News from Réunion Island / France

A summary of CIRAD research on pest control (*R. reniformis*, nematode, and *D. Brevipes*, mealybug associated with wilt), based on the stimulation of natural defenses of the MD2 pineapple in pesticide-free cropping system.

Alain Soler et al., Cirad, Réunion Island

Introduction

Rotylenchus reniformis are phytophagous nematodes spread over many pineapple production areas as Réunion (I.O.), but also in Hawaii, in Caribbean islands and many other areas. *R. reniformis* are semi-endoparasitic nematodes as their populations grow mainly in the soil surrounding the roots. Only the females attach themselves to the outside of the roots, take on a kidney shape full of eggs, which are laid in the ground. Large infestations reduce the nutrition of the pineapple and consequently can reduce yield by more than 50%.

Mealybugs are present everywhere in the pineapple production areas in Reunion (Nurbel et al, 2020), but also almost everywhere in the pineapple 'world'. In combination with Pineapple Mealybug Wilt associated Viruses (PMWaVs) they induce a disease called 'Pineapple Wilt' that has a strong impact on the yield and quality of fruits produced for export (Hu et al, 2005). Mealybugs and therefore PMWaVs are spread through contaminated plant material harvested from infested fields. The wind can also disseminate the young larvae called 'crawlers' in the fields. Finally, several species of ants have a mutualistic interaction with mealybugs, as they feed on the honeydew produced by the mealybugs; in turn, the ants protect the mealybugs from their predators.

Pineapple MD2 is a good host for both parasites. In conventional production systems, these pests are controlled by chemical applications. Consumers, buyers and authorities out of concern for the protection of health and the environment increasingly criticize these practices. Thus, pesticides are gradually banned in many producing countries, and producers are looking for alternatives to pest control.

Plants have evolved basic defenses against pests. In different plant species, many varieties have been observed to have developed the ability to strengthen these basic defenses against a wide range of pests. Cell surface receptors recognize the presence of a pathogen and, via specific signaling pathways, prepare the plant to react rapidly to new infestations (priming of systemic resistances). Priming can be triggered by the presence of certain parasites or by hormonal elicitation (chemicals or microorganisms). The plant is then able to reduce the ability of parasite populations to multiply and develop by regulating its own defense genes and the production of toxic metabolites. Priming can be induced either as systemic acquired resistance (SAR) dependent on the hormone salicylic acid, or as induced systemic resistance (ISR) dependent on the hormone jasmonic acid. SAR and ISR target interaction against biotrophic or necrotrophic parasites respectively. We hypothesized that priming pineapple plants could contribute to pest control in a more ecological production system based on rotation with cover crops to benefit from a low initial inoculum (Soler at 2013, a). Here, two examples of present experiments show the potential management of *R. reniformis* and *D. brevipes* on the MD2 variety with SAR and ISR induced by appropriate stimulations. The possibility of using them as components of pesticide-free biocontrols is discussed. This is a synthetic presentation of CIRAD's research on this subject; detailed data have recently been published (Soler et al., 2021) or will be published soon, such as during the next ISHS Pineapple Symposium in 2023.

Material and Methods

Vitroplants of MD2 (~100g) and suckers (~150g) from a MD2 nursery were used for separate experimentations for *R. reniformis* and *D. brevipes*. Inocula of 4000 nematodes per plant, and inocula of 5 to 20 individuals of mealybugs inoculated per plant were prepared from populations reared on *Vigna unguiculata* and on small vitroplants of MD2 pineapple, respectively for nematodes and mealybugs. Plant priming was done with salicylic acid (SAL, 1 mM) for SAR, or methyl jasmonate (JAME, 0.1 mM) for ISR, which were used as elicitors of SAR and ISR defense signaling pathways. Counting of populations for *R. reniformis* were made after extraction by an elutriation – sieving method of 50g soil under pineapple (Seinhorst, 1962). Counting of *D. brevipes* populations were done manually under a binocular magnifying device. Greenhouse experiments were done with stimulation repeated three times at 3-day intervals, and field experiments with monthly stimulations (six in total) between planting and forcing. The field experiments were conducted using a rotation with *Crotalaria sp.* in a system of production without pesticide to reduce soil borne parasites and other stresses before planting pineapple. Priming was characterized with three of the enzymatic markers as PAL, PR1 and PR3 for nematode and mealybug experiments, and three molecular markers, AcPAL, AcICS and AcMYB like, for mealybugs experiments.

