSTEMS

Chemically mediated multitrophic interactions occur both below and above ground between a diversity of organisms among four trophic levels in agro-ecosystems (Aartsma et al., 2019; Stelinski et al., 2019). Research on chemical interactions aiming at controlling insect pests are, however, usually focused on tritrophic systems typically composed of plants, herbivores and natural enemies (Price et al., 1980; Kaplan, 2012b; Mbaluto et al., 2020), and in this section we summarise these tritrophic chemically mediated interactions at above ground level (**Figure 1**). Organisms at each trophic level emit chemical cues (emitters) to mediate intraspecific and/or interspecific interactions with other organisms that detect the cues (receivers). These interactions can be direct or indirect (Wootton, 1994). A direct interaction occurs between two organisms without an intermediary, whereas an indirect interaction necessitates the presence of, or occurs through, at least one intermediary which is usually a third species (**Figure 1**) (Wootton, 1994). Plants (emitters) emit volatile organic compounds (VOCs) which are exploited by herbivores (receivers) to locate host plants (direct interactions) (Bruce and Pickett, 2011). Plants (emitters) also release HIPVs and OIPVs to attract natural enemies (receivers) for controlling herbivores (intermediary) (indirect interactions; **Figure 1**) (Hilker and Fatouros, 2015; Turlings and Erb, 2018). In addition to HIPVs and OIPVs that mediate the indirect plant defences, the role of zoophytophagous-induced plant volatiles (ZIPVs) in plant-insect chemical communication has recently gained attention (**Figure 1**) (Pappas et al., 2015; Pérez-Hedo et al., 2015). Zoophytophagous insects are sap sucking predators which can feed on plants and consequently induce the release of volatiles that attract conspecific and heterospecific predators, as well as parasitoids (Pérez-Hedo et al., 2015; Rim et al., 2018). This effect is like that induced by herbivores feeding on plants. Compared to volatiles of unexposed tomato plant (*Solanum lycopersicum* L. [Solanaceae]), ZIPVs from tomato plant volatiles induced by the mirid predator *Nesidiocoris tenus* (Reuter) (Hemiptera: Miridae) were found to attract conspecific males and females (Rim et al., 2018) and the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Pérez-Hedo et al., 2015). Undamaged plants can also perceive HIPVs, OIPVs, ZIPVs, and herbivore pheromones as signals to mount defences against herbivores to the benefit of the host plant community (known as plant priming defences) (**Figure 1**) (Dicke and Bruin, 2001; Frost et al., 2008, Pérez-Hedo et al., 2015), but the priming defence is not observed in all plant species as reported for cotton plants exposed to pheromones (Magalhães et al., 2019) and tea plants exposed to the HIPV (E)-4,8-dimethyl-1,3,7-nonatriene (Jing et al., 2020).

Natural enemies release pheromones that directly mediate interactions with their respective conspecifics (Ruther, 2013). While herbivores release pheromones to communicate with conspecific individuals to their benefit, their natural enemies eavesdrop on these communication cues to locate them to their disadvantage (Aukema and Raffa, 2005). Herbivores and their by-products such as larval frass, larval mandibular secretions, and honeydew also release chemical compounds that serve as kairomones for natural enemies in locating herbivores (Afsheen et al., 2008). Understanding chemical interactions between plants, herbivores, and natural enemies (**Figure 1**) is key to designing and implementing kairomone-based biological control measures in agro-ecosystems. In the following sections, we focus on above-ground chemical interactions in which natural enemies are receivers of pheromones and kairomones involved (**Figure 1**).

HOST PLANT AND HERBIVORE LOCATION BY NATURAL ENEMIES WITHIN THE ODOUR LANDSCAPE

Natural enemies foraging for herbivores locate infested host plants within the heterogeneous plant community using a sequence of behaviours (**Figure 2**). This is a tedious task since host plants are embedded in a semiochemically heterogeneous environment composed of diverse plant species and multiherbivore communities whose signals influence the detection of reliable kairomones during host or prey location (e.g., Schröder and Hilker, 2008; Randlkofer et al., 2010). This complex dynamic sensory environment has been described as an "odour landscape" which is composed of different odour plumes (an odour plume is a blend of volatile compounds carried by the wind) (Atema, 1996). How insects track relevant or resource-indicating odour plumes within the odour landscape has recently been reviewed (Beyaert and Hilker, 2014). Foraging insects can follow a single or several relevant odour plumes during which they shift from one odour plume to another if this conveys a more reliable signal (or indicates a more suitable resource), and they continue this behaviour until they find the target host plant (Beyaert and Hilker, 2014). During foraging, natural enemies rely on a diversity of resource-indicating odours to first locate the host plant within the plant community (section Role of Herbivore-Induced Plant Volatiles in long-Range Location of Host Plants), then find the potential infesting herbivores (section Herbivore Volatiles for Host or Prey Location), and finally select and accept the target host or prey upon landing on the host plant (section Herbivore Contact Kairomones for Host/Prey Recognition and Acceptance) (**Figure 2**).

Role of Herbivore-Induced Plant Volatiles in Long-Range Location of Host Plants

Plants emit VOCs while undamaged, known as constitutive VOCs, and herbivory or oviposition usually results in an increase in the constitutive VOCs and/or release of specific herbivore-induced volatiles, known as inducible VOCs (e.g., HIPVs and OIPVs). Although natural enemies may sometimes be attracted by odour plumes of constitutive VOCs, evidences have culminated in the literature that the odour plumes of HIPVs and OIPVs are those that play the key role in the foraging behaviour, serving as long-range signals that natural enemies exploit to locate plants infested by their hosts or prey within the plant community (**Figure 2**) (e.g., Kessler and Baldwin, 2001; Heil and Ton, 2008; Kaplan, 2012a; Beyaert and Hilker, 2014). Plants are usually infested by several herbivore species that are hosts or non-hosts for natural enemies (Vos et al., 2001). Infesting herbivore species may induce the same or different plant defence pathways (i.e., Salicylic Acid inducer [SA-inducer] and Jasmonic Acid-inducer [JA-inducer]), each affecting the composition of volatiles produced (Danner et al., 2018). The attraction of

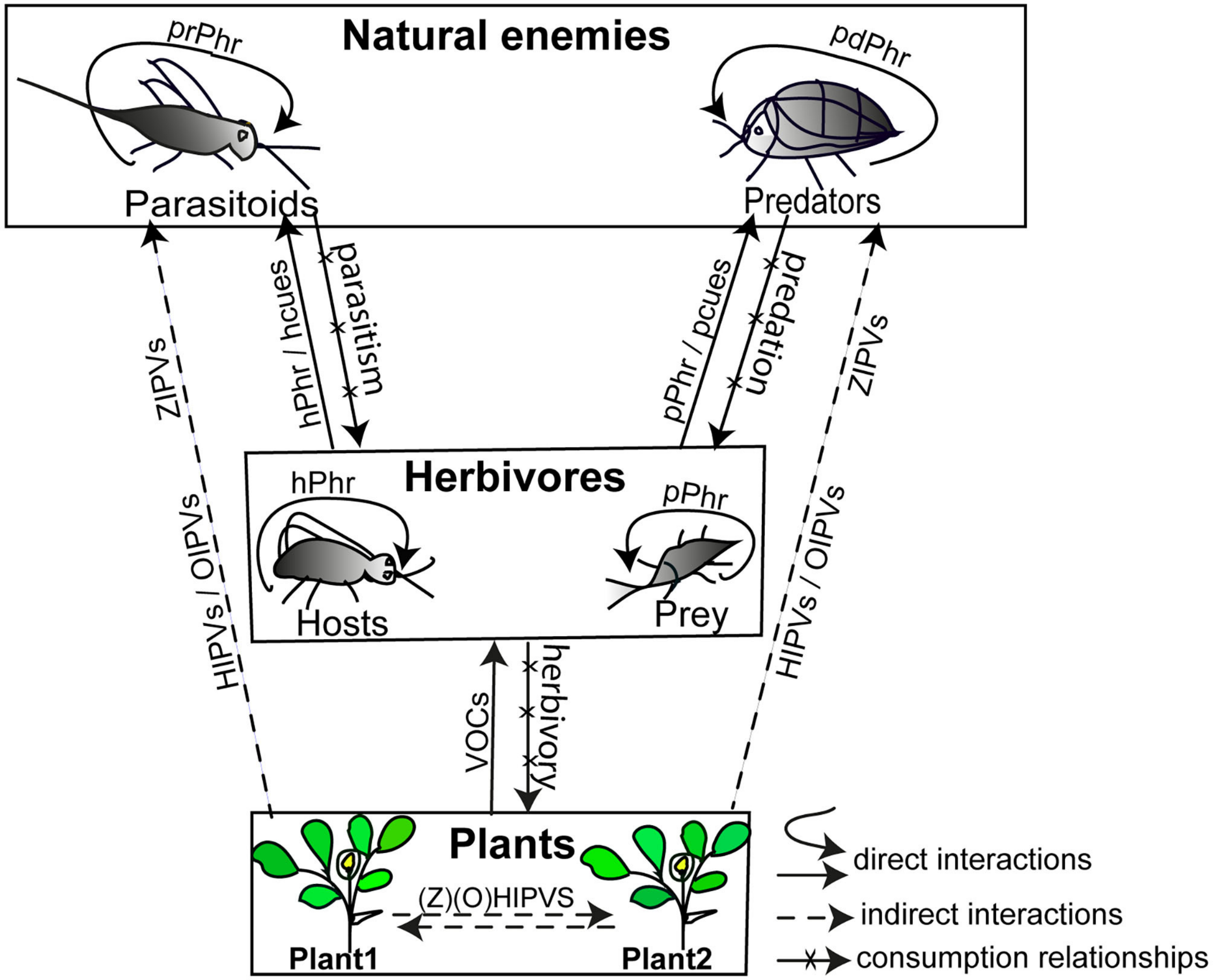

FIGURE 1 | Typical examples of above ground chemical interactions in a tri-trophic system. Solid and dotted lines represent kairomone-based direct and indirect interactions, respectively, in which the arrow bearing the kairomone is directed from the emitter to the receiver. The solid curves indicate pheromone-based direct interactions in which male and female individuals can be an emitter and/or a receiver. HIPVs, herbivory-induced plant volatiles; OIPVs, oviposition-induced plant volatiles; ZIPVs, zoophytophagous-induced plant volatiles; VOCs, volatile organic compounds; pPhr, prey pheromone; hPhr, host pheromone; pdPhr, predator pheromone; prPhr, parasitoid pheromone; pcues, prey-associated chemical cues; hcues, host-associated chemical cues.

natural enemies to infested plants depends on factors such as the infesting herbivores, i.e., single or multiple species; the suitability of the herbivores, i.e., hosts or non-hosts; the infesting herbivore density; and the activated plant defence pathways, i.e., SA and/or JA. For example, the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) was reported to be more attracted to volatiles of maize plants infested by only Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae), a JA-inducer, than plants infested by both *S. littoralis* and *Euscelidius variegatus* (Kirschbaum) (Hemiptera: Cicadellidae), an SA-inducer (Erb et al., 2010). In multi-herbivore systems, the location of target herbivore-infested plants by natural enemies may be disrupted by the co-infestation of non-target herbivores (Takabayashi et al., 2006). The parasitoid *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) were less attracted to volatile blends from cabbage plants simultaneously infested by caterpillars of both host (*Plutella xylostella* [L.] [Lepidoptera: Plutellidae]) and non-host (*Pieris rapae* [L.] [Lepidoptera: Pieridae]) species compared to volatiles released from host *P. xylostella*-infested plants (Shiojiri et al., 2001). However, this is not always the case, as illustrated by the fact that the larval parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) was more attracted to plants damaged by larvae of both host *P. rapae* and non-host *P. xylostella* compared to plants damaged by host *P. rapae* alone (Shiojiri et al., 2001). The herbivore-infestation level also affects the response of natural enemies to infested plants. The parasitoids *C. glomerata* and *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) were more attracted to volatiles of cabbage plants

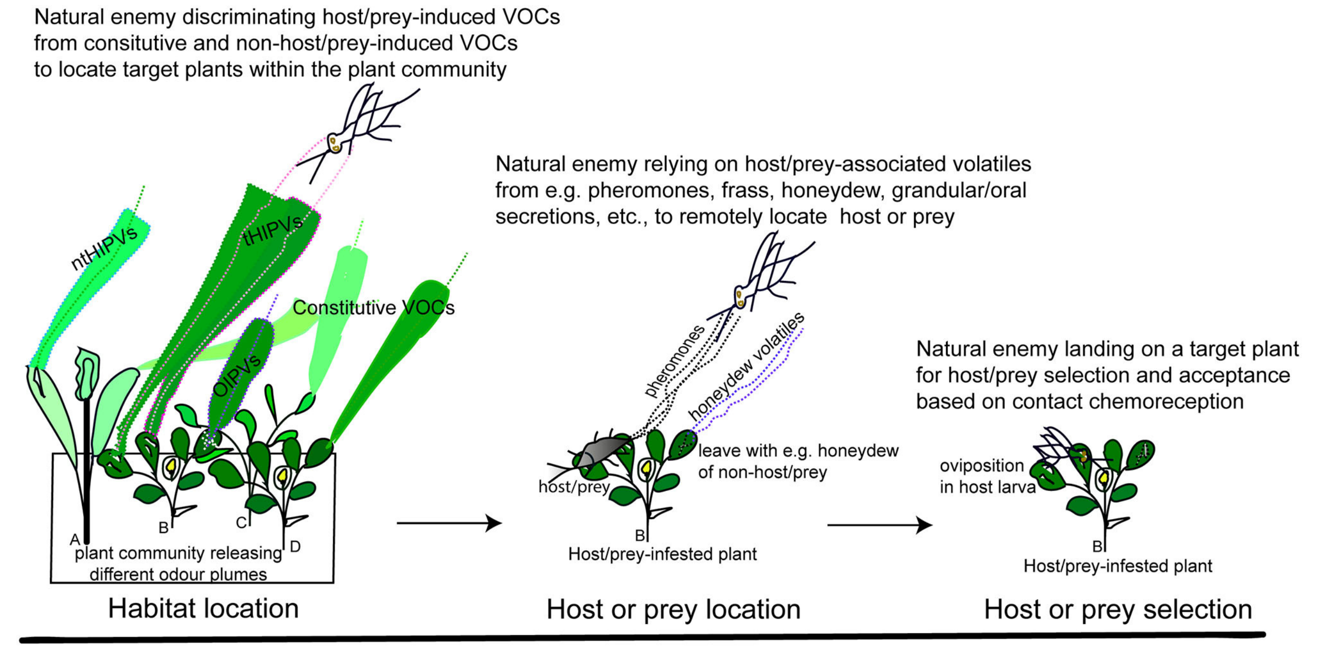

FIGURE 2 | Schematic description of the use of kairomones during foraging behavioural sequences involved in host/prey searching by parasitoids and predators. VOCs, volatile organic compounds; tHIPVs, target herbivore-induced plant volatiles; ntHIPVs, non-target herbivore-induced plant volatiles; OIPVs = oviposition-induced plant volatiles. Plant community composed of **(A)** infested non-host plants; **(B)** infested host plants; **(C)** uninfested non-host plants; and **(D)** uninfested host plants.

with high levels of *P. rapae* and *P. xylostella* larval infestation than those with low infestation levels (Shiojiri et al., 2010). Whereas, relative to volatiles of healthy plants, the predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) was reported to prefer volatiles of cabbage plants infested by an intermediate density of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) adults, but it showed no preference for plants infested with low and high densities of *M. persicae* (Yoon et al., 2010). Therefore, natural enemy species show different attraction responses to volatiles of infested plants depending on the specificity and density of infesting herbivores (De-Moraes et al., 1998; Shiojiri et al., 2010; McCormick et al., 2012).

The infesting herbivore species and its feeding mode (e.g., leaf chewing, leaf mining, phloem sap sucking, and cell content feeding) determine the plant defence signalling pathways to be activated (either SA or JA alone, or both). Emission of volatiles is regulated by the biosynthesis pathway(s) activated, and qualitative and quantitative differences are substantial between plant volatiles from SA- vs. JA-herbivore inducers (Danner et al., 2018). Chewing herbivores are known to activate the JA-pathway (Danner et al., 2018), while leaf mining larvae likely activate both JA- and SA-pathways (Yang et al., in press). For sap sucking herbivores, however, some species like whiteflies are known to induce a single pathway, i.e., SA (Zhang et al., 2013), whereas others like stink bugs induce both JA- and SA-pathways (Giacometti et al., 2016). A comparison of volatiles from tomato plants infested by the leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larva, and the phloem sap sucking feeder *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) adult, revealed that α- and β-copaene, as well as valencene, were released in higher amounts from plants infested by the sap sucking insect (Silva et al., 2017). Whereas (*Z*)-3-hexen-1-ol, (*E*)-β-ocimene, methyl salicylate, and β-caryophyllene were higher from plants infested by the leaf miner, in addition to some novel compounds such as (*Z*)-jasmone and (*Z*)-3-hexen-1-yl crotonate (Silva et al., 2017). Volatile compounds released by single and dual herbivore-infested plants also differ quantitatively and qualitatively. Infestation of tomato plants by both *T. absoluta* and *B. tabaci* led to increased emission of β-myrcene, limonene, γ-terpinene, and β-elemene than in plants infested only by *T. absoluta* or *B. tabaci,* whereas α-pinene emission increased in *T. absoluta*-infested plants (Silva et al., 2018). Conversely, cotton plants simultaneously infested by the leaf chewing larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and adults of *B. tabaci* emit lower amounts of HIPVs than plants infested only by *S. exigua* larvae, as a result of so-called "cross-talk" between SA- and JA-signalling pathways (Rodriguez-Saona et al., 2003). Natural enemies rely on these differences to find specific herbivore-infested plants (Mumm and Dicke, 2010; Shiojiri et al., 2010). Herbivores can also suppress the plant defence mechanisms and volatile emission when feeding, which could be a strategy to deceive natural enemies from locating herbivore-infested plants. The feeding and extracts of oral secretion of the leaf chewing larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) were found to suppress the emission of HIPVs in maize plants (De-Lange et al., 2020). Similarly, suppression of HIPV emission in host plants was reported upon herbivory by cell content feeders (e.g., spider mites and thrips) (Schausberger, 2018) and phloem sap sucking feeders (e.g., whiteflies and aphids) (Walling, 2008; Zhang et al., 2013). Suppression of HIPV emission by herbivores is known to occur through various mechanisms, but the front line mechanism is reported to operate through inoculation of the oral secretion-inhabiting bacteria during feeding, a phenomenon that deceives the plant to incorrectly perceive and identify the attacking herbivore, and to not activate the appropriate signalling defence (Felton et al., 2014; Schausberger, 2018). Unfortunately, volatile suppression, in general, is not found to prevent natural enemies from locating herbivore-infested plants (Sarmento et al., 2011; De-Lange et al., 2020), suggesting that the release rates of the specific attractive compounds or the ratios of the attractive compounds in the released HIPV blend are not necessarily affected.

Volatile compounds attractive to natural enemies include a subset of HIPV or OIPV compounds, from the same or different chemical classes (D'Alessandro and Turlings, 2006; McCormick et al., 2012). These are often mixtures of compounds in specific ratios (van Wijk et al., 2011), but sometimes these attractants are single compounds (Wei et al., 2008). For example, (*R*)-(+)-limonene released by the Mongolian oak, *Quercus mongolicus* Fisch. ex. Turcz. (Fagaceae) plants infested by the longhorned beetle, *Massicus raddei* (Blessig) (Coleoptera: Cerambycidae) elicited strong attraction in the predator *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) (Wei et al., 2008). The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was more attracted to the mixture of five compounds β-ocimene, (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and methyl salicylate than to the individual compounds or partial mixtures of these compounds identified from *Tetranychus urticae* Koch (Acari: Tetranychidae)-infested lima bean plants (van Wijk et al., 2011). The identification of attractant compounds from HIPVs and OIPVs is mostly specific to the tri-trophic system herbivore-plant-natural enemy and associated chemical interactions indicated in **Figure 1** (Mumm and Dicke, 2010; Kaplan, 2012b; McCormick et al., 2012).

Herbivore Volatiles for Host or Prey Location

Natural enemies locate herbivores via their emitted kairomones (**Figure 2**) (Vet and Dicke, 1992; Afsheen et al., 2008). Volatile compounds emitted by herbivores are generally distinct from plant field background odours and constitute the most reliable sources of information for natural enemies (Vet and Dicke, 1992; Rodriguez-Saona and Stelinski, 2009). Herbivore pheromones are species-specific and usually serve as kairomones for location of suitable herbivore species by natural enemies (Aukema and Raffa, 2005). The egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is highly attracted to (*Z*)-11-hexadecenyl and (*E*)-12-tetradecenyl acetates which are, respectively, components of the sex pheromones of *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) and *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) (Boo and Yang, 2000). Other natural enemy species are instead attracted to a blend of pheromone components, as seen in the predatory bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), which was only attracted to a mixture of 1:2.3 (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate, the major components of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) aggregation pheromone (Vaello et al., 2017).

Apart from pheromones, natural enemies eavesdrop on volatile compounds produced by herbivore by-products such as honeydew (Leroy et al., 2012; Watanabe et al., 2016), larval frass (Reddy et al., 2002; Wei et al., 2013), and glandular defensive secretions of larvae or adults (Kopf et al., 1997; Zverava and Rank, 2004). A single component, i.e., phenyl acetaldehyde identified from honeydew excreted by the aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), attracted the predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (Watanabe et al., 2016). Reddy et al. (2002) reported that allyl isothiocyanate, a volatile component of larval frass from *P. xylostella*-infested cabbage plants, was highly attractive to *P. xylostella* natural enemies, specifically the predator *Chrysoperla carnea* (Stepehens) (Neuroptera: Chrysopidae) and the parasitoids *C. plutellea* and *T. chilonis*. Moreover, the syrphid fly predator *Parasyrphus nigritarsis* (Zetterstedt) (Diptera: Syrphidae) was highly attracted to salicyl aldehyde, the main component of the larval secretion of the prey leaf beetle, *Phratora vitellinae* (L.) (Coleoptera: Chrysomelidae) (Kopf et al., 1997).

Herbivore Contact Kairomones for Host/Prey Recognition and Acceptance

Upon landing on a host plant, natural enemies have to select the target herbivore species and stages for oviposition or feeding (**Figure 2**) (Jaenike, 1978; Scheirs and De Bruyn, 2002; Gripenberg et al., 2010). This step of the foraging behaviour is crucial as it defines the survival of the forager and its offspring. Plants are usually infested by multiple herbivores, which makes it challenging for natural enemies to select the target species. Herbivore contact kairomones are often species-specific, and therefore natural enemies generally rely on these non-volatile signals, which are examined by antennating or probing for recognition of hosts or prey (Vinson, 1998; Bénédet et al., 2002). These cues originate from various sources such as adult/larva/nymph body surfaces, larvae oral secretions, egg coating, and wing scales (Afsheen et al., 2008; Kaiser et al., 2017). A typical example of this is *O*-caffeoylserine isolated from the body surface of *Phenacoccus herreni* Cox & Williams (Homoptera: Pseudococcidae) and which elicited strong drumming behaviour, thereby acting as a contact host-recognition kairomone in the parasitoids *Acerophagus coccois* Smith and *Aenasius vexans* Kerrich (both Hymenoptera: Encyrtidae) (Calatayud et al., 2001). Moreover, α-amylase from oral secretions of the host *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) larvae mediates oviposition in the parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) (Bichang'a et al., 2018). Hydrocarbons extracted from herbivores are also involved in host acceptance by parasitoids, as seen in *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) which uses non-adecane, a cuticular hydrocarbon from body extract of its stink bug host, *Nezaria viridula* (L.) (Hemiptera: Pentatomidae), to discriminate between male and female (Colazza et al., 2007) (see Kaiser et al., 2017, for a recent review on parasitoids). Gomes-Lagôa et al. (2019) further reported the crucial role of hydrocarbons in the selection of preferred stink bug host species by their parasitoids. These authors found that the parasitoid *T. basalis* preferred the hydrocarbons from the footprint extract of its preferred host, *N. viridula,* to those of *Dichelops melacanthus* (Dallas) and *Euschistus heros* (Fab.) (Hemiptera: Pentatomidae), and the parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) chose hydrocarbons of the footprint extract of its preferred host, *E. heros,* to those of the other two stink bug species. The selective responses of the parasitoids to hydrocarbons of their preferred hosts could be explained by differences in the composition of key hydrocarbons in the blend extract (Gomes-Lagôa et al., 2019) and by the co-evolutionary host-parasitoid interactions that have enabled parasitoids to easily recognise and eavesdrop on chemical stimuli of their suitable associated hosts (Peri et al., 2013).

Unlike in parasitoids, contact kairomones involved in prey recognition and acceptance by predatory insects have been explored in few species and little attention has been paid to the identification of the chemical cues involved. However, the predator *Cerceris fumipennis* Say (Hymenoptera: Crabronidae) was reported to exploit methyl-branch hydrocarbons from the cuticle of Buprestidae beetles to recognise prey from non-prey Chrysomelidae beetles (Rutledge et al., 2014). The author reported that washed buprestid prey that had been treated with their crude cuticular extract were accepted by the predator, whereas those treated with crude cuticular extract of non-prey beetles were rejected, suggesting that the predator uses cuticular hydrocarbons as contact kairomone to discriminate prey from non-prey.

USE OF KAIROMONES IN BIOLOGICAL CONTROL STRATEGIES

Biological control is a pest management method where natural enemies are used to reduce populations and damages of pest organisms (Eilenberg et al., 2001). There are three main forms of biological control: classical, augmentation, and conservation (Bale et al., 2008). Classical biological control aims at introducing and releasing a co-evolved exotic natural enemy—generally a parasitoid—of an invasive herbivore into invaded areas for permanent long-term control of the invasive herbivore (Eilenberg et al., 2001; Bale et al., 2008). Augmentation biological control involves periodical releases of a natural enemy when environmental conditions tend to limit its survival, reproduction, and establishment (van Lenteren, 2000; Collier and van Steenwyk, 2004). On the other hand, conservation biological control requires no release, but habitat manipulation or improvement of control practises are implemented to provide resources to maintain and enhance survival, fitness, and reproductive success of natural enemies (Gurr et al., 2000; Zhu et al., 2014). Biological control is one of the most promising alternatives to synthetic chemical insecticides, which are widely applied for the control of herbivores but unsustainable due to the development of herbivore resistance and associated with negative impacts on the environment and human health (Gay, 2012).

