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Essential Oil Trunk Injection Into Orchard Trees: Consequences on the Performance and Preference of Hemipteran Pests

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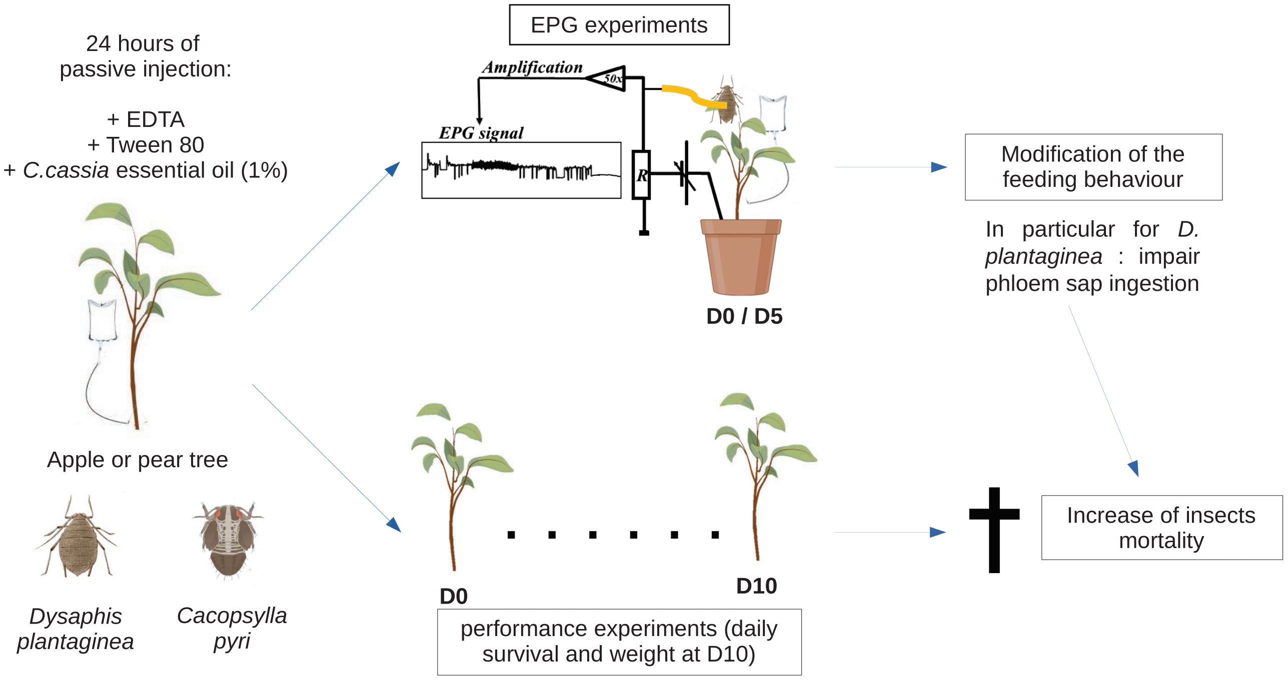
Abstract

Apples and pears are among the most widely cultivated fruit species in the world. Pesticides are commonly applied using ground sprayers in conventional orchards; however, most of it will not reach the target plant, increasing the contamination of nontarget organisms such as natural predators, pollinators, and decomposers. Trunk injection is an alternative method of pesticide application that could reduce risks to beneficials and workers. Essential oils represent a 'green' alternative to pesticides due to their reported insecticidal, antimicrobial, antiviral, nematicidal, and antifungal properties. The aim of this study was, therefore, to evaluate the impact that the injection of a cinnamon essential oil solution into the trunk of apple and pear trees could have on their respective pests, *Dysaphis plantaginea* and *Cacopsylla pyri,* respectively. The feeding behavior (preference), the life history traits (performance), and the timing of this effect were measured. The injection of an essential oil emulsion in trees impacted hemipteran host-plant colonization, as for both species a modification of their preference and of their performance was observed. The feeding behavior of *D. plantaginea* was altered as a significantly lower proportion of aphids ingested phloem sap on injected trees, suggesting that the aphids starved to death. On the contrary, the feeding behavior of the psyllids was little changed compared to the control condition, implying that the observed mortality was due to intoxication. The results presented here could theoretically be used to control these two orchard hemipteran pests, although the effectiveness in real conditions still has to be demonstrated.

Key words: *Cacopsylla pyri*, *Dysaphis plantaginea*, *Cinnamomum cassia*, trunk injection, electrical penetration graph

Graphical Abstract

Impact of essential oil tree-injection on the preference and the performance of two hemipteran orchard pests



Introduction

Apples and pears are among the most widely cultivated fruit species in the world. In 2020, apples were the 4th most important fruit crop in terms of number of tons produced in the world, with 87 million tons for a cultivated area of 4.7 million hectares (FAO 2022). World pear production was over 23.7 million tons for a cultivated area of over 1.29 million hectares (FAO 2022).

In commercial apple orchards, the use of chemicals is widespread for the control of a range of diseases and insect pests, in particular, the rosy apple aphid *Dysaphis plantaginea (Hemiptera: Aphididae)* (Passerini 1860) (Porcel et al. 2018). For pear orchards, pest population control is currently maintained based on integrated pest management that also may include the use of synthetic pesticides (Belien et al. 2021, DuPont et al. 2021). However, in recent years, concerns about the environmental risks associated with the use of pesticides, the emergence of pesticide-resistant populations, and the increasing public demand for environmentally friendly products have stimulated the research for new solutions to control these orchards pests (Tougeron et al. 2021).

The rosy apple aphid, *D. plantaginea*, and the pear psyllid, *Cacopsylla pyri* (Hemiptera: Psylloidea) (Linnaeus 1761), are both phloem sap sucking hemipteran insects and are both considered as important pests of their respective host plant. The *D. plantaginea* aphids cause significant damage, even at low densities, because their stings can provoke leaf curling and fruit deformation (Blommers et al. 2004). These can lead to important economic losses, with an estimate of 30% (Blommers et al. 2004) to 80% losses when aphid populations are not controlled (Qubbaj et al. 2005). The *C. pyri* psyllids can also be responsible for heavy economic losses (Civolani 2012). Although they do not cause fruit deformations, their feeding disrupts plant growth, and they are vectors of "*Candidatus Phytoplasma pyri*", a phytoplasma responsible for the pear decline disease (Süle and Jenser 2007, Civolani et al. 2011). Finally, both hemipteran insects produce a large amount of honeydew that favors the development of sooty mold (Dedryver et al. 2010, Civolani 2012).

Pesticides are commonly applied using ground sprayers in conventional orchards; (Wheeler et al. 2020); however, most of it will not reach the target pest (Steiner 1969, Reichard et al. n.d., Zhu et al. 2006, VanWoerkom et al. 2014), increasing the contamination of nontarget organisms such as natural predators, pollinators and decomposers. Trunk injection is an alternative method of pesticide application that could reduce risks to beneficials and workers (Fettig et al. 2014, Wise et al. 2014) and seems to be particularly well adapted to manage sap feeder pests. Indeed, the pesticide is delivered directly into the tree's vascular system and is absorbed by the xylem sap flow. This method allows the administration of many plant protection products, whether the characteristics of the injected product are compatible (or not) with apoplastic transport to achieve good absorption and minimize phytotoxic effects (Berger and Laurent 2019). This type of application also protects the active substances from UV or external biodegradation (Berger and Laurent 2019) as well as increasing their persistence (Doccola and Wild 2012, Fidgen et al. 2013). The injection of synthetic chemical pesticides has been shown to be effective in several biological models such as apple tree (Aćimović et al. 2014), avocado tree (Byrne et al. 2020), and date palm (Khalaf and Alrubeai 2016). Furthermore, Werrie et al. (2021) have recently demonstrated that the injection of biopesticides was possible in apple trees and proposed essential oil injection as a possible alternative strategy for pest control.