Result and Discussion

- **An ecological system of production**

An ecological pesticide-free system of production that included rotations with cover crops like *Crotalaria sp*., minimized inocula of *R. reniformis* populations and other soil pests like symphylidae before planting pineapple. The poor host status of Crotalaria species for the reniform nematodes is known to reduce their populations (Wang et al, 2003). Marie-Alphonsine et al (2017), and Soler et al. (2021) specifically tested *Crotalaria sp.* in pesticide-free production systems including rotation with pineapple to reduce *R. reniformis* but also Symphylidae. The biomass of crotalaria also contributed to high levels of soil organic matter (>50T fw/ha) with high levels of nitrogen (about 3%). They are also good cover crops reducing the impact of weeds on the next crop. We considered that these conditions were a key element of the production system to optimize the effectiveness of systemic resistances against pests because they reduce the stress level of pineapple (Soler et al, 2013a). A high level and/or number of stresses could reduce the effectiveness of systemic resistances by limiting the energy resources that the plant could mobilize to fight against a specific parasite (Choudhary et al., 2012).

- **Systemic resistances against the reniform nematode**

In greenhouse experiments on MD2 tissue culture plants, Soler et al. (2013) showed that the populations (eggs + adult vermiforms) were significantly reduced by both stimulations ISR and SAR, respectively -67.0% and -55.8% compared to controls. It was also noted that on the MD2 controls, nematode populations were higher than on the Smooth Cayenne controls (for example about 3x for eggs). The result indicated that, without priming, MD2 has been a better host than Smooth Cayenne for the reniform nematode.

In the same series of greenhouse experiments, Soler et al. have characterized the establishment of priming of ISR defenses with enzymatic markers such as Lipoxygenase, Superoxide dismutase, Peroxidase or Catalase (oxidative stress), and PAL, PR1 and PR2 (defense). A slight transient change in enzyme activities related to oxidative stress appeared within one hour after application of methyl jasmonate. This transient stress is normally observed with the setup of priming. Then two weeks after inoculation of nematode inoculum, during the multiplication of the reniform nematode, the activities of defense enzymes such as PAL, PR1 and PR3 were higher on the stimulated plants than on control plants (Soler et al., 2013, b). This is also related to an effective priming setup.

In a field experiment without pesticide, eggplants were grown before pineapple to increase the populations of reniform nematodes in the soil. Eggplants helped produce naturally a high inoculum of *R. reniformis (*Soler et al, 2021). The authors tested ISR stimulations (methyl-jasmonate) against *R. reniformis* on pineapple suckers of MD2 on this experimental plot. The results showed that eight months after planting pineapple, *R. reniformis* populations were significantly reduced by -58.4% in this field. The infestations of pineapple were respectively 4302 and 10332 individuals in 100g of soil for stimulated plants and non stimulated ones **(Figure 1)**.