Cases of successful implementation of biological control have been documented (e.g., Beddington et al., 1978; Collier and van Steenwyk, 2004). The parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) was used in an augmentation biological control and successfully controlled the red scale insect *Aonidiella aurantii* Maskell (Homoptera: Diaspididae) in citrus orchards (Moreno and Luck, 1992). However, many cases of failures in the biological control of insect pests have been observed (Lynch and Thomas, 2000; Collier and van Steenwyk, 2004). The main issues that cause failures include an insufficient population level of natural enemies in the target crop and the emigration or dispersal of natural enemies from cropping sites, limiting the control of insect pests in the target crops (Heimpel and Asplen, 2011). In this line, recent studies have highlighted the use of kairomone-based lures as a sound solution for limiting emigration of natural enemies and increasing their populations in target crops, in turn enhancing the efficacy of natural enemies for satisfactory control of insect pests (Kelly et al., 2014; Peri et al., 2018).

The effectiveness of kairomones in attracting natural enemies to enhance biological control of insect pests has been demonstrated in crop field conditions. The use of HIPVs/OIPVs in crop fields has generally led to a lower ratio of attracted natural enemies in kairomone-treated plots relative to control plots, than when pheromones are used (**Tables 1**, **2**). Unlike HIPVs or OIPVs, pheromones are species-specific (Aukema and Raffa, 2005; Ruther, 2013), and in most cases they are generally distinct from plant background odours and therefore reliable indicators for natural enemies searching for herbivorous insects (Vet and Dicke, 1992; Rodriguez-Saona and Stelinski, 2009). Insects are likely to detect and react to blends of odourant compounds in nature (Thomas-Danguin et al., 2014; Conchou et al., 2019), but reported studies have mainly focused on field applications of single kairomonal compounds (Kaplan, 2012a; **Tables 1**, **2**). The studies published in the last two decades documenting the use of HIPVs, OIPVs, and pheromones, whereby natural enemies have been reported to be more attracted to kairomone-baited plots compared to control plots are summarised in the following subsections.

Field Applications of Herbivore-Induced Plant Volatiles

The use of HIPVs and OIPVs as kairomones for the recruitment of natural enemies against insect pests in crop fields has been reported (e.g., Kaplan, 2012a; Peñaflor and Bento, 2013). Applications of methyl salicylate (MeSA) through dispensers in cranberry and soybean fields attracted more natural enemies and resulted in increased predation and parasitism rates, associated with a reduced number of herbivores on plants (e.g., Mallinger et al., 2011; Rodriguez-Saona et al., 2011). A pioneer review has addressed the role of (*Z*)-3-hexenol in attracting natural enemies into herbivore-infested field plants (*Arabidopsis thaliana, Nicotiana* spp., and *Phazeolus* spp.) (Wei et al., 2011). A typical example was reported in a study conducted by Kessler and Baldwin (2001), where

TABLE 1 | Summary of some synthetic herbivore-induced plant volatiles applied in fields to attract natural enemies.

Kairomone	Natural enemy	Type, order[a], and family of natural enemy	Host/prey species inducing release of the compound	Field crop species	Amount of kairomone	Ratio[b] of natural enemies caught between treated plot and control	Is the crop yield improved in the treated plot relative to control?	References[c]
Methyl salicylate	*Chrysopa nigricornis*	Predator (Neu.: Chrysopidae)	Not specified	*Vitis vinifera*	2 mL of neat compound	6	nd	James, 2003a
					5 g of neat compound	2	nd	James and Price, 2004
	Geocoris pallens	Predator (Hem.: Geocoridae)	Not specified	*Vitis vinifera*	1 mL of neat compound	7	nd	James, 2003b
	Orius tristicolor	Predator (Hem.: Anthocoridae)	Not specified	*Vitis vinifera*	5 g of neat compound	7	nd	James and Price, 2004
	Chrysopa oculata	Predator (Neu.: Chrysopidae)	*Tetranychus urticae*	*Vitis vinifera*	1 mL of neat compound	6	nd	James, 2006
	Stethorus punctum picipes	Predator (Col.: Coccinellidae)	Not specified	*Vitis vinifera*	5 g of neat compound	57	nd	James and Price, 2004
					1 mL of 1.5 µL/mL	2	nd	Maeda et al., 2015
	Coccinella septempunctata	Predator (Col.: Coccinellidae)	*Aphis glycines*	*Glycine max*	100 mg of neat compound	3	nd	Zhu and Park, 2005
	Predatory beetles and parasitoids[d]	–	Not specified	*Triticum aestivum*	120 mg of neat compound	2	Improved	Wang et al., 2011
	Toxomerus sp. & *Orius insidiosus*	Predators (Dip: Syrphidae) & (Hem.: Anthocoridae)	Not specified	*Phaseolus vulgaris*	3 mL of neat compound	7 & 2	Not improved	Salamanca et al., 2018
Mixture of Methyl salicylate, (*Z*)-3-hexenol and (*Z*)-3- hexenyl acetate	*Stethorus punctum picipes*	Predator (Col.: Coccinellidae)	Not specified	*Vitis vinifera*	1 mL of 1.5 µL/mL each (1:1:1 ratio)	2	nd	Maeda et al., 2015
(*Z*)-3-hexenyl acetate	*Orius tristicolor*	Predator (Hem.: Anthocoridae)	Not specified	*Vitis vinifera*	1 mL of neat compound	5	nd	James, 2003b
	Coccinella septempunctata	Predator (Col.: Coccinellidae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	9	nd	Yu et al., 2008
	Orius similes	Predator (Hem.: Anthocoridae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	4	nd	Yu et al., 2008
	Anaphes iole	Parasitoid (Hym.: Mymaridae)	*Lygus lineolaris*	*Gossypium hirsutum*	2 mL of neat compound	3^{++}	nd	Williams et al., 2008
2-Phenylethanol	*Chrysoperla carnea*	Predator (Neu.: Chrysopidae)	*Aphis glycines*	*Glycine max*	100 mg of neat compound	5	nd	Zhu and Park, 2005
	Eupeodes volucris or *Eupeodes fumipennis*	Predators (Dip: Syrphidae)	Not specified	Apple trees	1 mL of neat compound	8	nd	Jones et al., 2016

(Continued)

TABLE 1 | Continued

Kairomone	Natural enemy	Type, order[a], and family of natural enemy	Host/prey species inducing release of the compound	Field crop species	Amount of kairomone	Ratio[b] of natural enemies caught between treated plot and control	Is the crop yield improved in the treated plot relative to control?	References[c]
(*E*,*E*)-α-Farnesene	*Anaphes iole*	Parasitoid (Hym.: Mymaridae)	*Lygus lineolaris*	*Gossypium hirsutum*	2 mL of neat compound	4^{++}	nd	Williams et al., 2008
	Tetrastichus asparagi & *Paralispe infernalis*	Parasitoids (Hym.: Eulophidae)	*Crioceris asparagi*	*Phaseolus vulgaris*	0.75 mL of neat compound	2	nd	Ingrao et al., 2019
cis-α-Bergamotene	*Geocoris punctipes*	Predator (Hem.: Lygaeidae)	*Manduca sexta*	*Nicotiana attenuata*	40 µL of 0.1 nmol	4^{++}	nd	Halitschke et al., 2008
Octanal	*Deraeocoris punctulatus*	Predator (Hem.: Miridae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	8	nd	Yu et al., 2008
Nonanal	*Harmonia axyridis*	Predator (Col.: Coccinellidae)	Not specified	*Sophora japonica*	150 µL of 10 or 100 mg/mL	8 & 15	nd	Xiu et al., 2019a
3,7-Dimethyl-1,3,6-octatriene	*Orius similes*	Predator (Hem.: Anthocoridae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	3	nd	Yu et al., 2008
Isopropanol	*Chrysopa quadripunctata*	Predator (Neu.: Chrysopidae)	*Cotinis nitida*	*Malus domestica*	125 mL of 45% dilution	100*	nd	Pszczolkowski and Johnson, 2011
Squalene	*Chrysopa nigricornis*	Predator (Neu.: Chrysopidae)	Not specified	Apple trees	2 mL of neat compound	5	nd	Jones et al., 2011
Iridodial	*Chrysopa nigricornis*	Predator (Neu.: Chrysopidae)	Not specified	Apple trees	4 mg of neat compound	3	nd	Jones et al., 2011
Mixture of methyl salicylate and Iridodial	*Chrysopa oculata* & *C. nigricornis*	Predators (Neu.: Chrysopidae)	Not specified	Apple trees	2 mL: 4 mg ratio of neat compound	100* & 10	nd	Jones et al., 2011
Benzaldehyde	*Chrysoperla plorabunda*	Predator (Neu.: Chrysopidae)	Not specified	Apple trees	0.5 mL of neat compound	80	nd	Jones et al., 2011
Mixture of phenylacetonitrile, 2-phenylethanol and acetic acid	*Chrysoperla plorabunda*	Predator (Neu.: Chrysopidae)	*Pandemis pyrusana*	Apple trees	100 mg: 100 mg: 3 mL amounts of neat compounds	100*	nd	El-Sayed et al., 2018
Dimethyl disulfide	*Aleochara bilineata* & *A. bipustulata*	Predators (Col.: Staphilinidae)	*Delia radicum*	*Brassica oleracea*	500 µL neat compound: paraffin oil (1:250 ratio)	6 & 2	Not improved	Ferry et al., 2009
Indole	*Propylaea japonica*	Predator (Col.: Coccinillidae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL)	3	nd	Yu et al., 2017
Linalool	*Propylaea japonica*	Predator (Col.: Coccinillidae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	4	nd	Yu et al., 2017
α-Pinene	*Chrysoperla sinica*	Predator (Neu.: Chrysopidae)	Not specified	*Gossypium hirsutum*	1 mL of 10 mg/mL	4	nd	Yu et al., 2017
	Harmonia axyridis	Predator (Col.: Coccinellidae)	*Aphis gossypii*	*Gossipium hirsitum*	150 µL of 10 or 100 mg/mL	19 & 53	nd	Xiu et al., 2019b

(Continued)

TABLE 1 | Continued

Kairomone	Natural enemy	Type, order^a, and family of natural enemy	Host/prey species inducing release of the compound	Field crop species	Amount of kairomone	Ratio^b of natural enemies caught between treated plot and control	Is the crop yield improved in the treated plot relative to control?	References^c
(*E*)-4,8-dimethyl-1,3,7-non-atriene (DMNT)	*Oomyzus gallerucae*	Parasitoid (Hym.: Eulophidae)	*Xanthogaleruca luteola*	*Ulmus minor*	10 µL of 10 ng/µL	3	nd	Büchel et al., 2011
3-Methylbutyraldoxime	*Glyptapanteles liparidis*	Parasitoid (Hym: Braconidae)	*Lymantria dispar*	*Populus nigra*	10 µL of 10 µg/µL	9	nd	McCormick et al., 2014
m-Cymene^1 or 4′-Ethyl-acetophenone^2	*Peristenus spretus*	*Parasitoid* (Hym.: Braconidae)	*Apolygus lucorum*	*Gossipium hirsitum*	300 µL of 10 or 100 mg/µL	^{1}(7 & 3)++ or ^{2}(4 & 6)++	nd	Xiu et al., 2019c
Ocimene	*Tetrastichus asparagi* & *Paralispe infernalis*	Parasitoids (Hym.: Eulophidae)	*Crioceris asparagi*	*Phaseolus vulgaris*	500 µL of neat compound	2	nd	Ingrao et al., 2019

[a] *Orders abbreviated as Dip., Diptera; Hem., Hemiptera; Col., Coleoptera; Neu., Neuroptera; Hym., Hymenoptera;* [b] *Ratio as directly reported in the studies or expressed from the length of plots or graphs displaying the number of insects caught.* [c] *we included only studies conducted within nearby the two last decades and in which the number of the attracted natural enemy was reported to be significantly greater in kairomone-baited plots than in control plots, and where the attracted natural enemy has been identified to species level;* [d] *The predator species are C. septempunctata, H. axyridis and Propylaea japonica, and the parasitoids are Aphidius avenae and Aphidius gifuensis; *nearly zero insects caught in the control plots; ++ = egg predation or parasitism was measured; nd, not determined.*

plots of natural population of *Nicotiana attenuata* Torrey ex S. Watson (Solanaceae) plants with (*Z*)-3-hexenol applied in lanolin paste resulted in an 8-fold increase in eggs and larvae mortality thanks to the attractiveness of the predator *Geocoris pallens* Stål (Heteroptera: Geocoridae), which preyed on a high number of eggs and larvae of *Manduca quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), *Dicyphus minimus* Uhler (Heteroptera: Miridae), and *Epitrix hirtipennis* (Melsheimer) (Coleoptera: Chrysomelidae). **Table 1** provides some additional examples of field-based applications of herbivore-induced plant volatiles that resulted in significant increase in the number of attracted natural enemies in semiochemical-field plots compared to control plots.

Use of Pheromones as Field Attractants for Natural Enemies

The application of pheromones in agricultural crop fields to enhance host location behaviour by natural enemies for the control of insect pests has been the focus of great attention (Aukema and Raffa, 2005; Pfammatter et al., 2015). Pheromones (e.g., sex, aggregation, marking, alarm-pheromones) are environmentally safe for use in crop protection. Aggregation pheromones in bark beetle species are diversified, composed of mono or pluri-chemical compounds (ipsenol, ipsdienol, cis-verbenol, *trans*-verbenol, frontalin, exo-brevicomin, endo-brevicomin, etc.) (Symonds and Gitau-Clarke, 2016), some of which have shown kairomonal activity in attracting predators in crop fields. For example, high numbers of the predators *Thanasimus dubius* (Fabricius) (Coleoptera: Cleridae) and *Platysoma cylindrica* (Paykull) (Coleoptera: Histeridae) were attracted to 50%-ipsdienol-baited traps in red pine, *Pinus resinosa* Ait. (Pinaceae), plantations (Aukema and Raffa, 2005). Although many studies have focused on the role of herbivore pheromones in field recruitment of natural enemies, sex or aggregation pheromones of natural enemies can also be used as field attractants to manipulate the behaviour of conspecifics (Wu et al., 2019), thereby increasing the natural enemy population level which could indirectly be beneficial for the control of insect pests. **Table 2** presents some additional studies on the use of pheromones as field attractants for natural enemies. However, some insect pheromone components appeared to be plant volatile compounds (Tittiger and Blomquist, 2016). Such compounds were not considered in this analysis as they likely exert the same attraction effect as applied HIPVs/OIPVs under field conditions. For example, like HIPVs or OIPVs (**Table 1**), field application of the aphid-alarm pheromone (*E*)-β-farnesene, also known to be a plant compound, generally led to 2- to 3-fold more catches of aphid natural enemies in pheromone-treated plots relative to untreated plots (Vosteen et al., 2016, and reference therein).

AVENUE OF RESEARCH FOR IMPROVING KAIROMONE EFFICIENCIES AND THEIR ASSESSMENT IN BIOLOGICAL CONTROL

Applications of synthetic kairomone-based lures in biological control of insect pests continue to gain attention in the field

TABLE 2 | Summary of some pheromones applied in the field to attract natural enemies.

Kairomone	**Natural enemy**	**Type, order^a, and family of natural enemy**	**Host/prey species releasing the compound**	**Field crop species**	**Compound and its composition (amount)**	**Type of pheromone (sex of the emitter)**	**Ratio^b of insects caught between treated plot and control**	**Is the crop yield improved in the treated plot relative to control?**	**References^c**
Methyl (2*E*,4*Z*)-decadienoate	*Gymnoclytia occidentalis*	Parasitoid (Dip.: Tachinidae)	*Euschistus conspersus*	Apple trees	200 µL of neat compound	Aggregation (male)	19	nd	Krupke and Brunner, 2003
(2*E*,5*R*,6*E*,8*E*)-5,7-dimethyl-2,6,8-decatrien-4-one	*Hemerobius stigmaterus*	Predator (Neu: Hemerobiidae)	*Matsucoccus josephi*	*Pinus resinosa*	50 µg of neat compound	Sex (female)	100*	nd	Mendel et al., 2004
	Elatophilus hebraicus	Predator (Het.: Anthocoridae)	*Matsucoccus josephi*	*Pinus resinosa*	220 µg of neat compound	Sex (female)	100*	nd	Mendel et al., 2004
(2*E*, 4*E*)-4,6,10,12- Tetramethyl-2,4-tridecadien-7-one	*Hemerobius stigma*	Predator (Neu: Hemerobiidae)	*Matsucoccus matsumurae*	*Pinus* spp.	600 µg of neat compound	Sex (female)	100*	nd	Mendel et al., 2004; Branco et al., 2006
(3*S*,7*R*,8*E*, l0*E*)-3,7,9-trimethyl-8,10-dodecadien-6-one	*Elatophilus nigricornis*	Predator (Het.: Anthocoridae)	*Matsucoccus feytaudi*	*Pinus resinosa*	220 µg of neat compound	Sex (female	100*	nd	Mendel et al., 2004
	Elatophilus crassicornis	Predator (Het.: Anthocoridae)	*Matsucoccus feytaudi*	*Pinus pinaster*	220 µg of neat compound	Sex (female)	100*	nd	Branco et al., 2006
(*R*)-(C)-*Y*-decalactone	*Elater ferrugineus*	Predator (Col.: Elateridae)	*Osmoderma eremita*	*Quercus robur*	400 µL of neat compound	Sex (male)	20	nd	Svensson et al., 2004
(6*Z*,9*Z*,11*S*,12*S*)-11,12-Epoxyhenicosa-6,9-diene	*Telenomus euproctidis*	Parasitoid (Hym.: Scelionidae)	*Orgyia postica*	*Hibiscus* spp.	200 µL of 2.5 µg/µL	Sex (female)	44	nd	Arakaki et al., 2011
Mixture of *trans*-Verbenol and Exo-brevicomin	*Thanasimus dubius*	Predator (Col.: Cleridae)	*Dendroctonus ponderosae*	*Pinus resinosa*	150 mg of neat compound	Aggregation (male)	47	nd	Pfammatter et al., 2015
Cyclolavandulyl butyrate	*Anagyrus subalbipe*	Parasitoid (Hym: Encyrtidae)	*Planococcus kraunhia*	Persimmon and apple	10 µL of 0.16 µg/µL	Sex (-)	nd+	nd	Sugawara and Ueno, 2020
α-Terpineol**	*Podisus maculiventris*	Predator (Het: Pentatomidae)	Self	*Solanum tuberosum*	Not quantified	Aggregation (male)	nd$^+$	nd	Aldrich and Cantelo, 1999
Blend of (*E*)-2-octenal and (*E*)-2,7-octadienal	*Orius sauteri*	Predator (Het: Anthocoridae)	Self	grasses	1 mg of neat compound each (1:1 ratio)	Sex (female)	4	nd	Uehara et al., 2019
Blend of (*E*)-2-Hexenal, Benzyl alcohol, (*E*)-2-Hexen-1-ol and Benzaldehyde	*Arma chinensis*	Predator (Hem: Pentatomidae)	Self	Poplar trees	130 µL of neat compound in 5:5:2:1 ratio	Aggregation (male)	100*	nd	Wu et al., 2019

*[a]Orders abbreviated as Dip., Diptera; Hem., Hemiptera; Col., Coleoptera; Neu., Neuroptera; Hym., Hymenoptera; [b]Ratio as directly reported in the studies or expressed from the length of plots displaying the number of insects caught. [c]we included only studies conducted within nearby the two last decades and in which the number of the attracted natural enemy was reported to be significantly greater in pheromone-baited plots than in control plots, and where the attracted natural enemy has been identified to species level. *nearly zero insects caught in the control plots; **live insects producing the pheromone were used in the experiments; nd, not determined; nd+, not determined because there were no control plots in the experiments; self, the natural enemy itself is the pheromone emitter.*

of chemical ecology (Kaplan, 2012a; Kelly et al., 2014; Murali-Baskaran et al., 2018; Peri et al., 2018; Blassioli-Moraes et al., 2019; Peñaflor, 2019). Several questions will have to be addressed in future research to improve kairomone use and thereby to efficiently benefit biological control (Kaplan, 2012b; Gish et al., 2015). Here, we present some strategies and discuss gaps for consideration when designing a kairomone-oriented control strategy for the protection of field crops. This is particularly important because many previous applications of synthetic kairomones have not considered the direct benefits for plants, especially the reduction in pest populations and crop damage, and the increase in crop yields (Hiltpold and Turlings, 2012; Kaplan, 2012a; **Tables 1**, **2** of this review). It is therefore opportune to examine how kairomones have been implemented in the field and assess possible options to optimise their use.

An Optimised Attract and Reward Technique

The attract and reward technique consists of the combined use of an attractant—usually a kairomone—and a reward component such as flowering companion plants (Simpson et al., 2011). The technique has gained considerable attention since it promotes early establishment of natural enemies in crop fields and reduces their subsequent emigration from these cropping sites (Simpson et al., 2011; Parolin et al., 2012). The attract component stimulates the foraging behaviour and maintenance of natural enemies in target crops, whereas the reward component offers alternative foods such as nectar and pollen which supplement the diet of natural enemies. Consumption of nectar, which is source of carbohydrates, has proved to increase the longevity and fecundity of natural enemies (Araj and Wratten, 2015, and references therein). Apart from supplying extra-floral nectars and pollen, the reward component can also offer additional benefits such as shelter and supply of alternative hosts and prey to natural enemies.

The attract and reward combination significantly increases natural enemy populations in crop fields over the application of either of these components alone (Simpson et al., 2011; Salamanca et al., 2018). The combination of MeSA as attractant and buckwheat *Fagopyrum esculentum* Möench (Polygonaceae) as reward plant boosted the abundance of Scelonidae wasps on broccoli plants, and that of Eulophidae wasps on sweetcorn plants (Simpson et al., 2011). Salamanca et al. (2018) reported that the combination of MeSA and coriander plants also led to an increased abundance of the predatory thrips *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Aeolothripidae), predatory bugs *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and hoverflies, *Toxomerus* sp. (Diptera: Syrphidae), on bean plants, but no increase was found in the abundance of earwigs *Doru* sp. (Dermaptera: Forficulidae). However, the spatial arrangement of the attract and reward components influences the abundance of natural enemies and the control level of insect pests. For example, an increased abundance of the predator *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) and a decreased population of the aphid *Aphis citricola* van der Goot (Hemiptera: Aphididae) were noted when MeSA was placed inside and *Calendula officinalis* L. (Asteraceae) reward was placed at the edge of apple tree orchards compared to the opposite arrangement (reward inside and MeSA at the edge) and the control (no MeSA, no reward) (Jaworski et al., 2019). The efficacy of the spatial arrangement supports the design of the push-pull technique which promotes intercropping of companion plants, one placed inside the crop field for repelling herbivores, i.e., "push," and another placed at the edge of the crop field for attracting them, i.e., "pull" (Cook et al., 2007; Khan et al., 2010). Although the push-push itself increases the abundance of natural enemies compared to mono-cropping (Khan et al., 2008; Leslie et al., 2020), the combination of push-pull with the deployment of synthetic kairomone attractive to natural enemies could further increase the natural enemy population to a level required to reduce the insect pest density and crop damage to below economic threshold levels, which could be assessed in future studies.

Apart from increasing the abundance of natural enemies, the combination of attract and reward has a long-lasting effect, whereby more natural enemies can be sustained over time in a crop field with reward than those without reward. Simpson et al. (2011) used MeSA as attractant and buckwheat as reward and recorded an increase in populations of parasitoids; e.g., *Dolichogenidae tasmanica* (Cameron), *Micropiltis demolitor* Wilkinson (both Hymenoptera: Braconidae), *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae), and predators of Tachinidae family in crop fields with the reward compared to those without reward, especially as time elapsed. Jaworski et al. (2019) also recorded more predator *P. japonica* persisting in apple tree orchards bearing MeSA and the reward, *C. officinalis* plant, which resulted in long-term control of the pest *A. citricola* compared to control orchards.

Successful implementation of the attract and reward technique has some limitations, e.g., attraction of non-target organisms. Herbivores and hyper-parasitoids/predators can also be attracted into crop fields because most field-tested HIPVs are commonly emitted volatiles with broad-scale attraction effects, and the reward plants can also serve as habitat for herbivores (Simpson et al., 2011; Orre-Gordon et al., 2013; Sarkar et al., 2018). Methyl salicylate is a common plant volatile compound used to attract natural enemies. However, it was found to be attractive to untargeted insect species such as herbivorous insect pests (e.g., *P. rapae*, *P. xylostella, Scaptomyza flava*) and hyper-parasitoids (e.g., *Baryscapus galactopus* (Ratzeburg) (Hymenoptera: Eulophidae), *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae), and *Diplazon laetatorius* Fab. (Hymenoptera: Ichneumonidae) (Orre-Gordon et al., 2013). Buckwheat is a rewarding plant that is commonly used in an attract and reward technique. However, it was also reported to harbour herbivorous thrips such as *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and aphids (Orre-Gordon et al., 2013; Sarkar et al., 2018). Despite such unwanted effects, the attract and reward combination could potentially enhance conservation biological control through the recruitment of natural enemies and their retention into the vicinity of target crops (Orre-Gordon et al., 2013; Peñaflor and Bento, 2013). Ways to improve its implementation and overcome such negative impacts could include the selection of HIPVs that are attractive to a narrow range of natural enemies but not to their hosts or prey (Ferry et al., 2009), the use of natural enemy pheromone as the attractant (Ruther, 2013), reward plants which are non-hosts to the target insect pest(s) (Sarkar et al., 2018), an appropriate spatial arrangement of the attractant and reward (Jaworski et al., 2019), and the introduction of a herbivore-repellent component into the system (Cook et al., 2007; Xu et al., 2017a; Pålsson, 2019). Future studies are needed to investigate the integration of these tactics for an improved attract and reward technique to enhance biological control in agro-ecosystems.