Essential oils represent a 'green' alternative in the agricultural domain due to their reported insecticidal, antimicrobial, antiviral, nematicidal, and antifungal properties (Turek and Stintzing 2013).

They are composed of volatile secondary metabolites characterized by a strong odor. Many of these compounds have ecological functions such as internal messengers, defensive substances, or attractive substances for pollinators and natural enemies of pests (Harrewijn et al. 2001). Essential oils may contain about 20–60 components, present in different concentrations. There are one to three major components accounting for 20–70% of the oil, which generally determine its biological properties (Bakkali et al. 2008, Nollet and Rathore 2017, Reyes-Jurado et al. 2020). The properties of essential oils result not only from individual molecules, but also from the synergy/additivity between these different components. The effects of the oil are generally attributed to the major components, but the trace compounds participate in increasing these effects.

The essential oil used in our study was obtained from cinnamon (*Cinnamomum cassia*; Lauraceae). Several studies have already underlined its antifeeding and insecticidal effect against insect pests (Huang and Ho 1998, Kim et al. 2003, Lee et al. 2008) caused by its major compound, the *trans*-cinnamaldehyde.

The aim of this study was, therefore, to evaluate the impact that the injection of a cinnamon essential oil solution into the trunk of apple and pear trees could have on their respective pests, *D. plantaginea* and *C. pyri,* respectively. The feeding behavior (preference), the life history traits (performance), and the timing of this effect were then evaluated on injected and uninjected trees. Our hypothesis here is that the injection of cinnamon emulsion into the vascular system of trees will alter the feeding behavior of both aphids and psyllids on the injected trees, resulting in greater mortality or at least a decrease in mass of these insect pests.

Material and Methods

Insect Material

The population of *D. plantaginea* used in this study was developed from several wild clones collected in 2018 in the experimental orchards of the Proefcentrum voor Fruitteelt, Sint-Truiden (Belgium), while the *C. pyri* psyllid population was initially collected in 2013 in the experimental pear orchard of Proefcentrum voor Fruitteelt, Sint-Truiden (Belgium) and wild individuals from the same population have been added to the colony in 2018.

For both *D. plantaginea* and *C. pyri*, standardized mass rearing was performed on their respective host plant obtained by *in vitro* multiplication (*Malus. domestica* cv. 'Jonagold' and *Pyrus. communis* cv. 'Durondeau,' respectively) in growth chambers under 24 ± 1°C, 60 ± 5% RH, and a 16:8 (L:D) hr cycle. For both hemipteran species, synchronized first instar nymphs were obtained by placing adult females on plantlets for 48 hr before transferring them to a new plantlet. This protocol allowed us to produce offspring insects of known age/stage on plants.

Plant Material

All the experiments were performed using 40–60 cm high potted plants with 15–30 leaves and a trunk diameter of 5 mm in average, obtained by *in vitro* multiplication, *M. domestica* cv. 'Jonagold' for apple trees and *P. communis* cv. "Durondeau for pear trees. Plants were obtained from the 'centre wallon de recherche agronomique' (www.cra.wallonie.be). Apple and pear trees were grown in a climatic room under 22 ± 1°C, 60 ± 5% RH, and a 16:8 (L:D) hr.

Essential Oil

The cinnamon (*C. cassia* J. Presl) essential oil used in this study was purchased from Pranarôm (Pranarôm & Herbalgem, Ghislenghien, Belgium). Before formulation of the essential oils, the oil composition was analyzed by gas chromatography associated with mass spectrometry (GC-MS). These analyses were carried out on a 7890A-5975C GC-MS equipped with an HP-5MS 30 m × 0.25 mm × 0.25 µm capillary silica column (Agilent Technologies Inc., Santa Clara, USA). The operating conditions were the following: helium flow of 1.0 ml min^{-1}; the oven temperature was programmed at 40°C for 2 min, increased to 100°C at a rate of 5°C min^{-1}, increased to 120°C at a rate of 3°C min^{-1}, held for 3 min, increased to 220°C at a rate of 5°C min^{-1}, and finally increased to 310°C at a rate of 15°C min^{-1}. One microliter of a 1 mg ml^{-1} essential oil solution in hexane (HPLC grade, Merck KGaA, Darmstadt, Germany) was injected in splitless mode. The injector, quadrupole, and MS temperatures were 250, 150, and 230°C, respectively. The mass spectrometer (MS) ran in electron impact mode at an electron energy of 70 eV. Mass spectra were acquired in the range of 30–400 atomic mass units. GC-MS analysis of the essential oil demonstrated that *C. cassia* oil was composed of 79.49% *trans*-cinnamaldehyde. The detailed composition can be found in Supp Table 1 (online only).

Essential Oil Emulsion

To facilitate injection and diffusion of essential oils in the tree vascular tissue, a water-soluble, stable, and homogenous essential oil emulsion was prepared as described in Werrie et al. (2021). To prepare 100 ml of the 1% (v/v) essential oil/water emulsion, 2 ml of Tween 80 (CAS 9005-65-6, Merck KGaA, Darmstadt, Germany) and 20 ml of 100 mM ethylene diaminetetraacetic acid (EDTA) (Titriplex III, Merck KGaA, Darmstadt, Germany) solutions were added into 15 ml of water under constant agitation at 1,250 rpm. Water was then added to bring the final volume to 100 ml. After 5 min under constant agitation, the solution was then stabilized by high-speed homogenization for 6 min at 9,500 rpm (Ultra-Turrax T25, IKA WERKE, Staufenim Breisgau, Germany) and by high-pressure homogenization with eight cycles at 5,000 psi (FMC, Philadelphia, PA). The emulsion particle sizes distribution was analyzed with a particle sizer (Beckman Coulter DelsaTM Nano C Particle Analyser, CA and is presented in Supp Fig. 1 (online only) (mean distribution = 59.31 ± 0.69 nm).

Trunk Injection Procedure

The trunk injection procedure was adapted from Werrie et al. (2021). First, a single needle (L: 10 mm, Ø: 0.4 mm) was used to pierce the plant stem to the xylem at 2 cm from the bottom of the stem. Another needle (L: 10 mm, Ø: 0.4 mm) (Venofix) connected by a tube (L: 300 mm) to a plastic pocket containing 10 ml of emulsion (approximately 10 g) was then inserted into the hole previously made. Hot glue was then applied with a gun to hold the needle in place. Aluminum foil was placed around the plastic pocket to protect the emulsion from light. The device was then left in place for 24 hr. To estimate the amount of emulsion ultimately injected in the plant, the plastic pocket was weighed before and after the 24 hr of passive injection. Only trees that had received more than 0.02 g of emulsion were selected for the experiments. The mean intake of emulsion was 0.38 ± 0.08 g for apple trees (n = 33) and 0.39 ± 0.08 g for pear trees (n = 26).

Aphid and Psyllid Preference

The preference through feeding behavior was studied using the electrical penetration graph (EPG) method (Tjallingii 1985). An electrode consisting of a 2-cm long copper wire soldered to the head of a 2.2 mm diameter brass nail was used to wire the insects. The other end of the electrode consisted of a thin 20 µm diameter and 2-cm long gold wire glued to the copper wire portion of the electrode. A droplet of conductive water-based silver glue was used to attach the gold wire to the copper wire of the electrode, and another was deposited on the abdomen of individual aphids/psyllids to attach them to the extremity of the gold wire. Gold wire and silver glue were provided by EPG systems, Wageningen, Netherlands. Eight individual insects (adults for aphids and 4th instar larvae for psyllids) were connected to the Giga-8 DC-EPG amplifier (provided by EPG systems, Wageningen, Netherlands), each being placed on the leaf of an individual plant as described in Denoirjean et al. (2021) for aphids and Civolani et al. (2011) for psyllids. The recordings were performed continuously for 8 hr during the photophase inside a Faraday cage. The EPG data were digitized using a DatQ Instruments DI-710-UH analog-to-digital (A–D) board and recorded using the Stylet$^+$ software (EPG Systems, Wageningen, Netherlands), at a sample rate of 100 Hz, input impedances (Ri) of 10^9 Ohms (Ω), and DC substrate voltage. Analysis of the EPG waveforms was carried out using the Stylet$^+$ software. Parameters from the recorded waveforms were calculated with the EPG-Calc 6.1.7 software (Giordanengo 2014). They were based on different EPG waveforms, described by Tjallingii and Esch (1993) for aphids and Civolani et al. (2011) for psyllids, and corresponded to: (Pr) stylet activity within plant tissues; (C) stylet pathways in plant tissues except phloem and xylem; (E1) salivation in phloem elements; (E2) passive phloem sap ingestion; (G) active xylem sap ingestion; and (F) derailed stylet mechanics (the latter being described only in aphids).

