Comparison of biological methods to control *Aphis fabae* Scopoli (Hemiptera: Aphididae) on kalanchoe crops in East Africa

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Abstract

Aphids cause considerable damage to numerous crops all over the world and insecticides are the main means of controlling them, despite their detrimental impacts on human and environmental health. This study assessed the effectiveness of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae), a mixture of predatory ladybird beetles, *Hippodamia variegata* Goeze*,* *Chilocorus calvus* Chiccl, and *Cheilomenes propinqua* Mulsant (Coleoptera: Coccinellidae), and an entomopathogenic strain of *Aspergillus flavus* Link (Eurotiales: Trichocomaceae), collected locally in Tanzania, to control *Aphis fabae* Scopoli (Hemiptera: Aphididae). After assessing the predation and parasitism rates of these natural enemies at different aphid densities in laboratory experiments, their ability to control aphids on kalanchoe was assessed in a greenhouse experiment over two seasons. The largest number of *A. fabae* parasitized or consumed in the laboratory was found at a density of 160 aphids per predator, or parasitoid. At that density, an adult female of *A. colemani* parasitized 114 *A. fabae* per day, on average, and adults of *C. calvus*, *H. variegata*, and *C. propinqua* consumed 75, 72, and 85 aphids per day, respectively. *A. flavus* spores applied at $1x10^7$ spores ml^{-1} reduced the aphid population by 7.9 and 12.6 times within 10 days in the first and second seasons of the greenhouse experiments, respectively, as opposed to 2.8 and 2.5 times by releasing a mixture of the ladybirds at a rate of 5 adults/m^2, and by 3.3 and 9.5 times by releasing *A. colemani* at a rate of 2 adults/m^2. This study confirmed the potential of these locally collected bio-control agents for controlling *A. fabae*. However, use of the isolated *A. flavus* strain was undermined by its production of aflatoxin. Further research is therefore required to tap into the potential of a non-toxic strain of *A. flavus* and/or other entomopathogenic fungi.

Keywords: Tanzania; entomopathogenic fungus; parasitoid; predators; biological control; greenhouse

1. Introduction

Aphids cause considerable damage to numerous crops all over the world by (i) sucking plant sap resulting in leaf/fruit deformations, necrosis, gall formation, (ii) transmitting pathogenic viruses, and (iii) secreting honeydew, which promotes sooty mold development and reduces photosynthesis (Dedryver et al., 2010). Aphids can promptly reach damaging levels for plants because of their short life cycle, notably induced by their ability to telescope generations, since newborn aphids contain the embryo of their first grand-daughters (Leather et al., 2017). The appearance of winged morphs, which is believed to be induced by a combination of several factors, such as overcrowding, poor resources on host plants, the presence of natural enemies, and meteorological factors, promotes the rapid dispersion of aphids (Auad et al., 2009; Müller et al., 2001).

Several organophosphate and carbamate pesticides have been used to control aphids, but their negative impacts on human and environmental health gradually led to increasing reliance on pyrethroids and subsequently on neonicotinoids. Given their adverse impacts on pollinators, several neonicotinoid pesticides are gradually being banned or restricted, despite their effectiveness (Dewar and Denholm, 2017). The increasing concerns of consumers about pesticides, as well as the development of resistance to chemical pesticides in various aphid species, warrant alternative control methods (Foster et al., 2007).

Initial attempts to control aphids in greenhouses using parasitoids started more than a half-century ago and about ten parasitoid species from three families, Aphelinidae, Aphidiidae, and Braconidae, are currently available on the market (Boivin et al., 2012). Efforts to control aphids in greenhouses with predators also date back several decades with the release of species from different taxa, including Cecidomyiidae, Coccinellidae, and Syrphidae (Ofuya, 1995; Sutherland et al., 2001). Several entomopathogenic fungi, including *Lecanicillium* spp., *Beauveria* spp., *Metarhizium* spp. and *Isaria fumosorosea* are also currently marketed to control aphids (Aw and Hue, 2017; Hance et al., 2017; Kim et al., 2007).

A favorable and stable climate, access to water and fertile lands, along with a readily available workforce, have fostered the development of floriculture in several East African countries, including Kenya, Tanzania, Uganda, and Ethiopia. Most of the floriculture products in Tanzania are roses, but cuttings from the *Kalanchoe* genus have also started to become one of the horticultural products widely exported to Europe (Mwase, 2015). Kalanchoe crops grown in greenhouses are affected by two aphid species, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Aphis fabae* Scopoli (Hemiptera: Aphididae). Their control in the greenhouse is complicated by favorable climatic conditions, as well as a lack of natural enemies, which is conducive to their rapid outbreaks.

Several pests have been successfully controlled using biological control agents in East Africa, for instance, the diamondback moth (DBM) on cabbages with the parasitoid *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) (Gichini et al., 2008) or the red spider mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) with predatory mites, *Phytoseiulus persimilis* and *Neoseiulus* (*Amblyseius*) *californicus* McGregor (Mesostigmata: Phytoseiidae) in rose flowers farms. Although biological products have increasingly been marketed in the United States, Europe, or Asia for several decades (van Lenteren et al., 1997), their use in East Africa is hindered by a lack of suppliers, a limited number of registered products, improper storage conditions degrading product quality, and a lack of suitability for local climatic conditions. Using natural enemies that have been collected and bred locally appears to be a more suitable approach to overcoming these challenges.

This study was set out to assess the efficiency of locally occurring natural enemies, *i.e.*, predators, parasitoids, and entomopathogenic fungi, in controlling aphids on kalanchoe crops in a greenhouse production set-up. Efforts focused on the black aphid (*A. fabae*) which, unlike the green peach aphid (*M. persicae*), affects kalanchoe plants all year round in the study location. Once natural enemies collected from the field had been screened under laboratory conditions, their efficiency in controlling *A. fabae* was assessed on kalanchoe crops in a greenhouse trial over two consecutive seasons. The natural enemies assessed were: a parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae), a mixture of predatory ladybird beetles, *Hippodamia variegata* Goeze*, Chilocorus calvus* Chiccl*,* and

Cheilomenes propinqua Mulsant (Coleoptera: Coccinellidae), and an entomopathogenic strain of *Aspergillus flavus* Link (Eurotiales: Trichocomaceae).

