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# Assessment of the efficiency of different control programs to reduce *Trioza apicalis* Först. (Triozidae: Hemiptera) feeding damage and the spread of "*Candidatus* Liberibacter solanacearum" on carrots (*Daucus carota* ssp. *sativus* L.)

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## Abstract

The control of carrot psyllid *Trioza apicalis* Förster largely relies on chemical measures, and the current integrated pest management (IPM) strategy is based on pest scouting. The number of active ingredients available for psyllid control will further decline in the coming years. The need for alternative control measures is therefore urgent. In this work the efficiency of different control programs including a kaolin particle film and plant-derived, crude saponin extract, chemical control, insect net and IPM (kaolin in combination with insecticides) programs to reduce psyllid feeding damage, reproduction and the spread of "*Candidatus* Liberibacter solanacearum" (CLso) in carrots was studied. Each year in 2016–2018, four replicates of each treatment were randomised in a row-column design on a commercial carrot farm. After the treatments were executed, the psyllid nymphs and eggs were counted. At the harvest, the carrot roots and shoots were weighed, damages assessed, and samples were taken for CLso detection from 50 plants at each replicate. Kaolin treatment alone and in combination with insecticides effectively reduced the number of psyllid nymphs and eggs in all the years studied. Saponin was applied only on the first year, since it significantly increased the number of *T. apicalis* eggs compared to other treatments. The insect net was superior to other treatments in all the studied aspects in all the years. Under normal weather conditions, the highest root weight was harvested under the insect net, followed by the chemical control program consisting of pyrethroids, kaolin treatment, untreated control and saponin treatment. During extreme weather conditions, chemical control programs were not effective at protecting the carrots from psyllid feeding, which was reflected as low root yield. In all years, the carrot leaf damage percent negatively correlated with the root weight. Similarly, the shoot:root ratio increased as the leaf-curling percentage rose, which was even pronounced under drought stress. The effect of climatic stress should be considered when developing IPM strategies.

## 1 | INTRODUCTION

The carrot psyllid, *Trioza apicalis*, has been a serious carrot pest since the early decades of 1900 in northern Europe and in parts of central Europe (Bey, 1931; Burckhardt & Freuler, 2000; Krumrey & Wendland, 1973; Láska, 1974; Lundblad, 1929; Markkula, Laurema, & Tiittanen, 1976; Nehlin, Valterová, & Borg-Karlson, 1994; Rygg, 1977). Overwintered carrot psyllid adults damage carrots very quickly: leaf-curling symptoms become visible on average within 2 days (Markkula et al., 1976), and have been considered a gall-forming reaction (Hodkinson, 1984, 2009; Lundblad, 1929). Markkula et al. (1976) showed that one carrot psyllid confined on a carrot seedling for the whole summer totally prevented the root development. Further, Nissinen, Vanhala, Holopainen, and Tiilikkala (2007) and Nissinen et al. (2012) showed that one female psyllid feeding for 3 days at the cotyledon or one leaf stage led to a 35–50% root weight reduction. Therefore, the control measures should either have a quick knock down effect or they should prevent the psyllid feeding to be effective. In addition to the physical damage, infected carrot psyllids transmit "*Candidatus* Liberibacter solanacearum" (CLso), which was first detected in apiaceous plants, in carrots, and carrot psyllids in Finland (Munyaneza et al., 2010a,b). In carrots, CLso haplotype C causes leaf discolouration and phloem blockage leading to a reduction of sugars in the roots (Nissinen et al., 2012; Nissinen, Haapalainen, Jauhiainen, Lindman, & Pirhonen, 2014), whereas the leaf-curling symptoms are caused by the vector (Nissinen et al., 2014). In case of the other apiaceous haplotypes D and E occurring in Central Europe and the Mediterranean, the symptoms include shoot proliferation, discolouration and leaf-curling or twisting of the petioles (Hajri, Loiseau, Cousseau-Suhard, Renaudin, & Gentit, 2017; Mawassi et al., 2018; Teresani et al., 2014), however, no separate symptoms have been designated to the currently known psyllid vector *Bactericera trigonica* of these haplotypes.