To assess the suitability of essential oil injection for crop protection, the EPG experiments were first conducted on noninjected trees (blank treatment, no injection; named 'control'), and then on the same trees after the essential oil injection, directly after the 24 hr of passive injection (named 'D0') and five days after the end of the passive injection (named 'D5'). A total of 28 and 21 plants were used for the EPG measurements for aphids and psyllids, respectively. EPG records were obtained from 28 aphids and 19 psyllids on control trees, from 28 aphids and 18 psyllids on D0 injected trees and from 23 aphids and 21 psyllids on D5 injected trees.

To ensure that the effects observed were truly due to the essential oil and not to the piercing and/or the emulsifying solution alone, feeding behavior of aphids and psyllids was compared when feeding on noninjected trees (blank treatment, no injection; named 'control') and trees injected with the solution without the essential oil (negative control: emulsion devoid of essential oils; named 'injected control'). EPG records were obtained from 29 aphids and 19 psyllids on control trees and from 29 aphids and 20 psyllids on injected control trees.

Aphid and Psyllid Performance

To measure the impact of tree injection on aphids or psyllids survival, one clip-cage adapted from MacGillivray and Anderson (1957) and containing five synchronized first instar individuals was installed on a top leaf of an apple or pear tree that had been injected with the cinnamon 1% essential oil emulsion (HE modality) or not injected at all (Control modality). Ten trees were used per modality. For each clip-cage, the number of alive and dead individuals was assessed every 24 hr for 10 d. According to Abbott (1925), the mortality in injected trees was corrected against that in the control modality to calculate the actual mortality due to treatment, M corrected = ((M control − M treatment)/Mcontrol)*100.

At the end of the experiment, all the surviving individuals were weighed using an electronic precision balance (Mettler MT5, Max: 3 g Low: 0.1 mg). The average weight of the surviving individuals was then calculated for each clip-cage.

Statistical Analysis

Regarding aphids and psyllids feeding behavior, data on EPG parameters describing the number of occurrences of a particular phase (e.g. 'n_Pr') were not normally distributed (count data), accordingly a GLM was carried out using Poisson distribution. EPG data on feeding phase durations (e.g. duration of phloem sap ingestion 's_E2') were not normally distributed, so a GLM using a Gamma (link = 'inverse') distribution was carried out. The time to first probe (t.1Pr) was tested using a Cox proportional hazards (CPH) regression model, which is adapted to treat time-dependent parameters. The assumption of validity of proportional hazards was validated using the function 'coxph' (package R: 'survival', version 3.1.8: https://github.com/therneau/survival).

A nonlinear regression model and a linear regression were used to describe the evolution of the actual mortality caused by the treatment according to time, for *D. plantaginea* and *C. pyri* respectively. For *D. plantaginea*, a Beta growth and decline model was chosen to fit the data: Y = Ym*(1+ (Te − X)/(Te − Tm))*(X/Te)^(Te/(Te − Tm)), with Ym = the percentage of dead individuals at peak (same units as Y), Te is the time at which the percentage of dead individuals peaks (same units as X), and Tm is the time of inflexion point (same units as X).

Mann-Whitney tests were also used to compare the weight of surviving individuals (aphids and psyllids) at the end of the performance experiments between the control and injected trees.

Statistical analyses were performed using R version 4.1.0 R Core Team 2016 for Statistical Computing for Windows, and Prism 9 version 9.4.1 for macOS. All tests were applied using two-tailed hypotheses and significance level was set at 0.05.

Results

Aphid and Psyllid Preference

Aphids on D0 and D5 injected apple trees had a significantly shorter total duration of stylet probing (s_Pr), that lasted around 200 min, compared to aphids on control trees that lasted around 280 min (GLM using Gamma distribution, $\chi = 13.67$, $p = 0.001$) (Fig. 1). No significant difference was observed for the number of probes (n_Pr) (GLM using Poisson distribution, $\chi = 1.61$, $p = 0.45$). Aphids submitted to the D5 injected trees had a significantly greater time before the first probing (t.1Pr) (60 min) compared to that of aphids submitted to controls (30 min) (CPH, $\chi = 6.35$, $p = 0.04$).

Aphids on D5 injected trees had a significantly shorter total duration of pathway phase (s_C) compared to aphids on D0 injected trees (GLM using Gamma distribution, $\chi = 22.87$, $p < 0.001$). Indeed, aphids on the D5 injected trees performed pathway phase for *ca.* two hours, whereas on controls or D0 injected trees, aphids performed pathway phase for at least three hours.

Regarding phloem sap ingestion (s_E2), while no difference was observed for the total duration (GLM using Gamma distribution, $\chi = 2.81$, $p = 0.25$), a significant lower proportion of aphids did ingest phloem sap on both D0 and D5 injected trees compared to aphids on controls (χ^2 test, $\chi = 15.68$, $p < 0.001$). As shown on Fig. 2, ca. 80% of the aphids on noninjected trees ingested phloem sap, while only ca. 40% and ca. 25% of aphids submitted to D0 and D5 injected trees, respectively, ingested phloem sap.

Finally, no difference was observed for the total duration of xylem sap ingestion (s_G) (GLM using Gamma distribution, $\chi = 1.26$, $p = 0.53$), but a significantly greater proportion of aphids did ingest xylem sap on injected trees five days after injection compared to controls (χ^2 test, $\chi=9.02$, p=0.01). Ca. 7% of the aphids on control trees ingested xylem sap, while ca. 40% aphids submitted to D5 injected trees ingested phloem sap (Fig. 2). More preference

