PURE

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Collaborative Project
SEVENTH FRAMEWORK PROGRAMME

D11.4
Vibration mating disruption and biodegradable pheromone dispensers for mating disruption first prototypes

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1. Summary

The present WP aims at creating innovative and environmentally friendly tools, for practical applications against major agricultural pests. Two main objectives: (1) the development of a new method of mating disruption based on vibrations transmitted to grapevine plants against the phytoplasma vector *Scaphoideus titanus*; (2) the development of biodegradable pheromone dispensers to confuse the tomato leafminer *Tuta absoluta*.

1) The development of a new method of mating disruption is warranted because numerous insect pests, most notably leafhoppers and planthoppers (Hemiptera) do not rely on long-range chemical communication. In these insects mate recognition and localization of the partner are mediated exclusively via substrate-borne vibrational signals and their populations are currently managed primarily by insecticide treatments. Surprisingly, although males use special species-specific disruptive vibrational signals to interfere with the courtship of rivals, mating interruption by induced vibrations has been rarely considered even from a theoretical viewpoint and there has been virtually no research on how to exploit this common insect communication channel as a tool for pest control. Here we present the first implementation of mating disruption based on substrate-borne vibrations. The leafhopper *Scaphoideus titanus* Ball, a vector of a lethal grapevine disease Flavescence dorée, was chosen as a model pest species. We first analyzed the velocity characteristics of these vibrational signals. Next, we established whether disruptive vibrational signals can be applied to several grapevine plants simultaneously and whether under such circumstances these signals would mask male calls. Finally, we assessed copulation success of *S. titanus* in the presence of disruptive signals in a vineyard with mature, field growing grapevine plants. A prototype of a vibration dispenser was developed to vibrate the wire with disruptive signal that was transmitted as substrate vibrations to the plants in both potted and fully mature field grapevine plants. Further upgrading of the system and, in particular of the prototype are expected for 2013 season in order to make large scale tests in 2014.

2) The insect *Tuta absoluta* (Lepidopera) is a serious pest in tomato. The aim of this project is to develop biodegradable pheromone dispensers to confuse the insects (False Trail Following) resulting in control of the pest, reduced damage in tomato and lower chemical inputs. Slow release dispensers with *Tuta absoluta* pheromone have been produced in Wageningen and were sent to partner CNR (Massimo Giorgini and Emilio Guerrieri), to perform false trail following experiments in two tomato greenhouses in South Italy in 2012. Together with partner CNR a field trial plan and schedule were designed. Field experiments were performed and samples of dispensers from the field were sent regularly to DLO to estimate the release rate. Pheromone traps were used to check if any males would be captured in the treated fields. If the confusion of the insects would be effective it is expected that no males would be captured. Plant damage, insect counting and combination of sustainable control techniques against *Tuta absoluta* were performed by CNR and will be reported in detail in the specific WPs of PURE (WP7, WP9). In both experiments very few males were captured in the pheromone traps, indicating the efficacy of the dispensers in terms of release rate and durability. In 2013 the experiment will be repeated on a larger scale. Prototypes of biodegradable dispensers will then be tested in the field to check if their release of pheromone is comparable to the present effective dispenser. Finally in 2014 the best biodegradable dispenser will be tested for effectiveness in the field.

2. Objectives

1) To develop a novel mating disruption strategy based on substrate-borne vibrations, using *S. titanus* as a model insect species.
    - The prototype of an electromagnetic tool (shaker or vibration dispenser) to apply at the vineyard supporting wires, able at transmitting the disruptive signals into grapevine tissues.

2) Monitoring and pest control techniques (False Trail Following) based on innovative release method of semiochemicals. The case study chosen is *Tuta absoluta* in tomato.
    - Evaluated prototype of biodegradable dispensers with different pheromone quantities and release rates.