2. Material and Methods

2.1 Collection of local natural enemies

2.1.1 Collection of predators and parasitoids.

Adults and larvae of predators (ladybird beetles) and parasitized aphids were collected in September and October 2018 from fields of amaranth (*Amaranthus* spp.), common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp.), and okra (*Abelmoschus esculentus* (L.) Moench) infested by black aphids at the World Vegetable Center campus in Arusha, Tanzania (Latitude: 3.3753°S, Longitude: 36.805°E).

Predators (Coleoptera) and parasitoids (Hymenoptera) were separately reared in insect cages (80 x 80 x 80 cm) at 25 ± 2ºC and 60 ± 5% RH with a photoperiod of 12:12 (L: D) h. The insects were provided with potted cowpea plants infested with black aphids (originating from a previously reared pure culture) as a source of food and a breeding site for the predators and the parasitoids, respectively. The parasitoids were fed with honey smeared on pieces of sponge hung between the top of the cage and the top of the cowpea canopy. The predators and parasitoids were morphologically identified at the National Museum of Kenya using previously identified specimens, and specimens of individuals identified in the scope of this study were deposited in the collection of the International Centre of Insect Physiology and Ecology (ICIPE).

2.1.2 Collection of fungi

At the same time as the insect samples were collected, fungi were also collected and assessed in the laboratory for their effectiveness in controlling *A. fabae* in a prior study (Boni et al., 2020). This study showed that the most effective entomopathogenic fungus in controlling *A. fabae* was *Aspergillus flavus*. The laboratory experiments conducted in that previous study also showed that *A. flavus* spore suspensions at a concentration of 10^7 killed up to 90% of aphids. The strain was cultured on PDA medium at 28 ± 1°C in an incubator (Boni et al., 2020).

2.2 Laboratory assessment of parasitism and predation rates

2.2.1 Parasitism rate

A complete randomized experimental design was used to compare the parasitism rate of a single female wasp (*A. colemani*) over 24 h at different host densities. Individual female wasps were exposed to five different host density levels: 20, 40, 80, 160, and 320 second and third instar aphids per arena. Each treatment (*i.e*. host density) was replicated thirty times. The experiment was conducted in June 2019 at 25°C with a photoperiod of 16 h light and 8 h dark. Aphids were placed on three young cowpea leaves in a plastic box (1 liter) and one naive mated, 2 to 3-day-old *A. colemani* female was placed in each plastic box, which was then covered with a perforated lid for 24 h, and subsequently removed. *Aphidius colemani* individuals were sexed according to the abdomen shape. The female has a pointed abdomen with an ovipositor, while the male has a rounded abdomen (Khatri, 2017). The number of mummified aphids was recorded daily for 15 days.

2.2.2 Predation rate

The predation rates for adults of three ladybird beetle species, *H. variegata, C. calvus, and C. propinqua*, were assessed at different prey densities using a complete randomized experimental design. The individual ladybird adults of each species were exposed to four different prey density levels of 40, 80, 160, and 320 prey per arena, each being replicated thirty times. The experiment was conducted in August 2019 at 25°C with a photoperiod of 12:12 (L: D) h.

The prey, *A. fabae* (second and third instars) were placed on three young cowpea leaves in a 1L plastic box of similar characteristics to those used for the parasitoid experiment explained above. One adult of each of the three previously mentioned ladybird beetle species was placed in each plastic box containing the aphids. The number of missing aphids and those found partially consumed (body parts) in each plastic box was then recorded after 24 h.

2.3 Greenhouse trials to assess the efficiency of biological control agents against *Aphis fabae* on kalanchoe

2.3.1. The experimental site and design

A greenhouse experiment was conducted at the World Vegetable Center campus in Arusha over two seasons from May to October 2019 (dry season) and from November 2019 to March 2020 (rainy season). A Latin square experimental design with four treatments and four replications was used. The four treatments were (i) a mixture of three predatory ladybird beetle species (*H. variegata, C. calvus,* and *C. propinqua*), (ii) the *A. colemani* parasitoid, (iii) the *A. flavus* entomopathogenic fungus, and (iv) an untreated control. Each experimental unit was a separate greenhouse (a total of 16 greenhouses). All greenhouses were identical and measured 6 m wide, 14 m long and 3.5 m high, with straight walls and a half-moon roof. The walls and roofs of the greenhouses were covered with woven insect-proof nets with a 0.4 mm x 0.7 mm mesh size (AgroNet, AtoZ Textile Mills, Arusha, Tanzania). A double roof, 0.75 m above the first one, was covered with a polyethylene film and a shade net (50% shade) to protect the plants from rain and high solar radiation. The greenhouses were equipped with a double door system to restrict the movement of insect species to and from the greenhouses. Two varieties of kalanchoe (Perfecta White and Perfecta Rosa) were grown simultaneously in all the greenhouses in separate raised beds.

2.3.2 Crop management

Kalanchoe cuttings are produced all year round in the study location and can be harvested over several months after pinching in commercial conditions. The greenhouses were fitted with an electric lighting system to inhibit flowering of the kalanchoe plants by ensuring a 24 h photoperiod. Two lines of 9 bulbs (5 watts) running the length of the greenhouse were installed 2.5 m above the ground.

Well-decomposed cow manure was incorporated into the soil at 3 kg/m^2 during plowing. Unrooted kalanchoe (var. Perfecta White and Perfecta Rosa) cuttings were planted at a density of 100 plants per m^2 in two different raised beds, each measuring 1.5 m wide and 12 m long, spaced 1 m apart. The plants were irrigated daily with drippers to maintain high soil moisture, and were fertilized weekly by drenching with a solution of 17-17-17 NPK at 2 g per liter with a watering can. The plants were pinched four weeks after planting using blades to promote lateral branching and maximize their vegetative area for aphid infestation. Three weeks after pinching, the plants were infested with five adult *A. fabae* (second and third instars) per m². Natural enemies were released once the population of aphids had built up to an average of five adult aphids per plant in a random sampling of 20 plants per bed down its middle axis. Five aphid releases one week apart were required to build up a sufficient population (5 aphids per plant) to release natural enemies.

2.3.3. Application of natural enemies

A mixture of three adult ladybird beetle species, *H. variegata*, *C. calvus*, and *C. propinqua,* at a proportion of one third each, was released into the respective greenhouses to achieve a density of five insects per m², making a total of 140 individuals of each species released in a greenhouse of 84 m^2. *Aphidius colemani* adults were released into the respective greenhouses to achieve a density of two adults per m², making a total of 168 individuals (mix of males and females).