Due to the rapid damage formation caused by *T. apicalis*, the control measures have relied heavily on pyrethroids since the late 1980s. The number of treatments has been increasing ever since, and a suspicion of pyrethroid resistance in *T. apicalis* was reported in Norway (Meadow, 2010; Nehlin et al., 1994; Tiilikkala, Ketola, & Taivalmaa, 1996). Indeed, the intensive and repeated use of pyrethroids, has led to the development of resistance in *Cacopsylla* spp. (Buès, Boudinhon, & Toubon, 2003; Pree, Archibald, Ker, & Cole, 1990). In addition to the reduction of the root yield, the curled leaves protect the developing carrot psyllid nymphs from non-systemic pesticides. This in turn, ensures the emergence of the next overwintering generation leading to gradual population growth in the areas of intensive carrot cultivation, which has been observed by increasing trap catches during the last decades (Haapalainen et al., 2018; Nissinen et al., 2007; Tiilikkala et al., 1996). Therefore, not only the prevention of adult feeding, but

also the reduction of eggs and nymphs by the control measures is essential. The efficacy of pyrethroids to psyllid nymphs has been variable. Lambda-cyhalothrin had good efficacy against *B. cockerelli* nymphs (Berry, Walker, & Butler, 2009), whereas alpha-cypermethrin showed poor efficacy against pistachio psyllid nymphs (Saour, 2005). Thiacloprid and spirotetramat were included to the control programs in this study, since these active ingredients have been recently accepted for carrot psyllid control in Finland. They have different mode of action than pyrethroids, and they were expected to have good efficacy against psyllid nymphs (Berry et al., 2009). Currently, the demand for alternative control methods is urgent, since the number of active ingredients registered for carrot psyllid control in the northern Europe will decline in 2020.

The EU Parliament has adopted the Directive 2009/128/EC regarding the sustainable use of pesticides (European Parliament, 2009), in which the objective is to reduce dependence on, as well as the risks and adverse impacts of pesticide use on human health and the environment. According to the Directive, IPM is defined as the "Careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. IPM emphasises the growth of a healthy crop with the least possible disruption to agroecosystems and encourages natural pest control mechanisms".

The control of *T. apicalis* has exceeded the minimum threshold of IPM, that is, field scouting of the pest has been performed (Kogan, 1998) since the 1990s when Tiilikkala et al. (1996) observed substantial root damage by trap catches of one psyllid per trap per week, which has been used as an action threshold thereafter. Several components of more enhanced IPM strategies have been studied in *T. apicalis* control. The use of conifer sawdust has been shown to reduce the psyllid damage (Meadow, 2010; Nehlin et al., 1994), but this technique has not become common probably due to the laborious spreading of sawdust. Nissinen (2008) studied a push-pull strategy: an attractive carrot cultivar was found but not a repellent substance. Later, Rämert, Cotes, and Nilsson (2016) also studied the use of trap crops, but the difference in attractiveness between the trap crop and the cash crop was not high enough to provide adequate control of late migrating psyllids. Meadow (2010) suspected that exclusive fences would not be effective against carrot psyllids due to their flight height. Early sowing of the carrots as a means to avoid psyllid damages (Nissinen, 2008) cannot be utilised every year in the Nordic countries due to night frosts or soil wetness in the spring. The electrophysiological responses of carrot psyllids to host plant cues have been identified (Kristoffersen, Larsson, & Anderbrant, 2008; Rahmani et al., 2019), but the behavioural responses to these substances have not yet been

defined. The reduction of the carrot psyllid population by implementing novel IPM strategies would require the utilisation of several components of the strategy, such as trap crops and repellent substances, or trap crops and exclusive fences, simultaneously. As several of the novel psyllid management strategies have not yet reached the level of functionality needed for utilisation on the field scale, the current state of IPM does not yet allow the re-design of the ecosystem. Therefore, the methods studied here aim to advance the first level IPM, where the aim is to substitute insecticides with bio-rational compounds or physical means (Kogan, 1998. Previously, a kaolin particle film has been shown to reduce the number of *B. cockerelli* eggs and adults on tomatoes both in laboratory and field experiments (Peng, Trumble, Munyaneza, & Liuet, 2011). Saponins have been shown to have both toxic and deterrent effect on pea aphids (De Geyter, Smagghe, Rahbé, & Geelen, 2012).

The aim of this study was to assess the effect of different control programs, especially a kaolin particle film and plant-derived, crude saponin extract, on *T. apicalis* host plant selection, reproduction, feeding damage and CLso transmission on carrots. The aim was also to assess suitability of kaolin and saponin for control programs following IPM guidelines in order to suppress the use of insecticides.

