Selection of entomopathogenic fungi compatible with the attractive semio-chemical molecules of the thrips *Frankliniella occidentalis*

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Introduction and objectives

Frankliniella occidentalis, or Western Flower Thrips (WFT) is the most damaging species of thrips to crops worldwide. Feeding on 250 plant species in 60 crop families, it causes direct and indirect damage to plants, notably by transmitting viruses. In addition, these polyphagous insects deploy multiple metabolic pathways for detoxifying plant allelochemicals, allowing them to metabolise many insecticides. In addition, thrips produce numerous annual generations, through haplodiploid reproduction, with the consequence of rapidly emerging resistances.

The aim of this work was to create a collection of strains of entomopathogenic fungi that are virulent on *F. occidentalis* but tolerant to WFT semiochemical molecules, allowing to create an auto-inoculation trap.

Methodology

Isolation and identification of *Thysanoptera*

The *F. occidentalis* population used to build the rearing were obtained from bioprospection carried out during this study. Unknown *Thysanoptera* samples were taken in the Geneva area in Switzerland between February and March 2021, in greenhouse crops of *Cyclamen persicum*, *Chrysanthemum sp*., *Cannabis sp*. and *Zantedeschia sp*. showing symptoms of *Thysanoptera* damages. They were reared on *Phaseolus vulgaris* plants in Bugdorm 4 Woven nylon mesh microcosms placed in a climatic chamber at 25° C, with RH 60%-75% and 16:8 light:dark photoperiod. *Thysanoptera* were first identified down to the family clade by morphological characterization using a binocular magnifying glass. They were then genetically identified to the species level by amplification and sequencing of the COI taxonomic gene using the following pair of degenerate primers: COI-CO2/COI-CO4.

Isolation and identification of fungal strains

Corpses of thrips showing fungal sporulation have been sampled in rearings of thrips previously identified as *F. occidentalis*. The cadavers were selected for isolation, after binocular observation of the fungal fructifications and then incubated on a Petri dish of PDA medium supplemented with 200 mg/L of chlortetracycline and ampicillin in the dark for 7 days at 25°C. This procedure was followed until obtention of pure cultures. For genetic identification the ITS taxonomic gene region was amplified with the ITS4/ITS5 primer pair.

Tolerance to semio-chemical molecules

The inhibitory action of the following attractive molecules neryl (S)-2-methylbutanoate, nerol, geraniol, linalool, methyl isonicotinate and verbenone was observed on a fungal collection of 3 strains of *Beauveria bassiana* (1.1, 2.1, 11.4), 4 strains of *Metarhizium anisopliae* (10.1, 32.1, 33.1, 34.2), a strain of *Paecilomyces fumusoroseus* (28.2), a strain of *Akanthomyces lecanii* (Bb1) and the commercial strain of *B. Bassiana* (ATCC 74040). To assess the inhibitory effect of these molecules, the radial growth of fungal isolates was observed and compared to their negative control. For this purpose, a transplantation of fungal cultures less than 10 days old was performed using a 6 mm diameter cookie cutter to a PDA Petri dish, fitted with a 25 mm x 34 mm sterile microscope glass slide harboring a 5 mm diameter blotting paper containing 20µl of a solution of any of the tested semio-chemicals diluted to 1% or 10% in hexane or sterile distilled water for p-anisaldehyde. The test was then incubated in the dark at 25° C for 9 days. The experiments was conducted in duplicate. Statistical procedures, namely, a non-parametric Friedmann test supplemented by two-to-two comparisons using the parametric Student's T-test when possible, or in the opposite case a non-parametric Mann-Whitney test, were performed using the statistical program Minitab.

Virulence on *F. occidentalis*

The screening of the virulence potential of the fungal strains was assessed on the developmental stages of WFT. At this end, 20 females and two males were allowed to lay eggs in a Microbox® for 24 hours on a dwarf bean fruit. After this period, for each fungal strain, three beans previously cleaned of their adult thrips were immersed for 30 seconds in 20 mL of a concentrated spore solution at $2*10^6$ CFU/mL. After allowed to dry for 24 hours in sterile boxes, the beans were placed in a new sterile Microbox® and placed in rearing conditions. A negative control consisted of beans not exposed to a fungal strain. Positive controls were the commercial product diluted to 1%, containing the strain ATCC74040, the pure strain ATCC74040 at 10^6 CFU/mL and rapeseed oil diluted to 1%. The new generation of thrips was observable in the negative control 3 to 4 days after oviposition. From then on, the count of the number of living and dead individuals was carried out after 8 days. The parametric ANOVA procedures, complemented by Tukey's and Dunnet's multiple comparisons were performed using the Minitab statistical program.

Results and Discussion

Isolation and identification of organisms

Out of the 4 isolated *Thripidae* populations, three could be identified as *F. occidentalis*. In addition, the isolated nucleus of *Zantedeschia sp*. differed by 3.15% on the COI mitochondrial DNA demonstrating the presence of two cryptic subspecies. Thus, the latter was identified as belonging to subspecies G, while the population isolated from *Chrysanthemum sp*. and *Cannabis sp*. the cryptic subspecies L. Among the diverse microorganisms retrieved, one isolate of *Akanthomyces lecanii* (Bb1) represented an interesting entomophagous candidate..