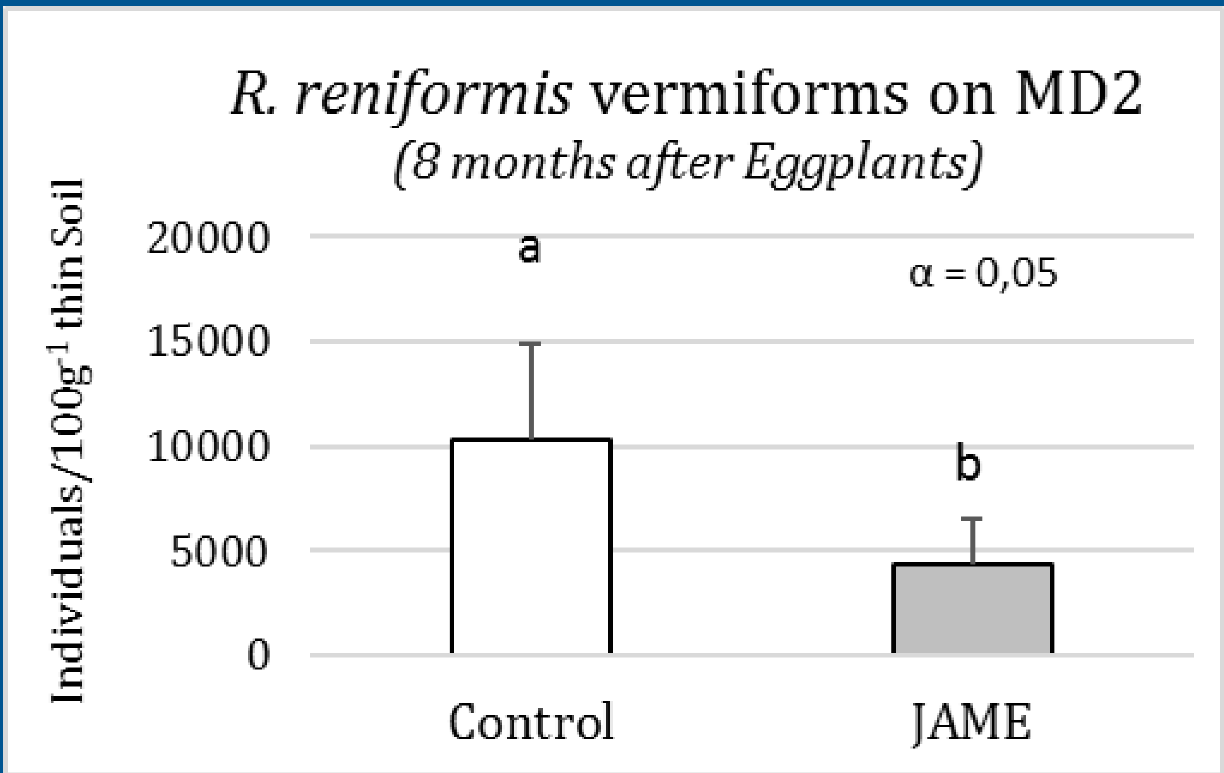

Figure 1. Decrease of populations of R. reniformis on MD2 in field experiment after ISR stimulation. [ISR induced by Methyl jasmonate (JAME) 0.1mM monthly application between planting and forcing (8), control = water; nematodes extracted and counted in 100g of soil. (different letters a,b indicate significant differences at α=0.05)]

In the same experimental field under ecological management with rotation, including *Crotalaria sp.,* before planting pineapple to minimize the nematode inoculum, the same authors obtained a significant reduction of the nematode populations evaluated just before forcing (8 months) on MD2 pineapple after *Crotalaria sp.* compared to populations on pineapple after eggplants. After *Crotalaria sp.* the populations of nematodes on pineapple were much lower, 3550 and 2408 individuals in 100g of thin soil respectively for control and ISR stimulated plants. These levels of nematode infestation were too low to reduce the plant growth as indicated by the 'D' leaves weight.

These results showed that the rotation system with *Crotalaria sp.,* without pesticides, was efficient per se to reduce nematodes populations, but although eggplants produced a higher inoculum in the soil, the ISR was able to maintain low multiplication of *R. reniformis* on pineapple compatible with a commercial production (Soler et al., 2019).