## 2 | MATERIALS AND METHODS

### 2.1 | Psyllids

In a series of greenhouse experiments, carrot psyllids originally collected from Forssa (60.815 N, 23.628 E) in July 2015, were used. They were continuously reared in a greenhouse (L20:D4, 20/15°C day/night, 50% RH) at Jokioinen, as described in Nissinen et al. (2007). In choice assays, the psyllids were collected in the morning in aspirators (made from pipette tips) in batches of 10 adults and released onto Petri dishes approximately 1 hr after collection. In a reproduction experiment, the psyllids were starved for 15–18 hr before release into cages. During the starvation period, the psyllids were provided with a water source to prevent mortality due to desiccation. In field experiments, carrots were exposed to the feeding of natural populations of *T. apicalis*. Psyllid flight was monitored using four sticky traps, one at each corner of the experimental plot. The traps were changed at least once a week.

### 2.2 | Kaolin and crude saponin extract

Kaolin (Imerys Polwhite E, importer: Bang & Bonsomer Group Oy, Helsinki) was received from a local retailer in a 20 kg sack. Saponin rich residue flour from the abrasive milling of quinoa seed was obtained from a local farmer (Lieto, Tarvasjoki, Finland, 60.583 N, 22.737 E). The quinoa seed flour (1.5 kg) was extracted using 50% methanol (12 L) in a plastic container. The container was shaken vigorously by hand several times during the working day. Extraction was carried out over 3 days, after which the slurry was filtered through a cheese cloth. Solids were then rinsed twice with 1.5 L of methanol. The methanolic extract was evaporated in a large-scale rotary evaporator (Heidolph Laborota 20) into a watery residue. The volume was adjusted to 3.2 L with distilled water and mixed carefully. This water extract was divided into plastic containers (*c.* 250 mL in each) and put into a freezer until the field study. In total, two batches were prepared and stored in a freezer. The extracts contained 0.4% saponins as α-hederin based on the HPLC-DAD analysis (analytical conditions described in Mattila et al., 2018).

### 2.3 | No-choice assays

To assess the concentration of kaolin and saponin to be used in the field experiments, no-choice bioassays with kaolin and saponin were conducted in Petri dishes (diam. 20 cm) with detached carrot leaves. Carrot cv. Fontana was grown in 1.1 L pots in patches of approximately five seedlings and treated at the 3–4-leaf stage with a crude saponin extract or kaolin at different concentrations. The control carrots were treated with water (milliQ). The concentrations studied were 0, 25, 37.5, 50 g L$^{-1}$ for kaolin and kaolin with 0.01% surfactant (Silwet Gold®). A surfactant was added to a kaolin suspension to achieve the even spreading of the suspension on the leaves, because without a surfactant the kaolin dried on the leaves into spots. For crude saponin extract, the dilutions studied were 0, 1:25, 1:10, and 1:5. After the treatments, the carrots were left to dry in a fume hood for 30 min. During that time, psyllids were collected. Thereafter, one leaf (the second leaf) was cut from each of the treated seedlings, the petiole was inserted into a water-filled Eppendorf tube and the tube was sealed with Parafilm®. Each treated leaf was set at the opposite side of an aspirator tip, from which 10 carrot psyllids were released. The settling of the psyllids on the leaves was checked after 24 hr. The Petri dishes holding the leaves were organised according to a row-column design (Williams, Matheson, & Harwood, 2002) into a greenhouse unit. Each of the concentrations was replicated in three Petri dishes, and each of the experiments was repeated two times, except kaolin without a surfactant, which was repeated three times.

### 2.4 | Reproduction experiment

The population development of carrot psyllids on treated plants was measured in a cage trial. The concentrations of kaolin and saponin used in the experiment were based on the results of no-choice assays. Carrot cv. Fontana seedlings, which were at the 2–2.75-leaf stage (third true leaf not yet fully opened), were treated with water (milliQ, control), kaolin 37.5 g L$^{-1}$ + 0.01% surfactant (SilwetGold®) or a plant-derived crude saponin extract diluted 1:5 in water and placed into insect cages (33 × 33 × 60 cm, *n* = 5 for each treatment). Thereafter, two gravid carrot psyllid females were released into each of the cages. The treatments were repeated two times by hand sprayer (Plastex®, Oy Plastex Ab, Lohja, Finland) while the adult psyllids were in the cages, first 11 days and second 20 days after the psyllid release. These latter treatments were targeted against developing nymphs. The emerging second-generation adults were collected separately from each cage with an aspirator at two to three-day intervals over a