The parasitoids and predators were released twice, 98 and 109 days after transplanting in the first season and once only, 129 days after transplanting, in the second season. A knapsack sprayer was used to apply a spore suspension of the entomopathogenic fungus, *A. flavus*, on kalanchoe plants at a concentration of 1×10^{10} spores L^{-1}, which was the concentration level that showed the greatest efficiency in controlling *A. fabae* under laboratory conditions (Boni et al., 2020), at 0.2 L m^{-2}. Spores of the entomopathogenic fungus were sprayed only once in the first and second seasons, 98 and 129 days after transplanting, respectively.

2.3.4. Data collection

Weather: Hourly rainfall records were extracted from a complete weather station (Vantage PRO2, Davis Instruments, California, The USA) installed outside the greenhouses and the temperature and relative humidity were recorded every 30 minutes inside each greenhouse using data loggers (HOBO

Pro v2 U23-001, Onset Computer Corporation, Massachusetts, USA). The loggers were placed in the middle of the greenhouse at a height of 1.8 m, and were covered with perforated white shelters with a wide open bottom to avoid direct exposure to the sun.

Insect sampling: The total number of aphids at all development stages, of mummies, and of Coccinellidae larvae were monitored twice a week in all the greenhouses on the 20 plants previously selected from the two varieties tested.

2.4 Statistical analysis

All statistical analyses were carried out using R software (version 3.6.1) (R Development Core Team, 2012) with the agricolae (De Mendiburu, 2014), the lme4 (Bates et al., 2007), and the multcomp (Hothorn et al., 2016) packages.

Generalized linear models (GLM) with a quasipoisson distribution were used to compare the number of mummified aphids and the number of consumed aphids in laboratory experiments. Post-hoc analyses were carried out to compare treatment means when significant differences were established using the Tukey's honestly significant difference.

Poisson generalized mixed models (Poisson GLMM) including 'days after treatment' as fixed effects and 'plots' as random effects (since counts from the same greenhouse are correlated) were carried out to compare the number of Coccinellidae larvae and mummies per plant between the measurement date and the previous date. Backward selection, using likelihood ratio (LR) tests, were performed to assess the significance of the fixed effects.

The number of aphids per plant was analyzed by fitting Poisson GLMM including 'seasons', 'varieties', 'treatments', 'days after treatment' as fixed effects, and 'plots' as random effects. Post-hoc analyses were carried out at each date of measurements to compare treatments means using the Tuckey test.

Generalized linear models (GLM) with a quasipoisson distribution were used to compare the total of aphids counted on twenty plants over the experiment between treatments and varieties. Post-hoc analyses were carried out to compare treatment means when significant differences were established using the Tukey's honestly significant difference.

3. Results

3.1. The richness of collected natural enemies.

At time of the sample collection, several species of aphids were observed on amaranth, common bean, cowpea, and okra, including the black bean aphid (*A. fabae*), the cowpea aphid (*A. craccivora*) and the peach aphid (*M. persicae*). Mummies infested by *A. colemani,* and by a secondary parasitoid *Dendrocerus* sp. Ratzeburg (Hymenoptera: Megaspilidae), were found in collections from open-field vegetable crops on the WorldVeg campus (Table 1). Several Coccinellidae adults, *i.e*., *H. variegata*, *C. calvus*, and *C. propinqua*, and an unidentified hoverfly species were also found.

3.2. Assessment of the parasitism and predation rates in the laboratory

The mean number of *A. fabae* hosts parasitized over 24 h increased linearly from 16.4 to 114.3 (p<2.2 x 10^{-16}, df=4) by increasing the host density from 20 to 160 (Table 2). An increase in the host density from 160 to 320 did not significantly increase (p=0.334, df=1) the number of hosts parasitized. No significant differences in the parasitism rates were observed between host densities from 20 to 160 (p=0.459, df=3), with a mean value of 77.0%. In line with previous results, an increase in host density to 320 significantly decreased (p=7.58 x 10^{-7}, df=1) the parasitism rate to 39.2% (Table 2). The number of aphids consumed per day varied with the prey density (p<2.2 x 10^{-16}, df=3) (Table 3) but no significant variations were established between the Coccinellid species studied (p=0.759, df=2).

The mean number of aphids consumed per day increased linearly from 29.1 to 74.5 (p=1.52 x 10^{-8}, df=3), from 25.8 to 72.1 (p=1.36 x 10^{-13}, df=3), and from 28.4 to 84.8 (p<2.2 x 10^{-16}, df= 3) by increasing the prey density from 40 to 160 for *C. calvus*, *H. variegata*, and *C. propinqua*, respectively. An increase in the prey density from 160 to 320 did not significantly increase the number of aphids consumed by *C. calvus* (p=0.559, df=1), *H. variegata* (p=0.586, df=1), and *C. propinqua* (p=0.109, df=1). The predation rates for *C. calvus*, *H. variegata*, and *C. propinqua* significantly declined from 72.8 to 21.8% (p<2.2 x 10^{-16}, df=3), from 64.6 to 21.1% (p<2.2 x 10^{-16}, df=3), and from 71.1 to 21.8% (p<2.2 x 10^{-16}, df=3), respectively, by increasing the prey density from 40 to 320 (Table 3).

3.3. Greenhouse experiments

The second season of the greenhouse experiment was wetter, *i.e*., 723 mm as opposed to 122 mm of rainfall, and warmer, *i.e*., 23.7°C as opposed to 20.8°C on average, than in the first season. The temperature varied from 15.6 to 29.8°C and from 17.7 and 34.1°C, on average, in the first and second seasons, respectively (Figure 1). The number of mummies and Coccinellidae larvae varied over time in the greenhouses into which predators and parasitoids were released (Figure 2). Population of parasitoids was quicker to build up than Coccinellidae, as shown by the earlier appearance of mummies than Coccinellidae larvae.

The number of aphids per plant before imposing treatments was significantly higher (p<2.2 x 10^{-16}, df=1) on the 'Perfecta Rosa' variety, *i.e*., 4.3 and 7.2 on average during the first and the second season respectively, than on the 'Perfecta White' variety, *i.e*., 0.1 and 0.02 on average during the first and the second season, respectively. The total number of aphids on plants was also significantly higher (p<2.2 x 10^{-16}, df=1) on the 'Perfecta Rosa' variety than on the 'Perfecta White' variety (Table 4). Further analyses did not include data on the 'Perfecta White' variety because of the poor aphid establishment and hence focused only on the 'Perfecta Rosa' variety (Figure 2).