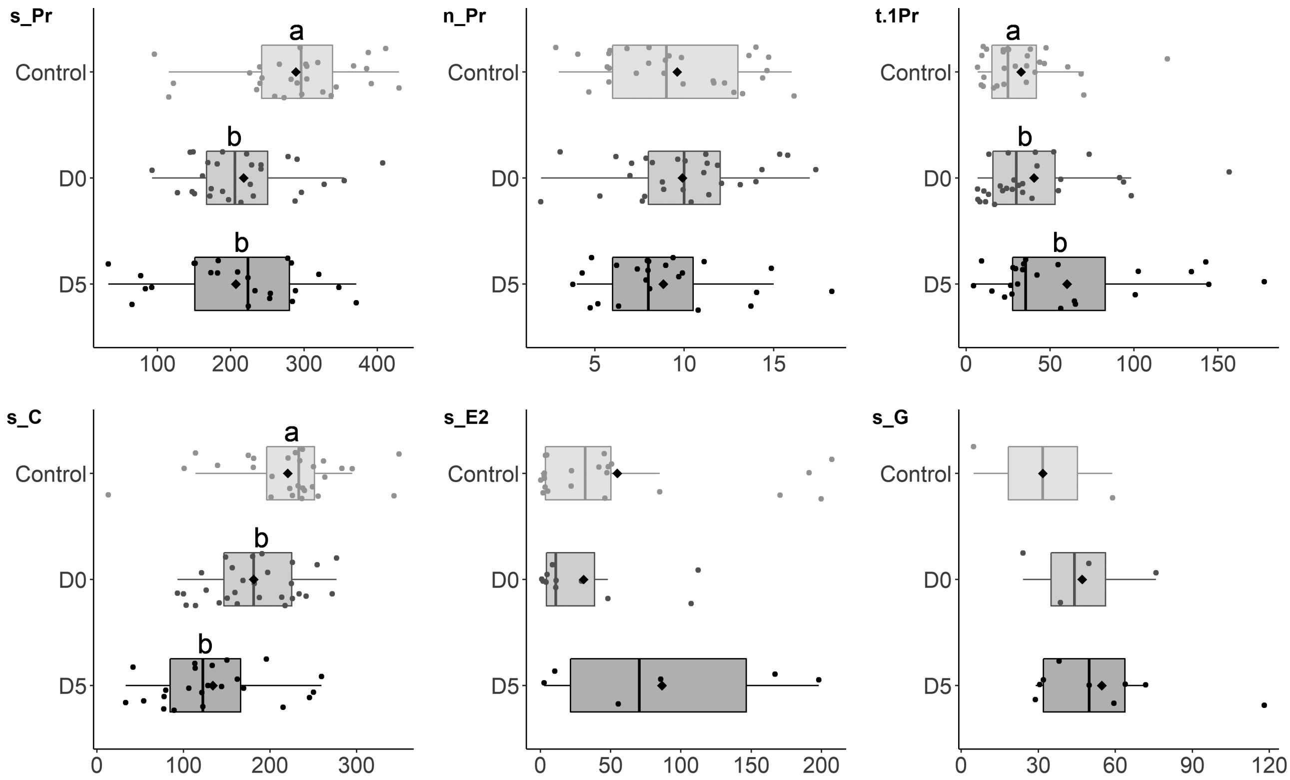


Fig. 1. Feeding behavior parameters of *Dysaphis plantaginea* adults submitted to control apple trees (light grey) or D0 (grey) and D5 (black) injected trees. (s_Pr) Total duration of probing; (n_Pr) Number on stylet probes; (t.1PR) Time to first probe; (s_C) Total duration of pathway phase; (s_E2) Total duration of phloem sap ingestion;(s_G) Total duration of xylem sap ingestion. Controls consisted of trees that had not been injected. Each tree that had been injected with the essential oil was tested directly at the end of the 24h of passive injection (D0) then five days later (D5). Box plots show median (line) and 25–75% percentiles (box). Black dots show means. Different letters indicate significant differences.

parameters are detailed in the supplementary material in the Supp Table 1 (online only).

For psyllids, the total duration of stylet probing (s_Pr) lasted around 400 min and no significant difference was observed among treatments (GLM using Gamma distribution, $\chi = 5.11$, $p = 0.08$) (Fig. 3). Psyllids on injected trees performed a greater number of probes (n_Pr), as controls did *ca.* 4 probes whereas psyllids on D0 and D5 injected trees performed ca. 6 and 7 probes, respectively (GLM using Poisson distribution, $\chi = 15.20$, $p < 0.001$). Psyllids submitted to the D0 and D5 injected trees had a greater time before the first probing (t.1Pr) compared to psyllids on controls (CPH, $\chi = 6.23$, $p = 0.04$), ca. 25 and ca. 9 min respectively. No difference was observed in terms of total duration of pathway phase (s_C) (GLM using Gamma distribution, $\chi = 0.72$, $p = 0.70$). Regarding phloem sap ingestion (s_E2), no difference was observed for both the total duration (GLM using Gamma distribution, $\chi = 0.79$, $p = 0.67$) and proportion of psyllids performing this phase (χ^2 test, $\chi = 2.19$, $p = 0.33$) (Fig. 2). Finally, no difference was observed for the total duration of xylem sap ingestion (s_G) (GLM using Gamma distribution, $\chi = 2.69$, $p = 0.26$) or for the proportion of psyllids ingesting xylem sap (χ^2 test, $\chi = 5.01$, $p = 0.08$) (Fig. 2). More preference parameters are detailed in the supplementary material in Supp Tables 2 and 3 (online only).

There was no impact of the injection of the emulsion solution without the essential oil on the feeding behavior of aphids and psyllids when compared to aphids and psyllids on control (noninjected trees) (Supp Tables 4 and 5 [online only]).

Aphid and Psyllid Performance

For both insects, our results showed that trunk injection significantly affected the individuals' survival (Fig. 4). Indeed, for *D. plantaginea*, the nonlinear regression model showed that the mortality increased linearly from the beginning of the experiment, reaching a peak of a 25% death rate on day 7 ($R^2 = 0.08$, $N = 100$, Ym ± CI = 25.96 ± 13.86, Te ± CI = 6.88 ± 2.08, Tm ± CI = 4.30 ± 4.26). This mortality then decreased to 0 from day 9 (Fig. 4). For *C.pyri*, the mortality due to treatment increased linearly from the beginning of the experiment to reach a maximum death rate of 46% at day 10 ($R^2 = 0.22$, $N = 100$, Y ± CI = 0.78 ± 9.24, X ± CI = 4.63 ± 1.74) (Fig. 4).

Regarding the final weight of surviving individuals, no difference was observed between the two tested modalities, either for the aphids ($U = 33.50$, $P > 0.05$) or the psyllids ($U = 25.00$, $P > 0.05$). The average weight of *D. plantaginea* and *C. pyri* at the end of the experiment was ca. 120 µg and 236 µg, respectively.

Discussion

The results obtained in our study confirmed our hypotheses. Indeed, the injection of an essential oil emulsion in trees impacted host-plant colonization by Hemiptera, as for both species a modification of their preference and performance was observed.

Concerning *D. plantaginea*, we observed that the injection of a 1% cinnamon essential oil emulsion in the host plant trunk induced a disturbance of aphid feeding behavior. Indeed, the injection of cinnamon essential oil emulsion shortened the duration and increased the latency time of stylet probing, while their number remained similar for all modalities. Short probing duration indicates the presence of negative factors in the epidermis and/or mesophyll, which cause the stylets to withdraw (Slesak et al. 2001, Crompton and Ode 2010, Kordan et al. 2012, Dancewicz et al. 2016, Marchetti et al. n.d.). The longer latency time recorded here may indicate