Incorporating Olfactory Associative Learning to Improve the Efficiency of Foraging Behaviour by Natural Enemies

Olfactory associative learning is a training process that involves conditioning an insect to learn and recognise to associate a particular odour with the presence of a reward (Arthur, 1971; Turlings et al., 1993). This process tends to modify the insect's innate host preference (Weiss, 1997), which is established during larval development (Hopkins, 1917; Barron, 2001) or shortly after adult emergence (Jaenike, 1983; Barron, 2001). It is likely that insect foragers learn and use odour stimuli that increase their chances of finding suitable hosts (Jaenike, 1983; Vet et al., 1995; Scheirs and De Bruyn, 2002). In conditioned insects, olfactory associative learning develops their ability to exhibit a strong response upon perception of the learned odour (Vet and Papaj, 1992; Faber et al., 1999). Therefore, odour learning has emerged as a strategy to enhance foraging success and to consequently minimise interference of environmental background odours (Vet and Papaj, 1992; Arenas et al., 2007).

The use of olfactory associative learning in natural enemies to improve biological control has recently been a focus of great interest, especially by training or conditioning them on kairomones during the mass rearing process before their release in fields [reviewed in Giunti et al. (2015), Kruidhof et al. (2019)]. Exposure of parasitoid *Hyssopus pallidus* (Askew) (Hymenoptera: Eulophidae) larvae to the apple fruit extract odour led to an adult preference for apple fruit extract, whereas unexposed individuals did not show any preference (Gandolf et al., 2003). Unlike naïve individuals of the predator *Anthocoris nemoralis* (Fab.) (Hemiptera: Anthocoridae), individuals that had experience on MeSA-treated prey showed a pronounced preference for the volatile (Drukker et al., 2000). Interestingly, Meiners et al. (2003) showed that parasitoids that had learned to recognise a mixture of compounds could also respond to the individual blend compounds.

The efficiency of olfactory associative learning has also been reported for improving host location behaviour by natural enemies in crop fields. Females of the parasitoids *C. glomerata* and *D. semiclausum* with previous experience on HIPVs from Brassicaceae plants infested by *P. brassicae* and *P. xylostella* successfully oriented towards host-infested plants even in the presence of surrounding non-host and alternative host plant vegetation, whereas unexperienced parasitoids failed to preferentially locate the host-infested plants (Tibor et al., 2007; Kruidhof et al., 2015). Moreover, Hare et al. (1997) reported that the parasitoid *A. melinus* previously trained on *O*-caffeoyltyrosine during rearing achieved greater egg parasitisation on field populations of *A. aurantii* than naïve parasitoids. Similarly, the predators *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae), *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae), and *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) trained on mint oil, a commercial volatile blend predominantly consisting of menthol, menthone, (+)-menthyl acetate, menthofuran, isomenthone, limonene, and 1,8-cineole, consumed more eggs of *Thyrinteina leucoceraea* (Rindge) (Lepidoptera: Geometridae) and *Diatraea saccharalis* (Fab.) (Lepidoptera: Pyralidae) in field plots bearing the mint oil than in control plots (Janssen et al., 2014).

Although olfactory associative learning enhances host finding success by natural enemies, it remains unknown how long the learned odour could persist in the insect's memory. Natural enemy species differ in their intrinsic capacity to conserve information over time (short-term, mid-term, and long-term memories) (Vet and Dicke, 1992; Hoedjes et al., 2011; Mery, 2013). Moreover, it is worth considering to what extent acquired information could be conserved under natural conditions, knowing that environmental variation has been reported as one of the major factors underlying variation in learning and memory retention (Mery, 2013; Smid and Vet, 2016). Future studies should therefore be focused on investigating how to fine-tune odour learning in natural enemies for long-term memory consolidation to the benefit of their foraging success. However, periodic releases of natural enemies trained on kairomones during rearing could provide an efficient control of insect pests in crop fields (Hare et al., 1997; Giunti et al., 2015; Kruidhof et al., 2019).

Applying Volatile Kairomones Based on Field Background Odour of Target Crop

Plant field background odour is one factor that could mask the relevance of kairomone-based lures to natural enemies in agricultural fields (Schröder and Hilker, 2008; Cai et al., 2017; Xu et al., 2017b). Some HIPVs and OIPVs are common in the volatile profiles of host plants, but they are often released in different ratios between plant species. Plants therefore have different potentials for shaping the attraction of natural enemies to field applied synthetic HIPVs/OIPVs. Braasch et al. (2012) evaluated the effects of target crop species on parasitoid attraction to MeSA and found that more hymenopteran parasitoids were attracted to MeSA-baited traps in field soybean plants than in corn plants. Similarly, when (*E*)-β-farnesene and (*E*)-β-caryophyllene were deployed in beet, bean, and wheat crops, predatory hoverflies of the Syrphidae family were significantly attracted to (*E*)-β-caryophyllene-baited traps only in beet crops, and to (*E*)-β-farnesene-baited traps only in bean crops compared to unbaited controls (Heuskin et al., 2012).

Field HIPV-based lures are usually applied in crops which also release the HIPV of interest, and this may increase interference with the successful location of lures by natural enemies. Flint et al. (1979) found that the attraction of the green predatory lacewing, *C. carnea,* to synthetic caryophyllene applied on cotton plants reduced as the plants grew and released the compound, and detection of the lure thereby waned. Caryophyllene was, however, not attractive to *C. carnea* in wheat plants (Dean and Satasook, 1983) for which it is one of the most abundant volatile compounds (Jiménez-Martínez et al., 2004). In such cases, the use of concentrations beyond that of the target HIPV in the plant field background odour or in specific blends could help pinpoint the relevant concentration or blend composition that would best attract natural enemies to kairomone-based lures (Szendrei and Rodriguez-Saona, 2010; Xu et al., 2017b). Field application of a synthetic volatile blend composed of ocimene, limonene, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate attracted tea leafhoppers in autumn when the tea field background odour contained very low quantity of these compounds, yet the blend was unattractive to the insects in summer when the individual compounds were present in much higher concentrations in the background odour (Xu et al., 2017b).

An alternative strategy for reducing this interplay between the kairomone-based lure and volatile emission by the target field crops could be to use an attractant HIPV in crop fields where it is not released or only released in very low quantity to enhance the detection of the lure within the crop background odour. Field traps baited with phenylacetaldehyde attracted 10–100-fold more predator *C. carnea* than unbaited traps when deployed in cherry and peach orchards (Tóth et al., 2006). Interestingly, the volatile profiles of cherry and peach plants contain minor amounts or no phenylacetaldehyde (Najar-Rodriguez et al., 2013; Bandeira-Reidel et al., 2017; Ye et al., 2017; Maatallah et al., 2020). Therefore, unlike caryophyllene, it is likely that the predator *C. carnea* would be attracted to phenylacetaldehyde on cotton and wheat plants of which the headspace volatiles lack this compound (Thompson et al., 1971; Rodriguez-Saona et al., 2001; Jiménez-Martínez et al., 2004; Starr et al., 2015). Two synthetic blends which differed in their benzaldehyde and ethyl benzoate content attracted tea leafhoppers in the laboratory, but the results differed when they were deployed in tea field plantations. The blend that contained ethyl benzoate that was absent in the tea field background odour attracted more tea leafhoppers in field experiments compared to a paraffin oil control solvent (Cai et al., 2017). The blend that contained benzaldehyde, a compound which dominated the tea background odour, was no longer attractive to tea leafhoppers in crop fields (Cai et al., 2017).

Standardising Kairomone-Based Field Implementation Design to Be Able to Draw Generic Conclusions

Kairomones have been validated for the recruitment of natural enemies in crop fields across diverse agro-ecosystems (James and Price, 2004; Tóth et al., 2009; Jones et al., 2016; Cai et al., 2017; Peñaflor, 2019). However, the approaches used in these studies varied, and this may lead to some discrepancies. Although abiotic factors are hardly standardised across landscapes (Almekinders et al., 1995), a number of biotic factors and kairomone implementation practises could be made uniform in studies to strengthen the conclusions drawn from the findings of spatial and temporal studies. There is a need to improve how kairomones have been applied and assessed regarding their effectiveness with the aim of enhancing biological control strategies.

Firstly, there is a need to optimise the concentrations, compositions, and release rates of kairomones under field conditions. Since natural enemies display concentration-dependent responses in nature (James, 2006; Ferry et al., 2007; Kaplan, 2012a), conclusions drawn from studies considering only one concentration of the test kairomone are to be interpreted with caution. Field studies have demonstrated that population levels of attracted natural enemies differed according to the kairomone concentrations (**Tables 1**, **2**). Therefore, it is crucial to test a range of concentrations to better assess the effectiveness of kairomone-based lures and their possible optimisation for field application. Using MeSA-baited yellow sticky cards in vineyards, James (2006) recorded more than 2-fold increase in green predatory lacewing *Chrysopa oculata* Say (Neuroptera: Chrysopidae) populations in plots baited with 99% MeSA compared to plots baited with 10 and 1% MeSA, while both of the latter did not differ from the control. Similarly, field application of different dimethyl disulfide concentrations (0.1, 1, 10, and 100% v/v dimethyl sulphide/paraffin oil) revealed that a 10% dilution caught the highest number of predators, *Aleochara bipustulata* (L.) and *Aleochara bilineata* Gyllenhal (both Coleoptera: Staphilinidae). Whereas more predatory carabid beetles were caught with a 1% concentration compared with a 100% concentration and the control (Ferry et al., 2007). It is also important that future studies focus on the use of odourant mixtures as field attractants for the recruitment of natural enemies. In a simulated model, Chan et al. (2018) showed that odourant mixtures elicit faster olfactory processing responses in insects (e.g., honeybees) and are more reliable for insect olfaction than single odourants. Insects are likely to exploit blend of odourants when foraging in nature (Thomas-Danguin et al., 2014; Conchou et al., 2019). Jones et al. (2011) reported high numbers of the predators *C. nigricornis* and *C. oculata* in apple orchard plots with traps baited with the blend of MeSA and iridodial compared to plots with traps baited with either compound alone. The release rate of kairomone-based lures should also be taken into consideration, as it can provide insight on their diffusion and the frequency of lure replacement during the study period. The solvent used to formulate the lure, the dispenser used to deliver it, and some abiotic factors (e.g., temperature and relative humidity) affect the release rate of kairomone and consequently its long-lasting effect and effectiveness in attracting natural enemies in crop fields (Jones et al., 2011; Heuskin et al., 2012; Kaplan, 2012a; Xu et al., 2017b). James (2006) used a 99% MeSA lure replaced at weekly intervals over a 5-week period and observed that the number of captured *C. oculata* was 5–8-fold higher in MeSA-treated plots than in the controls during the first 3 weeks, but then the captures declined to a low level in the final 2 weeks. Likewise, more predatory ladybeetle and hoverfly adults were recorded on wheat plants when the applied pheromone (*E*)-β-farnesene-based lure was formulated in paraffin oil compared with an alginate bead-based formulation (Xu et al., 2017b). The observed difference was explained by the effect of the experimental conditions whereby the diffusion of (*E*)-β-farnesene from alginate beads was limited by the high relative humidity that prevailed, as reported by Heuskin et al. (2012).

Secondly, there is need for an implementation design that enables a uniform distribution of the attracted natural enemies within the semiochemical-treated field. Studies investigating the effects of semiochemical-based lures have been implemented in diverse crop fields, but little attention has been paid to the distribution of natural enemies within the target field, which is nevertheless a key factor with regard to herbivorous insect pest control (Rodriguez-Saona et al., 2011; Braasch and Kaplan, 2012; Lim and Mainali, 2013; Vidal and Murphy, 2018). A simulated model conducted by Kaplan and Lewis (2014) revealed that the size of the kairomone field had a marked effect on the number and distribution of attracted predators, with more predators attracted to small fields compared to large fields, and with abundance of attracted predators within the target field decreasing with increasing distance from the field edge. In these scenarios, the core of the target field becomes an enemy-free space, and therefore an unprotected zone where herbivore outbreaks could occur. Apart from the field size, the density of kairomone-release points and the distance over which the formulated lure can be effective also affect also the distribution of attracted natural enemies for successful control of insect pests. By tracking the abundance of natural enemies over a gradient of distances from the HIPV lure-releasing point, 90d Predalure (AgBio Inc. Westminstewr, CO, USA), a commercially available MeSA lure, was reported to be active over a small spatial scale of 1.5 to 2.5 m (i.e., increased abundance only in a localised area surrounding the emission source) in soybean and cranberries crop fields (Mallinger et al., 2011; Rodriguez-Saona et al., 2011). A 40d Predalure was active over a broad spatial scale of 8–10 m (i.e., increased abundance both at the immediate emission source as well as in neighbouring areas) in tomato crop fields (Kelly et al., 2014). Similarly, phenylethyl alcohol and an induced maize volatile blend were also active at a broad spatial scale of 8–10 m in soybean and maize crop fields (Ockroy et al., 2001; Braasch and Kaplan, 2012). The bioactive attraction range of a formulated kairomone could generate insight on the experimental field size and the adequate lure density (i.e., number of lure sources per unit area), both of which have a profound effect on the abundance of natural enemies and the insect pest control level. For example, over a transect distance of 36 m from pheromone releasing-points, Lim and Mainali (2013) recorded high abundance of the parasitoids *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) and *Gryon japonicum* (Ashmead) (Hymenoptera: Scelionidae) associated with a high parasitism rate up to 18 m in soybean fields baited with the pheromone [blend of (*E*)-2-hexenyl (*E*)-2-hexenoate and (*E*)-2-hexenyl (*Z*)-3-hexenoate] identified from the host, *Riptortus pedestris* (Fab.) (Hemiptera: Alydidae). Using MeSA in hop yards and vineyards, abundance of predators increased with less lure density (James and Price, 2004), but such relationship may depend on the attracted natural enemy species and family (Gadino et al., 2012). Under field conditions, the odour concentration decreases with increasing distance from its source (Beyaert and Hilker, 2014). Therefore, when designing a kairomone-based lure field experiment, it is also important to create enough space between kairomone and kairomone-free zones to prevent interference of odours and emigration of natural enemies between both zones. Although there is no rule on how to define the space of such interzone (buffer zone), we suggest a minimum of 2-fold the distance over which the kairomone-based lure is bioactive. However, future studies are needed to be able to draw evidence-based recommendations.

Thirdly, when used to enhance conservation biological control strategies, a kairomone should not only show effectiveness in attracting natural enemies, but its application should also lead to reduction in pest populations and crop damage through increased parasitism and predation rates, and to an increase in crop yields (Kaplan, 2012a; Salamanca et al., 2018). This may require assessment of kairomone effectiveness shifting from simply looking at natural enemy catches to direct field observation when possible, or at least a combination of the two methods. The field application of synthetic HIPVs is known to prime HIPV production in neighbouring undamaged plants, hence triggering indirect defences against herbivores (Engelberth et al., 2004; von Mérey et al., 2011). James and Price (2004) reported that canopy shake samples from hop yards resulted in a 7- and 57-fold increase in *Orius tristicolor* (White) (Hemiptera: Anthocoridae) and *Stethorus punctum picipes* (Casey) (Coleoptera: Coccinellidae), respectively, in MeSA-baited compared to unbaited plots. Although it is widely acknowledged that kairomones increase the abundance of natural enemies in crop fields, it is debatable whether their increase leads to a reduction in crop damage by pests, and a subsequent increase in crop yields. In a 2-year study, Wang et al. (2011) recorded an increase in the abundance of predatory lady beetles (*C. septempunctata, H. axyridis,* and *P. japonica*) associated with a reduced density of the grain aphid, *Sitobion avenae* (Fab.) (Hemiptera: Aphididae), and increases in the parasitism rate and wheat yield in MeSA-baited wheat plots compared to unbaited plots. Although, Salamanca et al. (2018) reported an increased abundance of biocontrol agents (*Toxomerus* sp. and *O. insidiosus*) with a reduced spider mite damage to bean plants, the yield of beans was not improved by the application of MeSA compared to control plots. Application of MeSA also did not reduce pest populations or increase strawberry and grapevine yields (Lee, 2010; Simpson et al., 2011). We speculate that, in the latter studies, the population levels of the attracted natural enemies may not have been enough to cause a reduction of crop damage to under the economic threshold level, or the attracted natural enemies may not have been those of the primary insect pests of the target crops. The application of kairomone-based lures may sometimes lead to sex-biased recruitment of natural enemies, whereby one sex (male or female) predominantly responds to the lure, which affects the mating status and may influence natural enemy population dynamics in the target field (Jones et al., 2011; Kaplan, 2012a). One way of dealing with this would be to combine sex-specific attractants to target both sexes of natural enemies. Although the use of semiochemicals has evolved as a novel tool for environmentally friendly pest control, farmers who are the end-users continue to primarily rely on the use of chemical insecticides for crop protection. We suggest that future studies investigate the effectiveness of applying semiochemical-based lures vs. use of chemical insecticides in terms of the reduction of herbivore populations and damage, the improvement of crop yield, and cost-benefit analysis. Above all, the use of semiochemical-based lures offers great potentials for reducing crop damage by pests and improving crop productivity, and the effectiveness of their application could be further improved by taking into account the different aspects discussed earlier in this section.

CONCLUDING REMARKS

Over the last two decades, application of kairomones in crop fields has evolved as a novel approach to enhance biological control strategies through the attraction and retention of natural enemies on crops. To date, effective application measures and more effective lures are needed to increase kairomone implementation and boost its effectiveness under field conditions. The efficiency of kairomone-based lures under field conditions could be improved on the basis of the formulation, concentration, ratio, and release rate of the kairomone. Moreover, kairomone detection by natural enemies could be facilitated by taking into consideration the kairomone concentration and the crop field background odour. The control of insect pests by natural enemies could be further enhanced with the use of kairomone-based lures, the release of odour-based experienced natural enemies, and the combination of kairomone with reward plants set up in an appropriate spatial arrangement. Several field experiments have tested the use of single kairomonal compounds, and it is important that future studies assess the use of compound blends in recruiting natural enemies in crop fields. In addition to the attraction of natural enemies, future studies should be focused on evaluating the reduction of pest populations and crop damage when using kairomones compared to pesticide application, as well as the increase in crop yields, so as to be able to address and take full advantage of kairomone-based field pest control.

AUTHOR CONTRIBUTIONS

PA and ED conceptualised the structure of the review. PA wrote the first draft of the review manuscript. CP, AY, AC, SM, and ED contributed to the development of the review content. All authors provided intellectual inputs, proofread the manuscript, and approved the final version for submission.

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REFERENCES

Aartsma, Y., Cusumano, A., de Bobadilla, F. M., Rusman, Q., Vosteen, V., and Poelman, E. H. (2019). Understanding insect foraging in complex habitats by comparing trophic levels: insights from specialist host-parasitoid-hyperparasitoid systems. *Cur. Opin. Insect Sci.* 32, 54–60. doi: 10.1016/j.cois.2018.11.001

Afsheen, S., Wang, X., Li, R., Zhu, C.-S., and Lou, Y.-G. (2008). Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. *Insect Sci.* 15, 381–397. doi: 10.1111/j.1744-7917.2008.00225.x

Aldrich, J. R., and Cantelo, W. W. (1999). Suppression of colorado potato beetle infestation by pheromone-mediated augmentation of the predatory spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). *Agric. For. Entomol.* 1, 209–217. doi: 10.1046/j.1461-9563.1999.00026.x

Almekinders, C. J. M., Fresco, L. O., and Struik, P. C. (1995). The need to study and manage variation in agroecosystems. *Neth. J. Agric. Sci.* 43, 127–142. doi: 10.18174/njas.v43i2.572

Araj, S. E., and Wratten, S. D. (2015). Comparing existing weeds and commonly used insectary plants as floral resources for a parasitoid. *Biol. Control* 81, 15–20. doi: 10.1016/j.biocontrol.2014.11.003

Arakaki, N., Yamazawa, H., and Wakamura, S. (2011). The egg parasitoid *Telenomus euproctidis* (Hymenoptera: Scelionidae) uses sex pheromone released by immobile female tussock moth *Orgyia postica* (Lepidoptera: Lymantriidae) as kairomone. *Appl. Entomol. Zool.* 46, 195–200. doi: 10.1007/s13355-011-0031-4

Arenas, A., Fernández, V. M., and Farina, W. M. (2007). Floral odor learning within the hive affects honeybees' foraging decisions. *Naturwissenschaften* 94, 218–222. doi: 10.1007/s00114-006-0176-0

Arthur, A. P. (1971). Associative learning by *Nemeritis canescens* (Hymenoptera: Ichneumonidae). *Can. Entomol.* 103, 1137–1141. doi: 10.4039/Ent10 31137-8

Atema, J. (1996). Eddy chemotaxis and odor landscapes: exploration of nature with animal sensors. *Biol. Bull.* 191, 129–138. doi: 10.2307/1543074

Aukema, B. H., and Raffa, K. F. (2005). Selective manipulation of predators using pheromones: responses to frontalin and ipsdienol pheromone components of bark beetles in the Great Lakes Region. *Agric. For. Entomol.* 7, 193–200. doi: 10.1111/j.1461-9555.2005.00250.x

Bale, J. S., van Lenteren, J. C., and Bigler, F. (2008). Biological control and sustainable food production. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 761–776. doi: 10.1098/rstb.2007.2182

Bandeira-Reidel, R. V., Cioni, P. L., and Pistelli, L. (2017). Volatile emission of different plant parts and fruit development from Italian cherry plums

(*Prunus cerasifera* and *P. cerasifera* “*Pissardii*”). *Biochem. Syst. Ecol.* 75, 10–17. doi: 10.1016/j.bse.2017.10.001

Barron, A. B. (2001). The life and death of hopkins’ host-selection principle. *J. Insect Behav.* 14, 725–737. doi: 10.1023/A:1013033332535

Beddington, J. R., Free, C. A., and Lawton, J. H. (1978). Characteristics of successful natural enemies in models of biological control of insect pests. *Nature* 273, 513–519. doi: 10.1038/273513a0

Bénédet, F., Leroy, T., Gauthier, N., Thibaudeau, C., Thibout, E., and Renault, S. (2002). Gustatory sensilla sensitive to protein kairomones trigger host acceptance by an endoparasitoid. *Proc. R. Soc. Lond. B Biol. Sci.* 269, 1879–1886. doi: 10.1098/rspb.2002.2077

Beyaert, I., and Hilker, M. (2014). Plant odour plumes as mediators of plant-insect interactions. *Biol. Rev.* 89, 68–81. doi: 10.1111/brv.12043

Bichang’a, G., Da Lage, J.-L., Capdevielle-Dulac, C., Zivy, M., Balliau, T., Sambai, K., et al. (2018). α-Amylase mediates host acceptance in the braconid parasitoid *Cotesia flavipes*. *J. Chem. Ecol.* 44, 1030–1039. doi: 10.1007/s10886-018-1002-9

Blassioli-Moraes, M. C., Laumann, R. A., Michereff, M. F. F., and Borges, M. (2019). “Semiochemicals for integrated pest management,” in *Sustainable Agrochemistry*, ed S. Vaz Jr (Cham: Springer Nature Switzerland), 85–112. doi: 10.1007/978-3-030-17891-8_3

Boo, K. S., and Yang, J. P. (2000). Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *J. Chem. Ecol.* 26, 359–375. doi: 10.1023/A:1005453220792

Braasch, J., and Kaplan, I. (2012). Over what distance are plant volatiles bioactive? Estimating the spatial dimensions of attraction in an arthropod assemblage. *Entomol. Exp. Appl.* 145, 115–123. doi: 10.1111/j.1570-7458.2012.01317.x

Braasch, J., Wimp, G. M., and Kaplan, I. (2012). Testing for phytochemical synergism: arthropod community responses to induced plant volatile blends across crops. *J. Chem. Ecol.* 38, 1264–1275. doi: 10.1007/s10886-012-0202-y

Branco, M., Franco, J. C., Dunkelblum, E., Assael, F., Protasov, A., Ofer, D., et al. (2006). A common mode of attraction of larvae and adults of insect predators to the sex pheromone of their prey (Hemiptera: Matsucoccidae). *Bull. Entomol. Res.* 96, 179–185. doi: 10.1079/BER2005415

Bruce, T. J. A., and Pickett, J. A. (2011). Perception of plant volatile blends by herbivorous insects - finding the right mix. *Phytochemistry* 72, 1605–1611. doi: 10.1016/j.phytochem.2011.04.011

Büchel, K., Malskies, S., Mayer, M., Fenning, T. F., Gershenzon, J., Hilker, M., et al. (2011). How plants give early herbivore alert: volatile terpenoids attract parasitoids to egg-infested elms. *Basic Appl. Ecol.* 12, 403–412. doi: 10.1016/j.baae.2011.06.002

Cabello, T., Rodriguez-Manzaneque, M. A., and Gallego, J. R. (2017). Can the pheromones of predators modulate responses to herbivore-induced plant volatiles? *Ann. Appl. Biol.* 170, 369–378. doi: 10.1111/aab.12341

Cai, X., Bian, L., Xu, X., Luo, Z., Li, Z., and Chen, Z. (2017). Field background odour should be taken into account when formulating a pest attractant based on plant volatiles. *Sci. Rep.* 7, 1–10. doi: 10.1038/srep41818

Calatayud, P.-A., Auger, J., Thibout, E., Rousset, S., Caicedo, A. M., Calatayud, S., et al. (2001). Identification and synthesis of a kairomone mediating host location by two parasitoids species of the casava mealybug *Phenacoccus herreni*. *J. Chem. Ecol.* 27, 2203–2217. doi: 10.1023/A:1012274703197

Chan, K. H., Hersperger, F., Marachlian, E., Smith, B. H., Locatelli, F., Szyszka, P., et al. (2018). Odorant mixtures elicit less variable and faster responses than pure odorants. *PLoS Comput. Biol.* 14:e1006536. doi: 10.1371/journal.pcbi.1006536

Colazza, S., Aquila, G., De Pasquale, C., Peri, E., and Millar, J. G. (2007). The egg parasitoid *Trissolcus basalis* uses n-nonadecane, a cuticular hydrocarbon from its stink bug host *Nezara viridula*, to discriminate between female and male hosts. *J. Chem. Ecol.* 33, 1405–1420. doi: 10.1007/s10886-007-9300-7