Large temporal variations in the aphid populations were recorded in the untreated control greenhouses in the first season, despite no treatment being applied (Figure 3). All of the tested biological control agents significantly reduced the aphid populations during the first (p=0.015, df=3) and the second season (p=3.92 x 10^{-5}, df=3). After the application of *A. flavus* spores, the average number of aphids per plant significantly declined by 7.9 (p<2.2 x 10^{-16}, df=1) and 12.6 (p<2.2 x 10^{-16}, df=1) times within 10 days in the first and second seasons, respectively. One month after *A. flavus* spores were applied, the aphid population started to rise again (Figure 2). The aphid population significantly declined by 2.75 (p<2.2 x 10^{-16}, df=1) and 2.5 (p<2.2 x 10^{-16}, df=1) times within 10 days after releasing the mixture of ladybird beetles in the first and second seasons, respectively. In contrast to the greenhouses where *A. flavus* spores were applied, no increase in aphid population was observed after the release of predators (Figure 3).

In the greenhouses where the parasitoid *A. colemani* was released, the number of aphids significantly declined by 3.3 ($p<2.2 \times 10^{-16}$, df=1) and 9.5 ($p<2.2 \times 10^{-16}$, df=1) times within 10 days in the first and the second seasons, respectively (Figure 3).

The minimum number of aphids per plants in the greenhouses where fungus spores were applied was 0.0 and 0.01 in the first and second seasons, respectively, as opposed to 1.31 and 0.73 in the greenhouses where parasitoids were released, 0.0 and 0.38 in the greenhouses where ladybird beetles were released, and 2.33 and 5.68 in the untreated control greenhouses. In line with the analysis of the temporal changes of aphid infestation (Figure 2), the total number of aphids counted over the experiment (Table 4) significantly varied among the treatments ($p<2.2 \times 10^{-16}$, df=3).

4. Discussion

All the biological control agents collected locally and tested in this study were highly efficient in controlling *A. fabae* on kalanchoe crop grown in greenhouses. Further studies would be required to identify underlying factors related to the greater attraction of plants of the Perfecta Rosa variety to aphids than plants of the Perfecta White variety.

The parasitoid identified as *A. colemani* tested in this study is a solitary, koinobiont endoparasitoid of aphids, and is one of the most successful commercial biological control agents used in greenhouse crops. *Aphidius colemani* is already marketed in Europe and the United States to control different aphid species, including *A. fabae, A. gossypii*, and *M. persicae* (Hance et al., 2017). The mean parasitism rate of 77.0% recorded on *A. fabae* over 24 h for host densities ranging from 20 to 160 in this study was higher than the rates previously reported on *A. gossypii* (56%) and on *M. persicae*, (50%) at 25°C for a density of 100 individuals (Zamani et al., 2007). The higher parasitism rate in this experiment may be attributable to the smaller size of the arena used (1-liter plastic box) compared to the one used by the previous authors (35 x 35 x 50 cm cages), thereby increasing the spatial density of hosts and the efficiency of the parasitoid (Walde and Murdoch, 1988).

The linear increase in the number of parasitized *A. fabae* by *A. colemani* at host densities from 20 to 160 to reach a maximum measured suggest a type I functional response. Previous studies rather reported a type III functional response of *A. colemani* on *Myzus persicae* (Byeon et al., 2011) and a type II on *Aphis gossypii* (Zamani et al., 2006). As previously discussed, the small size of the arena used might affect the host-parasitoid interaction and further work would be therefore required to better understand the functional response of *A. colemani* on *A. fabae*.

Our field results confirmed previous studies reporting the efficiency of *Aphidius* spp. in controlling aphids on various greenhouse crops, including sweet pepper, cucumber and beans (Hance et al., 2017).

Our results showed that the parasitoid did not eradicate aphids but only reduced their population to a certain threshold. This was consistent with results from the laboratory experiments recording no significant difference in the parasitism rate at aphid densities ranging from 20 to 160 (Table 2).

Even though *A. colemani* is known to be more resistant to warm climates than *Aphidius* matricariae Haliday (Zamani et al., 2006), its development may have been undermined by temperature exceeding 30°C during the experiments (Goh et al., 2001). Although daily average temperature did not exceed 25°C during the experiments due to cold nights, higher temperature range during the second season with maximum temperature exceeding 35°C (Figure 1) may have impeded the development of *A. colemani*. A previous study called for caution regarding the ability of *A. colemani* to control aphids in the presence of the hyperparasitoid (*Dendrocerus* sp.), which was observed in the surrounding plots during the collection campaign (Nagasaka et al., 2010), but this insect was not observed in the greenhouses during the study.

Our greenhouse results were consistent with those from previous studies reporting the merits of *H. variegata* (Jafari, 2011; Madadi et al., 2011) and *C. propinqua* (Sæthre et al., 2011) as predators of aphids. Our results provided proof of the merits of using *C. calvus* to control *A. fabae*. Other species of the same genus, such as *Chilocorus nigritus* (F.) (Ponsonby, 2009) and *Chilocorus bipustulatus* L. (Eliopoulos et al., 2010) have been reported as biological control agents against scale insects.

Our laboratory results showed that adults of *H. variegata* can consume more than 80 *A. fabae* per day when the resource is not limiting. Lower voracity was reported in a previous study indicating that male and female adults of *H. variegata* can consume up to 18 and 45 *A. fabae* per day, respectively (Farhadi et al., 2010b). It was estimated by a later study that the larvae of *H. variegata* can consume 142 *A. fabae* adults throughout their development (Skouras and Stathas, 2015). Since a similar predation rate was recorded in this study for *C. propinqua* and *C. calvus*, it was decided to release a mixture of the three ladybird beetles, assuming that it would increase the chances of their establishment in the greenhouses. Our field results showed that ladybird beetles successfully controlled *A. fabae* population in the greenhouse over several weeks. *Hippodamia variegata* has already been successfully used against *A. gossypii* in cucumber greenhouses (El Habi et al., 2000) and against *M. persicae* on sweet pepper, despite the fact that better results were reported with *Adalia bipunctata* L. (Beltrà et al., 2018). Our results showed that the release of coccinellids steadily reduced the aphid population over the long term, in contrast to parasitoids. This may be attributable to the fact that coccinellids consume aphids throughout their development, and to their longer longevity compared to parasitoids, *i.e*., up to 55 days for a female of *H. variegata* (Jafari, 2011) as opposed to around 10 days for *A. colemani* (Wäckers et al., 2008).