3. Deliverable procedure

3.1 Objective 1: Mating disruption of *Scaphoideus titanus* with Vibrational Signals

3.1.1 Insects

We collected *S. titanus* eggs from organic farms in Villazzano (Trento, Italy) and from them reared the adults used in field trials on grapevine. Eggs were hatched in a controlled environment chamber (25+1°C, L16:D8, RH: 75+5%). Nymphs and adults were housed in plastic boxes and fed with vine leaf dishes (Fig. 1). To ensure that leafhoppers were sexually mature and receptive, all tests were done with virgin males and females that were at least six and ten days old, respectively.

3.1.2 Signal transmission through grapevine plants

A *S. titanus* male calling song (MCS) used in transmission study was recorded with a laser vibrometer (Ometron, Bruel and Kjaer, Denmark, Fig. 2a) in the laboratory with male singing at 0.5 cm distance from the recording point. To verify the characteristics of this signal, we also recorded it with a prepolarized free-field 1/2" microphone (mod. 4189, sensitivity 50 mV/Pa; Brüel & Kjær). This enabled to simultaneously record both air-borne sounds and substrate-borne vibrations produced by the insect. The disruption signal was a pre-recorded natural disruptive signal (also termed disturbance noise, DN) recorded from a rival *S. titanus* male with the above mentioned laser vibrometer during rivalry encounters on a single grapevine leaf. An exemplar with the best signal-to-noise ratio was chosen from a library of recordings at Fondazione Edmund Mach (Italy).
Field tests in a grape producing vineyard were conducted at Fondazione Edmund Mach (Italy) in July and August 2011 and 2012. Mature rooted grapevine plants (height ~ 1.5 m) grew in a row at distances 70 cm from each other with stems tied to a supporting metal wire (Fig. 2b). In 2011 a single MP3 driven electromagnetic shaker used as source of disruptive signals was applied to a vineyard wire (Fig. 2c). A second improved version of the prototype, with powered signal and different coupling device was created in 2012 (Fig. 2d).

3.1.3 Tests of mating disruption

In field experiments, a shoot from the middle part of each plant (with approximately 20 leaves) was isolated in a nylon-netting sleeve (30x70 cm) (Bugdorm Insect Rearing Sleeves) with closable openings to release and collect the insects.

- **Season 2011: First prototype and validation of the method's principles**

Since most mating activity in *S. titanus* occurs during twilight or during the night, all trials were made between 5 pm and 10 am the following day when insects were recollected from the cages/sleeves. Plants were chosen 100, 310, 520, 730 and 940 cm distant (Figure 2b) from the shaker. In each overnight trial one virgin male and female *S. titanus* were put on separated leaves of each grapevine plant. When a male or a female could not be found or when one individual was dead, the replicate was discarded. Collected females were placed individually in rearing containers without access to egg laying sites and dissected 10 days later.
In total, 24 trials were made with 129 females subjected to DN treatment and 23 control females (Fig. 3). After dissection, 118 of the DN treated females were virgin and 11 females were mated. In the control plants, 18 females were mated while 5 were virgin.

- **Season 2012: Second prototype and spatial/temporal questions. Further acquisition of insect mating behaviour knowledge**

The intensity (measured directly as substrate velocity) that a *S. titanus* male receives from the reply of a female was recorded along a grapevine cutting consisting in three leaves. The aim was to investigate if males adjust their behaviour according to the perceived intensity of the female reply and if they are able to make directional decisions after receiving a female reply. There was no statistical difference in intensity between male leaf, empty leaf and stem, whereas the intensity level of female pulse was significantly increased at the petiole, base and lamina female leaf. These results show that the male associates the higher intensity level with presence of female when being at her leaf and that he extracts directional information from the female signal in order to make a correct decision on her location.