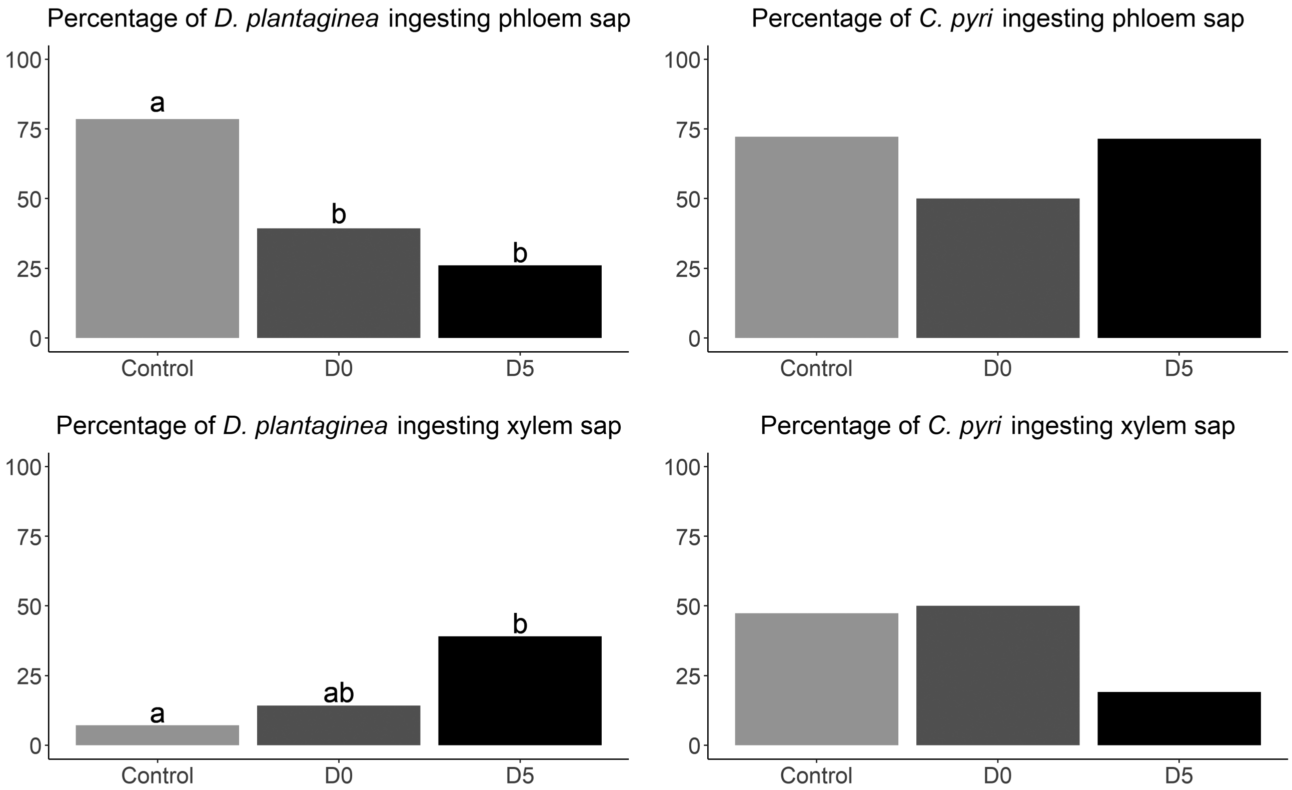


Fig. 2. Percentages of *Dysaphis plantaginea* and *Cacopsylla pyri* ingesting phloem or xylem sap on control trees (light grey) or D0 (grey) and D5 (black) injected trees. Controls consisted of trees that had not been injected. Each tree that had been injected with the essential oil was tested directly at the end of the 24h of passive injection (D0) then five days later (D5). Different letters indicate significant differences.

that the aphids were reluctant to probe. Knowing that they were restricted to a limited space, due to our experimental set-up, this suggests that they might have looked for another place to feed if they had not been constrained (Slesak et al. 2001, Marchetti et al. n.d.). This increase in latency time of the first probe could be due to a surface repellent effect causing plant rejection prior to stylet insertion (Slesak et al. 2001, Powell et al. 2006, Crompton and Ode 2010, Marchetti et al. n.d.). In our experiment, a likely explanation could be a change in the olfactory information from the plant, caused directly or indirectly by the presence of the essential oil. However, Werrie et al. (2021) also injected cinnamon essential oil into apple tree and showed that *trans*-cinnamaldehyde, the major compound in cinnamon essential oil, was not found in the blend of volatile organic compounds (VOCs) emitted from the injected apple trees. Another possibility is a change in VOCs emitted by the plant due to stress induced by trunk injection via the activation of systemic acquired resistance (SAR) (Perina et al. 2019). Werrie et al. (2021) showed that the injury caused by an injection did not result in changes in VOC emission. Therefore, the effect of the trunk injection on this phase would be rather due to a triggering of the plant's defense systems by the injection of the essential oil, which would act as an elicitor.

Furthermore, the results of the feeding behavior experiments revealed that the injection of the emulsion without the essential oil had no impact in comparison with the noninjected control, thus the piercing of the trunk and the injection of the emulsifier did not seem to have deeply, or visibly, altered the physiology of the plant. This is in accordance with the results of Werrie et al. (2021) which showed, with the same modalities (only on apple trees), that the emission of VOCs and the photosynthetic capacity remain unchanged.

Werrie et al. (2021) also showed that higher quantities of a series of sesquiterpenes (caryophyllene, germacrene, α-farnesene, bergamotene, and (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT)), as well as monoterpenes (terpinen-4-ol and linalool) and (*E*)-4,8-dimethyl-nonatriene (DMNT), were emitted from apple trees injected with cinnamon essential oil. These compounds could be the cause of the behavioral change we recorded here, as several studies have shown their repulsive or toxic effect on several aphid species (Bruce et al. 2005, Abbassy et al. 2009). Beside this suggested impact on volatiles metabolites, an experiment regarding plant defense elicitation using qRT-PCR on major apple defense pathways highlighted an increased gene expression following cinnamon essential oil application similar to SAR-inducer Bion 50 WG (SYNGENTA, active substance Acibenzolar-S-methyl) (Werrie et al. 2022). Moreover, prolonged induction of genes from pathogenesis-related proteins, parietal modification, and phenylpropanoids pathway was observed. Metabolites from the phenylpropanoid pathways hydroxycinnamic acids, particularly 4- caffeoylquinic acid (4-CQA) and 4-p-coumaroylquinic acid (4-pCoQA), were identified as the major players in *D. plantaginea*-resistant apple cultivars (Berrueta et al. 2018). Therefore, studying activation of the plant defense mechanism by the cinnamon essential oil, as well as evaluating the timing of this effect and specific secondary metabolites production, such as VOCs, hydroxycinnamic acids, or dibenzofurans could clarify the physiological cause leading to *D. plantaginea* mortality. The fact that a difference was observed at D5 and not at D0 suggests that this effect took time to develop. If the activation of the plant's defense systems was indeed the cause of the effect observed at D5, then this may explain the lack of effect at D0, as there was a lag period between the injection and the release of the volatile substances (Dudareva et

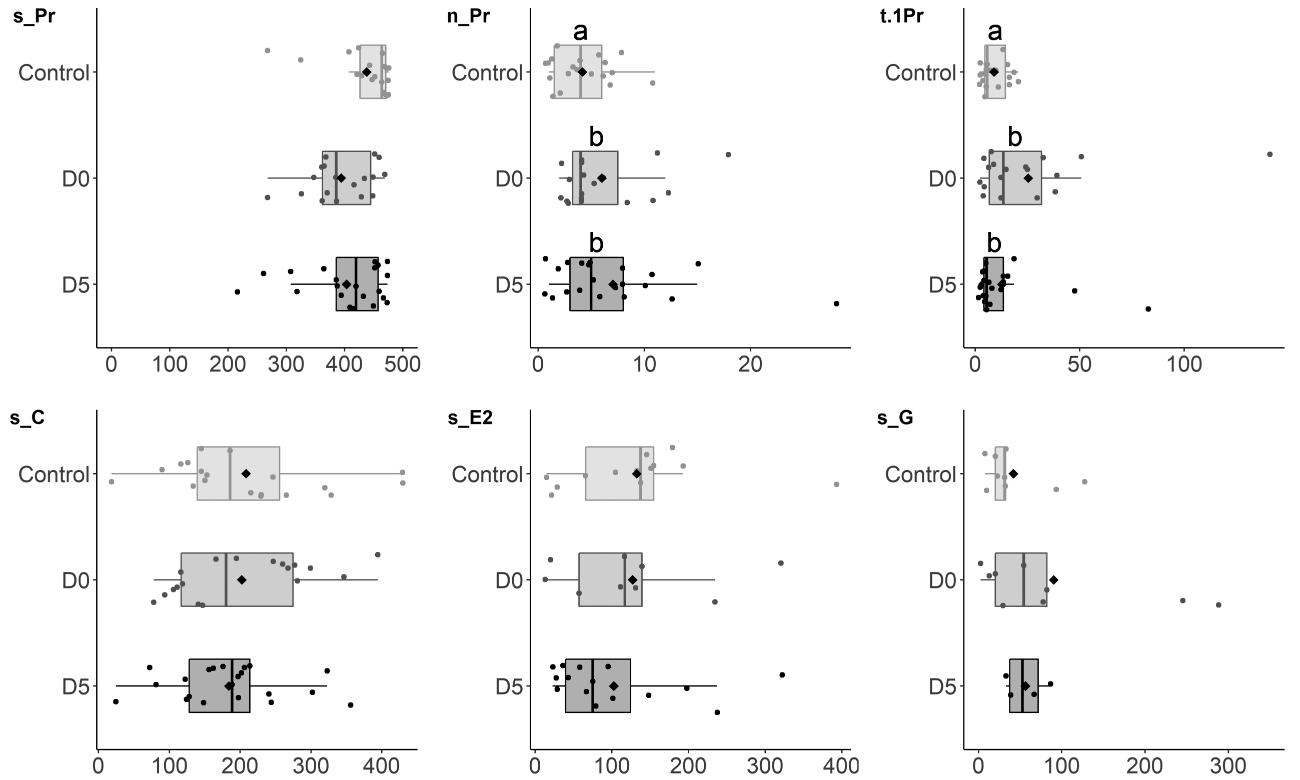


Fig. 3. Feeding behavior parameters of *Cacopsylla pyri* adults submitted to control pear trees (light grey) or D0 (grey) and D5 (black) injected trees. (s_Pr) Total duration of probing; (n_Pr) Number on stylet probes; (t.1PR) Time to first probe; (s_C) Total duration of pathway phase; (s_E2) Total duration of phloem sap ingestion; (s_G) Total duration of xylem sap ingestion. Controls consisted of trees that had not been injected. Each tree that had been injected with the essential oil was tested directly at the end of the 24h of passive injection (D0) then five days later (D5). Box plots show median (line) and 25–75% percentiles (box). Black dots show means. Different letters indicate significant differences.