- ***Systemic resistances against the mealybugs D. brevipes associated with the Wilt disease***

In a greenhouse experiment on MD2 tissue culture plants, the SAR defenses induced by salicylic acid (SAL) application before inoculation of mealybugs significantly reduced their multiplication (-91.8%)

compared to the control **(Figure 2).** However, ISR defenses induced by methyl jasmonate (JAME) could not significantly reduce mealybug multiplication. These results were consistent with the trophic status of mealybugs, which, as biotrophic agents, induce and respond better to SAR defenses than to ISR defenses. The last ones are related to necrotrophic agents like caterpillars, or to symbiotic microorganisms like mycorrhiza and several nonpathogenic bacteria.

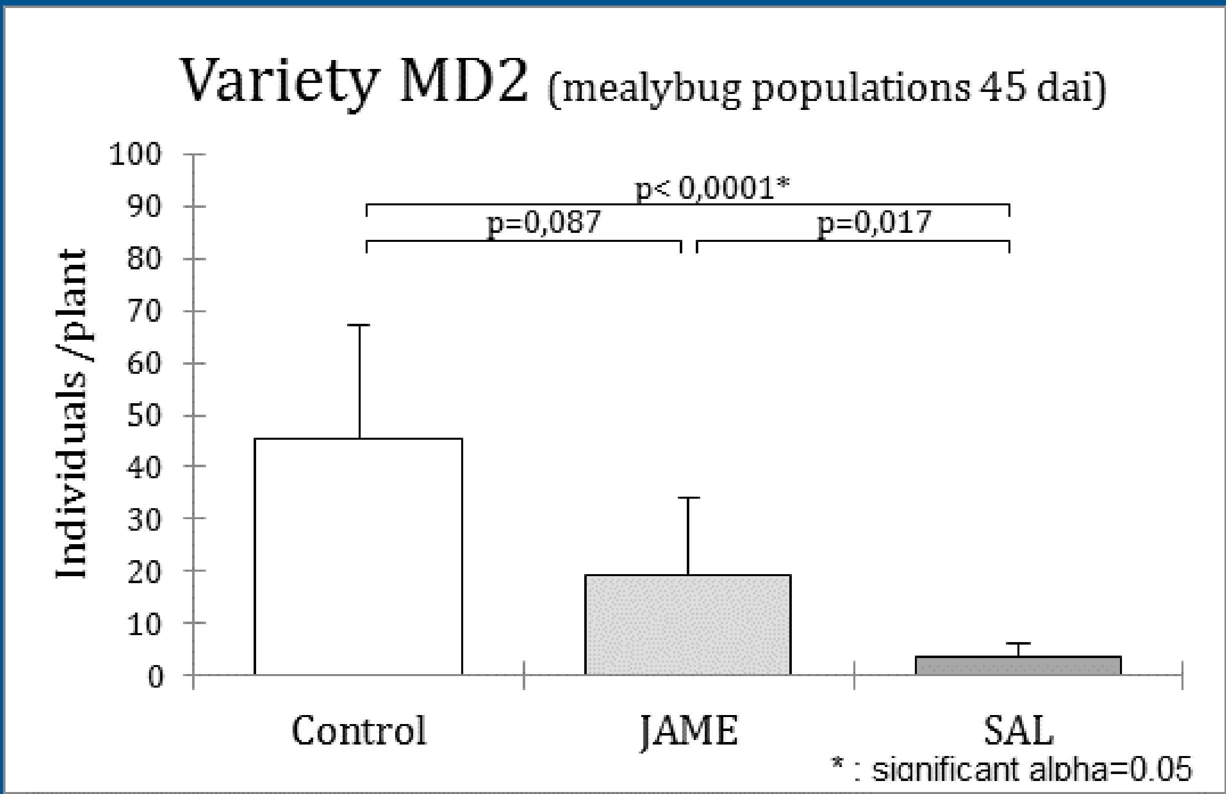

Figure 2. Evolution of D. brevipes populations on MD2 in greenhouse experiment after ISR and SAR stimulations. [ISR by Methyl jasmonate (JAME) 0.1mM, and Salicylic acid (SAL) 1mM, and water (Control) applications; counting 45 days after inoculation.]