Colazza, S., and Wanjberg, E. (2013). “Chemical ecology of insect parasitoids: towards a new area,” in *Chemical Ecology of Insect Parasitoids,* eds E. Wanjberg and S. Colazza (Wiley-Blackwell: John Wiley & Sons, Ltd), 1–8. doi: 10.1002/9781118409589.ch1

Collier, T., and van Steenwyk, R. (2004). A critical evaluation of augmentative biological control. *Biol. Control* 32, 245–256. doi: 10.1016/j.biocontrol.2004.05.001

Conchou, L., Lucas, P., Meslin, C., Proffit, M., Staudt, M., and Renou, M. (2019). Insect odorscapes: from plant volatiles to natural olfactory scenes. *Front. Physiol.* 10:972. doi: 10.3389/fphys.2019.00972

Cook, S. M., Khan, Z. R., and Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annu. Rev. Entomol.* 52, 375–400. doi: 10.1146/annurev.ento.52.110405.091407

D’Alessandro, M., and Turlings, T. C. J. (2006). Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *Analyst* 131, 24–32. doi: 10.1039/B507589K

Danner, H., Desurmont, G. A., Cristescu, S. M., and van Dam, N. M. (2018). Herbivore-induced plant volatiles accurately predict history of coexistence, diet breadth, and feeding mode of herbivores. *New Phytol.* 220, 726–738. doi: 10.1111/nph.14428

Dean, G. J., and Satasook, C. (1983). Response of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to some potential attractants. *Bull. Entomol. Res.* 73, 619–624. doi: 10.1017/S0007485300009226

De-Lange, E. S. D., Laplanche, D., Guo, H., Xu, W., Vlimant, M., Erb, M., et al. (2020). *Spodoptera frugiperda* caterpillars suppress herbivore-induced volatile emissions in maize. *J. Chem. Ecol.* 46, 344–360. doi: 10.1007/s10886-020-01153-x

De-Moraes, C. M., Lewis, W. J., Paré, P. W., Alborn, H. T., and Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature* 393, 570–573. doi: 10.1038/31219

Dicke, M., and Bruin, J. (2001). Chemical information transfer between plants: back to the future. *Biochem. Syst. Ecol.* 29, 981–994. doi: 10.1016/S0305-1978(01)00045-X

Dicke, M., and Sabelis, M. W. (1988). Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.* 2, 131–139. doi: 10.2307/2389687

Dicke, M., and van Loon, J. J. A. (2000). Multitrophic effects of herbivore-induced plant volatile in an evolutionary context. *Entomol. Exp. Appl.* 97, 237–249. doi: 10.1046/j.1570-7458.2000.00736.x

Drukker, B., Bruin, J., and Sabelis, M. W. (2000). Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiol. Entomol.* 25, 260–265. doi: 10.1046/j.1365-3032.2000.00190.x

Eilenberg, J., Hajek, A., and Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl* 46, 387–400. doi: 10.1023/A:1014193329979

El-Sayed, A. M., Knight, A. L., Basoalto, E., and Suckling, D. M. (2018). Caterpillar-induced plant volatiles attract conspecific herbivores and a generalist predator. *J. Appl. Entomol.* 142, 495–503. doi: 10.1111/jen.12495

Engelberth, J., Alborn, H. T., Schmelz, E. A., and Tumlinson, J. H. (2004). Airborne signals prime plants against insect herbivore attack. *PNAS* 101, 1781–1785. doi: 10.1073/pnas.0308037100

Erb, M., Foresti, N., and Turlings, T. C. J. (2010). A tritrophic signal that attracts parasitoids to host-damaged plants withstands disruption by non-host herbivores. *BMC Plant Biol.* 10:247. doi: 10.1186/1471-2229-10-247

Faber, T., Joerges, J., and Menzel, R. (1999). Associative learning modifies neural representations of odors in the insect brain. *Nat. Neurosci.* 2, 74–78. doi: 10.1038/4576

Fatouros, N. E., Dicke, M., Mumm, R., Meiners, T., and Hilker, M. (2008). Foraging behavior of egg parasitoids exploiting chemical information. *Behav. Ecol.* 19, 677–689. doi: 10.1093/beheco/arn011

Fellowes, M. D. E., van Alphen, J. J. M., and Jervis, M. A. (2007). “Foraging behaviour,” in *Insects as Natural Enemies: A Pratical Perspective*, ed M. A. Jervis (Dordrecht: Springer), 1–71. doi: 10.1007/978-1-4020-2625-6_1

Felton, G. W., Chung, S. H., Hernandez, M. G. E., Louis, J., Peiffer, M., and Tian, D. (2014). “Herbivore oral secretions are the first line of protection against plant-induced defences,” in *Annual Plant Reviews, Insect Plant-Interactions*, eds C. Voelckel and G. Jander (Oxford: Wiley), 37–76. doi: 10.1002/9781119312994.apr0506

Ferry, A., Dugravot, S., Delattre, T., Bagnères, A.-G., Poinsot, D., and Cortesero, A. M. (2007). Identification of a widespread monomolecular odor differentially attractive to several delia radicum ground-dwelling predators in the field. *J. Chem. Ecol.* 33, 2064–2077. doi: 10.1007/s10886-007-9373-3

Ferry, A., Tron, L. E., Dugravot, S., and Cortesero, A. M. (2009). Field evaluation of the combined deterrent and attractive effects of dimethyl disulfide on *Delia radicum* and its natural enemies. *Biol. Control* 49, 219–226. doi: 10.1016/j.biocontrol.2009.01.013

Flint, H. M., Salter, S. S., and Walters, S. (1979). Caryophyllene: an attractant for the green lacewing. *Environ. Entomol.* 8, 1123–1125. doi: 10.1093/ee/8.6.1123

Frost, C. J., Mescher, M. C., Carlson, J. E., and De Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146, 818–824. doi: 10.1104/pp.107.113027

Gadino, A. N., Walton, V. M., and Lee, J. C. (2012). Evaluation of methyl salicylate lures on populations of *Typhlodromus pyri* (Acari: Phytoseiidae) and other natural enemies in Western Oregon vineyards. *Biol. Control* 63, 48–55. doi: 10.1016/j.biocontrol.2012.06.006

Gandolf, M., Mattiacci, L., and Dorn, S. (2003). Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Royal Soc.* 270, 2623–2629. doi: 10.1098/rspb.2003.2541

Gay, H. (2012). Before and after silent spring : from chemical pesticides to biological control and integrated pest management — Britain, 1945–1980. *Ambix* 59, 88–108. doi: 10.1179/174582312X13345259995930

Giacometti, R., Barneto, J., Barriga, L. G., Pedro, M. S., Balestrasse, K., Andrade, A. M., et al. (2016). Early perception of stink bug damage in developing seeds of field-grown soybean induces chemical defences and reduces bug attack. *Pest Manag. Sci.* 72, 1585–1594. doi: 10.1002/ps.4192

Gish, M., De-Moraes, C. M., and Mescher, M. C. (2015). Herbivore-induced plant volatiles in natural and agricultural ecosystems: open questions and future prospects. *Cur. Opin. Insect Sci.* 9, 1–6. doi: 10.1016/j.cois.2015.04.001

Giunti, G., Canale, A., Messing, R. H., Donati, E., Stefanini, C., Michaud, J. P., et al. (2015). Parasitoid learning : current knowledge and implications for biological control. *Biol. Control.* 90, 208–219. doi: 10.1016/j.biocontrol.2015.06.007

Gomes-Lagôa, A. C., Blassioli-Moraes, M. C., Borges, M., and Laumann, R. A. (2019). Selective responses of *Trissolcus basalis* and *Telenomus podisi* to chemical footprints of preferred hosts. *Physiol. Entomol.* 45, 60–71. doi: 10.1111/phen.12316

Gripenberg, S., Mayhew, P. J., Parnell, M., and Roslin, T. (2010). A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol. Lett.* 13, 383–393. doi: 10.1111/j.1461-0248.2009.01433.x

Gross, H. R., Lewis, W. J., Jones, R. L., and Nordlund, D. A. (1975). Kairomones and their use for management of entomophagous insects: III. stimulation of *Trichogramma achaeae*, *T. pretiosum*, and *Microplitis croceipes* with host-seeking stimuli at time of release to improve their efficiency. *J. Chem. Ecol.* 1, 431–438. doi: 10.1007/BF00988584

Gunton, R. M., and Pöyry, J. (2016). Scale-specific spatial density dependence in parasitoids: a multi-factor meta-analysis. *Funct. Ecol.* 30, 1501–1510. doi: 10.1111/1365-2435.12627

Gurr, G. M., Wratten, S. D., and Barbosa, P. (2000). "Success in conservation biological control of arthropods," In *Measures of Success in Biological Control*, eds G. Gurr and S. Wratten (Dordrecht: Kluwer Academic Publishers), 105–132. doi: 10.1007/978-94-011-4014-0_4

Halitschke, R., Stenberg, J. A., Kessler, D., Kessler, A., and Baldwin, I. T. (2008). Shared signals - 'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecol. Lett.* 11, 24–34. doi: 10.1111/j.1461-0248.2007.01123.x

Hare, J. D., Morgan, D. J. W., and Nguyun, T. (1997). Increased parasitization in the field after exposing its parasitoid, *Aphytis melinus*, to a synthetic kairomone. *Entomol. Exp. Appl.* 82, 73–81. doi: 10.1046/j.1570-7458.1997.00115.x

Heil, M., and Ton, J. (2008). Long-distance signalling in plant defence. *Trends Plant Sci.* 13, 264–272. doi: 10.1016/j.tplants.2008.03.005

Heimpel, G. E., and Asplen, M. K. (2011). A 'Goldilocks' hypothesis for dispersal of biological control agents. *BioControl* 56, 441–450. doi: 10.1007/s10526-011-9381-7

Heuskin, S., Lorge, S., Lognay, G., Wathelet, J.-P., Bera, F., Leroy, P., et al. (2012). A semiochemical slow-release formulation in a biological control approach to attract hoverflies. *J. Environ. Ecol.* 3, 72–85. doi: 10.5296/jee.v3i1.1725

Hilker, M., and Fatouros, N. E. (2015). Plant responses to insect egg deposition. *Annu. Rev. Entomol.* 60, 493–515. doi: 10.1146/annurev-ento-010814-020620

Hiltpold, I., and Turlings, T. C. J. (2012). Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *J. Chem. Ecol.* 38, 641–650. doi: 10.1007/s10886-012-0131-9

Hoedjes, K. M., Kruidhof, H. M., Huigens, M. E., Dicke, M., Vet, L. E. M., and Smid, H. M. (2011). Natural variation in learning rate and memory dynamics in parasitoid wasps : opportunities for converging ecology and neuroscience. *Proc. Royal Soc. B: Biol. Sci.* 278, 889–897. doi: 10.1098/rspb.2010.2199

Hopkins, A. D. (1917). A discussion of H. G. Hewitt's paper on insect behaviour. *J. Econ. Entomol.* 10, 92–93.

Ingrao, A. J., Walters, J., and Szendrei, Z. (2019). Biological control of *Asparagus* pests using synthetic herbivore-induced volatiles. *Environ. Entomol.* 48, 202–210. doi: 10.1093/ee/nvy171

Jaenike, J. (1978). On optimal oviposition behavior in phytophagous insects. *Theoretical Popul. Biol.* 14, 350–356. doi: 10.1016/0040-5809(78)90012-6

Jaenike, J. (1983). Induction of host preference in *Drosophila melanogaster*. *Oecologia* 58, 320–325. doi: 10.1007/BF00385230

James, D. G. (2003a). Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29, 1601–1609. doi: 10.1023/a:1024270713493

James, D. G. (2003b). Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environ. Entomol.* 32, 977–982. doi: 10.1603/0046-225X-32.5.977

James, D. G. (2006). Methyl salicylate is a field attractant for the goldeneyed lacewing, *Chrysopa oculata*. *Biocontrol Sci. Technol.* 16, 107–110. doi: 10.1080/09583150500188510

James, D. G., and Price, T. S. (2004). Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *J. Chem. Ecol.* 30, 1613–1628. doi: 10.1023/B:JOEC.0000042072.18151.6f

Janssen, A., Fonseca, J. O., Colares, F., Silva, L., Pedrosa, A. R. P., Lima, E. R., et al. (2014). Time scales of associating food and odor by predator communities in the field. *Behav. Ecol.* 25, 1123–1130. doi: 10.1093/beheco/aru094

Jaworski, C. C., Xiao, D., Xu, Q., Ramirez-Romero, R., Guo, X., Wang, S., et al. (2019). varying the spatial arrangement of synthetic herbivore-induced plant volatiles and companion plants to improve conservation biological control. *J. Appl. Ecol.* 56, 1176–1788. doi: 10.1111/1365-2664.13353

Jiménez-Martínez, E. S., Bosque-Pérez, N. A., Berger, P. H., Zemetra, R. S., Ding, H., and Eigenbrode, S. D. (2004). Volatile cues influence the response of rhopalosiphum padi (Homoptera: Aphididae) to barley yellow dwarf virus–infected transgenic and untransformed wheat. *J. Chem. Ecol.* 33, 1207–1216. doi: 10.1603/0046-225X-33.5.1207

Jing, T., Du, W., Gao, T., Wu, Y., Zhang, N., Zhao, M. J., et al. (2020). Herbivore-induced DMNT catalyzed by CYP82D47 plays an important role in the induction of JA-dependent herbivore resistance of neighboring tea plants. *Plant Cell Environ.* 44, 1178–1191. doi: 10.1111/pce.13861

Jones, V. P., Horton, D. R., Mills, N. J., Unruh, T. R., Baker, C. C., Melton, T. D., et al. (2016). Evaluating plant volatiles for monitoring natural enemies in apple, pear and walnut orchards. *Biol. Control.* 102, 53–65. doi: 10.1016/j.biocontrol.2015.03.009

Jones, V. P., Steffan, S. A., Wiman, N. G., Horton, D. R., Miliczky, E., Zhang, Q.-H., et al. (2011). Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards. *Biol. Control* 56, 98–105. doi: 10.1016/j.biocontrol.2010.10.001

Kaiser, L., Ode, P., van Nouhuys, S., Calatayud, P. A., Colazza, S., Cortesero, A. M., et al. (2017). "The plant as a habitat for entomophagous insects," in *Insect-Plant Interactions in a Crop Protection Perspective. Advances in Botanical Research*, eds N. Sauvion, P-A. Calatayud, and D. Thiéry (Londres: Elsevier Ltd.), 179–223. doi: 10.1016/bs.abr.2016.09.006

Kaplan, I. (2012a). Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? *Biol. Control.* 60, 77–89. doi: 10.1016/j.biocontrol.2011.10.017

Kaplan, I. (2012b). Trophic complexity and the adaptive value of damage-induced plant volatiles. *PLoS Biol.* 10:e1001437. doi: 10.1371/journal.pbio.1001437

Kaplan, I., and Lewis, D. (2014). What happens when crops are turned on? Simulating constitutive volatiles for tritrophic pest suppression across an agricultural landscape. *Pest Manag. Sci.* 71, 139–150. doi: 10.1002/ps.3779

Kelly, J. L., Hagler, J. R., and Kaplan, I. (2014). Semiochemical lures reduce emigration and enhance pest control services in open-field predator augmentation. *Biol. Control* 71, 70–77. doi: 10.1016/j.biocontrol.2014.01.010

Kessler, A., and Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291, 2141–2244. doi: 10.1126/science.291.5511.2141

Khan, Z. R., James, D. G., Midega, C. A. O., and Pickett, J. A. (2008). Chemical ecology and conservation biological control. *Biol. Control* 45, 210–224. doi: 10.1016/j.biocontrol.2007.11.009

Khan, Z. R., Midega, C. A. O., Bruce, T. J. A., Hooper, A. M., and Pickett, J. A. (2010). Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. *J. Exp. Bot.* 61, 4185–4196. doi: 10.1093/jxb/erq229

Kopf, A., Rank, N. E., Roininen, H., and Tahvanainen, J. (1997). Defensive larval secretions of leaf beetles attract a specialist predator parasyrphus nigritarsis. *Ecol. Entomol.* 22, 176–183. doi: 10.1046/j.1365-2311.1997.t01-1-00061.x

Kpongbe, H., van den Berg, J., Khamis, F., Tam,ò, M., and Torto, B. (2019). Isopentyl butanoate : aggregation pheromone of the brown spiny bug, *Clavigralla tomentosicollis* (Hemiptera: Coreidae), and kairomone for the egg parasitoid *Gryon* sp. (Hymenoptera: Scelionidae). *J. Chem. Ecol.* 45, 570–578. doi: 10.1007/s10886-019-01081-5

Kramer, D. L. (2001). "Foraging behavior," in *Evolutionary Ecology, Concepts and Cases Studies*, eds C. W. Fox, D. A. Roff, and D. J. Fairbairn (Oxford: Oxford University Press), 232–246. doi: 10.1093/oso/9780195131543.003.0024

Krost, C. (2008). "Chemical communication," in *Encyclopedia of Ecology*, eds S. E. Jorgensen and B. D. Fath (Oxford: Elsevier), 557–575. doi: 10.1016/B978-008045405-4.00036-7

Kruidhof, H. M., Kostenko, O., Smid, H. M., and Vet, L. E. M. (2019). Integrating parasitoid olfactory conditioning in augmentative biological control: potential impact, possibilities, and challenges. *Front. Ecol. Evol.* 7:84. doi: 10.3389/fevo.2019.00084

Kruidhof, H. M., Roberts, A. L., Magdaraog, P., Gols, D. M. R., Vet, L. E. M., Hoffmeister, T. S., et al. (2015). Habitat complexity reduces parasitoid foraging efficiency, but does not prevent orientation towards learned host plant odours. *Oecologia* 179, 353–361. doi: 10.1007/s00442-015-3346-y

Krupke, C. H., and Brunner, J. F. (2003). Parasitoids of the consperse stink bug (Hemiptera: Pentatomidae) in North Central Washington and attractiveness of a host-produced pheromone component. *J. Entomol. Sci.* 38, 84–92. doi: 10.18474/0749-8004-38.1.84

Lee, J. C. (2010). Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *J. Chem. Ecol.* 39, 653–660. doi: 10.1603/EN09279

Leroy, P. D., Heuskin, S., Sabri, A., Verheggen, F. J., Farmakidis, J., Lognay, G., et al. (2012). Honeydew volatile emission acts as a kairomonal message for the Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *Insect Sci.* 19, 498–506. doi: 10.1111/j.1744-7917.2011.01467.x

Leslie, A. W., Hamby, K. A., McCluen, S. R., and Hooks, C. R. R. (2020). Evaluating a push-pull tactic for management of *Epilachna varivestis* mulsant and enhancement of beneficial arthropods in *Phaseolus lunatus* L. *Ecol. Eng.* 7:105660. doi: 10.1016/j.ecoleng.2019.105660

Lewis, W. J., Jones, R. L., Nordlund, D. A., and Sparks, A. N. (1975). Kairomones and their use for management of entomophagous insects: I. evaluation for increasing rates of parasitization by *Trichogramma* spp. in the field. *J. Chem. Ecol.* 1, 343–347. doi: 10.1007/BF00988836

Lim, U. T., and Ben-Yakir, D. (2020). Visual sensory systems of predatory and parasitic arthropods. *Biocontrol Sci. Technol.* 30, 728–739. doi: 10.1080/09583157.2020.1752362

Lim, U. T., and Mainali, B. P. (2013). Effect of aggregation pheromone trap of *Riptortus pedestris* (Hemiptera: Alydidae) on the distribution and composition of its egg parasitoids. *Biol. Microb. Control* 106, 1973–1978. doi: 10.1603/EC13154

Lynch, L. D., and Thomas, M. B. (2000). Nontarget effects in the biocontrol of insects with insects, nematodes and microbial agents: the evidence. *Biocontrol News Inform.* 21, 117N−130N.

Maatallah, S., Dabbou, S., Castagna, A., Guizani, M., Hajlaoui, H., Ranieri, A. M., et al. (2020). *Prunus persica* by-products: a source of minerals, phenols and volatile compounds. *Sci. Hortic.* 261:109016. doi: 10.1016/j.scienta.2019.109016

Maeda, T., Kishimoto, H., Wright, L. C., and James, D. G. (2015). Mixture of synthetic herbivore-induced plant volatiles attracts more stethorus punctum picipes (Casey) (Coleoptera: Coccinellidae) than a single volatile. *J. Insect Behav.* 28, 126–137. doi: 10.1007/s10905-015-9489-1

Magalhães, D. M., da Silva, I. T. F. A., Borges, M., Laumann, R. A., and Blassioli-Moraes, M. C. (2019). *Anthonomus grandis* aggregation pheromone induces cotton indirect defence and attracts the parasitic wasp *Bracon vulgaris*. *J. Exp. Bot.* 70, 1891–1901. doi: 10.1093/jxb/erz040

Mallinger, R. E., Hogg, D. B., and Gratton, C. (2011). Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *J. Econ. Entomol.* 104, 115–124. doi: 10.1603/EC10253

Mbaluto, C. M., Ayelo, P. M., Duffy, A. G., Erdei, A. L., Tallon, A. K., Xia, S., et al. (2020). Insect chemical ecology: chemically mediated interactions and novel applications in agriculture. *Arthropod-Plant Interact.* 14, 671–684. doi: 10.1007/s11829-020-09791-4

McCormick, A. C., Irmisch, S., Reinecke, A., Boeckler, G. A., Veit, D., Reichelt, M., et al. (2014). Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. *Plant Cell Environ.* 37, 1909–1923. doi: 10.1111/pce.12287

McCormick, A. C., Unsicker, S. B., and Gershenzon, J. (2012). The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends Plant Sci.* 17, 303–310. doi: 10.1016/j.tplants.2012.03.012

Meiners, T., Wäckers, F., and Lewis, W. J. (2003). Associative learning of complex odours in parasitoid host location. *Chem. Senses* 28, 231–236. doi: 10.1093/chemse/28.3.231

Mendel, Z., Assael, F., and Dunkelblum, E. (2004). Kairomonal attraction of predatory bugs (Heteroptera: Anthocoridae) and brown lacewings (Neuroptera: Hemerobiidae) to sex pheromones of *Matsucoccus* species (Hemiptera: Matsucoccidae). *Biol. Control* 30, 134–440. doi: 10.1016/j.biocontrol.2003.06.001

Mery, F. (2013). Natural variation in learning and memory. *Cur. Opin. Neurobiol.* 23, 52–56. doi: 10.1016/j.conb.2012.09.001

Mills, N. J., and Heimpel, G. E. (2018). Could increased understanding of foraging behavior help to predict the success of biological control? *Cur. Opin. Insect Sci.* 27, 26–31. doi: 10.1016/j.cois.2018.02.013

Mills, N. J., and Wajnberg, E. (2008). "Optimal foraging behavior and efficient biological control methods," in *Behavioral Ecology of Insect Parasitoids: From Theoretical Approaches to Field Applications,* eds E. Wanjberg, C. Bernstein, and J. J. M. van Alphen (Blackwell: Oxford), 3–30. doi: 10.1002/9780470696200.ch1

Moreno, D. S., and Luck, R. F. (1992). Augmentative releases of *Aphytis melinus* (Hymenoptera: Aphelinidae) to suppress california red scale (Homoptera: Diaspididae) in Southern California lemon orchards. *J. Econ. Entomol.* 85, 1112–1119. doi: 10.1093/jee/85.4.1112

Mumm, R., and Dicke, M. (2010). Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Can. J. Zool.* 88, 628–667. doi: 10.1139/Z10-032

Murali-Baskaran, R. K., Chander, K., Kaushal, P., Kumar, J., Parthiban, P., Senthil-Nathan, S., et al. (2018). Role of kairomone in biological control of crop pests - a review. *Physiol. Mol. Plant Pathol.* 101, 3–15. doi: 10.1016/j.pmpp.2017.07.004

Najar-Rodriguez, A., Orschel, B., and Dorn, S. (2013). Season-long volatile emissions from peach and pear trees *in situ*, overlapping profiles, and olfactory attraction of an oligophagous fruit moth in the laboratory. *J. Chem. Ecol.* 39, 418–429. doi: 10.1007/s10886-013-0262-7

Ockroy, M. L. B., Turlings, T. C. J., Edwards, P. J., Fritzsche-hoballah, M. E., Ambrosetti, L., Bassetti, P., et al. (2001). Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). *Agric. For. Entomol.* 3, 201–209. doi: 10.1046/j.1461-9555.2001.00107.x

Orre-Gordon, G. U. S., Wratten, S. D., Jonsson, J., Simpson, M., and Hale, R. (2013). 'Attract and Reward': combining a herbivore-induced plant volatile with floral resource supplementation - multi-trophic level effects. *Biol. Control* 64, 106–115. doi: 10.1016/j.biocontrol.2012.10.003

Pålsson, J. (2019). *Attract, Reward and Disrupt: Combining Habitat Manipulation and Semiochemicals to Enhance Pest Control in Apple Orchards*. [PhD Thesis]: Swedish University of Agricultural Sciences.