al. 2006). In addition to the development of biopesticides with direct (insecticidal) and indirect toxic properties on aphids (by stimulating defense mechanisms), investigating the roles of these metabolites in insect-plant interactions could assist in the development of targeted plant breeding.

The injection also induced a decrease in the search time in the mesophyll. These results are quite surprising since Werrie et al. (2021) found that *trans*-cinnamaldehyde of treated trees accumulated in the leaves, and that the amounts of other repellent or insecticidal compounds such as caryophyllene significantly increased in the leaves (Werrie et al. 2021). It is possible that the essential oil emulsion delivered by xylem into leaf tissues may partition into phloem and other cells including the mesophyll as suggested for volatile in plant-plant communication (Sugimoto et al. 2016). It would be interesting to carry out a histological study to determine where these compounds can be found in the leaf, and whether they are found in the punctured cells. Using aphids as sensors (Dancewicz et al. 2016), have indirectly demonstrated that exogenous volatiles may penetrate the plant cuticle and epidermis, and pass into deeper tissue layers.

The higher proportion of individuals ingesting xylem sap in injected trees was a sign that the aphids were suffering from water stress, which is increased by starvation (Spiller et al. 1990, Ramírez and Niemeyer 2000, Pompon et al. 2010). Moreover, the injection system used in the present study allowed the emulsion to reach the leaves via the xylem. The fact that the aphids ingested more xylem on the injected trees, combined with the toxic effects observed, would therefore reinforce the idea that the mortality observed in our experiments could have been not only due a direct intoxication by the presence of the essential oil itself in the xylem (the vessel in which the essential oil was injected) and possibly in the phloem (where the essential oil could have been partitioned), but also to a reaction of the plant.

In our study, there was no significant difference in the proportion of individuals performing a stylet derailment phase (F) between treated and untreated trees. As this phase indicates a mechanical resistance of the plant tissues (Dardouri 2018), this suggests that either there was no increase in this mechanical resistance, or the aphids were not exposed to it, or were not sensitive to it.

The EPG results allow a better understanding of the results of the experiment on population dynamics. For aphids, the results for survival show an effect of the injection, with a lower survival on treated trees. These results are consistent with the EPG data, the absence of phloem sap ingestion led to aphid death by starvation. On the other hand, the presence of toxic compounds in the phloem, as suggested by the increased salivation recorded, could have led to death by intoxication. We observed a significant difference in survival from the fifth day onwards. This difference coincides with the EPG observations and corresponds to the time when an increase in time to first probing, a decrease in probing time in the mesophyll and a decrease in total salivation time were observed.

For psyllids, their feeding behavior was little changed on the treated trees compared to controls since only their probing behavior was affected (number and latency time). Psyllid honeydew plays an important role in the ecology of these insects as it is used as a defense against predators (Ge et al. 2020) and parasitoids (Le Goff et al. 2021, Tougeron et al. 2021). It is, therefore, possible that the psyllids needed to feed on the plant to produce this protection, even if the plant had been stressed by the injection and did not correspond to a perfect host.

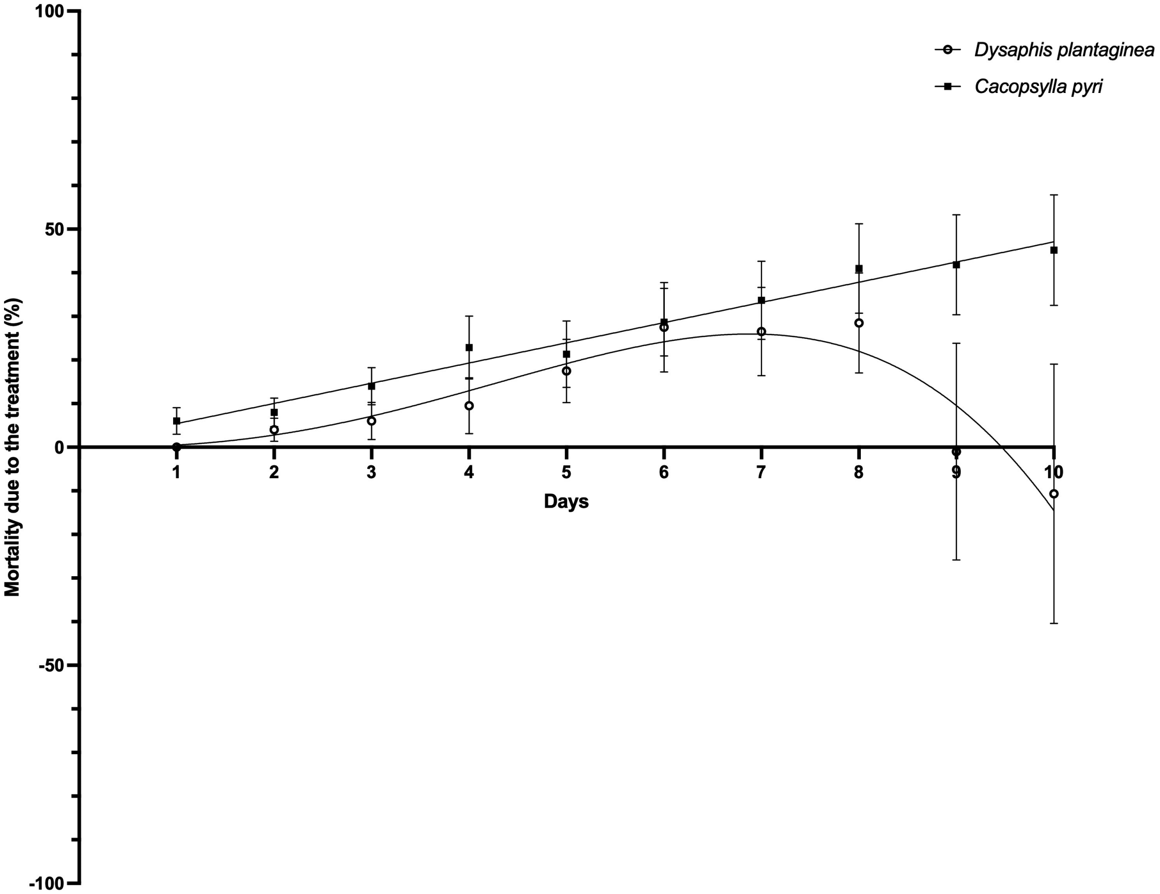


Fig. 4. Dynamics of the mortality rate due to treatment for *Dysaphis plantaginea* and *Cacopsylla pyri.* Error bars indicate standard error bars ($n = 10$).

The survival profile of psyllids was the same as for aphids with a significantly higher mortality from the third day onwards. The fact that the feeding behavior of the psyllids was little changed compared to the control condition suggests that the observed mortality was possibly mainly due to intoxication.