To increase the efficacy of the vibrational mating disruption method, the following two aims were tested: the duration of the prototype shaker transmitting disturbance noise (DN) to the plants (test 1), and at an increased distance from 5-45 m (test 2). In addition to test 2, we looked at the transmission efficacy of disturbance signal along the grapevine wire during three different periods of the day: duration of 24 h, 21 h, 19 h, 12 h and 8 h. As a control, one pair of insects was put on a plant without disturbance signal for 24 h. Females remained virgin on plants subjected to DN for 19-24 h. On the plant with 19 h of DN there were 14/17 virgin females, on 21 h; 25/26 were virgin and when DN was on for 24 h as many as 33/36 females were virgin. This is a significant result of mating disruption when compared to the silent control plant with 25/34 mated females. The high percentage of virgin females in plants where DN was transmitted for 21 h shows that the system may be safely turned off from 12 - 15 pm to keep 21 hours effective disruption and still remain as effective as if the duration was 24 h.

3.2 Objective 2: New biodegradable pheromone dispensers to confuse *Tuta absoluta*

For the first experiments in 2012, field trials were designed with a pheromone dispenser density of 2000 dispensers/ha (annex II). Dispensers were made of polyethylene vials (Kartell, Italy) (Fig. 4a) containing 10 mg of *Tuta absoluta* pheromone (2 components). Vials were hung in the crop (Fig. 4b) and were replaced twice per season. On a regular base vials were sent to Wageningen to determine the actual release in the field.

In 2012 the first prototype biodegradable pheromone dispensers have been designed and produced (Fig. 4c) however these could not be tested in the field yet.

The effect of the confusion of male *Tuta absoluta* by the pheromone dispensers was checked by placing pheromone traps in the treated and untreated fields (Figure 5a, 5b). The results showed that in the pheromone treated fields almost no males were captured whereas in the control fields high numbers were caught. This indicates that the confusion was effective and the pheromone release sufficient.

Analyses of the dispensers that were sampled from the field showed the actual release pattern in the field (Figure 6). After approximately 30 days the dispensers had released almost all of their content.

4. Conclusions

Our results indicate that the principles from which the mating disruption with vibrational signals are valid and applicable even at field level, on mature plants. The disruption has been effective on more than 90% of tested pairs when some conditions were respected. In particular we found that mating is almost totally prevented when the device is working for periods of more than 19 hrs. Indeed we detected important losses and dispersion of the signal, due to numerous points of contact between vibrating wires/plants/poles. This limit must be eliminated or strongly reduced, by for instance by passing the signal or using dampers in correspondence of such critical points.

The main conclusions are:

1) The method has a very high potential of success for field applications and the first prototype of the full device is already available.
2) However, it will be necessary to find soon some new elements of implementation, in order to solve limits due to the progressive loss of signal intensity along the distance, in order to make the technology available for open field experiments in 2014.

As for the development of biodegradable pheromone dispensers to confuse *Tuta absoluta* the main conclusions, from the first two field trials, are:

3) The application of the pheromone dispensers at the present density and release rate resulted in effective confusion of males.

4) Based on these results in 2013 we will repeat the experiment on a larger scale and test at the same time the release rate of biodegradable dispenser prototypes.

5. Annex I - Figures

Figure 1: Rearing chamber. In greenhouse *S. titanus* individuals were reared up from eggs (included in 2 years old grapevine shoots, fig. 1a) to adults inside plastic boxes, with a grapevine leaf disc laid over a 2 cm layer of technical agar solution (figs. 2b-d).

Figure 2. Laser vibrometer (a) and field test overview (b): net sleeves were mounted around grapevine shoots at different distances from the shakers. The first model of shaker (2011, c) was fixed with a screwdriver to the wire; the second model (2012, d) was hung with a special hook.