Pappas, M. L., Steppuhn, A., Geuss, D., Topalidou, N., Zografou, A., Sabelis, M. W., et al. (2015). Beyond predation: the zoophytophagous predator Macrolophus pygmaeus induces tomato resistance against spider mites. *PLoS ONE* 10:e0127251. doi: 10.1371/journal.pone.0127251

Parolin, P., Bresch, C., Desneux, N., Brun, R., Bout, A., Boll, R., et al. (2012). Secondary plants used in biological control: a review. *Int. J. Pest Manag.* 58, 91–100. doi: 10.1080/09670874.2012.659229

Peñaflor, M. F. G. V. (2019). "Use of semiochemical-based strategies to enhance biological control," in *Natural Enemies of Insect Pests in Neotropical Agroecosystems: Biological Control and Functional Biodiversity*, eds B. Souza, L. L. Vazquez, and R. C. Marucci (Cham: Springer Nature Switzerland), 479–488. doi: 10.1007/978-3-030-24733-1_41

Peñaflor, M. F. G. V., and Bento, J. M. S. (2013). Herbivore-induced plant volatiles to enhance biological control in agriculture. *Neotrop. Entomol.* 42, 331–343. doi: 10.1007/s13744-013-0147-z

Pérez-Hedo, M., Urbaneja-Bernat, P., Jaques, J. A., Flors, V., and Urbaneja, A. (2015). Defensive plant responses induced by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants. *J. Pest Sci.* 88, 543–554. doi: 10.1007/s10340-014-0640-0

Peri, E., Frati, F., Salerno, G., Conti, E., and Colazza, S. (2013). Host chemical footprints induce host sex discrimination ability in egg parasitoids. *PLoS ONE* 8:e79054. doi: 10.1371/journal.pone.0079054

Peri, E., Moujahed, R., Wajnberg, E., and Colazza, S. (2018). "Applied chemical ecology to enhance insect parasitoid efficacy in the biological control of crop pests," in *Chemical Ecology of Insects: Applications and Associations with Plants and Microbes*, ed J. Tabata (London; New York, NY: Taylor & Francis), 234–267. doi: 10.1201/9781351228398-10

Pervez, A., and Yadav, M. (2018). Foraging behaviour of predaceous ladybird beetles: a review. *Eur. J. Environ. Sci.* 8, 102–108. doi: 10.14712/23361964.2018.14

Pfammatter, J. A., Krause, A., and Raffa, K. F. (2015). Evaluating predators and competitors in wisconsin red pine forests for attraction to mountain pine beetle pheromones for anticipatory biological control. *J. Chem. Ecol.* 44, 1161–1171. doi: 10.1093/ee/nvv091

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., and Weis, A. E. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11, 41–65. doi: 10.1146/annurev.es.11.110180.000353

Pszczolkowski, M. A., and Johnson, D. T. (2011). Isopropanol attracts the green lacewing, *Chrysopa quadripunctata* (Neuroptera: Chrysopidae). *Biocontrol Sci. Techn.* 21, 47–50. doi: 10.1080/09583157.2010.524294

Randlkofer, B., Obermaier, E., Hilker, M., and Meiners, T. (2010). Vegetation complexity-the influence of plant species diversity and plant structures on plant chemical complexity and arthropods. *Basic Appl. Ecol.* 11, 383–395. doi: 10.1016/j.baae.2010.03.003

Reddy, G. V. P., Holopainen, J. K., and Guerrero, A. (2002). Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *J. Chem. Ecol.* 28, 131–143. doi: 10.1023/a:1013519003944

Rim, H., Uefune, M., Ozawa, R., and Takabayashi, J. (2018). An omnivorous arthropod, *Nesidiocoris tenuis*, induces gender-specific plant volatiles to which conspecific males and females respond differently. *Arthropod-Plant Interact.* 12, 495–503. doi: 10.1007/s11829-018-9612-2

Rodriguez-Saona, C., Crafts-Brandner, S. J., and Canas, L. A. (2003). Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *J. Chem. Ecol.* 29, 2539–2550. doi: 10.1023/A:1026314102866

Rodriguez-Saona, C., Crafts-Brandner, S. J., Paré, P. W., and Henneberry, T. J. (2001). Exogenous methyl jasmonate induces volatile emissions in cotton plants. *J. Chem. Ecol.* 27, 679–695. doi: 10.1023/A:1010393700918

Rodriguez-Saona, C., Kaplan, I., Braasch, J., Chinnasamy, D., and Williams, L. (2011). Field responses of predaceous arthropods to methyl salicylate: a meta-analysis and case study in cranberries. *Biol. Control* 59, 294–303. doi: 10.1016/j.biocontrol.2011.06.017

Rodriguez-Saona, C., and Stelinski, L. L. (2009). "Behavior-modifying strategies in IPM: theory and practice," in *Integrated Pest Management: Innovation and Development*, ed A. K. D. R. Peshin (New York, NY: Springer), 261–312. doi: 10.1007/978-1-4020-8992-3_11

Ruther, J. (2013). "Novel insights into pheromone-mediated communication in parasitic hymenopterans," in *Recent Advances in Chemical Ecology of Insect Parasitoids*, eds E. Wajnberg and S. Colazza (Wiley-Blackwell: John Wiley and Sons, Ltd.), 112–144. doi: 10.1002/9781118409589.ch6

Rutledge, C. E., Silk, P. J., and Mayo, P. (2014). Use of contact chemical cues in prey discrimination by *Cerceris fumipennis*. *Entomol. Exp. Appl.* 153, 93–105. doi: 10.1111/eea.12233

Salamanca, J., Souza, B., and Rodriguez-Saona, C. (2018). Cascading effects of combining synthetic herbivore-induced plant volatiles with companion plants to manipulate natural enemies in agro-ecosystem. *Pest Manag. Sci.* 74, 2133–2145. doi: 10.1002/ps.4910

Sarkar, S. C., Wang, E., Wu, S., and Lei, Z. (2018). Application of trap cropping as companion plants for the management of agricultural pests: a review. *Insects* 9:128. doi: 10.3390/insects9040128

Sarmento, R. A., Lemos, F., Bleeker, P. M., Schuurink, R. C., Pallini, A., Olivera, A. M. G., et al. (2011). A herbivore that manipulates plant defence. *Ecol. Lett.* 14, 229–236. doi: 10.1111/j.1461-0248.2010.01575.x

Schausberger, P. (2018). Herbivore-associated bacteria as potential mediators and modifiers of induced plant defense against spider mites and thrips. *Front. Plant Sci.* 9:1107. doi: 10.3389/fpls.2018.01107

Scheirs, J., and De Bruyn, L. (2002). Integrating optimal foraging and optimal oviposition theory in plant–insect research. *Oikos* 96, 187–191. doi: 10.1034/j.1600-0706.2002.960121.x

Schröder, R., and Hilker, M. (2008). The relevance of background odor in resource location by insects: a behavioral approach. *BioScience* 58, 308–316. doi: 10.1641/B580406

Shiojiri, K., Ozawa, R., Kugimiya, S., Uefune, M., van Wijk, M., Sabelis, M. W., et al. (2010). Herbivore-specific, density-dependent induction of plant volatiles: honest or 'cry wolf' signals? *PLoS One* 5:e121614. doi: 10.1371/journal.pone.0012161

Shiojiri, K., Takabayashi, J., and Yano, S. (2001). Infochemically mediated tritrophic interaction webs on cabbage plants. *Popul. Ecol.* 43, 23–29. doi: 10.1007/PL00012011

Silva, D. B., Bueno, V. H. P., van Loon, J. J. A., Peñaflor, M. F. G. V., Bento, J. M. S., and van Lenteren, J. C. (2018). Attraction of three mirid predators to tomato infested by both the tomato leaf mining moth tuta absoluta and the whitefly *Bemisia tabaci*. *J. Chem. Ecol.* 44, 29–39. doi: 10.1007/s10886-017-0909-x

Silva, D. B., Weldegergis, B. T., van Loon, J. J. A., and Bueno, V. H. P. (2017). Qualitative and quantitative differences in herbivore-induced plant volatile blends from tomato plants infested by either *Tuta absoluta* or *Bemisia tabaci*. *J. Chem. Ecol.* 43, 53–65. doi: 10.1007/s10886-016-0807-7

Simpson, M., Gurr, G. M., Simmons, A. T., Wratten, S. D., James, D. G., Leeson, G., et al. (2011). Attract and reward: combining chemical ecology and habitat manipulation to enhance biological control in field crops. *J. Appl. Ecol.* 48, 580–590. doi: 10.1111/j.1365-2664.2010.01946.x

Smid, H. M., and Vet, L. E. M. (2016). The complexity of learning, memory and neural processes in an evolutionary ecological context. *Cur. Opin. Insect Sci.* 15, 61–69. doi: 10.1016/j.cois.2016.03.008

Starr, G., Petersen, M. A., Jespersen, B. M., and Hansen, A. S. (2015). Variation of volatile compounds among wheat varieties and landraces. *Food Chem.* 174, 527–237. doi: 10.1016/j.foodchem.2014.11.077

Stelinski, L. L., Willett, D., Rivera, M. J., and Ali, J. G. (2019). "Tuning" communication among four trophic levels of the root biome to facilitate biological control. *Biol. Control* 131, 49–53. doi: 10.1016/j.biocontrol.2019.01.006

Sugawara, Y., and Ueno, T. (2020). Field evaluation of CLB as an attractant for beneficial parasitoid wasps attacking mealybug pests. *J. Entomol. Zool. Stud.* 8, 192–196. doi: 10.22271/j.ento

Svensson, G. P., Larsson, M. C., and Hedin, J. (2004). Attraction of the larval predator *Elater ferrugineus* to the sex-pheromone of its prey, *Osmoderma eremita*, and its implication for conservation biology. *J. Chem. Ecol.* 30, 353–363. doi: 10.1023/B:JOEC.0000017982.51642.8c

Symonds, M. R. E., and Gitau-Clarke, C. W. (2016). "The evolution of aggregation pheromone diversity in bark beetles," In *Advances in Insect Physiology*, eds C. Tittiger and G. J. Blomquist (Amsterdam: Elsevier Ltd.), 195–234. doi: 10.1016/bs.aiip.2015.12.003

Szendrei, Z., and Rodriguez-Saona, C. (2010). A meta-analysis of insect pest behavioral manipulation with plant volatiles. *Entomol. Exp. Appl.* 134, 201–210. doi: 10.1111/j.1570-7458.2009.00954.x

Takabayashi, J., Sabelis, M. W., Janssen, A., Shiojiri, K., and van Wijk, M. (2006). Can plants betray the presence of multiple herbivore species to predators and parasitoids? The role of learning in phytochemical information networks. *Ecol. Res.* 21, 3–8. doi: 10.1007/s11284-005-0129-7

Thomas-Danguin, T., Sinding, C., Romagny, S., Mountassir, F. E., Atanasova, B., Le Berre, E., et al. (2014). The perception of odor objects in everyday life: a review on the processing of odor mixtures. *Front. Physiol.* 5:504. doi: 10.3389/fpsyg.2014.00504

Thompson, A. C., Baker, D. N., Gueldner, R. C., and Hedin, P. A. (1971). Identification and quantitative analysis of the volatile substances emitted by maturing cotton in the field. *Plant Physiol.* 48, 50–52. doi: 10.1104/pp.48.1.50

Tibor, B., Gols, R., Hemerik, L., van Lenteren, J. C., and Vet, L. E. M. (2007). Time allocation of a parasitoid foraging in heterogeneous vegetation: implications for host–parasitoid interactions. *J. Anim. Ecol.* 76, 845–853. doi: 10.1111/j.1365-2656.2007.01259.x

Tittiger, C., and Blomquist, G. J. (2016). Pheromone production in pine bark beetles. *Adv. Insect Physiol.* 50, 235–263. doi: 10.1016/bs.aiip.2016.02.002

Tóth, M., Bozsik, A., Szentkirályi, F., Letardi, A., Tabilio, M. R., Verdinelli, M., et al. (2006). Phenylacetaldehyde: a chemical attractant for common green lacewings (*Chrysoperla carnea* s.l., Neuroptera: Chrysopidae). *Eur. J. Entomol.* 103, 267–271. doi: 10.14411/eje.2006.033

Tóth, M., Szentkirályi, F., Vuts, J., Letardi, A., Tabilio, R. M., Jaastad, G., et al. (2009). Optimization of a phenylacetaldehyde-based attractant for common green optimization of a phenylacetaldehyde-based attractant for common green lacewings (*Chrysoperla carnea,* s.l.). *J. Chem. Ecol.* 35, 449–458. doi: 10.1007/s10886-009-9614-8

Turlings, T. C. J., and Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annu. Rev. Entomol.* 63, 433–452. doi: 10.1146/annurev-ento-020117-043507

Turlings, T. C. J., Wackers, F. L., Vet, L. E. M., Lewis, W. J., and Tumlinson, J. H. (1993). "Learning of host-finding cues by hymenopterous parasitoids," in *Insect Learning*, ed D. R. Papaj (Dordrecht: Springer Science), 51–78. doi: 10.1007/978-1-4615-2814-2_3

Uehara, T., Maeda, T., Shimoda, M., Fujiwara-Tsujii, N., and Yasui, H. (2019). Identification and characterization of the pheromones in the minute pirate bug *Orius sauteri* (Heteroptera: Anthocoridae). *J. Chem. Ecol.* 45, 811–817. doi: 10.1007/s10886-019-01104-1

Vaello, T., Casas, J. L., Pineda, A., De Alfonso, I., and Marcos-García, M. Á. (2017). Olfactory response of the predatory bug *Orius laevigatus* (Hemiptera: Anthocoridae) to the aggregation pheromone of its prey, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Environ. Entomol.* 46, 1115–1119. doi: 10.1093/ee/nvx141

van Lenteren, J. C. (2000). "Success in biological control of arthropods by augmentation of natural enemies," in *Biological Control: Measures of Success*, eds G. Gurr and S. Wratten (Dordrecht: Kluwer Academic Publishers), 77–103. doi: 10.1007/978-94-011-4014-0_3

van Wijk, M., De Bruijn, P. J. A., and Sabelis, M. W. (2011). Complex odor from plants under attack : herbivore's enemies react to the whole, not its parts. *PLoS ONE* 6:e21742. doi: 10.1371/journal.pone.0021742

Vet, L. E. M., and Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37, 141–172. doi: 10.1146/annurev.en.37.010192.001041

Vet, L. E. M., Lewis, W. J., and Carde, R. T. (1995). "Parasitoid foraging and learning," in *Chemical Ecology of Insect 2*, ed R. T. Carde (New York, NY: Springer), 65–101. doi: 10.1007/978-1-4615-1765-8_3

Vet, L. E. M., and Papaj, D. R. (1992). Effects of experience on parasitoid movement in odour plumes. *Physiol. Entomol.* 17, 90–96. doi: 10.1111/j.1365-3032.1992.tb00994.x

Vidal, M. C., and Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecol. Lett.* 21, 138–150. doi: 10.1111/ele.12874

Vinson, S. B. (1998). The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control* 11, 79–96. doi: 10.1006/bcon.1997.0601

von Mérey, G., Veyrat, N., Mahuku, G., Valdez, L. R., Turlings, T. C. J., and D'Alessandro, M. (2011). Dispensing synthetic green leaf volatiles in maize fields increases the release of sesquiterpenes by the plants, but has little effect on the attraction of pest and beneficial insects. *Phytochemistry* 72, 1838–1847. doi: 10.1016/j.phytochem.2011.04.022

Vos, M., Berrocal, S. M., Karamaouna, F., Hemerik, L., and Vet, L. E. M. (2001). Plant-mediated indirect effects and the persistence of parasitoid-herbiore communities. *Ecol. Lett.* 4, 38–45. doi: 10.1046/j.1461-0248.2001.00191.x

Vosteen, I., Weisser, W. W., and Kunert, G. (2016). Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? *Ecol. Entomol.* 41, 1–12. doi: 10.1111/een.12271

Walling, L. L. (2008). Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiol.* 146, 859–866. doi: 10.1104/pp.107.113142

Wang, G., Cui, L. L., Dong, J., Francis, F., Liu, Y., and Tooker, J. (2011). Combining intercropping with semiochemical releases: optimization of alternative control of *Sitobion avenae* in wheat crops in China. *Entomol. Exp. Appl.* 140, 189–195. doi: 10.1111/j.1570-7458.2011.01150.x

Watanabe, H., Yano, E., Higashida, K., Hasegawa, S., and Ozawa, R. (2016). An attractant of the aphidophagous gall midge *Aphidoletes aphidimyza* from honeydew of *Aphis gossypii*. *J. Chem. Ecol.* 42, 149–155. doi: 10.1007/s10886-016-0666-2

Wei, J., Kang, L., Wei, J., and Kang, L. (2011). Roles of (Z)-3-hexenol in plant-insect interactions. *Plant Signal. Behav.* 6, 369–371. doi: 10.4161/psb.6.3.14452

Wei, J.-R., Lu, X.-P., and Jiang, L. (2013). Monoterpenes from larval frass of two cerambycids as chemical cues for a parasitoid, *Dastarcus helophoroides*. *J. Insect Sci.* 13:59. doi: 10.1673/031.013.5901

Wei, J.-R., Yang, Z.-Q., Hao, H.-L., and Du, J.-W. (2008). (R)-(+)-Limonene, kairomone for *Dastarcus helophoroides*, a natural enemy of longhorned beetles. *Agric. For. Entomol.* 10, 323–330. doi: 10.1111/j.1461-9563.2008.00384.x

Weiss, M. R. (1997). Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Anim. Behav.* 53, 1043–1052. doi: 10.1006/anbe.1996.0357

Williams, I. I. I., L., Rodriguez-Saona, C., Castle, S. C., and Zhu, S.u. (2008). EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *J. Chem. Ecol.* 34, 1190–1201. doi: 10.1007/s10886-008-9520-5

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.* 25, 443–466. doi: 10.1146/annurev.es.25.110194.002303

Wu, H., Coudron, T. A., Zhang, L., Aldrich, J. R., Xu, W., Xu, J., et al. (2019). Identification and field verification of aggregation-sex pheromone from the predaceous bug, *Arma chinensis*. *Chemoecology* 29, 235–245. doi: 10.1007/s00049-019-00292-2

Xiu, C., Dai, W., Pan, H., Zhang, W., Luo, S., Wyckhuys, K. A. G., et al. (2019c). Herbivore-induced plant volatiles enhance field-level parasitism of the mirid bug *Apolygus lucorum*. *Biol. Control* 135, 41–47. doi: 10.1016/j.biocontrol.2019.05.004

Xiu, C., Xu, B., Pan, H., Zhang, W., Yan, Y., and Lu, Y. (2019b). Volatiles from *Sophora japonica* flowers attract *Harmonia axyridis* adults (Coleoptera: Coccinellidae). *J. Integ. Agric.* 18, 873–883. doi: 10.1016/S2095-3119(18)61927-6

Xiu, C., Zhang, W., Xu, B., Wyckhuys, K. A. G., Cai, X., and Lu, Y. (2019a). Volatiles from aphid-infested plants attract adults of the multicolored asian lady beetle *Harmonia axyridis*. *Biol. Control* 129, 1–11. doi: 10.1016/j.biocontrol.2018.11.008

Xu, Q., Hatt, S., Lopes, T., Zhang, Y., Bodson, B., Chen, J., et al. (2017a). A push – pull strategy to control aphids combines intercropping with semiochemical releases. *J. Pest Sci.* 91, 93–103 doi: 10.1007/s10340-017-0888-2

Xu, X., Cai, X., Bian, L., Luo, Z., Li, Z., and Chen, Z. (2017b). Does background odor in tea gardens mask attractants? Screening and application of attractants for *Empoasca onukii* matsuda. *J. Econ. Entomol.* 110, 2357–2363. doi: 10.1093/jee/tox269

Yang, J.-N., Wei, J.-N., and Kang, L. (in press). Feeding of pea leafminer larvae simultaneously activates jasmonic and salicylic acid pathways in plants to release a terpenoid for indirect defense. *Insect Sci.* 1–43. doi: 10.1111/1744-7917.12820

Ye, L., Yang, C., Li, W., Hao, J., Sun, M., Zhang, J., et al. (2017). Evaluation of volatile compounds from chinese dwarf cherry (*Cerasus humilis* (Bge.) Sok.) germplasms by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Food Chem.* 217, 389–397. doi: 10.1016/j.foodchem.2016.08.122

Yoon, C., Seo, D.-K., Yang, J.-O., Kang, S.-H., and Kim, G.-H. (2010). Attraction of the predator, harmonia axyridis (Coleoptera: Coccinellidae), to its prey, *Myzus persicae* (Hemiptera: Aphididae), feeding on chinese cabbage. *J. Asia-Pac. Entomol.* 13, 255–260. doi: 10.1016/j.aspen.2010.06.009

Yu, H., Khashaveh, A., Li, Y., Li, X., and Zhang, Y. (2017). Field trapping of predaceous insects with synthetic herbivore-induced plant volatiles in cotton fields. *Environ. Entomol.* 47, 114–120. doi: 10.1093/ee/nvx201

Yu, H., Zhang, Y., Wu, K., Gao, X. W., and Guo, Y. Y. (2008). Field-testing of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Chem. Ecol.* 37, 1410–1415. doi: 10.1603/0046-225X-37.6.1410

Zhang, P.-J., Li, W.-D., Huang, F., Zhang, J.-M., Xu, F.-C., and Lu, Y.-B. (2013). Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid aignaling. *J. Chem. Ecol.* 39, 612–619. doi: 10.1007/s10886-013-0283-2

Zhu, J., and Park, K. C. (2005). Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. *J. Chem. Ecol.* 31, 1733–1746. doi: 10.1007/s10886-005-5923-8

Zhu, P., Lu, Z., Heong, K., Chen, G., Zheng, X., Xu, H., et al. (2014). Selection of nectar plants for use in ecological engineering to promote biological control of rice pests by the predatory bug, *Cyrtorhinus lividipennis* (Heteroptera: Miridae). *PLoS ONE* 9:e108669. doi: 10.1371/journal.pone.0108669

Zverava, E. L., and Rank, N. E. (2004). Fly parasitoid *Megaselia opacicornis* uses defensive secretions of the leaf beetle *Chrysomela lapponica* to locate its host. *Oecologia* 140, 516–522. doi: 10.1007/s00442-004-1602-7

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CHEMOECOLOGY

REVIEW

Prospects for repellent in pest control: current developments and future challenges

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Abstract The overall interest in environmentally safe pest control methods and the rise of insecticide resistance in pest populations have prompted medical and agricultural entomology research on insect repellents in recent years. However, conducting research on repellent is challenging for several reasons: (1) the different repellent phenomena are not well defined; (2) it is difficult to test for and quantify repellent; (3) the physiological mechanisms are poorly known; (4) the field efficacy appears to be highly variable. Here, we identified five different types of repellent: expellency, irritancy, deterrency, odor masking and visual masking, and described behavioral bioassays to differentiate between them. Although these categories are currently defined by their behavioral response to different stimuli, we suggest new definitions based on their mechanism of action. We put forward three main hypotheses on the physiological mechanism: (1) a dose effect that modifies the behavior, (2) a repellent mechanism with specific receptors, or (3) inhibition of the transduction of neural information.

Keywords Deterrent · Antifeeding · Odorant receptor · Olfaction · Gustation · DEET · Pest management · Vector control

Introduction

The plant kingdom produces several hundred thousand chemical substances, which affect insect behavior (Fraenkel 1959). For instance, their secondary compounds are involved in the recognition and acceptability of plants by insects. The reception of host cues encompasses a wide variety of stimuli (olfactory, visual, tactile and gustatory) (Thorsteinson 1960), and host selection is believed to proceed in a hierarchical manner. The first step, 'choice', involves seeking and recognition of the host using olfactory and visual cues. It generally takes place over a distance. The second step, 'selection', is the acceptability of the host on the basis of gustatory cues (Visser 1988) and it generally takes place upon contact. A similar hierarchical sequence operates for insects feeding on human or animal hosts.

The overall interest of environmentally safe pest control methods and the increasing number of insecticide-resistant pest populations have recently prompted research on insect repellents in medical (including veterinary) and agricultural entomology. Hence, for the past decade, research on insect repellent increased steadily, with nearly 250 articles published in 2015 on this topic (154 on insect repellents, 42 on insect antifeedants and 52 on insect deterrents) according to the ISI 'Web of Knowledge'.

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A repellent is currently defined as a behavioral response to a stimulus (Miller et al. 2009) and the differences between behavioral responses can be subjective. Historically, the word 'repellent' has indeed been used to characterize many different phenomena. In addition, comparisons of results from different studies worldwide are difficult due to the variability in the methods used to assess repellent, i.e., authors use different apparatuses and/or conditions, variables and parameters.

The word repellent derives from the Latin verb *repellere*, meaning 'to reject'. Hence, strictly speaking, a substance should only be considered as a repellent when it causes an organism to make oriented movements away from its source (Nordlund 1981). In this review, we use a broader definition of repellent, i.e., a phenomenon that prevents a pest's ability to track, locate and/or recognize its host. Hence, a repellent phenomenon can be a movement away from an odor source, but also an inability to find the host. A mechanism that nullifies the attraction of an insect to an odor source should also be considered as a repellent (Ramirez et al. 2012).

Using this broad definition, we can identify five types of repellent on the basis of the observed insect behavior: (1) true repellent (also called expellent, spatial repellent), which corresponds to an oriented movement of the insect away from an odor source without direct contact, (2) odor masking (also called attraction inhibition), which is either a reduction in the attractiveness of the host or a disruption of the localization of the host by the odor cue, (3) contact irritancy (also called landing inhibition or excito-repellent), an oriented movement of the insect away from a chemical after direct contact, (4) deterrence (also called antifeeding, suppressant, anorexigenic and anti-appetant), which corresponds to a disruption of feeding activity by contact with or ingestion of a chemical and (5) visual masking, which defines a reduction in the attractiveness of the host or a disruption of the localization of the host by a visual cue (Fig. 1).

Here, we review these repellent phenomena by giving each a precise definition and illustrating this with experimental findings. Then, after summarizing the olfaction and gustatory pathways, we discuss the potential action mechanisms of repellents. To illustrate the potential of repellent in insect management to protect humans or plants, we review two examples of well-known strategies, i.e., the use of impregnated bed nets for disease control and the push–pull strategy for crop pest management.

Repellent phenomena

True repellent: expellent

A true repellent—sometimes named expellent in medical entomology (Achee et al. 2012)—is a substance that causes oriented movement away from the odor source (Matthews and Matthews 1978; Bernier et al. 2007; Nerio et al. 2010). One example is the aphid alarm pheromone—including (E)-β-farnesene which is the main, if not only, component in most species—which induces the dispersion of the aphids (Montgomery and Nault 1977). Movement away from the odor source can be innate or acquired by experience (White 2007). In pest management, the aim of a true repellent is to create an odor barrier to prevent an arthropod from entering a space occupied by a potential host, as a 'safe zone' to reduce encounters between the insect and the host (Brown and Hebert 1997). In the case of a pathogen vector, the probability of pathogen transmission could be reduced or even eliminated (Achee et al. 2012).