For the mass, there was no difference between the two modalities for either aphids or psyllids. This can be explained by the fact that only the individuals remaining at the end of the experiment, i.e., those that resisted the injection, were weighed. If they resisted the injection treatment, then their diet may not have changed, or may have changed only slightly, and therefore their mass may not have changed either.

In this study, we showed that the injection of cinnamon essential oil into the trunk of apple and pear trees resulted in changes in the feeding behavior of their respective pests and ultimately increased their mortality rate.

In terms of agricultural application, trunk injection and EO applications are rarely used (Aćimović et al. 2019). However, while the results of this study are encouraging, we must highlight that more works need to be done before an application of the tree injection method by horticulturists. Indeed, some grey areas are still to be clarified such as the effect of this methodology on the health of rootstocks (growth, fruit production), the movement of essential oil within the mature tree (young/old leaves, fruits) or the impact of this treatment on other pests and beneficial insects, This method could, therefore, theoretically be used in the future to control these two orchard hemipteran pests, but its effectiveness in real conditions still has to be demonstrated.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.

Abbassy, M. A., S. A. M. Abdelgaleil, and R. Y. A. Rabie. 2009. Insecticidal and synergistic effects of *Majorana hortensis* essential oil and some of its major constituents. *Entomol. Exp. Appl.* 131: 225–232. doi: 10.1111/j.1570-7458.2009.00854.x

Aćimović, S. G., A. H. VanWoerkom, P. D. Reeb, C. Vandervoort, T. Garavaglia, B. M. Cregg, and J. C. Wise. 2014. Spatial and temporal distribution of trunk-injected imidacloprid in apple tree canopies: Distribution of trunk-injected imidacloprid in apple tree canopy. *Pest Manag. Sci*. 70: 1751–1760. doi: 10.1002/ps.3747

Aćimović, S. G., D. K. H. Martin, R. M. Turcotte, C. L. Meredith, and I. A. Munck. 2019. Choosing an adequate pesticide delivery system for managing pathogens with difficult biologies: case studies on *Diplodia corticola*, *Venturia inaequalis* and *Erwinia amylovora*. *In Plant Diseases - Current Threats and Management Trends*. IntechOpen.

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar. 2008. Biological effects of essential oils – A review. *Food Chem. Toxicol*. 46: 446–475. doi: 10.1016/j.fct.2007.09.106

Belien, T., S. Raymaekers, M. Eeraerts, V. Mommaerts, G. Claus, C. Bogen, N. Piot, G. Smagghe, P. Spanoghe, and D. Bylemans. 2021. Towards integrated pest and pollinator management in intensive pear cultivation: a case study from Belgium. *Insects*. 12: 901. doi: 10.3390/insects12100901

Berger, C., and F. Laurent. 2019. Trunk injection of plant protection products to protect trees from pests and diseases. *Crop Prot*. 124: 104831. doi: 10.1016/j.cropro.2019.05.025

Berrueta, L. A., A. Sasía-Arriba, M. Miñarro, M. J. Antón, R. M. Alonso-Salces, D. Micheletti, B. Gallo, and E. Dapena. 2018. Relationship between hydroxycinnamic acids and the resistance of apple cultivars to rosy apple aphid. *Talanta*. 187: 330–336. doi: 10.1016/j.talanta.2018.05.040

Blommers, L. H. M., H. H. M. Helsen, and F. W. N. M. Vaal. 2004. Life history data of the rosy apple aphid *Dysaphis plantaginea* (Pass.) (Homopt., Aphididae) on plantain and as migrant to apple. *J. Pest Sci*. 77: 155–163.

Bruce, T. J., M. A. Birkett, J. Blande, A. M. Hooper, J. L. Martin, B. Khambay, I. Prosser, L. E. Smart, and L. J. Wadhams. 2005. Response of economically important aphids to components of *Hemizygia petiolata* essential oil. *Pest Manag. Sci*. 61: 1115–1121. doi: 10.1002/ps.1102

Byrne, F. J., J. Almanzor, I. Tellez, A. Eskalen, D. M. Grosman, and J. G. Morse. 2020. Evaluation of trunk-injected emamectin benzoate as a potential management strategy for Kuroshio shot hole borer in avocado trees. *Crop Prot*. 132: 105136. doi: 10.1016/j.cropro.2020.105136

Civolani, S. 2012. The past and present of pear protection against the pear psylla, *Cacopsylla pyri* L. *In* F. Perveen (ed.), *Insecticides – pest engineering*. InTech.

Civolani, S., M. Leis, G. Grandi, E. Garzo, E. Pasqualini, S. Musacchi, M. Chicca, G. Castaldelli, R. Rossi, and W. Freddy Tjallingii. 2011. Stylet penetration of *Cacopsylla pyri*; an electrical penetration graph (EPG) study. *J. Insect Physiol*. 57: 1407–1419.

Crompton, D. S., and P. J. Ode. 2010. Feeding behavior analysis of the soybean aphid (Hemiptera: Aphididae) on resistant Soybean 'Dowling'. *J. Econ. Entomol*. 103: 648–653. doi: 10.1603/ec09370

Dancewicz, K., K. Sznajder, D. Załuski, B. Kordan, and B. Gabryś. 2016. Behavioral sensitivity of *Myzus persicae* to volatile isoprenoids in plant tissues. *Entomol. Exp. Appl*. 160: 229–240.

Dardouri, T. 2018. Implication des composés organiques volatils dans la capacité des plantes de service à perturber le comportement et les performances de *Myzus persicae* (sulzer) le puceron vert du verger. *Ph.D. dissertation*, Université d'Avignon, France.

Dedryver, C. -A., A. Le Ralec, and F. Fabre. 2010. The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *C.R. Biol*. 333: 539–553. doi: 10.1016/j.crvi.2010.03.009

Denoirjean, T., G. Doury, A. Cornille, X. Chen, T. Hance, and A. Ameline. 2021. Genetic structure of *Malus sylvestris* and potential link with preference/performance by the rosy apple aphid pest *Dysaphis plantaginea*. *Sci. Rep*. 11: 5732. doi: 10.1038/s41598-021-85014-x

Doccola, J. J., and P. M. Wild. 2012. Tree injection as an alternative method of insecticide application. *In* S. Soloneski and M. Larramendy (eds.), *Insecticides – basic and other applications*. IntechOpen.

Dudareva, N., F. Negre, D. A. Nagegowda, and I. Orlova. 2006. Plant volatiles: recent advances and future perspectives. *Crit. Rev. Plant Sci*. 25: 417–440. doi: 10.1080/07352680600899973

DuPont, S. T., C. Strohm, L. Nottingham, and D. Rendon. 2021. Evaluation of an integrated pest management program for central Washington pear orchards. *Biol. Control*. 152: 104390.

FAO. 2022. FAO. https://www.fao.org/faostat/en/#data/QCL.

Fettig, C. J., A. S. Munson, D. M. Grosman, and P. B. Bush. 2014. Evaluations of emamectin benzoate and propiconazole for protecting individual *Pinus contorta* from mortality attributed to colonization by *Dendroctonus ponderosae* and associated fungi. *Pest Manag. Sci*. 70: 771–778. doi: 10.1002/ps.3612

Fidgen, J. G., N. T. Kittelson, T. Eckberg, J. Doccola, and C. Randall. 2013. Field note: Emamectin benzoate reduces defoliation by *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae) on three host species. *West. J. Appl. For*. 28: 170–173.

Ge, Y., P. Liu, L. Zhang, W. E. Snyder, O. M. Smith, and W. Shi. 2020. A sticky situation: honeydew of the pear psylla disrupts feeding by its predator *Orius sauteri*. *Pest Manag. Sci*. 76: 75–84. doi: 10.1002/ps.5498

Giordanengo, P. 2014. EPG-Calc: a PHP-based script to calculate electrical penetration graph (EPG) parameters. *Arthropod-Plant Interact*. 8: 163–169.

Harrewijn, P., A. Oosten, and P. G. M. Piron. 2001. *Natural terpenoids as messengers. A multicisciplinary study of their production, biological functions and practical applications*. Kluwer. ed, Ann. Bot. Dordrecht.