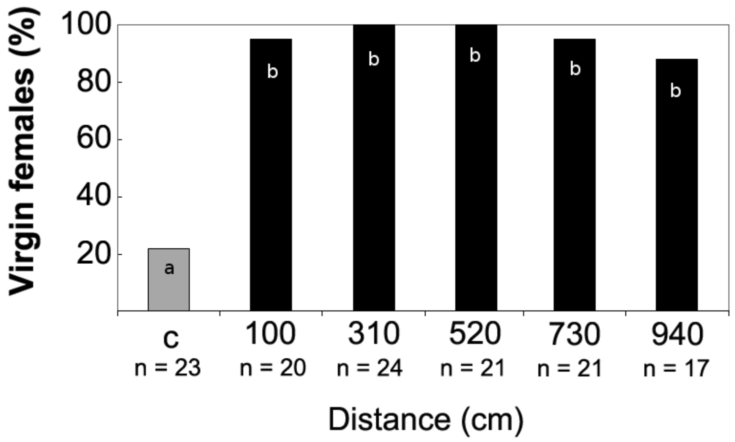
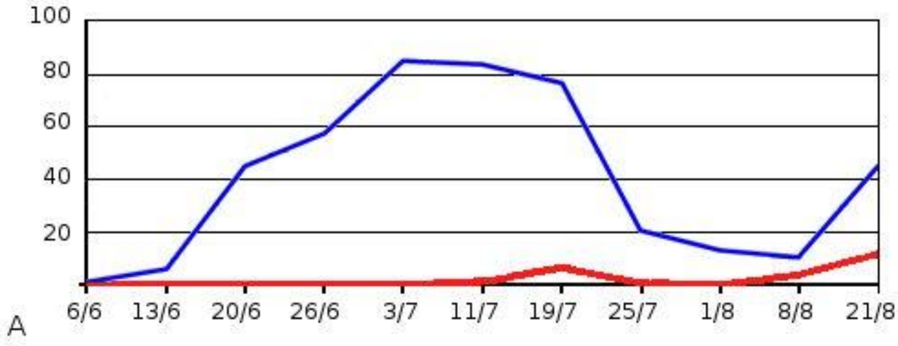
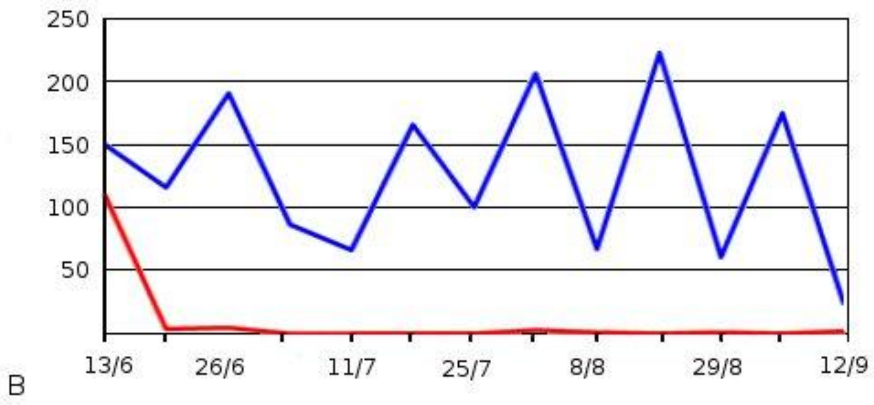


Figure 3. Number of virgin and mated females found on vibrated and non-vibrated grapevine plants in a field trial with mature grapevine plants in 2012. Black and gray bars show virgin females from plants at increasing distances from the source of disruptive signals and from control plants in the absence of these signals, respectively. Different letters indicate significant differences ($P < 0.0001$) between treatments after G-test for contingency table (William's corrected) followed by a Ryan multiple comparison of proportions test. The number of replicates (n) at each distance from the source of disruptive signals and for controls is given.

Figure 4. (a) Kartell vial used as pheromone dispenser for field trials; (b) pheromone dispenser in tomato crop in field experiment in South Italy, 2012; (c) Prototype biodegradable pheromone dispenser.

Figure 5. Number of male *Tuta absoluta* caught in pheromone traps in two tomato greenhouses (A, B) in South Italy. Upper blue line control field, lower red line pheromone treated field.