True repellent should be tested: (1) using a bioassay that prevents contact between the insect and the stimulus and (2) in the absence of the host, because it can have a masking effect and/or disturb the tested insect. The repellent can be studied with a tubular olfactometer oriented vertically or horizontally with flowing or still air (Deletre et al. 2013; Deletre et al. 2015; Abtew et al. 2015). A new tubular olfactometer system was recently designed by Steck et al. (2012) for assaying *Drosophila melanogaster*. Five tubes were aligned horizontally with one insect inside. Using air flow, the insect was exposed to repeated odor pulses. Its position was visually tracked: repellent odors evoke decreased activity followed by downwind movement, whereas attractive odor elicits directed upwind movement. Using this system, Steck et al. (2012) showed that benzaldehyde, a well-known repellent compound, elicited a true repellent response. Conversely, most other aversive compounds elicited neither attraction nor aversion. The four-arm olfactometer, first designed by Pettersson (1970), can also be used to study attractive or repellent odor. One arm delivers the test odor and the three other arms are used as controls (Pettersson 1970; Vet et al. 1983; Abdullah et al. 2014). The same scheme can be used for testing a repellent (Bruce et al. 2015). It is hard to study a repellent with a Y olfactometer, because the insect will not go all the way to the choice zone if the compound is a true repellent. To get around this issue, we can either put the insect directly in the choice area or use a T-maze (Stensmyr et al. 2012). For example, Grieco et al. (2005) put *Aedes aegypti* mosquitoes in the central part of a cylinder divided into three parts. The mosquitoes chose between a treated (repellent odor) and a non-treated chamber. Video recording technology (e.g., Ethovision® software from Noldus) facilitates data collection in behavioral assays for the repellent. This bioassay is now recommended by the World Health Organization (WHO 2013).

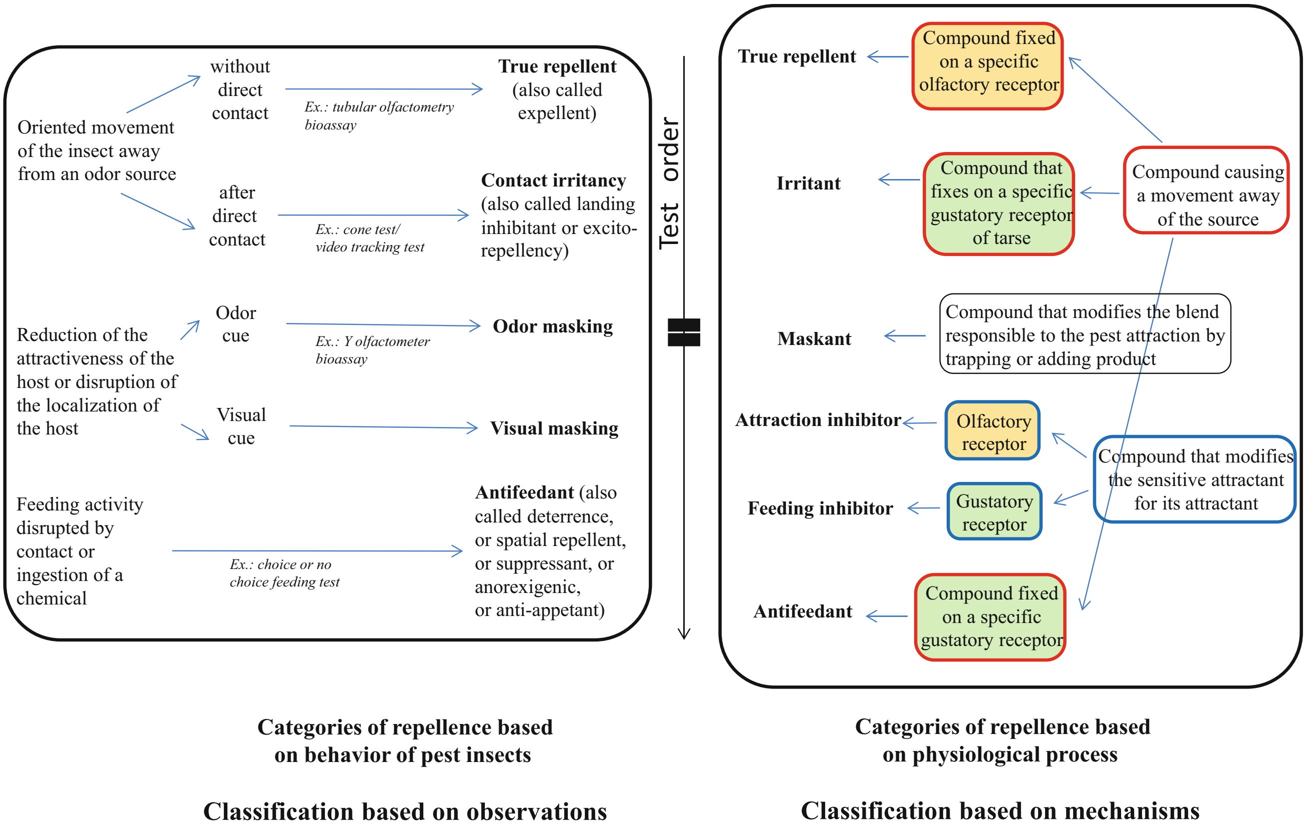


Fig. 1 From the repellent definitions based on behavioral responses to definitions based on their neural mechanism. The test order as a function of the behavioral response enables to determine the properties of a candidate repellent product

Odor masking: attraction inhibition

A masking odor interferes with the host detection/localization or decreases the attractiveness of the host (Nolen et al. 2002; Bernier et al. 2007; WHO 2013). Such compounds are therefore not repellent alone. One example is the N,N-diethyl-3-methylbenzamide (DEET) which inhibits the lactic acid attraction of *Aedes aegypti* mosquitoes (Dogan et al. 1999). Masking odors can also decrease the host attractiveness by changing the host chemical, thereby impeding an insect's host-seeking activity. For instance, DEET can reduce 3-octenol release, hence changing the chemical host profile and disrupting host-seeking behavior (Syed and Leal 2008). In pest management, the aim of an odor-masking agent is to hide the host from the insect pest.

The masking effect should be evaluated: (1) using a bioassay that prevents contact between the insect and the stimulus and (2) with the host volatile. Y and four-arm olfactometers can be used to assess masking effects (Togni et al. 2010). For the Y-tube, one arm contains the host—animal or plant—attractant odor and the other contains both the host attractant odor and the tested product. This test is currently recommended by WHO (2013). For the four-arm olfactometer, all the arms contain the host odor but one arm also contains the repellent odor (Irmisch et al. 2014). If the product seems to be a maskant, the best is to test the product alone—i.e., in the absence of the host—to eliminate a potential expellent property.

Irritability (excito-repellent, landing inhibition, contact disengagement)

An irritant causes movement away from the stimulus after the insect has physically contacted it (Grieco et al. 2005; Miller et al. 2009). In medical entomology, this behavior is also called landing inhibition (WHO 2013) or excito-repellent for the pyrethroids, because they increase mosquito activity after contact (White 2007). In pest management, the aim of an irritant product is to break the physical association between insects and hosts, hence reducing the probability of feeding and the risk of pathogen transmission (Achee et al. 2012).

By definition, the irritant effect must be tested using an experimental arena: (1) allowing contact between the insect and the tested product and (2) in the absence of the host. For crawling insects, Petri dish bioassays are common (Jilani and Saxena 1990). A filter paper is divided into two equal areas, one with the test stimulus and the other with solvent only. The insects are released at the center of the Petri dish, and the number of insects found in the two areas and/or the time spent on each area is compared (Nilsson and Bengtsson 2004; Wang et al. 2006; Martin et al. 2013). Because a stimulus could be toxic, the challenge is to work at a sub-lethal concentration (Schrek 1977). For flying insects, such as mosquitoes, a plastic cone where a treated material is placed at the base of the cone is used. After a short acclimation time, the mosquito lands on the treated material and then the time before takeoff is recorded. Another method involves the use of a cylinder divided into two parts: a chamber lined with a treated paper and another chamber lined with a non-treated paper separated by a butterfly valve (Grieco et al. 2005; Deletre et al. 2013). The mosquitoes are placed in the treated chamber, the valve is opened and the number of mosquitoes in the non-treated chamber is recorded 10 min later to characterize the irritant effect. This should be compared to the effect with a grid avoiding contact with treated paper using the same apparatus to differentiate between compounds having only irritant effect and compounds with irritant and expellent effect.

Antifeeding (suppressant, deterrent, anorexigenic, attraction antagonist, feeding inhibitor)

An antifeedant product prevents, interrupts or otherwise disrupts feeding behavior (Koul 2008). This change in feeding behavior either occurs after the insect contacts the antifeedant or as postgustatory effects of the antifeedant. Here, we focus on the first cause. Contrary to an irritant, an antifeedant may not cause the insect to move away from the product, but only inhibit its feeding behavior. A suppressant inhibits the initiation of feeding, a deterrent impedes the continuation of this behavior and an anorexigenic causes a loss of appetite (Warthen and Morgan 1990). For example, the tobacco hornworm, *Manduca sexta*, approached the deterrent *Grindella*-treated diet but rejected it within 6 s of initiating biting (Glendinning et al. 1998). In medical entomology, a product interfering with feeding behavior is called a feeding inhibitor (WHO 2013), a substance that interrupts the blood-feeding process is an attraction antagonist (Bernier et al. 2007) and the term deterrent is used in a broad context, i.e., prevention of mosquitoes from bite or even entering a house (White 2007). In pest management, antifeedants reduce or stop feeding behavior (Foster and Harris 1997).

To test the antifeeding effect, the insect must be (1) in contact with the test compound and (2) the host simultaneously. For such studies, it is advisable to use both choice and no-choice assays, because data are complementary and often provide a clearer view of the response. For example, Blaney et al. (1990) showed that compounds recorded as active antifeedants in the choice bioassay were not always as active in the no-choice test. Response variables measured in antifeeding studies include the amount of diet consumed (Abdelgaleil et al. 2002), weight gain of insects, duration of feeding activity (Cameron et al. 2016) and behavioral reflexes (Amrein and Thorne 2005).

Visual masking

A compound or an object can modify the shape or color of a crop or hide the host, hence ensuring a visual masking effect. In pest management, the aim is to disrupt host plant recognition. The visual stimulant may be used as an attractant stimulant combined with insecticides or glue to trap them. For example, blue and black traps are used to control or survey cattle flies, but it is possible that some colors could be repelling (Gibson and Torr 1999). It seems difficult to change the visual properties of a plant to decrease its attractiveness but, for example, gibberellic acid has been used to keep grapefruit green, which is less attractive than yellow fruit to fruit flies (Foster and Harris 1997). The use of UV-absorbing plastics as greenhouse covers may also reduce the spread of insect-borne virus diseases (Raviv and Antignus 2004). This is because these UV-absorbing plastics modify insect behavior: (1) in a high UV reflectance environment, anthophilous thrips are repelled from the surface of attractive colors, (2) in the whitefly, *B. tabaci,* adult dispersion was hindered with UV light filtration and (3) in an UV-deficient environment, the flight activity of the aphid, *Myzus persicae*, was reduced. Moreover, colored shade netting may be effective against insect pests: whiteflies landed on yellow nets but did not penetrate to reach the plant, and thrips were less likely to penetrate through blue and yellow nets (Weintraub 2009). Finally, maize is often used to dissimulate some crops to their pests and can therefore be considered as a visual masking plant (Smith and McSorley 2000). Studies of visual effects should take care to eliminate or otherwise control for effects of olfactory cues.

Potential action mechanisms

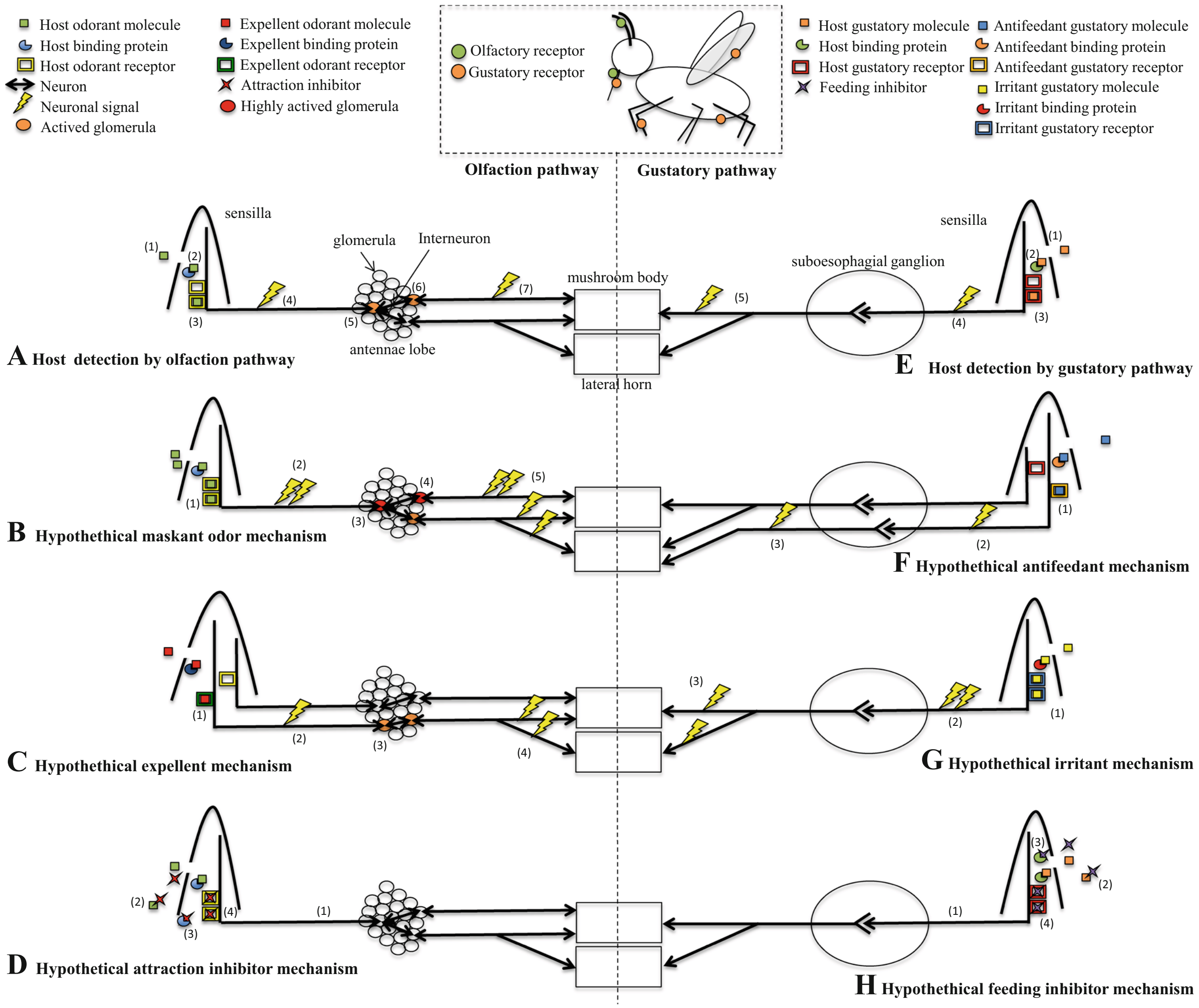
The sources of potential repellent products are diverse (Foster and Harris 1997), but can be classified into three categories: (1) plant sources, (2) insect sources and (3) synthetic compounds. Repellents can originate from plants for several reasons. First, several plants have specialized organs to repel enemies: the nettle *Urtica* has trichomes, lemon possesses oil vacuoles in the peel, mint has extracellular glands and pine has resin canals (Hossaert-McKey and Bagnères-Urbany 2012). Plants also emit volatile organic compounds to attract pollinators, predators and parasitoids or to repel pests, e.g., tobacco plants release herbivore-induced volatiles that are repellent for *Heliothis virescens* female moths (De Moraes et al. 2001; Kessler and Baldwin 2001; Unsicker et al. 2009). These induced secondary metabolites of plants can be antidigestive and antinutritive proteins (Baldwin et al. 2001). Finally, the essential oils of the major plant families (Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae) may be irritants, repellents, antifeedants, or maskants (Regnault-Roger 1997; Tawatsin et al. 2001; Isman 2006; Moore et al. 2007a; Regnault-Roger et al. 2012; Nerio et al. 2010). Insects are another source of repellent products. Some of them produce defense secretions such as alarm pheromones (Pickett et al. 1992). Another famous example concerns ant cues that affect landing behavior of fruit flies (Van Mele et al. 2009). Synthetic compounds are the third source of repellent products; these include DEET, IR3535, DEPA (N,N-diethyl phenylacetamide), PMD (p-menthane-3,8-diol), picaridin and some pyrethroids (Moore and Debboun 2007b). How do these repellent products, synthetic or extracted from plants and animals, act on the insects?

Olfaction pathway

Insect olfactory organs, the antennae and maxillary/labial palps, are covered with different types of sensilla. A sensillum is a sensory structure housing one to four olfactory receptor neurons (Dethier 1954). Each olfactory receptor neuron expresses a unique combination of olfactory receptors with olfactory co-receptors (Vosshall et al. 2000; Krieger et al. 2003; Vosshall and Hansson 2011) and projects axons into a single olfactory glomerulus in the antennal lobe (Vassar et al. 1994). In the antennal lobe, interneurons are located between glomeruli and projection neurons (PNs) (Jefferis et al. 2001; Marin et al. 2002). Projection neurons link the antennal lobe to higher-order processing centers: mushroom bodies and the lateral horn (Christensen and White 2000). Olfactory signal transduction is detailed in Fig. 2. Insects recognize their hosts by their odors, characterized by: (1) blends of volatile compounds, (2) their ratio and (3) their spatiotemporal diffusion (Sachse and Galizia 2003; Bruce et al. 2005).

The first step in odor recognition is odorant receptor activation (Suh et al. 2014). This could be due to the odor alone or to the binding protein–odorant complex (Buck and Axel 1991; Nakagawa et al. 2005; Leal 2013). Binding proteins contribute to the overall odorant specificity and sensitivity of the insect olfactory system (Vogt and Riddiford 1981; Leal 2013). Olfactory receptors play a significant role in odorant sensitivity, because of the available number, their specificity and affinity (Ha and Smith 2009; Gomez-Martin et al. 2010). Olfactory co-receptors take part in signal transduction and assist in receptor trafficking, targeting and tuning (Larsson et al. 2004; Hansson and Stensmyr 2011; Kaupp 2010). For example, in *Anopheles gambiae*, De Genarro et al. (2013) showed that Orco mutant mosquitoes were less attracted to honey and did not respond to human scent in the absence of CO_2, because the spontaneous activity and odor responses of the Orco mutant olfactory neurons were reduced.

All olfactory receptor neurons expressing the same receptor converge onto one glomerulus (Laissue et al. 1999; Gomez-Martin et al. 2010). However, one olfactory receptor can recognize multiple odors and one odor can be recognized by multiple olfactory receptors. Consequently, a blend of odors activates different combinations of olfactory receptors (De Bruyne et al. 1999; De Bruyne et al. 2001; Hallem and Carlson 2006). Odors can also inhibit odorant receptors (De Bruyne et al. 1999; Hallem and Carlson 2006) and elicit different temporal responses, so temporal coding also enhances the insect ability to recognize odors (Kaupp 2010). Odorant receptors have different activation thresholds and glomeruli have different levels of excitation (Hallem and Carlson 2006). Odor information is therefore coded by the distribution of several activated glomeruli, corresponding to activated olfactory receptor neurons by the odorant molecule (Malnic et al. 1999; Séjourné et al. 2011). Odor information is treated by local interneurons that are the link between olfactory receptor neurons and projection neurons. They modulate, by inhibition or activation, the glomeruli, thus improving the signal from the initial noise that results from odor code conveyed by the projection neurons (Jefferis et al. 2001; Marin et al. 2002; Silbering and Galizia, 2007; Cunningham, 2012). Cortical representations of odor information created in the antennal lobe are not known (Touhara and Vosshall, 2009). Mushroom bodies are probably sites for olfactory learning and memory and experience-dependent modulation of olfactory behavior, whereas the lateral horn appears to be a site for innate, experience-independent modulation of olfactory behavior (de Belle and Heisenberg 1994; Heisenberg 2003; Gomez-Martin et al. 2010). Pheromone or general odor receptors differ with regard to specificity and selectivity, while being narrowly or broadly tuned and linked to specialist or generalist olfactory receptor neurons, so the generating combinatory code in the antennal lobe is different (Christensen and Hildebrand 2002; Hallem and Carlson 2006; Touhara and Vosshall 2009). An odor-like plant volatile can activate different types of olfactory receptors in various neurons converging on different glomeruli, contrary to pheromone receptors which are very specialized (Touhara and Vosshall 2009; Leal 2013). But some olfactory receptor neurons involved in host volatiles can also be highly specialized, depending on their concentration, number and ecological relevance (Hansson and Stensmyr 2011). For example, scarab beetles



have selective olfactory receptor neurons for specific host volatiles that can elicit either repellent or no behavior. How might a single compound acting on this olfactory system result in a repellent? The molecular targets and signaling pathways involved in sensing insect repellents as well as antifeedants are poorly understood (Kim 2013).

Specific receptors or neurons?

Here, we hypothesize that the activation of specific olfactory receptor neurons innervating one glomerulus can be responsible for an innate repellent behavior. Accordingly, Knaden et al. (2012) found some glomeruli that responded discriminately to attractive and aversive odors: DA4 and DC3 glomeruli were identified as ‘aversive specific’, both at the input and output levels. For example, geosmin—a compound produced by fungi and bacteria—is a repellent for *Drosophila melanogaster* through an innate avoidance pathway (Stensmyr et al. 2012). The molecule activates ab4B neurons which are specific only to geosmin and elicit a response from only two PNs (Stensmyr et al. 2012). Moreover, *D. melanogaster* exhibited strong avoidance to odors (CO_2) released by stressed flies, and CO_2 activated only a single specific glomerulus in the antennal lobe (Suh et al. 2004). Avoidance is lost when this specific glomerulus is inhibited (Suh et al. 2004). Moreover, DEET is detected by the sacculus (antennal structure) of *D. melanogaster* innervated by axons of Ir40a-expressing neurons (Kain et al. 2013). When synaptic transmission in these neurons was blocked, DEET repellent was decreased for these flies, suggesting that Ir40a neurons are required for repellent triggering. Note that Ir40a is a highly conserved receptor among insects, which could explain the effectiveness of DEET in a wide variety of species.

As defined above, a true repellent (expellent) is a compound that causes movement away from the odor

◀**Fig. 2** Hypothetical mecanisms of the different kinds of repellents. **a** Signal transduction through the olfaction pathway. Odorant molecules pass through the sensilla pore tubule^1, diffuse into the endolymph by means of binding proteins^2 which carry the molecule to olfactory receptors^3 (Leal 2013). Binding of the odorant to olfactory receptors leads to the opening of^4 olfactory receptor-associated ion channels and subsequent depolarization of olfactory receptor neurons^4 (Kaupp 2010). In the antennal lobe, the activated glomeruli activate interneurons^5 that modulate the activity of projection neurons^6 and depolarization in projection neurons conveys information to mushroom bodies and the lateral horn^7 (Ache and Young 2005). **c** Hypothetical expellent mechanism. True repellents (expellents) remotely cause movement away from the odor source independently of the context. An expellent would activate specific olfactory receptor^1 and the depolarization^2 of the olfactory receptor neuron would activate a specific glomeruli^3 with labeled lines. Then a projection neuron would convey the information^4 that codes for a movement away. **b** Hypothetical maskant odor mechanism. Maskant odor compounds decrease the attractiveness of the host and are not repellent by themselves, but depend on the context. This compound induces different behavior as a function of their concentration and the presence of other molecules. When the concentration is high, a higher number of olfactory receptors are recruited^1 so the depolarization of olfactory neurons is more important^2. There is a different threshold activation for glomeruli^3, which causes different modulation by interneurons^4. The odor code conveyed by projection neurons^5 to the higher brain is different from the low concentration code, resulting in different behavior. **d** Hypothetical attraction inhibitor mechanism. Attraction inhibitors interfere with host detection and localization. These compounds inhibit the olfactory receptor neuron response^1 by affecting the odorant^2, binding proteins^3 or olfactory receptors^4. There is no signal, so there is no behavior. **e** Signal transduction through the gustatory pathway. Gustatory compounds pass through the unipore^1 and diffuse in the endolymph via the host binding protein^2, which carries them to the gustatory receptor^3 (Amrein and Thorne 2005; Isono and Morita 2010). Gustatory receptor activation results in opening the ion channel of gustatory receptors and then depolarization of gustatory receptor neurons^4; their axons project directly without synapsing into the subesophageal ganglion (Schoonhoven and Van Loon 2002). Finally, interneurons are activated^5 and taste information is sent from the subesophageal ganglion to the mushroom body and the lateral horn (Vosshall and Stocker 2007). **f** Hypothetical antifeedant mechanism. Antifeedant compounds act on the feeding behavior. Antifeedant compounds activate a specific gustatory receptor^1 on mouthparts. Depolarization of gustatory receptor neurons^2 conveys information to the subesophagial ganglion, which gets the information out to the higher brain^3 for a nonfeeding behavior. Moreover, these compounds activate deterrent cells, inhibit the proboscis extension reflex and thus the feeding activity, but they do not cause a movement away. **g** Hypothetical irritant mechanism. Irritant compounds cause a movement away from the source after physical contact with it. They would activate a specific gustatory receptor^1 on tarses. Depolarization of gustatory receptor neurons^2 conveys the information to the subesophagial ganglion, which gets the information out to the higher brain^3 for a movement away. The difference between an expellent and an irritant is that the movement away is a distance-mediated behavior through specific olfactory receptors and contact-mediated behavior through specific gustatory rceptors, respectively. **h** Hypothetical feeding inhibitor mechanism. Antifeedant compounds act on the feeding behavior. Feeding inhibitors inhibit the gustatory receptor neuron response^1 by affecting odorants^2, binding proteins^3 or gustatory receptors^4

source independently of the context. It could therefore be a compound that has a specific olfactory receptor and glomeruli with labeled lines that code for a movement away (Galizia and Rossler 2010) (Fig. 2).