The Coccinellid species studied exhibited a type I functional response on different prey density although previous studies on *H. variegata* reported a type II functional response on *A. fabae* (Farhadi et al., 2010a; Jafari and Goldasteh, 2009). As previously discussed for *A. colemani*, the discrepancy in findings with previous studies may be attributed to the size of the arena used.

The *A. flavus* strain selected after laboratory results for its effectiveness in controlling *A. fabae* (Boni et al., 2020), was found to be particularly effective in our greenhouse experiments. Other studies have reported on the ability of *A. flavus* to control aphids (Seye et al., 2014) and the tomato leaf miner, *Tuta absoluta* Meyrick (Zekeya et al., 2019). Despite its widespread presence in tropical regions, the potential use of this fungus as a biological control agent is undermined by the aflatoxins produced by several strains (Scheidegger and Payne, 2003). Further studies should therefore focus on atoxigenic strains of *A. flavus* (*i.e*., lacking the ability to produce aflatoxins), or on other species of entomopathogenic fungi (Moral et al., 2020).

The greatest obstacles to using biological control agents remain the cost of mass-rearing insects and the availability of quality products. Rearing parasitoids and predators is labor-intensive and expensive, since it takes more man-days to multiply not only the natural enemies, but also their hosts/prey and their food (plants). Producing parasitoids on aphids that are themselves reared on artificial media was reported as a suitable alternative for reducing the costs and time involved in *in vivo* production methods (Jingwen et al., 2018). Coccinellid species are usually mass-produced by feeding them with aphids, *Trichogramma chilonis* Ishii. pupae, or *Ephestia kuehniella* Zeller eggs

(Cheng et al., 2018; Mahyoub et al., 2013), but good results have also been reported with artificial diets (Cheng et al., 2018; Sarwar and Saqib, 2010).

In terms of production costs, ease of storage and application, entomopathogenic fungi seem to be the most suitable alternative to pesticides. In contrast to predators and parasitoids, which tend to disperse after release, the use of fungi is not restricted to confined spaces. Consequently, further work should tap into the potential of entomopathogenic fungi as biopesticides in aphid management.

5. Conclusions

Laboratory and greenhouse experiments confirmed the ability of the parasitoid (*A. colemani*), the predators (*H. variegata*, *C. calvus*, and *C. propinqua*) and the entomopathogenic fungus (*A. flavus*), collected locally in Arusha, Tanzania to control the bean aphid, *A. fabae* on kalanchoe crops. Given its efficiency, low production costs and ease of storage and application, the entomopathogenic fungus was found to be of special interest. However, use of the isolated strain was undermined by its production of aflatoxin. Further research is therefore required to tap into potential, locally occurring and non-toxic entomopathogenic fungi.

Tables and Figures

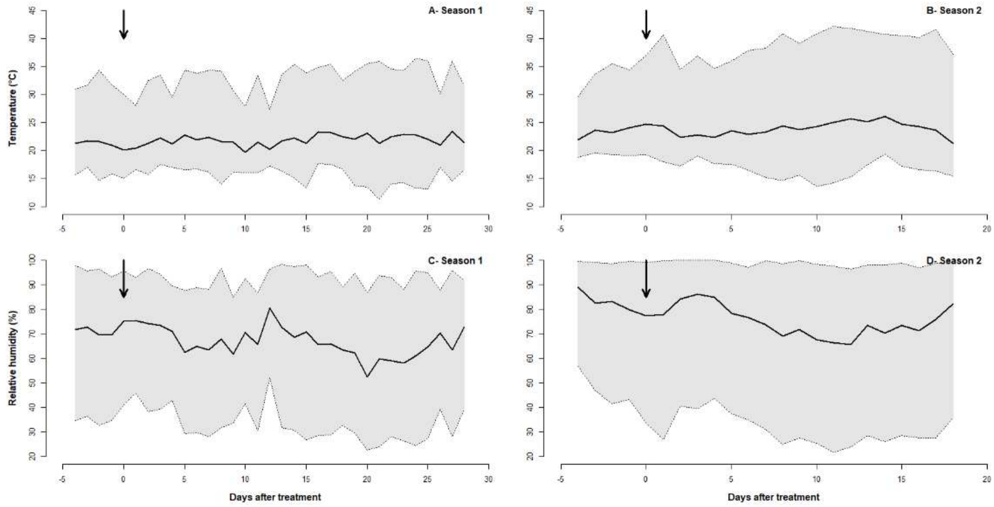

Figure 1: Temperature (A and B, in °C) and relative humidity (C and D, in %) inside the greenhouses over the two seasons of the field experiments. Solid lines represent the daily average values and the dashed lines represent daily minimum and maximum values. Arrows represent the dates of the first release of biological control agents.

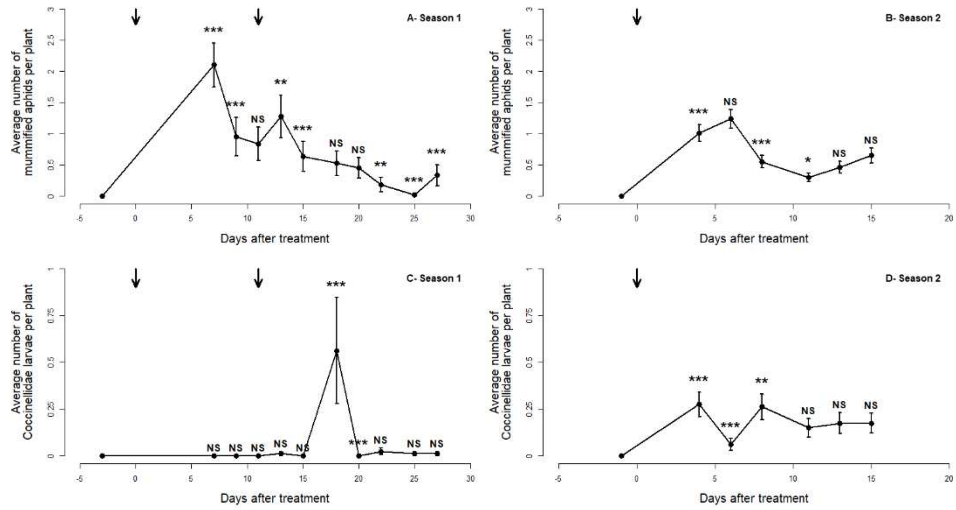

Figure 2: Comparison of the mean number (± standard errors, N=4) of mummified aphids (A and B) and Coccinellidae larvae (*Chilocorus calvus*, *Cheilomenes propinqua*, and *Hippodamia variegata*) (C and D) monitored on twenty kalanchoe plants (Perfecta Rosa variety) in greenhouses after releasing parasitoids (*Aphidius colemani*) and Coccinellidae. Arrows represent the dates on which predators and parasitoids were released. “NS”, “*”, “**”, and “***” mean no significant difference and significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, in the number of mummified aphids, or the number of Coccinellidae larvae since the previous measurement date.