Different enzymatic markers characterized the SAR priming and defenses. Activities of Phenylalanin ammonia lyase (PAL), and Pathogenesis related proteins (PR3 and PR1) showed significant differences (α =0.05) between stimulated (S I) and non-stimulated (NS I) plants after inoculation of the mealybugs **(Figure 3)**. PAL is the enzymatic activity responsible of salicylic acid biosynthesis by the plants, and leads to biosynthesis of phenolic compounds. PR3 and PR1 are directly related to the defense of the plants.

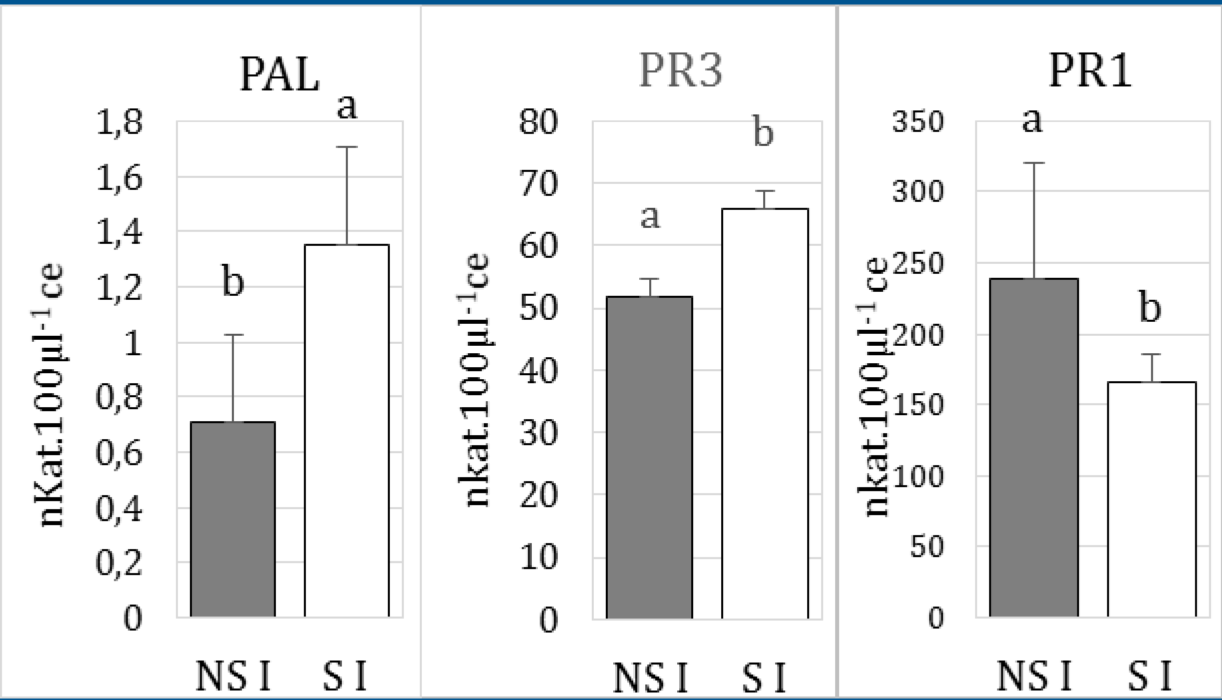

Figure 3. Enzymatic markers of SAR on MD2 pineapple, two weeks after SAR induction and mealybugs inoculation. [SAR induced by salicylic acid (1mM) 2 weeks after inoculation of 20 mealybugs. (different letters a,b indicate significant differences at α=0.05)]

Molecular markers can also characterize the SAR defenses **(Figure 4)**. The regulation, up or down, of the expression of genes of defense such as AcPAL and AcICS2, two genes related to the synthesis of salicylic acid, and one transcription factor AcMYB-like involved in the control of the expression of the AcPAL were evaluated. The results showed that the gene expressions linked to PAL and salicylic acid biosynthesis were strongly upregulated by SAR stimulation of the plants, and the inoculation of the pathogens (S I) compared to the control only inoculated with mealybugs (NS I).