**TABLE 1** Trial treatment descriptions 2016–2018. The order of the treatments is indicated in Table 2

| | AI | Formulation | Concentration | Dose rate |
|---|---|---|---|---|
| A | Kaolin + Organosilicone adjuvant | DU | 99% | 11.25 kg ha$^{-1}$ 0.01–0.1% |
| B | Deltamethrin | EW | 50 g L$^{-1}$ | 0.1–0.2 L ha$^{-1}$ |
| C | Thiacloprid | SC | 480 g L$^{-1}$ | 0.2 L ha$^{-1}$ |
| D | Lambda-cyhalothrin | CS | 100 g L$^{-1}$ | 0.075–0.1 L ha$^{-1}$ |
| E | Tau-fluvalinate | EW | 240 g L$^{-1}$ | 0.2 L ha$^{-1}$ |
| F | Spirotetramate | SC | 100 g L$^{-1}$ | 0.75 L ha$^{-1}$ |
| G | Esfenvalerate | EW | 50 g L$^{-1}$ | 0.038–0.4 L ha$^{-1}$ |
| H | Sypermethrin | EC | 250 g L$^{-1}$L | 0.1 L ha$^{-1}$ |
| S | 0.4% saponins as α-hederin | L | 0.8 g L$^{-1}$ | 240 g ha$^{-1}$ |
| W | Water | | 1 kg L$^{-1}$ | 300 L ha$^{-1}$ |

Abbreviations: AI, active ingredient; CS, capsule suspension; DU, dust or powder; EC, emulsifiable concentrate; EW, emulsion, oil in water; L, liquid; SC, suspension concentrate.

**TABLE 2** Control programs compared in experiments 2016–2018. The treatments indicated by different letters are explained in Table 1

| | Program 1 | Program 2 | Program 3 | Program 4 | Program 5 |
|---|---|---|---|---|---|
| 2016 | WWWWWW | BDDHGG | net | AAAAAA | SSSSSS |
| 2017 | WWWWWW | EDCEDC | net | AAAAAA | AADECA |
| 2018 | WWWWWWWWWWWWW | BGDCDFBFEBGEC | net | AAAAAAAAAAAAA | AADCDFBFAAAAA |

period of 8 weeks. The psyllids were killed in a freezer at −20°C, and thereafter, the number of females and males were counted.

## 2.5 | Field experiments

In May 2016, a field experiment was established on a commercial carrot farm to study the efficiency of different control programs against carrot psyllid. The compared treatments were (a) a control (water treatment), (b) a chemical control program consisting of pyrethroids, (c) an insect net (mesh 0.8 mm, Crop Solutions Ltd, Perth, United Kingdom), (d) kaolin at the level of 37.5 g L$^{-1}$ with 0.01% surfactant and (e) a crude saponin extract diluted in water 1:5 (Tables 1 and 2). The concentrations of kaolin and crude saponin extract were selected based on the greenhouse experiments. In 2017 and 2018, the plant-derived, crude saponin extract was excluded from the further experiments, because it significantly increased the number of T. apicalis eggs compared to the other treatments. It was replaced by program 5 (the combination of insecticides and kaolin) to evaluate the suitability of kaolin in an IPM program. In 2018, the chemical control program consisted of pyrethroids, thiacloprid and spirotetramat. The control measures applied and the number of sprayings performed each year are listed in Tables 1 and 2.

In all the years, four replicates of each treatment were randomised in a row-column design (Figure S1), which took into account within-field variation in two directions (Williams et al., 2002). Randomization was performed using the CycDesigN-software (VSNI, Hemel Hempstead, England, United Kingdom). All treatments except the insect net, which covered the vegetation for approximately 2 months, were applied six times per season in 2016 and 2017, and 13 times in 2018 (Table 2)

according to weekly trap catches of carrot psyllids. The action threshold value for T. apicalis control in Finland is one psyllid per trap per week (Tiilikkala et al., 1996). During the flight peak the traps are recommended to be changed at least twice a week, since the feeding damage occurs very quickly—within 3 days—when the seedlings are small (Nissinen et al., 2007, 2012). The treatments were applied with a field plot sprayer (Schachtner PSGF 4.3, Schachtner Gerätetechnik GmbH, Ludwigsburg, Germany) using Agrotop airmix 10-025 nozzles (Kramp GmbH, Strullendorf, Germany) at pressure of 2.4 bar.

Six plants were sampled diagonally throughout each replicate after all the treatments were applied (in the latter half of July) to count the number of psyllid eggs and nymphs. The whole foliage of the plants was inspected under stereomicroscopes, since the psyllid eggs and nymphs from different phases of flight are found at different levels of the leaves, that is, the psyllid early flight descendants are found in the early true leaves and the late flight descendants are found in the more recently developed true leaves. Each year in September, 50 plants were harvested in the middle of each replicate. For each plant, the root and leaves were weighed. The leaves were assessed for psyllid feeding damage, discolouration symptoms, and other possible damage. Thereafter, the root top was pressed onto an FTA card to preserve DNA from the phloem sap for PCR analyses of the CLso.