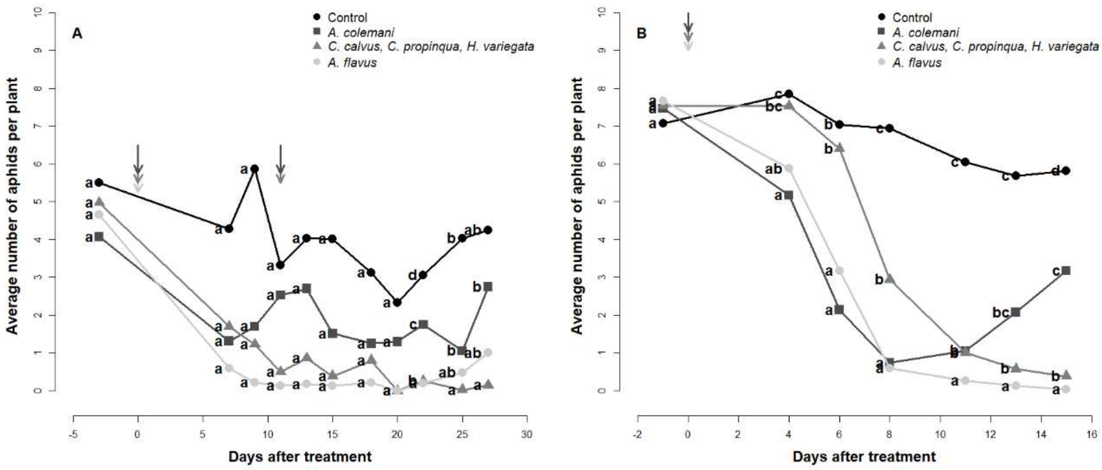

Figure 3: Comparison of the mean number (N=20) of black aphids (*A. fabae*) counted on kalanchoe plants (Perfecta Rosa variety) per greenhouse between the different treatments, *i.e.*, control (no biological control agent), fungus (*A. flavus*), parasitoid (*A. colemani*), and predator (a mixture of *Chilocorus calvus*, *Cheilomenes propinqua*, and *Hippodamia variegata*), over two seasons (A: first season; B: second season). Arrows represent the treatment date (light gray = fungus, black = parasitoid, and dark gray = predator). Different lower case letters mean significant differences between treatments at $P < 0.05$ between treatments on different dates.

Table 1. List of *Aphis fabae* natural enemies found on open-field vegetable crops at the WorldVeg campus, Arusha, Tanzania

Natural enemies	Family	Species
Parasitoids	Braconidae	*Aphidius colemani*
	Megaspilidae	*Dendrocerus* sp
Predators	Coccinellidae	*Hippodamia variegata*
		Chilocorus calvus
		Cheilomenes propinqua
	Syrphidae	Undetermined species

Table 2. Comparison of the number of aphids parasitized by *Aphidius colemani* and parasitism rates over 24 hours on different densities of *Aphidius fabae*. Different lowercase letters mean significant differences ($P < 0.05$) in the number of aphids parasitized, or in the parasitism rate, between aphid densities in the same experiment. The data are means (± standard errors, N=30).

Aphid density	**Number of aphids parasitized**	**Parasitism rate (%)**
20	16.4 ± 1.0 d	82.0 ± 4.8 a
40	31.0 ± 1.8 c	77.4 ± 4.5 a
80	61.7 ± 2.9 b	77.1 ± 3.7 a
160	114.3 ± 6.2 a	71.4 ± 3.9 a
320	125.6 ± 10.1 a	39.2 ± 3.2 b

Table 3. Comparison of prey (*Aphis fabae*) eaten and the predation rates of three ladybird beetles (*Chilocorus calvus*, *Cheilomenes propinqua*, and *Hippodamia variegata*) according to different prey densities. The data are means ± standard errors (N = 30). Different lower case letters mean significant differences (P < 0.05) in the number of prey eaten or in the predation rates between the three ladybird beetles for a similar prey density, whereas different upper case letters mean significant differences ($P < 0.05$) in the number of prey eaten or in the predation rate between the different prey densities for the same species of ladybird beetle.

Prey density	**Number of aphids eaten by *Chilocorus calvus***	**Number of aphids eaten by *Hippodamia variegata***	**Number of aphids eaten by *Cheilomenes propinqua***	**Predation rate by *Chilocorus calvus* (%)**	**Predation rate by *Hippodamia variegata* (%)**	**Predation rate by *Cheilomenes propinqua* (%)**
40	29.1 ± 1.6 a C	25.8 ± 1.7 a C	28,4 ± 1.6 a C	72. 8 ± 4.0 a A	64.6 ± 4.4 a A	71.1 ± 4.1 a A
80	44.3 ± 2.4 ab B	48.7 ± 2.4 a B	39.6 ± 2.4 b B	55.3 ± 3.0 ab B	60.9 ± 3.0 a A	49.5 ± 3.0 b B
160	74.5 ± 6.0 a A	72.1 ± 4.4 a A	84.8 ± 4.7 a A	46.6 ± 3.7 a B	45.1 ± 2.7 a B	53.0 ± 3.0 a B
320	69.7 ± 4.5 a A	67,4 ± 5.9 a A	69.7 ± 6.1 a A	21.8 ± 1.4 a C	21.1 ± 1.8 a C	21.8 ± 1.9 a C

Table 4. Comparison of the average of the total number of aphids at all development stages (± standard deviation, N=4) counted on twenty plants over the experiment per variety (Perfecta White and Perfecta Rosa) and per greenhouse between treatments (control = no biological agent, fungus, predator, and parasitoid). Different letters mean significant differences in Tukey's HSD test (p=0.05).