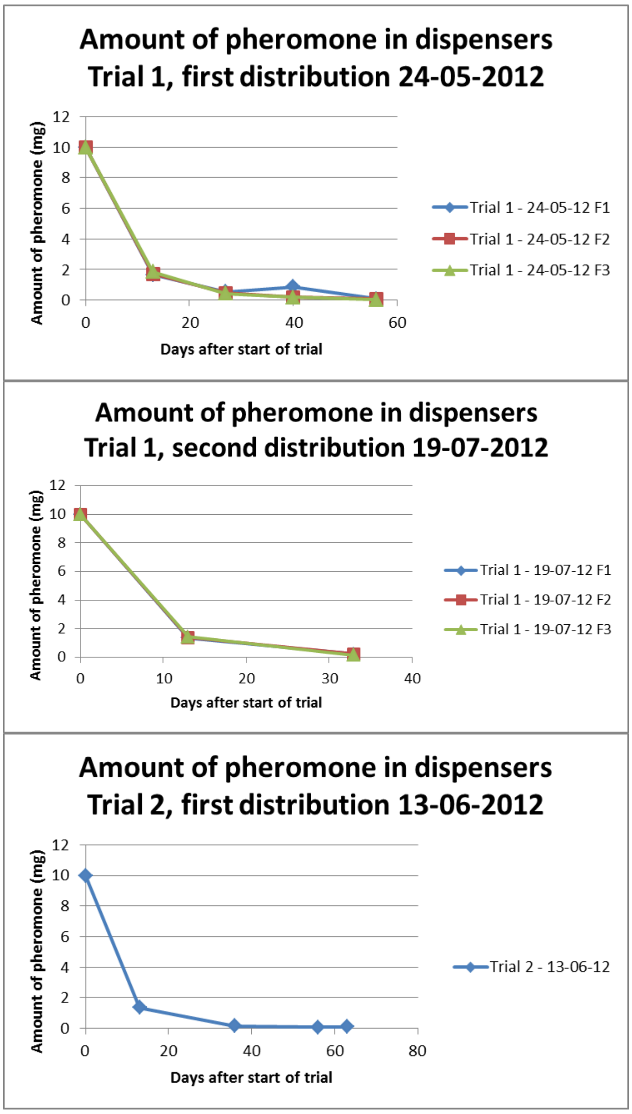


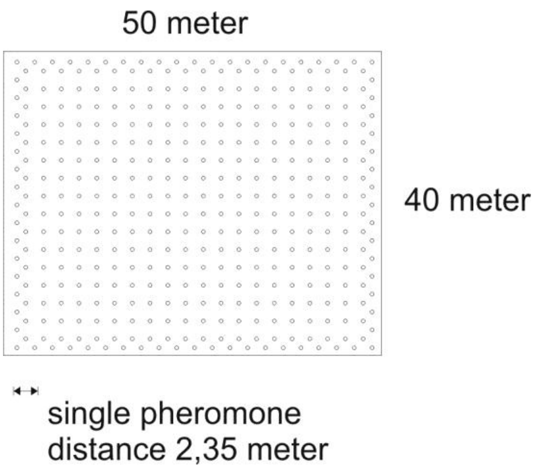
Figure 6. Release rate of pheromone dispensers from field trials in Southern Italy, 2012.

6. Annex II - **FTF Tuta absoluta Italy 2012 – protocol**

Aim: test Tuta false trail following/mating disruption with a high number of dispensers/ha. Per hectare 2000 dispensers are used loaded with 10 mg pheromone. Change vials 2 times per season.

Hang vials in the crop with the aid of a piece of wire or a clip. Do not open the vials! They must remain closed. The pheromone will diffuse through the walls of the vial.

Distribute the vials evenly over the surface of the greenhouse/field. Apply higher density along the edges of the greenhouse/field.
Distance between vials approx.: 2.35 meters



Hang on one side a few vials extra in order to sample 2 vials every 2 weeks (send them to Wageningen).