A matter of concentration and/or ratio?

Here, we hypothesize that a compound could provoke different behaviors as a function of its concentration and the presence of other molecules. Depending on the concentration and molecule ratio, activated olfactory receptors, and thus activated glomeruli, result in a different odor code by a different type of interneuron modulation (Knaden et al. 2012). This different odor codes conveyed by the PNs to the higher brain result in different behaviors. Most stimuli that are attractant at low concentration can become repellent at high concentration (Foster and Harris 1997). At high concentration, most fragrant molecules activate multiple receptors, whereas at low concentration fewer receptors are activated (Malnic et al. 1999; Hallem and Carlson 2006). The recruitment of additional glomeruli has been proposed as a mechanism mediating this switch in behavioral response. Hence, in *D. melanogaster* two glomeruli, DM1 and VA2, were identified as mediators of attraction to vinegar at low concentration. At high concentration, vinegar became aversive and active on another glomerulus, DM5, which mediates the decreased attraction (Semmelhack and Wang 2009). Actually, odorant receptors have different activation thresholds and glomeruli also have different levels of excitation (Hallem and Carlson 2006). Individual odors activate distinct subsets of olfactory receptors, resulting in the construction of a glomerular activation pattern—odor map—but different concentrations result in different patterns (Knaden et al. 2012). The different glomerular activation pattern initiates different projection neuron responses via local interneuron activity (Sachse and Galizia 2003; Knaden et al. 2012).The response increased in amplitude and duration with increasing odor concentration up to a saturated maximum for input and output neurons. Interestingly, the behavioral response did not depend only on the concentration of a compound, but also on its ratio with other compounds. Several volatiles which are attractant as a blend can be each individually repellent. For instance, the black bean aphid, *Aphis fabae*, is attracted by the volatile blend produced by *Vicia faba* (host cue), but when the volatiles were presented alone they were not bioactive (Webster et al. 2010). A similar scenario is exhibited in the context of masking odors, e.g., in the case of herbivore-induced plant volatiles, an increase in some volatiles decreases the host attractiveness (Turlings et al. 2002). Thus, the context results in a different odor code by a different type of interneuron modulation (Fig. 2). Insects can differentiate between host and non-host plants, healthy vs stressed plants and unripe vs ripe fruits, despite cues with overlapping patterns of odors, through the processing of odor information by interneurons in antennal lobes (Cunningham 2012). So, a repellent might not be due to only one compound and its concentration, i.e., the context and presence of other compounds are also important.

Information blocker?

Repellent compounds could affect the olfactory receptor function by modifying or blocking the response of olfactory receptor neurons sensitive to attractants (Davis 1985). For example, inhibitors of the mosquito CO_2 receptor can mask attraction to human odors (Ray 2015). Bohbot et al. (2011) showed that IR3535 and DEET inhibited the response of a complex olfactory receptor (AaOr8+AaOr7) of the mosquito *Aedes aegypti* to octenol, an attractant. In this mosquito, DEET decreases the sensitivity of sensitive olfactory receptor neurons to lactic acid, a compound in human sweat (Davis and Sokolove, 1976). Tsitoura et al. (2015) also showed that specific mosquito repellent such as ethyl cinnamate inhibits odorant receptor function through a functional blocking of olfactory co-receptors and Ditzen et al. (2008) showed that DEET blocked the response of olfactory neurons by decreasing the current mediated by the olfactory receptor due to a change in ion permeability.

We have defined a masking odor as a compound that interferes with host detection and localization and can modify the host profile. It could therefore correspond to a compound that inhibits the olfactory receptor neuron response by affecting olfactory receptors, binding proteins or by trapping the attractant (Fig. 2). Acting on the attractant is actually another way to block the information. For example, DEET decreases octenol release, lowering its stimulation on specific olfactory receptors, which in turn changes the chemical host profile and reduces the host attractiveness. DEET might therefore be viewed as an attractant trap (Syed and Leal 2008). But this mode of action is controversial.

Gustatory pathway

Taste organs are distributed over multiple body parts: mouthparts (maxillary palps, oral cavity and pharynx), legs and wings (De Boer 2006; Vosshall and Stocker 2007). Sensilla on these organs enable the insect to sample potential food sources without consuming them (Sturcow 1959; Montell 2009). External gustatory sensilla are uniporous, contrary to olfactory sensilla, which are multiporous (Altner and Prillinger 1980; Fig. 2). Gustatory sensilla have two kinds of gustatory receptor neuron cells responding to attractive tastants or aversive tastants, or four kinds of gustatory receptor neuron cells responding to sugar (S cells), tap water (W cells), low salt concentrations (L1 cells), high salt concentrations and bitterness (L2 cells) (Rodrigues and Siddiqi 1981). They include one chemosensory neuron and several types of accessory cells (Amrein and Thorne 2005; Montell 2009). Odorant-binding proteins are also expressed in the lymph of gustatory sensilla and have the same role as odorant-binding proteins in the olfactory system (Amrein and Thorne 2005). Like olfactory receptors, gustatory receptors form heterodimers with other receptors as a common co-receptor such as Gr64f (Isono and Morita 2010). Transduction of chemical information occurs in the mouthparts via gustatory receptor neurons when molecules make contact with taste receptor cells composed of trans-membrane proteins (Isono and Morita 2010). In the proboscis, taste receptor neuron axons project directly without synapsing into the subesophageal ganglion, which provides motor output to those mouthparts (Schoonhoven and Van Loon 2002). The subesophageal ganglion contains no morphologically apparent structural subdivisions, such as glomeruli in the antenna lobe (Vosshall and Stocker 2007). But projections of gustatory receptor neurons into the subesophageal ganglions terminate in spatially segregated domains (Dunipace et al. 2001; Thorne et al. 2004; Wang et al. 2004; Isono and Morita 2010). Gustatory afferents from the pharynx, labellum and legs traveling through different nerves terminate in distinct areas of the subesophageal ganglion, and some of these spatially distinct afferents express the same receptors, suggesting that a given tastant may trigger different behaviors depending on the stimulation site (Vosshall and Stocker 2007). Interneurons also link the subesophageal ganglion to the mushroom body (Melcher and Pankratz 2005; Vosshall and Stocker 2007). Taste information is sent to higher brain centers, while simple reflexes, such as proboscis extension or food ingestion, may rely on local circuitry with fairly limited processing.

Like the olfactory system, the gustatory system codes quantitatively, qualitatively and by the compound's ratio as well as the compound's presence in space and time (Schoonhoven and Van Loon 2002; Koul 2008). For example, glucose-sensitive cells in *Manduca sexta* responded differently to sucrose or glucose. The differences could be attributed to the topographical binding-site characteristic of gustatory receptors (Lam and Frazier 1991). Many gustatory receptors are co-expressed in the same gustatory receptor neurons (Montell 2009). In contrast to one receptor = one neuron in the olfactory system, different gustatory receptor subsets are expressed in one taste neuron. Multiple receptor expression may be able to expand the ligand spectrum, but decrease the discrimination performance (Isono and Morita 2010). Finally, there are three types of sensory coding: (1) each neuron conveys a specific message to the central nervous system, (2) the global message is contained in a neural activity pattern (input) transmitted by several receptors and (3) the stimulus quality affects nerve impulse patterns (output) and the adaptation rate, which may contain additional information (Schoonhoven et al. 1992).

How could a compound acting on this gustatory system result in a repellent behavior? A first response is that behavioral rejection is not due to the ingested product, but to external receptors because there is no link between feeding deterrence and internal toxicity (Koul 2008). Since the gustatory system is quite similar to the olfactory system, the same hypothesis regarding the mode of physiological action could be put forward to explain the repellent effects, such as antifeeding and irritancy.

A matter of concentration, ratio or time?

Drosophila spp. are attracted by low salt concentration. Conversely, they are averted by high salt concentration. Two categories of neurons respond to NaCl: Gr5a for low concentrations and Gr66a for high concentrations (Thorne et al. 2004; Wang et al. 2004; Marella et al. 2006). All compounds activating Gr5a neurons are attractive to flies and all those activating Gr66a neurons are aversive. But the Gr66a neuron alone did not explain the salt aversion, because its ablation did not significantly affect salt avoidance (Yarmolinsky et al. 2009).

Host plant selection depends on the balance of phagostimulant (e.g., sugars) and deterrent (e.g., plant secondary compounds) inputs, so the selection depends on the compound's concentration (Schoonhoven 1987; Chapman 2003). Actually, plant secondary compounds are usually, deterrent but they also stimulate phagostimulatory cells. For example, in the Colorado potato beetle, *Leptinotarsa decemlineata*, epipharyngeal taste sensillum is innervated by five neurons (one responds to water, one to sucrose and three to two antifeedants (drimane, sinigrin), but sucrose-sensitive cells are also strongly inhibited by drimane (Messchendorp et al. 1998). One hypothesis is that electrical signals from receptor cells sensitive to antifeedants are subtracted algebraically to electrical signals from receptor cells sensitive to feeding stimulants in the central nervous system (Schoonhoven and Van Loon 1988).

The concentration, and particularly the exposure time can play a key role in stimulation or inhibition of the neuronal response. For example, taste cells in the butterfly *Pieris brassicae* responding to phagostimulants showed a gradual decrease in sensitivity to drimane (antifeedant) at high dosage for periods up to 30 min (Schoonhoven and Van Loon 2002).

Specific receptors or neurons?

All phytophagous insects have deterrent receptors which, upon stimulation, reduce or fully stop feeding activity (Schoonhoven and Van Loon 2002). Like the olfactory system, the gustatory system could be modulated by learning and memory (Vosshall and Stocker 2007). Moreover, evolution has led to the selection of specific receptors to deterrents. Deterrent cells generally show greater latency in their response than phagostimulatory cells (Schoonhoven and Van Loon 2002). For example, in the labellum, bitter aversive compounds are primarily detected via L2 cells and they also inhibit S and W cells (Montell 2009). Moreover, at the labellum, pharynx and subesophageal ganglion level, DEET can activate bitter-sensing deterrent neurons in *D. melanogaster*, which could explain its efficiency in repelling insects (Lee et al. 2010; Sanford et al. 2013; Kain et al. 2013).

We defined an antifeedant as a peripheral-mediated behavior-modifying substance resulting in feeding deterrence (Isman 1994). An antifeedant could be a compound that has a specific gustatory receptor that codes for a non-feeding behavior (Fig. 2). Actually, the compounds that activate deterrent cells inhibit the proboscis extension reflex and thus the feeding activity, without causing the insect's movement away from the stimulus.

Gamma aminobutyric acid (GABA) stimulates feeding and triggers a taste cell response among herbivorous insects, and the antagonists of GABA causes feeding deterrence along with hyperexcitation of the central nervous system and has an excitoirritant effect (Rattan 2010). We have defined an irritant as a compound that causes a movement away from the source after physical contact with it. An irritant could therefore be a compound that has a specific gustatory receptor coding for movement away (Fig. 2). The difference between an expellent and an irritant is that the movement away is a distance-mediated behavior through specific olfactory receptors and a contact-mediated behavior through specific gustatory receptors, respectively. De Gennarro et al. (2013) showed that even in the presence of DEET, Orco mutant female mosquitoes were still attracted to human hosts, but were repelled upon contact. This indicates that olfactory- and contact-mediated effects of DEET are mechanistically distinct, thus supporting our hypothesis that irritancy is coded by gustatory receptors on the tarsus.

Information blocker?

We hypothesize that a compound can block the stimulant taste receptor. According to Koul (2008), the sensory code may be altered due to the stimulation of specialized receptors and also to modulation of the activity of receptors tuned to other compounds. Although antifeedant compounds do not stimulate any neurons within a sensillum, they may decrease the responsiveness of a cell responding to a nutrient (Schoonhoven and Van Loon 2002). For example, deterrents may inhibit S cells (Schoonhoven and Van Loon 2002). In mouthparts of lepidopteran larvae, terpenes block the stimulatory effects of glucose on chemosensory cells, thus inducing an antifeedant effect (Rattan 2010). Azadirachtin, the main active compound of neem oil, stimulates deterrent receptors in various insects (Schoonhoven 1988), but also appears to inhibit sugar or inositol receptors in other species (Schoonhoven 1988). Sinigrin inhibits inositol cells in the moth *Heliothis virescens* (Bernays and Chapman 2000).

We have defined an antifeedant as a compound acting on feeding behavior. An antifeedant that we have called a feeding inhibitor could therefore be a compound that inhibits the gustatory receptor neuron response by affecting gustatory receptors (Fig. 2).

As clearly outlined by Dickens and Bohbot (2013), the mode of action of mosquito repellents has been widely studied, particularly for DEET, the potential molecular targets and their numerous neurophysiological pathways. But it is still not clear which target initiates the repellent phenomenon. We should also stress the plasticity of the sensory system and the faculty of insects to adapt to rapid environmental changes. The avoidance behavioral response can actually be modulated by experience and memorized in the short and long term (Séjourné et al. 2011). In the mushroom body, MB-V2 neurons are specifically required to retrieve aversive olfactory memory. Aversive odor represses their activity and the reduced response could lead to enhanced avoidance to the aversive odor, resulting in lower inhibition of olfactory signaling mediated by the lateral horn. Other neurons provide the output for appetitive memory, but the process could be the same.

Receptor types other than olfactory and gustatory receptors could be involved in repellent phenomena. Transient receptor potential (TRP) channels are non-selective ion channels and participate in the detection of pain, hot temperature, gravity sensation, sound sensation, visual transduction and chemosensation (Kim 2013). For example, TRPA1 is required for avoiding the volatile insect repellent citronellal (Kim 2013). In *Drosophila,* citronellal interacts with TRPA1, modifying the activity of the $Ca2^+$-activated potassium channel, but TRPA1 is directly activated by citronellal in *An. gambiae* (Kwon et al. 2010).

Two examples of repellent use

Advantages/disadvantages of repellent use

One advantage of using repellents is the durability of their efficacy over time (Achee et al. 2012). The selection pressure from contact-mediated toxicity, like chemical insecticides, is generally high and rapidly selects for resistance (REX Consortium 2013), while the selection pressure from a repellent that modifies behavior may be lower. This is because repellents concomitantly increase the predation risk and the energy expenditure, but also allows the insects to find an alternative host (Achee et al. 2012). The lower risk of resistance could be also explained by higher fitness costs associated with resistance to repellents. For these reasons and because repellents are currently underutilized in public health and domestic hygiene, no case of resistance to these products has been documented. On the contrary, data suggest that changes occur in the efficiency of repellents on insecticide-resistant populations. For instance, *Anopheles gambiae*-, *kdr*- and *ace1*-resistant populations were less irritated by pyrethroids, but more irritated by repellents than the susceptible reference strain (Chandre et al. 2000; Deletre et al. 2016). Populations of the German cockroach have rapidly evolved an adaptive behavioral aversion to glucose, the phagostimulatory component of baits (Wada-Katsumata et al. 2013). In both wild-type and glucose-averse cockroaches, D-fructose and D-glucose stimulated sugar-gustatory receptor neurons, whereas the deterrent caffeine stimulated bitter-gustatory receptor neurons (Wada-Katsumata et al. 2013). In contrast, in glucose-averse cockroaches, D-glucose also stimulated bitter-gustatory receptor neurons and suppressed the responses of sugar-gustatory receptor neurons.

One disadvantage of using repellents is that insects can lose their sensitivity to them or they can change their mode of feeding after repeated and prolonged exposure to these products, especially to antifeedants (Jermy 1990; Foster and Harris 1997). For example, when rice plants were treated with neem extracts, *Nephotetettix virescens* fed on xylem instead on phloem (Saxena and Khan 1985). Several possible mechanisms could explain the behavioral changes, including sensory adaptation, motor fatigue and habituation (Akhtar et al. 2003). Habituation is a learning or temporal desensitization phenomenon, whereas sensory adaptation can be a persistent synaptic change in specific neural pathway (Bernays and Chapman 2000, Koul 2008). Glendinning et al. (2001) showed that the adapted aversive response (exposure-induced adaptation) to caffeine of the moth *Manduca sexta* is directly mediated by desensitized taste cells.

The push–pull strategy in crop protection

The push–pull strategy developed by Pike et al. (1987) is a concept that uses attractive and repellent stimuli simultaneously to modify the abundance and distribution of insect pests or beneficial insects (Cook et al. 2007). This method repels the insects away from the resource (by making it hard to locate, unattractive or unsuitable) while simultaneously attracting the insects to another area (Ratnadass et al. 2012). The source (push) depends on the management tactics on visual and/or chemical cues both at short (e.g., through antifeedants, oviposition deterrents, deterring pheromones) and long range (through synthetic repellents, non-host volatiles, host volatiles and anti-aggregation, sex or alarm pheromones). The stimulus is generally a plant-produced compound(s), but it can also be a synthetic blend (Cook et al. 2007).

The most famous example of a push–pull strategy is the control of stem borers in maize and sorghum (Khan and Pickett 2004). The push stimulus involves intercropping with a repellent non-host plant such as *Desmodium uncinatum, Desmodium intortum* or *Melinis minutiflora,* while the pull stimulus is an attractant trap plant such as *Sorghum vulgare sudanense* or *Pennisetum purpureum* (Khan et al. 1997a). Intercropping is based on the odor masking offered by plants with the aim of decreasing the attractiveness of the host crop or hiding this crop to the insect pests via another crop (Cook et al. 2007). For instance, the repellent molasses volatile (E)-β-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene produced by plants damaged by phytophagous insects (Kimani et al. 2000) reduces stem borer infestation and increases parasitism of these borers by the wasp *Cotesia sesamiae* (Khan et al. 1997b). The intercropping principle and efficiency have been questioned by Finch and Collier (2012). At a few meters from the source, specialist insects respond only to volatile chemicals released by their host plants, even when emitted in small quantities. Tosh and Brogan (2014) suggested that generalist insects such as *Bemisia tabaci* with a super-abundance of volatiles by intercropping could have a confusion effect. Another example is the control of *L. decemlineata* in potato *(Solanum tuberosum)* crops (Martel et al. 2005a). The push stimulus involves the use of neem-based antifeedants on the crop (Martel et al. 2005a), while the pull stimulus is ensured by combining attractants (Z)-3-hexenyl acetate, (R,S)-linalool and methyl salicylate on the potato crop, with insecticide applications (Martel et al. 2005b). To improve this system, the beetle aggregation pheromone (S-3,7-dimethyl-2-oxo-6-octene-1,3- diol) could be used to concentrate the beetle in the trap crop (Dickens et al. 2002; Dickens 2006).

The push–pull strategy is used in horticulture (Miller and Cowles 1990), forestry (Borden 1997) and for control of veterinary and medical pests (Nalyana et al. 2000). This strategy maximizes the control efficacy, sustainability and yield and minimizes negative environmental impacts (Miller and Cowles 1990; Cook et al. 2007). The combined effect between the pull and push stimuli increases the efficiency of some stimuli, like antifeedants (Jermy 1990). Another advantage of this strategy is that resistance management is facilitated (Foster et al. 2005). Unfortunately, this strategy is often not as effective as the use of a broad-spectrum chemical insecticide at reducing pest numbers (Cook et al. 2007). This lower efficacy in pest control is therefore not always economically profitable, especially for crops with low economic incomes. Indeed, this strategy is operationally complex, requiring monitoring and decision systems and thus induces higher operational costs than conventional systems. The lack of general knowledge about insect/plant interactions is also a factor limiting its adoption by small farmers (Ratnadass et al. 2012).

The use of pyrethroids in human health protection

Pyrethroids were originally derived from natural pyrethrin extracted from *Chrysanthemum cinerariaefolium* (Elliott et al. 1978). Since then, many other pyrethroids have been synthesized, such as permethrin, bifenthrin, deltamethrin, lambda-cyhalothrin and alpha-cypermethrin. These insecticides are very toxic against most insects due to a modification of the gating kinetics of the voltage-dependent sodium channel (Narahashi 1971). Moreover, they are odorless, resistant to degradation by UV, heat and hydrolysis, and displayed a low toxicity for mammals. All these properties make pyrethroids a powerful and suitable means of pest control in a wide variety of situations.

For instance, bed nets, clothes (e.g., battle dress) and hammocks can be treated by pyrethroids, thus avoiding mosquito bites (Pennetier et al. 2010; Hougard et al. 2007; McCain and Leach 2007). Treated bed nets are recommended by WHO (WHO 2002) against malaria vectors, especially endophagic species responsible for indoor transmission such as *Anopheles gambiae* and *Anopheles funestus*. Martin et al. (2013, 2014) recently documented the effect of pyrethroid-treated nettings in agriculture to protect cabbage and tomato crops against aphids and whiteflies. These insecticides are indeed irritating (excitorepellent) for the several insects, which therefore avoid landing on the treated surfaces. If insects alight many times on treated areas, they are either knocked down or killed. The knockdown effect involves rapid incapacitation, sometimes with metabolic recovery (White 2007).

In field experiments, the number of mosquitoes entering huts with treated nets was usually lower compared to huts with untreated nets (Adeogun et al. 2012), indicating an expellent or masking effect. The observed withdrawal of female mosquitoes could be explained by the loss of response to host cues rather than repellent (Siegert et al. 2009). Apart from transfluthrin and metofluthrin, most pyrethroids lack the volatility to function as expellents at concentration necessary to be active against mosquitoes under ambient conditions (Achee et al. 2009; Deletre et al. 2013). However, these compounds can still be heated or aerosolized in a room to obtain a sufficient gaseous or suspended concentration to result in expellency (Chadwick and Lord 1977).

Unfortunately, vector management by pyrethroids is jeopardized by the selection of resistance mechanisms. In *An. gambiae,* pyrethroid resistance can be due to a mutation in the sodium channel sequence (Hemingway et al. 2004), which decreases the irritant effects of these molecules (Chandre et al. 2000). Through this mutation or other resistance mechanisms, *An. gambiae* resistance to pyrethroids has been reported in 27 countries in sub-Saharan Africa, thus underscoring the urgent need to find alternatives to these insecticides (Zaim et al. 2000; WHO 2011; Ranson et al. 2011; Temu et al. 2013). The prioritization of toxic actions over spatial repellent and contact irritant actions should be balanced with the higher risk of rapid selection for resistance to the active compounds (Achee et al. 2009). Moreover, pyrethroids are commonly used to protect crop fields adjacent to *An. gambiae* habitats, thus contributing to the selection of resistant mosquitoes (Yadouleton et al. 2011).

Conclusion and perspectives

(1) In this paper, we define insect repellent as a phenomenon that prevents a pest's ability to track, locate and/or recognize its host. We also subdivide repellent into five phenomena: true repellent, odor masking, irritability, antifeeding and visual masking.

(2) The greatest problem with repellent research is the use of bioassays that do not adequately discriminate between these five types of repellents. Consideration of the stimulus that produces these reactions may facilitate this discrimination. For example, Junker et al. (2015) summarized the mode of action and DEET and performed different kinds of bioassays to discriminate the different modes of action. Understanding the mode of action of insect repellents and the ways by which they modulate receptor activity will allow us to design potent formulations aimed at interfering with insect sensory signaling and, ultimately, disrupt their cognitive processes (Dickens and Bohbot 2013).

(3) Insect repellents exert their effects through interactions with olfactory (antennae and maxillary palps) and gustatory (maxillary palps, mouthparts, tarsi and wind) receptors. With greater knowledge on neural mechanisms, the repellent definitions could be based on their modes of action rather than behavioral responses, as highlighted in Fig. 1.

(4) Many potential repellents are available, but their use is still limited. A better understanding of the chemical ecology of pests would be helpful for identifying candidate semiochemicals that could then be used in attractant and repellent formulations (Dickens and Bohbot 2013). The development of repellents might however remain impeded by the economic costs that must be paid to identify those molecules and to perform analyses to ensure that they can safely be used in health and agriculture (Kain et al. 2013). Nevertheless, the discovery and design of new repellents could be achieved more rapidly and at lower costs by molecular-based chemical prospecting (Leal 2007) and by computer-aided molecular modeling (Gupta and Bhattacharjee 2007; Tauxe et al. 2013) confirmed by behavioral assays. In this vein, Kain et al. (2013) developed a high-throughput chemical informatics screen using a structure–activity approach to discover new repellents. Using this technique, they identified a new repellent that activated the same chemosensory pathways as DEET. The need to preserve our environment and to provide efficient and sustainable tools for IPM could strongly foster future prospects on repellent. Chemosensory-based insect control like repellent/antifeedant, trap and the mating disruption strategies might become the most used insect pest management strategies in the near future.