Season	Variety	Treatment	Number of aphids
1	Perfecta Rosa	Control	1296.8 ± 1740.9 e
		Fungus	318.5 ± 96.9 c
		Predator	311.5 ± 216.3 c
		Parasitoid	716.5 ± 280.0 d
	Perfecta White	Control	3.8 ± 6.8 a
		Fungus	10.0 ± 19.3 b
		Predator	4.0 ± 3.8 a
		Parasitoid	3.2 ± 6.5 a
2	Perfecta Rosa	Control	1289.5 ± 694.9 e
		Fungus	350.0 ± 97.4 b
		Predator	513.5 ± 67.6 d
		Parasitoid	425.0 ± 81.1 c
	Perfecta White	Control	4.2 ± 8.5 a
		Fungus	0.0 ± 0.0
		Predator	0.0 ± 0.0
		Parasitoid	0.0 ± 0.0

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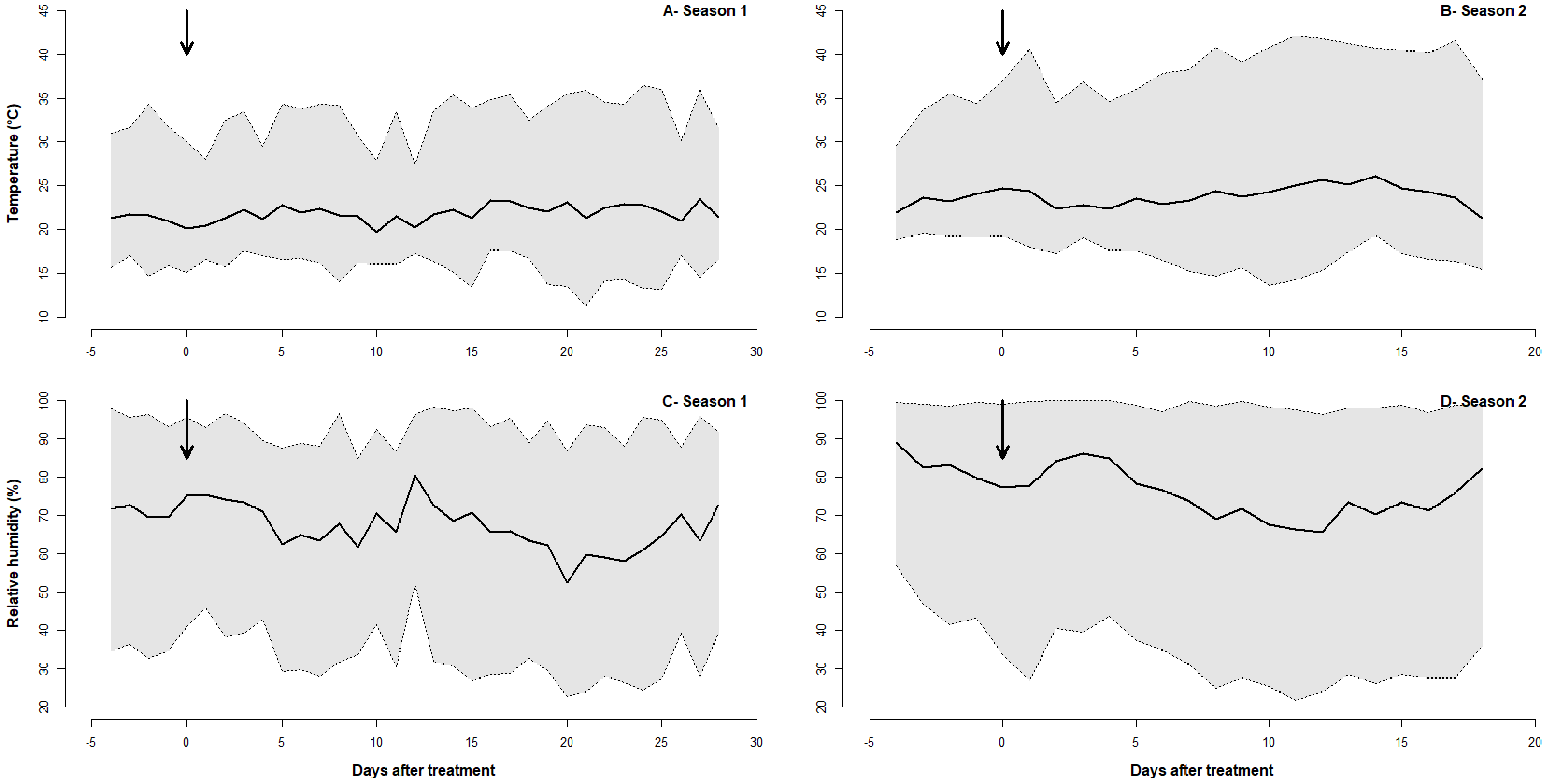
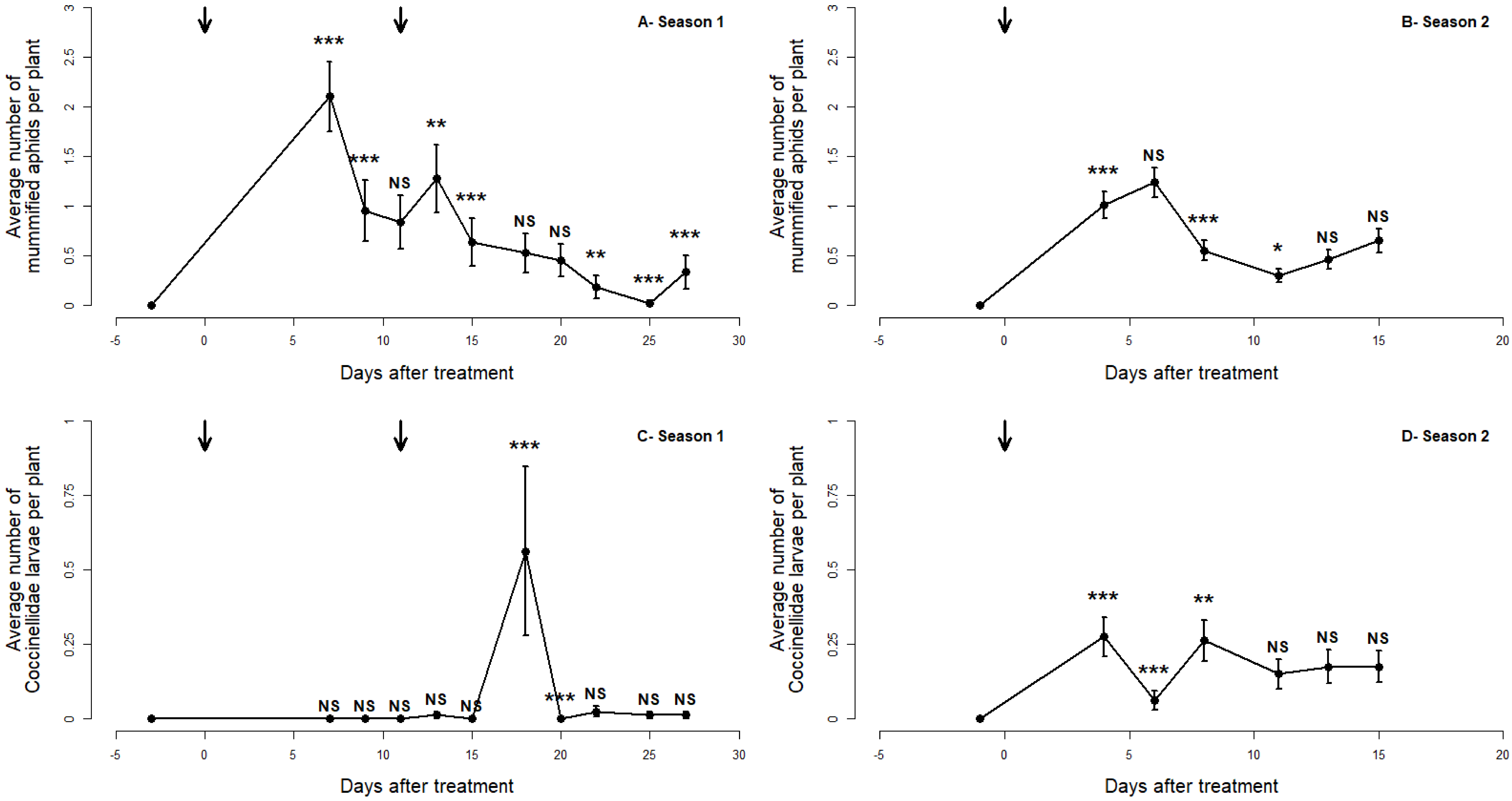