Huang, Y., and S. H. Ho. 1998. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res*. 34: 11–17.

Khalaf, M. Z., and H. F. Alrubeai. 2016. Chemical control of date palm tree borers, Orycytes species (Coleoptera: scarabidae:Dynastinae). *Pak. Entomol*. 38: 1–5.

Kim, S. -I., J. -Y. Roh, D. -H. Kim, H. -S. Lee, and Y. -J. Ahn. 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res*. 39: 293–303.

Kordan, B., K. Dancewicz, A. Wróblewska, and B. Gabryś. 2012. Intraspecific variation in alkaloid profile of four lupine species with implications for the pea aphid probing behaviour. *Phytochem. Lett*. 5: 71–77. doi: 10.1016/j.phytol.2011.10.003

Le Goff, G. J., J. Berthe, K. Tougeron, B. Dochy, O. Lebbe, F. Renoz, and T. Hance. 2021. Effect of the instar of the pear psyllid *Cacopsylla pyri* (Hemiptera: Psyllidae) on the behaviour and fitness of the parasitoid *Trechnites insidiosus* (Hymenoptera: Encyrtidae). *Eur. J. Entomol*. 118: 279–287. doi: 10.14411/eje.2021.028

Lee, E. -J., J. -R. Kim, D. -R. Choi, and Y. -J. Ahn. 2008. Toxicity of cassia and cinnamon oil compounds and cinnamaldehyde-related compounds to *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Econ. Entomol*. 101: 1960–1966. doi: 10.1603/0022-0493-101.6.1960

MacGillivray, M. E., and G. B. Anderson. 1957. Three useful insect cages1. *Can. Entomol*. 89: 43–46. doi: 10.4039/ent8943-1

Marchetti, E., S. Civolani, M. Leis, M. Chicca, W. F. Tjallingii, E. Pasqualini, and P. Baronio. n.d. Tissue location of resistance in apple to the rosy apple aphid established by electrical penetration graphs. *Bull. Insectol*. 62: 203–208.

Nollet, L. M. L., and H. S. Rathore. 2017. *Green pesticides handbook*, 1st ed. CRC Press, Boca Raton: Taylor & Francis, CRC Press, 2017.

Perina, F. J., C. C. L. de Andrade, S. I. Moreira, E. M. Nery, C. Ogoshi, and E. Alves. 2019. *Cinnamomun zeylanicum* oil and trans-cinnamaldehyde against Alternaria brown spot in tangerine: direct effects and induced resistance. *Phytoparasitica*. 47: 575–589. doi: 10.1007/s12600-019-00754-x

Pompon, J., D. Quiring, P. Giordanengo, and Y. Pelletier. 2010. Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). *J. Insect Physiol*. 56: 610–615. doi: 10.1016/j.jinsphys.2009.12.009

Porcel, M., G. K. S. Andersson, J. Pålsson, and M. Tasin. 2018. Organic management in apple orchards: higher impacts on biological control than on pollination. *J. Appl. Ecol*. 55: 2779–2789.

Powell, G., C. R. Tosh, and J. Hardie. 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol*. 51: 309–330. doi: 10.1146/annurev.ento.51.110104.151107

Qubbaj, T., A. Reineke, and C. P. W. Zebitz. 2005. Molecular interactions between rosy apple aphids, *Dysaphis plantaginea*, and resistant and susceptible cultivars of its primary host *Malus domestica*. *Entomol. Exp. Appl*. 115: 145–152. doi: 10.1111/j.1570-7458.2005.00255.x

Ramírez, C. C., and H. M. Niemeyer. 2000. The influence of previous experience and starvation on aphid feeding behavior. *J. Insect Behav*. 13: 699–709.

Reichard, D. L., R. D. Fox, R. D. Brazee, and F. R. Hall. n.d. *Air velocities delivered by orchard air sprayers*. Transactions of the ASAE. 22. 22.

Reyes-Jurado, F., A. R. Navarro-Cruz, C. E. Ochoa-Velasco, E. Palou, A. López-Malo, and R. Ávila-Sosa. 2020. Essential oils in vapor phase as alternative antimicrobials: a review. *Crit. Rev. Food Sci. Nutr*. 60: 1641–1650. doi: 10.1080/10408398.2019.1586641

Slesak, E., M. Slesak, and B. Gabrys. 2001. Effect of methyl jasmonate on hydroxamic acid content, protease activity, and bird cherry–oat aphid *Rhopalosiphum padi* (L.) probing behavior. *J. Chem. Ecol*. 27: 2529–2543. doi: 10.1023/a:1013635717049

Spiller, N. J., L. Koenders, and W. F. Tjallingii. 1990. Xylem ingestion by aphids – a strategy for maintaining water balance. *Entomol. Exp. Appl*. 55: 101–104.

Steiner, P.W. 1969. The distribution of spray material between target and nontarget areas of a mature apple orchard by Airblast equipment. *Masters Thesis*, Cornell University, pp. 1e57.

Sugimoto, K., K. Matsui, and J. Takabayashi. 2016. Uptake and conversion of volatile compounds in plant–plant communication, pp. 305–316. *In* J. D. Blande and R. Glinwood (eds.), *Deciphering chemical language of plant communication, signaling and communication in plants*. Springer International Publishing, Cham.

Süle, S., and G. Jenser. 2007. Management of pear decline caused by 'Candidatus *Phytoplasma pyri*' in Hungary. *Bull. Insectol*. 60: 319–320.

Tjallingii, W. F. 1985. Electrical nature of recorded signals during stylet penetration by aphids. *Entomol. Exp. Appl*. 38: 177–186. doi: 10.1111/j.1570-7458.1985.tb03516.x

Tjallingii, W. F., and T. H. Esch. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiol. Entomol*. 18: 317–328. doi: 10.1111/j.1365-3032.1993.tb00604.x

Tougeron, K., C. Iltis, F. Renoz, L. Albittar, T. Hance, S. Demeter, and G. J. Le Goff. 2021. Ecology and biology of the parasitoid *Trechnites insidiosus* and its potential for biological control of pear psyllids. *Pest Manag. Sci*. 77: 4836–4847.

Turek, C., and F. C. Stintzing. 2013. Stability of essential oils: a review. *Compr. Rev. Food Sci. Food Saf*. 12: 40–53.

VanWoerkom, A. H., S. G. Aćimović, G. W. Sundin, B. M. Cregg, D. Mota-Sanchez, C. Vandervoort, and J. C. Wise. 2014. Trunk injection: an alternative technique for pesticide delivery in apples. *Crop Prot*. 65: 173–185.

Werrie, P. -Y., C. Burgeon, G. J. Le Goff, T. Hance, and M. -L. Fauconnier. 2021. Biopesticide trunk injection into apple trees: a proof of concept for the systemic movement of mint and cinnamon essential oils. *Front. Plant Sci*. 12: 650132. doi: 10.3389/fpls.2021.650132

Werrie, P. -Y., A. Juillard, C. Heintz, M. -N. Brisset, and M. -L. Fauconnier. 2022. Phytotoxicity and plant defence induction by *Cinnamomum cassia* essential oil application on *Malus domestica* tree: a molecular approach. *Agronomy*. 12: 512. doi: 10.3390/agronomy12020512

Wheeler, C. E., C. Vandervoort, and J. C. Wise. 2020. Organic control of pear psylla in pear with trunk injection. *Insects*. 11: 650. doi: 10.3390/insects11090650

Wise, J., A. Vanwoerkom, S. Aćimović, G. Sundin, B. Cregg, and C. Vandervoort. 2014. Trunk injection: a discriminating delivering system for horticulture crop IPM. *Entomol. Ornithol. Herpetol*. 3: 126.

Zhu, H., R. C. Derksen, H. Guler, C. R. Krause, and H. E. Ozkan. 2006. Foliar deposition and off-target loss with different spray techniques in nursery applications. *Trans. ASABE*. 49: 325–e334.