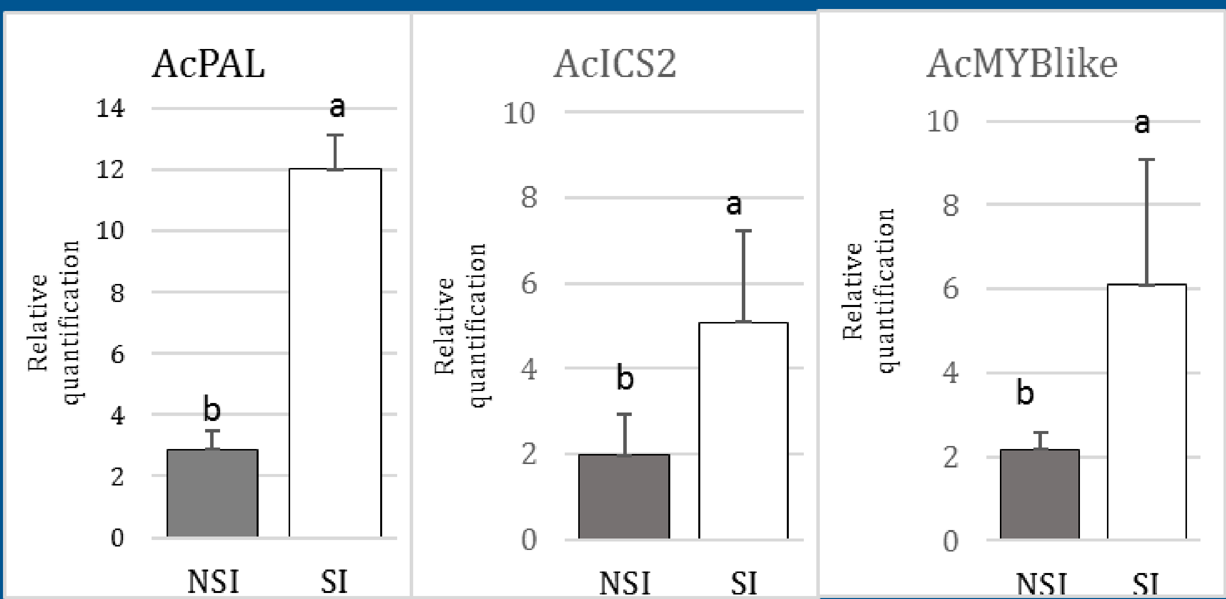

Figure 4. Molecular markers of SAR on MD2 pineapple, one day after SAR induction and mealybugs inoculation. [SAR induced by salicylic acid (1mM), then gene expression analyzed 24h after inoculation of 20 mealybugs/plant. (letters a,b indicate significant differences at α=0.05), SI = stimulated then inoculated, NS I = inoculated control]

These results showed that the priming of MD2 pineapple with salicylic acid induced SAR defenses able to control mealybug multiplication in controlled conditions. Few tests in a rotation system as previously described have been successfully done, but results must be consolidated.

Conclusion

The CIRAD researches presented here show that the management of mealybugs associated with wilt and nematodes by introducing systemic resistance into cropping practices is a promising tool for integrated pest management in MD2 pineapple. Large amounts of pesticide are still currently used on pineapple. Thus, a zero pesticides should be a target for ecological production systems. The inducers of systemic resistances currently tested work at very low concentrations, giving quantities per ha counted in a few g. Ongoing studies focus on the methods of application for producers (number of applications, optimal concentrations of inducers, time of application and others), and the actual effectiveness of systemic resistances in real field conditions. Sources of natural inducers or microorganisms are also targets of current Cirad research. Systemic resistances are involved in the multi-stress tolerance (biotic and abiotic) of plants. Therefore, integrating them into an IPM system without pesticides is necessary for better efficiency of systemic resistances directed against specific pathogens before their populations become too high.

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