A- Season 1
B- Season 2
C- Season 1
D- Season 2
Temperature (°C)
Relative humidity (%)
Days after treatment
Days after treatment



A- Season 1
B- Season 2
C- Season 1
D- Season 2
Average number of mummified aphids per plant
Average number of Coccinellidae larvae per plant
Days after treatment
***
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NS
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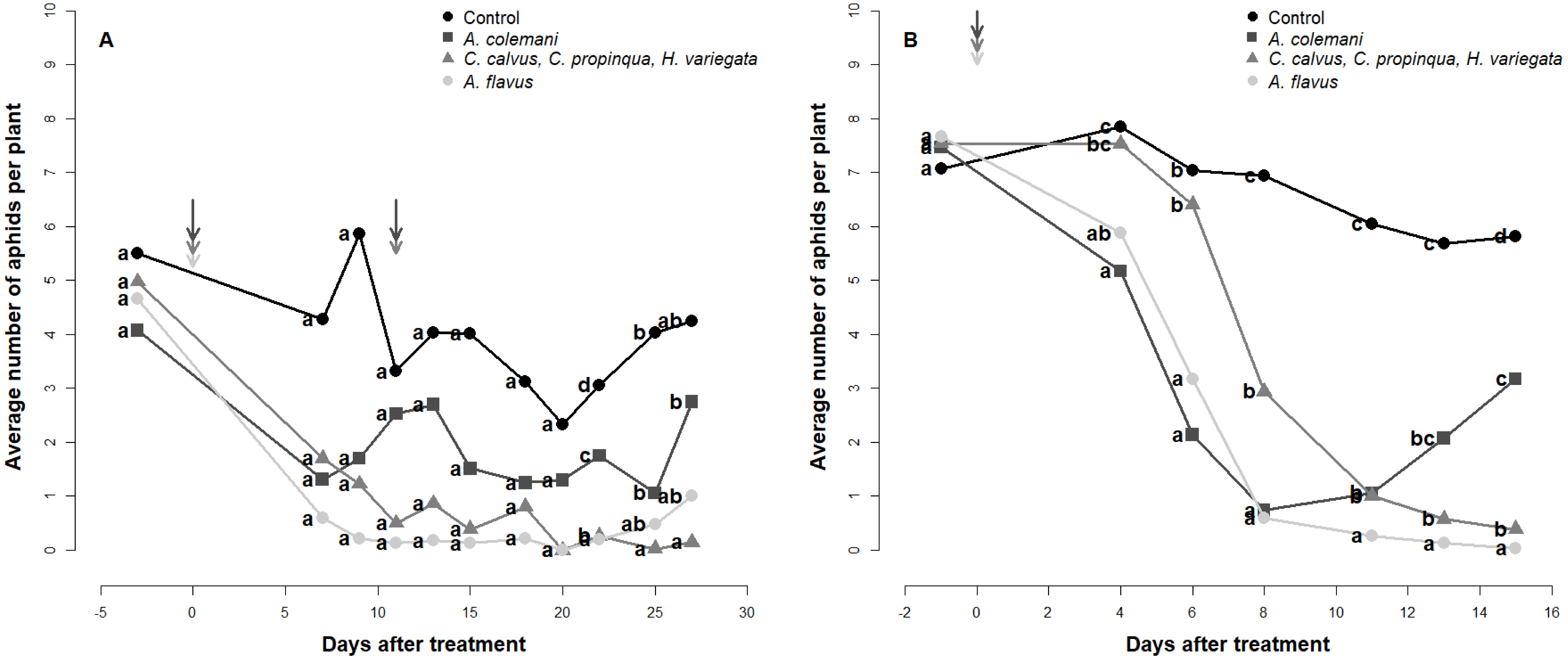

A
B
Control
A. colemani
C. calvus, C. propinqua, H. variegata
A. flavus
Average number of aphids per plant
Days after treatment