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References

Abdelgaleil SAM, El-Aswad AF, Nakatani M (2002) Molluscidal and anti-feedant activities of diterpenes from *Euphorbia paralias* L. Pest Manag Sci 58:479–482

Abdullah ZS, Ficken KJ, Greenfield BP, Butt TM (2014) Innate responses to putative ancestral hosts: is the attraction of Western flower thrips to pine pollen a result of relict olfactory receptors? J Chem Ecol 40:534–540

Abtew A, Subramanian S, Cheseto X, Kreiter S, Tropea Garzia G, Martin T (2015) Repellency of plant extracts against the legume flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). Insects 6:608–625

Ache BW, Young JM (2005) Olfaction: diverse species, conserved principles. Neuron 48(3):417–430

Achee N, Sardelis M, Dusfour I, Chauchan K, Grieco J (2009) Characterization of spatial repellent, contact irritant, and toxicant chemical actions of standard vector control compounds. J Am Mosquito Contr 25:156–167

Achee N, Bangs M, Farlow R, Killeen G, Lindsay S, Logan J, Moore S, Rowland M, Sweeney K, Torr S, Zwiebel L, Grieco J (2012) Spatial repellents: from discovery and development to evidence-based validation. Malaria J 11:164–182

Adeogun AO, Olojede JB, Oduola AO, Awolola TS (2012) Village-scale evaluation of PermaNet 3.0: anenhanced efficacy combination long-lasting insecticidal net against resistant populations of *Anopheles gambiae* s.s. MCCE 1:1–9

Akhtar Y, Hankin CH, Isman MB (2003) Decreased response to feeding deterrents following prolonged exposure in the larvae of a generalist herbivore, *Trichoplusia ni* (Lepidoptera: Noctuidae). J Insect Behav 16:811–831

Altner H, Prillinger L (1980) Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. Int Rev Cytol 67:69–139

Amrein H, Thorne N (2005) Gustatory perception and behavior in *Drosophila melanogaster*. Curr Biol 15:673–684

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant-insect interactions. Curr Opin Plant Biol 4:351–358

Bernays EA, Chapman RF (2000) A neurophysiological study of sensitivity to a feeding deterrent in two sister species of *Heliothis* with different diet breadths. J Insect Physiol 46:905–912

Bernier UR, Kline DL, Posey KH (2007) Human emanations and related natural compound that inhibit mosquito host-finding abilities. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. New York, USA, CRC Press, Taylor and Francis Group, pp 77–100

Blaney WM, Simmonds MSJ, Ley SV, Anderson JC, Toogood PL (1990) Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae. Entomol Exp appl 743 55(2):149–160

Bohbot JD, Fu L, Le TC, Chauchan KR, Cantrell CL, Dickens JC (2011) Multiple activities of insect repellents on odorant receptors in mosquitoes. Med Vet Entomol 25:436–444

Borden JH (1997) Disruption of semiochemical-mediated aggregation in bark beetles. In: Cardé ED, Minds AK. Insect pheromone research: new directions. Chapman and Hall, New York, pp 421–438

Brown M, Hebert AA (1997) Insect repellents: an overview. J Am Acad Dermatol 36:243–249

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274

Bruce TJA, Aradottir GI, Smart LE, Martin JL, Caulfield JC, Doherty A, Sparks CA, Woodcock CM, Birkett MA, Napier JA, Jones HD (2015) The first crop plant genetically engineered to release an insect pheromone for defence. Scientific reports 5

Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 651:175–187

Cameron R, Hopper L, Alvarez JM (2016) Use of fluorescence to determine reduction in *Bemisia tabaci* (Hemiptera: Aleyrodidae) nymph feeding when exposed to cyantraniliprole and imidacloprid through systemic applications. Crop Prot 84:21–26

Chadwick PR, Lord CJ (1977) Tests of pyrethroid vaporising mats against *Aedes aegypti* (L.) (Diptera: Culicidae). Bull Entomol Res 67:667–674

Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P (2000) Modifications of pyrethroid effects associated with kdr mutation in *Anopheles gambiae*. Med Vet Entomol 14:81–88

Chapman RF (2003) Contact chemoreception in feeding by phytophagous insects. Ann Rev Entomol 48:455–484

Christensen TA, Hildebrand JG (2002) Pheromonal and host-odor processing in the insect antennal lobe: how different? Curr Opin Neurobiol 12:393–399

Christensen TA, White J (2000) Representation of olfactory information in the brain. In: Bryant BP, Silver WL. The neurobiology of taste and smell. Wiley-Liss, New York, pp 201–232

Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Ann Rev Entomol 52:375–400

Cunningham JP (2012) Can mechanism help explain insect host choice? J Evol Biol 25:244–251

Davis EE (1985) Insect repellents: Concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). J Med Entomol 22:237–243

Davis EE, Sokolove PG (1976) Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*. J Comp Physiol 105:43–54

de Belle JS, Heisenberg M (1994) Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. Science 263(5147):692–695

De Boer G (2006) The role of the antennae and maxillary palps in mediating food preference by larvae of the tobacco hornworm, *Manduca sexta*. Entomol Exp Appl 119:29–38

De Bruyne M, Clyne PJ, Carlson JR (1999) Odor coding in a model olfactory organ: the Drosophila maxillary palp. J Neurosci 19:4520–4532

De Bruyne M, Foster K, Carlson JR (2001) Odor coding in the Drosophila antenna. Neuron 30:537–552

De Gennaro M, McBride CS, Seeholzer L, Nakagawa T, Dennis EJ, Goldman C, Jasinskiene N, James AA, Vosshall LB (2013) Orco mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. Nature 498:487–491

De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410:577–580

Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C, Bonafos R, Chandre F (2013) Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. PLoS ONE 8:12

Deletre E, Chandre F, Barkman B, Menut C, Martin T (2015) Naturally occurring bioactive compounds from four essential oils against *Bemisia tabaci* whiteflies. Pest Manag Sci. doi:10.1002/ps.3987

Deletre E, Martin T, Duménil C, Chandre F (2016) DEET and natrural compounds are more effective on *Anopheles gambiae* resistant strains

Dethier VG (1954) The physiology of olfaction in insects. Ann NY Acad Sci 58(2):139–157

Dickens JC (2006) Plant volatiles moderate response to aggregation pheromone in Colorado potato beetle. J Appl Entomol 130:26–31

Dickens JC, Bohbot JD (2013) Mini review: Mode of action of mosquitoes repellents. Pestic Biochem Phys 106(3):149–155

Dickens JC, Oliver JE, Hollister B, Davis JC, Klun JA (2002) Breaking a paradigm: male produced aggregation pheromone for the Colorado potato beetle. J Exp Biol 205:1925–1933

Ditzen M, Pellegrino M, Vosshall LB (2008) Insect odorant receptors are molecular targets of the insect repellent DEET. Sciences 319:1838–1842

Dogan EB, Ayres JW, Rossignol PA (1999) Behavioural mode of action of DEET: inhibition of lactic acid attraction. Med Vet Entomol 13:97–100

Dunipace L, Meister S, McNealy C, Amrein H (2001) Spatially restricted expression of candidate taste receptors in the Drosophila gustatory system. Curr Biol 11:822–835

Elliott M, Janes NF, Potter C (1978) The future of pyrethroids in insect control. Ann Rev Entomol 23:443–469

Finch S, Collier RH (2012) The influence of host and non-host companion plants on the behavior of pest insects in field crops. Entomol Exp Appl 142:87–96

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Ann Rev Entomol 42:123–146

Foster SP, Denholm I, Thompson R, Poppy GM, Powell W (2005) Reduced response of insecticide resistant aphids and attraction of parasitoids to aphid alarm pheromone; a potential fitness trade-off. Bull Entomol Res 95:37–46

Fraenkel GS (1959) The 'raison d'être' of secondary plant substances. Science 129:1466–1470

Galizia CG, Rössler W (2010) Parallel olfactory systems in insects: anatomy and function. Ann Rev Entomol 55:399–420

Gibson G, Torr SJ (1999) Visual and olfactory responses of haematophagous Diptera to host stimuli. Med Vet Entomol 13:2–23

Glendinning JL, Valcic S, Timmermann BN (1998) Maxillary palps can mediate taste rejection of plant allelochemicals by caterpillars. J Comp Phys 183:35–43

Glendinning JI, Brown H, Capoor M, Davis A, Gbedemah A, Long E (2001) A peripheral mechanism for behavioral adaptation to specific “bitter” taste stimuli in an insect. J Neurosci 21:3688–3696

Gomez-Martin A, Duistermars BJ, Frye MA, Matthieu LM (2010) Mechanisms of odor-tracking: multiple sensors for enhanced perception and behavior. Front Cell Neurosci 4:1–15

Grieco J, Achee N, Sardelis M, Chauchan K, Roberts D (2005) A novel high-troughput screening system to evaluate the behavioural response of adult mosquitoes to chemical. J Am Mosquito Contr 21:404–411

Gupta RK, Bhattacharjee AK (2007) Discovery and design of new arthropod/insect repellents by computer-aided molecular modling. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. CRC Press, Taylor and Francis Group, New York, pp 195–228

Ha TS, Smith DP (2009) Odorant and pheromone receptors in insects. Front cell Neurosci 3(10):55–60

Hallem EA, Carlson JR (2006) Coding of odors by a receptor repertoire. Cell 125:143–160

Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. Neuron 72:698–711

Heisenberg M (2003) Mushroom body memoir: from maps to models. Nat Rev Neurosci 4(4):266–275

Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance inmosquitoes. Insect Biochem Mol Biol 34:653–665

Hossaert-McKey M, Bagnères-Urbany AG (2012) Ecologie chimique le language de la nature. France, Le Cherche Midi, Chabreuil A. Lefabvre V

Hougard JM, Martin T, Guillet PF, Coosemans M, Itoh T, Akogbeto M, Chandre F (2007) Preliminary field testing of a long-lasting insecticide-treated hammock against *Anopheles gambiae* and Mansonia spp. (Diptera: Culicidae) in West Africa. J Med Entomol 44:651–655

Irmisch S, Clavijo McCormick A, Günther J, Schmidt A, Boeckler GA, Gershenzon J, Unsicker SB, Köllner TG (2014) Herbivore-induced poplar cytochrome P450 enzymes of the CYP71 family convert aldoximes to nitriles which repel a generalist caterpillar. Plant J 80(6):1095–1107

Isman MB (1994) Botanical insecticides and antifeedants: new sources and perspectives. Pestic Res J 849(6):11–19

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Ann Rev Entomol 51:45–56

Isono K, Morita H (2010) Molecular and cellular designs of insect taste receptor system. Front cell Neurosci 4:20

Jefferis GS, Marin EC, Stocker RF, Luo L (2001) Target neuron prespecification in the olfactory map of Drosophila. Nature 414(6860):204–208

Jermy T (1990) Prospects of antifeedant approach to pest control—a critical review. J Chem Ecol 16:3151–3166

Jilani G, Saxena RC (1990) Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). J Econ Entomol 83(2):629–634

Junker RR, Klupsch K, Paulus J (2015) Prior exposure to DEET interrupts positive and negative responses to olfactory cues in *Drosophila melanogaster*. J Insect Behav 28(1):1–14

Kain P, Boyle SM, Tharadra SK, Guda T, Pham C, Dahanukar A, Ray A (2013) Odour receptors and neurons for DEET and new insect repellents. Nature 000:1–8

Kaupp UB (2010) Olfactory signaling in vertebrates and insects: differences and commonalities. Nat Rev Neurosci 11:188–200

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144

Khan ZR, Pickett JA (2004) The “push-pull” strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. In: Gurr GM, Wratten SD, Altieri MA. ecological engineering for pest management: advances in habitat manipulation for arthropods. CABI, Wallington, pp 155–164

Khan ZR, Chiliswa P, Ampong-Nyarko K, Smart LE, Polaszek A, Wandera J, Mulaa MA (1997a) Utilisation of wild gramineous plants for management of cereal stemborers in Africa. Int J Trop Insect Sci 17:143–150

Khan ZR, Ampong-Nyarko K, Chiliswa P, Hassanali A, Kimani S, lwande WA, Overholt WA, Pickett JA, Smart LE, Woodcock CM (1997) Intercropping increases parasitism of pests. Nature 388:631–632

Kim SH (2013) Insect GPCRs and TRP channels: putative targets for insect repellents. Interdi Bio Central 6–12

Kimani SM, Chhabra SC, Lwande W, Khan ZR, Hassanali A, Pickett JA (2000) Airborne volatiles from *Melinis minutiflora* P. Beauv, a non-host plot of the two spotted stem borer. J Essent Oils Res 882(12):221–224

Knaden K, Strutz A, Ahsan J, Sachse S, Hansson BS (2012) Spatial representation of odorant valence in an insect brain. Cell 1:392–399

Koul O (2008) Phytochemical and insect control: an antifeedant approach. Crit Rev Plant Sci 27:1–24

Krieger J, Klink O, Mohl C, Raming K, Breer H (2003) A candidate olfactory receptor subtype highly conserved across different insect orders. J Comp Physiol A 189:519–526

Kwon Y, Kim SH, Ronderos DS, Lee Y, Akitake B, Woodward OM, Guggino WB, Smith DP, Montell C (2010) Drosophila TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. Curr Biol 20:1672–1678

Laissue PP, Reiter C, Hiesinger PR, Halter S, Fischbach KF, Stocker RF (1999) Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. J Comp Neurol 405(4):543–552

Lam PYS, Frazier JL (1991) Rational approach to glucose taste chemoreceptor inhibitors as novel insect antifeedants. ACS Symp 443:400–412

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB (2004) Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43:703–714

Leal WS (2007) Molecular-based chemical prospecting of mosquito attractants and repellents. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. CRC Press, Taylor and Francis Group, New York, pp 229–248

Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Ann Rev Entomol 58:373–391

Lee Y, Kim SH, Montell C (2010) Avoiding DEET through insect gustatory receptors. Neuron 67–61:903

Mac Cain WC, Leach GJ (2007) Repellents used in fabric: the experience of the US military. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. Taylor and Francis Group, New York, pp 103–110

Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96(5):713–723

Marella S, Fischler W, Kong P, Asgarian S, Rueckert E, Scott K (2006) Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. Neuron 49:285–295

Marin EC, Jefferis GS, Komiyama T, Zhu H, Luo L (2002) Representation of the glomerular olfactory map in the Drosophila brain. Cell 109(2):243–255

Martel JW, Alford AR, Dickens JC (2005a) Laboratory and greenhouse evaluation oa synthetic host volatile attractant for Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Agr For Entomol 7:71–78

Martel JW, Alford AR, Dickens JC (2005b) Synthetic host volatiles increase efficacy of trap cropping system for management of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Agr For Entomol 7:79–86

Martin T, Palix R, Kamal A, Deletre E, Bonafos R, Simon S, Ngouajio M (2013) A repellent net as a new technology to protect cabbage crop. J Econ Entomol 106:1699–1706

Martin T, Gogo EO, Saidi M, Kamal A, Deletre E, Bonafos R, Simon S, Ngouajio M (2014) Repellent effect of an alphacypermethrin treated net against the whitefly *Bemisia tabaci* Gennadius. J Econ Entomol 107:684–690

Matthews RW, Matthews JR (1978) Insect behavior. USA, Wiley, New York

Melcher C, Pankratz MJ (2005) Candidate gustatory interneurons modulating feeding behavior in the Drosophila brain. PLoS Biol 3:e305

Messchendorp L, Smid HM, Van Loon JJA (1998) The role of an epipharyngeal sensillum in the perception of feeding deterrents by *Leptinotarsa decemlineata* larvae. J Comp Physiol 183:255–264

Miller JR, Cowles RS (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. J Chem Ecol 16:3197–3212

Miller JR, Siegert PY, Amimo FA, Walker ED (2009) Designation of chemical in terms of the locomotor responses they elicit from insects: an update of Dethier et al. (1960). Ecol Behav.102:2056–2060

Montell C (2009) A taste of the Drosophila gustatory receptors. Curr Opin Neurobiol 19:345–353

Montgomery ME, Nault LR (1977) Comparative response of aphids aphids to the alarm pheromone, (E)—ß farnesene. Entomol Exp Appl 22(3):236–242

Moore SJ, Debboun M (2007) History of insect repellent. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. CRC Press, Taylor and Francis Group, New York, 940:3–29

Moore SJ, Lenglet A, Hill N (2007) Plant-based insect repellents. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. CRC Press, Taylor and Francis Group, New York

Nakagawa T, Sakurai T, Nishioka T, Touhara K (2005) Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307:1638–1642

Nalyana G, Moore CB, Schal C (2000) Integration of repellents, attractants, and insecticides in a push pull strategy for managing the cockroach (Dictyoptera: Blatellidae) populations. J Med Entomol 37:427–434

Narahashi T (1971) Mode of action of pyrethroids. Bull WHO 44:337–345

Nerio LS, Olivero-Verbel J, Stashenko E (2010) Repellent activity of essential oils: a review. Bioresour Technol 101:372–378

Nilsson E, Bengtsson G (2004) Endogenous free fat y acids repel and attract collembolan. J Chem Ecol 30:1431–1443

Nolen JA, Bedoukian RH, Maloney RE, Kline DL (2002) Method, apparatus and compositions for inhibiting the human scent tracking ability of mosquitoes in environmentally defined three dimensional spaces. US Patent No. 6,362,235. Patent issued March 26, 2002

Nordlund DA (1981) Semiochemicals: a review of the terminology. In: Nordlund DA, Jones RL, Lewis WJ. Semiochemicals: their role in pest control. John Wiley and Sons, New York, pp 13–28

Pennetier C, Chabi J, Martin T, Chandre F, Rogier C, Hougard JM, Pages F (2010) New protective battle-dress impregnated against mosquito vector bites. Parasite Vector 3:81

Pettersson J (1970) An aphid sex attractant. Insect Syst Evol 1(1):63–73

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Ann Rev 971 Entomol 37:67–90

Pike B, Rice M, Sabine B, Zalucki MP (1987) The push-pull strategy-behavioral control of *Heliothis*. Australian Cotton Grow, May-July, pp 7–9

Ramirez GIJ, Logan JG, Loza-Reyes E, Stashenko E, Moores GD (2012) Repellents inhibit P450 enzymes in Stegomyia (*Aedes aegypti*). PLoS ONE 7:1–8

Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V (2011) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 27:91–98

Ratnadass A, Frenandes P, Avelino J (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron Sust Dev 32:273–303

Rattan RS (2010) Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot 29:913–920

Raviv M, Antignus Y (2004) UV radiation effects on pathogens and insect pests of greenhouse-grown crops. Photochem Photobiol 79:219–226

Ray A (2015) Reception of odors and repellents in mosquitoes. Curr Opin Neurobiol 34:158–164

Regnault-Roger C (1997) The potential of botanical essential oils for insect pest control. Int Pest Manag Rev 2:25–34

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: low-risk products in a high-stakes world. Ann Rev Entomol 57:405–424

REX Consortium (2013) Heterogeneity of selection and the evolution of resistance. Trends Ecol Evol 28:110–118

Rodrigues V, Siddiqi O (1981) A gustatory mutant of Drosophila defective in pyranose receptors. Mol Gen Genet 181:406–408

Sachse S, Galizia CG (2003) The coding of odor-intensivity in the honeybee antennal lobe: local computation optimizes odour representation. Eur J Neurosci 18:2119–2132

Sanford JL, Shields VDC, Dickens JC (2013) Gustatory receptor neuron responds to DEET and other insect repellents in the yellow fever mosquito, *Aedes aegypti*. Naturwissenschaften 100:269–273

Saxena RC, Khan ZR (1985) Electronically recorded disturbance in feeding behavior of *Nephotettix virescens* (Homoptera: Cicadellidae) on neem oil-treated rice plants. J Econ Entomol 78:22–226

Schoonhoven LM (1987) What makes a caterpillar eat? The sensory codes underlying feeding behaviour. In: Chapman RF, Bernays EA, Stoffo JG. Advances in Chemoreception and Behavior. Springer, New York, pp 69–97

Schoonhoven LM (1988) Stereoselective perception of antifeedants in insects. In: Ariens EJ, Van Rensen JJS, Welling W. Stereoselectivity of Pesticides: biological and Chemical Problems. Elsevier, Amsterdam, pp 289–302

Schoonhoven LM, Van Loon JJA (1988) Chemoreception and feeding behavior in a caterpillar: towards a model of brain functioning in insects. Entomol Exp Appl 49:123–129

Schoonhoven LM, Van Loon JJA (2002) An inventory of taste in caterpillars: each species its own key. Acta Zool Acad Sci H 48:215–263

Schoonhoven LM, Blaney WM, Simmonds MSJ (1992) Sensory coding of feeding deterrents in phytophagous insects. In: Bernays EA. insect-plant interactions: feeding and oviposition. CRC Press, Boca Raton, pp 59–79

Schrek CE (1977) Techniques for the evaluation of insect repellents: a critical review. Ann Rev Entomol 22:101–119

Séjourné J, Plaçais PY, Aso Y, Siwanowicz I, Trannoy S, Thoma V, Tedjakumala SR, Rubin GM, Tchénio P, Ito K, Isabel G, Tanimoto H, Preat T (2011) Mushroom body efferent neurons responsible for aversive olfactory memory retrieval in drosophila. Nat Neurosci 14-903–910

Semmelhack JL, Wang JW (2009) Select Drosophila glomeruli mediate innate olfactory attraction and aversion. Nature 459:218–223

Siegert PY, Walker E, Miller JR (2009) Differential behavioral responses of *Anopheles gambiae* (Diptera: Culicidae) modulate mortality caused by pyrethroid-treated bednets. J Econ Entomolol 102:2061–2071

Silbering AF, Galizia CG (2007) Processing of odor mixtures in the Drosophila antennal lobe reveals both global inhibition and glomerulus-specific interactions. J Neurosci 27(44):11966–11977

Smith HA, McSorley R (2000) Potential of field corn as barrier crop and eggplant as a trap crop for management of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on common bean in north Florida. Fla Entomol 83:145–158

Steck K, Veit D, Grandy R, Bermudez i, Badia S, Mathews Z, Verschure P, Hansson BS, Knaden M (2012) A high-throughput behavioral paradigm for Drosophila olfaction—the Flywalk. Nat Sci Re. 1031(2):1–9

Stensmyr MC, Dweck HKM, Farhan A, Ibba I, Strutz A, Mukunda L, Linz J, Grabe V, Steck K, Lavista- Llanos S, Wicher D, Sachse S, Knaden M, Becher PG, Seki Y, Hansson BS (2012) A conserved dedicated olfactory circuit for detecting harmful microbes in Drosophila. Cell 151:1345–1357

Sturcow B (1959) Ueber den Geschmackssinn und den Tastinn von *Leptinotarsa decemlineata* Say (Chrysomelidae). Z Vergl Physiol 42:255–302

Suh GS, Wong AM, Hergarden AC, Wang JW, Simon AF, Benzer S, Axel R, Anderson DJ (2004) A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature 431:854–859

Suh E, Bohbot JZ, Zwiebel LJ (2014) Peripheral olfactory signaling in insects. Curr Opin Insect Sci 6:86–92

Syed Z, Leal WS (2008) Mosquitoes smell and avoid the insect repellent DEET. PNAS 36:13598–13603

Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A (2013) Targeting a dual detector of skin and CO_2 to modify mosquito host seeking. Cell 155:1365–1379

Tawatsin A, Wratten SD, Scott RD, Thavara U, Techadamrongsin Y (2001) Repellency of volatile oils from plants against three mosquito vectors. J Vector Ecol 26:76–82

Temu EA, Maxwell C, Munyekenye G, Howard AFV, Munga S, Avicor SW, Poupardin R, Jones JJ, Allan R, Kleinschimidt I, Ranson H (2013) Pyrethroid resistance in *Anopheles gambiae*, in Bomi County, Liberia, compromises malaria vector control. PLoS ONE 7:9

Thorne N, Chromey C, Bray S, Amrein H (2004) Taste perception and coding in Drosophila. Curr Biol 14:1065–1079

Thorsteinson AJ (1960) Host selection in phytophagous insects. Ann Rev Entomol 5:193–218

Togni P, Laumann R, Medeiros M, Sujii E (2010) Odour masking of tomatoe volatiles in host plant selection of *Bemisia tabaci* biotype B. Entomol Exp Appl 136:164–173

Tosh CR, Brogan B. 2014. Control of tomato whiteflies using the confusion effect of plant odours. Agron Sustain Dev 1–11

Touhara K, Vosshall LB (2009) Sensing odorants and pheromones with chemosensory receptors. Ann Rev Physiol 71:307–332

Tsitoura P, Koussis K, Iatrou K (2015) Inhibition of Anopheles gambiae odorant receptor function by mosquito repellents. J Biol Chem 290(12):7961–7972

Turlings TCJ, Gouinguené S, Degen T, Fritzsche-Hoballah ME (2002) The chemical ecology of plant-caterpillar–parasitoid interactions. In: Tscharntke T, Hawkins BA. Multitrophic level interactions. Cambridge University Press, Cambridge, pp 148–173

Unsicker SB, Kunert G, Gershenzon J (2009) Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. Curr Op Plant Biol 12:479–485

Van Mele P, Vayssieres JF, Adandonon A, Sinzogan A (2009) Ant cues affect the oviposition behaviour of fruit flies (Diptera: Tephritidae) in Africa. Physiol Entomol 34:256–261

Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R (1994) Topographic organization of sensory projections to the olfactory bulb. Cell 79:981–991

Vet LE, Lenteren JV, Heymans M, Meelis E (1983) An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol Entomol 8(1):97–106

Visser JH (1988) Host-plant finding by insects—orientation, sensory input and search patterns. J Insect Physiol 34:259–268

Vogt RG, Riddiford LM (1981) Pheromone binding and inactivation by moth antennae. Nature 293:161–163

Vosshall LB, Hansson BS (2011) A unified nomenclature system for the insect olfactory coreceptor. Chem Sens 36(6):497–498

Vosshall LB, Stocker RF (2007) Molecular architecture of smell and taste in Drosophilla. Ann Rev Neurosci 30:505–533

Vosshall LB, Wong AM, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102:147–159

Wada-Katsumata A, Silverman J, Schal C (2013) Changes in taste neuron support the emergence of an adaptative behavior in cockroaches. Science 340:972–975

Wang Z, Singhvi A, Kong P, Scott K (2004) Taste representations in the Drosophila brain. Cell 117:981–991

Warthen JD, Morgan ED (1990) Insect feeding deterrents. In: Morgan ED, Mandava NB. CRC Handbook of natural pesticide, Boca Raton, pp 23–134

Webster B, Bruce T, Pickett J, Hardie J (2010) Volatiles functioning as host cues in a blend odor become nonhost cues when presented alone to the black bean aphid. Anim Behav 79:451–457

Weintraub PG (2009) Physical control: an important tool in pest management programs. In: Ishaaya I, Horowitz AR. Biorational control of arthropods pests. Springer Science, Amsterdan

White GB (2007) Terminology of insect repellents, Chapter 2. In: Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods and uses. CRC Press, Taylor and Francis Group, New York

WHO (2002) Scaling-up insecticide-treated netting programs in Africa. WHO/CDS/RBM/2002.43

WHO (2013) Guidelines for efficacy testing of spatial repellent, Control of neglected tropical diseases. WHO pesticide evaluation scheme, p 58

Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbenou L, Dabire R, Aikpon R, Boko M, Glitho I, Akogbeto M (2011) Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin. Parasite Vector 4:60–69

Yarmolinsky DA, Zuker CS, Ryba NJ (2009) Common sense about taste: from mammals to insects. Cell 139:234–244

Zaim M, Aitio A, Nakashima N (2000) Safety of pyrethroid-treated mosquito nets. Med Vet Entomol 14:1–5