Virulence against *F. occidentalis*

Cumulative mortality showed differences between treatments with strains 10.1, 11.4, 32.1, 33.1, 34.2, Bb1, the commercial product and its strain, rapeseed oil and the control (Fig.1). However only isolate Bb1, the commercial product and rapeseed oil showed a statistical difference of 5% in the ANOVA procedure. However, the strain of the commercial product ATCC 74040 only showed a 12% mortality. Indeed, the higher mortality in the commercial product is explained by the liquiid formulation (Fig.1).

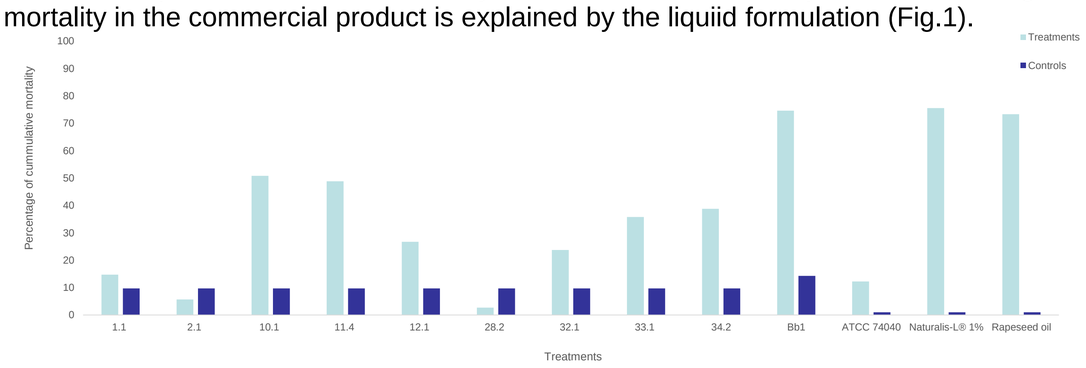

Figure 1. Percentage of cumulative mortality of treatments compared to their respective negative controls.

Tolerance to semio-chemical molecules

The attractive molecules tested here showed differences in the average percentage of growth inhibition for all strains tested. Indeed, linalool, nerol, and geraniol were found to be particularly deleterious when applied at 10% concentration, with 82%, 71%, and 42% average inhibition, respectively (Fig.2). In addition, Friedmann non parametric tests showed a statistically significant difference in inhibition at the 1‰ threshold (p-value<0.001) between treatments of the same concentrations. Thus, pairwise comparisons showed statistically significant inhibition at the 5% (p-value<0.003) threshold for all molecules and concentrations applied except verbenone at 1% and p-anisaldehyde at 10%. Furthermore, linalool, nerol and geraniol were statistically more inhibitory when the concentration was increased. Moreover, statistical differences at the 5% threshold were observed according to the molecules and their concentration applied, on the strains.

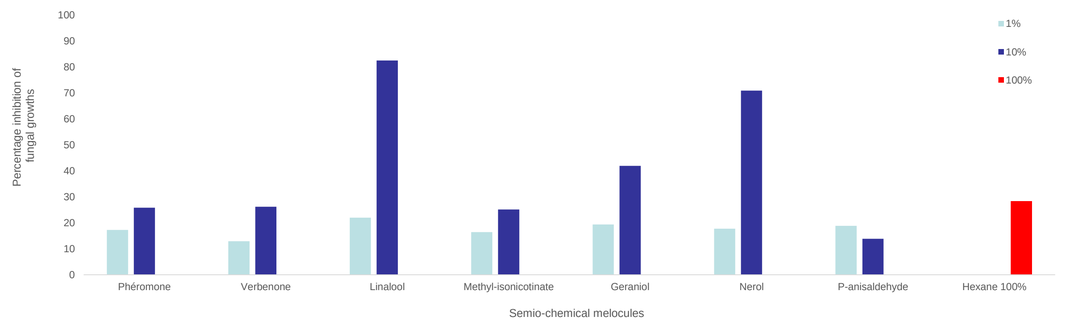

Figure 2. Average inhibition effect (in %) of semio-chemical molecules on all fungal strains combined.

Conclusion

This work allowed the isolation and characterisation of the Bb1 fungal strain. In addition, its virulence on *F. occidentalis* could be established, as well as its compatibility with the semio-chemical molecules of WFT. In parallel, 4 of these compounds were selected for their compatibility with strains of entomopathogenic fungi. These are p-anisaldehyde, neryl (S)-2-methylbutanoate, methyl isonictoniate and verbenone. These results are highly promising for the development of a self-inoculation trap against *F. occidentalis*.

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ABIM, Annual Biocontrol Industry Meeting
23-25 October 2023, Basel Switzerland