## 2.6 | Sample preparation using FTA cards and PCR analyses

The carrot root top was cut off with a knife and the cut surface was pressed manually onto a sample area of a Whatman FTA™ MicroCard

(GE Healthcare, Buckinghamshire, United Kingdom) for 30 s. The FTA cards were dried for 4 hr at room temperature. Sample discs were taken from the site of the vascular bundle in the carrot root prints on FTA cards using a Whatman Uni-Core™ 2.00 mm punch (GE Healthcare) and placed into a 96-well plate (Thermo Fisher Scientific, United Kingdom). The cutting edge of the punch was cleaned between each sample by dipping it first into a 2% sodium hypochlorite (NaClO) solution, followed by dipping in absolute EtOH according to the instructions by GE Healthcare. The discs were washed once with 200 μL of an FTA purification reagent (GE Healthcare) followed by one wash with 200 μL of TE-buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA), after which the water was removed from the wells and the discs were used directly for PCR.

The carrot samples on FTA discs were tested for CLso by PCR with primers OA2 (GCGCTTATTTTTAATAGGAGCGGCA, Liefting et al., 2009) and Lsc2 (GCCTCACGACTTCGCAACCCAT, Haapalainen et al., 2017). The PCR reactions with the FTA sample discs (in 50 μL reaction volume) contained 500 nM of each primer, 0.2 nM of each dNTP, a Phire Hot Start II DNA Polymerase and the reaction buffer according to the instructions supplied by the manufacturer (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). The PCR program used was as follows: an initial denaturation step was conducted at 98°C for 3 min, then 35 amplification cycles were carried out with denaturation at 98°C for 10 s, followed by primer annealing at 68°C for 10 s and elongation at 72°C for 30 s, lastly followed by a final elongation step at 72°C for 5 min. The reactions were run on 96-well plates covered with a MicroAmp™ Optical Adhesive Film (Applied Biosystems by Thermo Fisher Scientific, Carlsbad, CA) in Biorad S1000 Thermal Cycler (Biorad, Hercules, CA). The size and quality of the PCR products were analysed by electrophoresis on a 1% agarose gel in a TBE buffer. Gels were stained with ethidium bromide and visualised under UV-transilluminator (SCIE-PLAS, Cambridge, United Kingdom).

## 2.7 | Statistical analyses

The settling of the psyllids on the leaves was analysed in no-choice assays using the SAS Generalised Mixed Model Procedure (Glimmix) with the following statistical model based on the row-column experimental design:

$$\text{logit}(y_{ijk}) = \mu + E_i + R_{j(i)} + C_{k(i)} + T_l$$

where $\mu$ is the intercept, $E_i$, $R_{j(i)}$, and $C_{k(i)}$ are the random effects of the experiment, the row within the experiment and the column within the experiment, respectively. $T_l$ represents the fixed effects of the lth treatment factor. Here $\text{logit}(y_{ijk})$ is the logit of the probabilities found for the settled and non-settled psyllids on the leaves after 24 hr (=$\log(p_{ijkl}/[1 - p_{ijkl}])$). The underlying distribution was assumed to be binomial.

The data from reproduction experiment was square root transformed before the statistical analysis because of the underlying skewed distribution of the numbers of the emerged females and males. Thereafter, a SAS Mixed Model Procedure and a two-way analysis of variance

model were used to test the difference between treatments and sexes, as well as the treatment-by-sex interaction.

In the field experiments, the leaf fresh weight, root fresh weight, number of leaves, percentage of damaged leaves, number of discoloured leaves, shoot:root ratio and CLso infection were analysed using the SAS Mixed Model Procedure or Generalised Mixed Model Procedure (CLso infection rate assumed to be binomial distributed) with the following statistical model based on the row column experimental design:

$$y_{ijk} = \mu + R_i + C_j + T_k + \varepsilon_{ijk}$$

where $\mu$ is the intercept, $R_i$ and $C_j$ are the random effects of the row and column, respectively. $T_k$ represents the fixed effects of the kth treatment factor and $\varepsilon_{ijk}$ is the normally distributed residual error. Before the analysis of the results, the root fresh weight values were square-rooted, while the leaf fresh weight values and the shoot to root ratios were transformed by taking their logarithm. Transformations were needed because the original variables were not normally distributed as the used model assumed. In 2018, all experimental units from one row of the design had to be excluded from the analyses due to exceptionally late germination as a consequence of drought.

Correlations between the variables in the field experiments were calculated using the SAS Corr Procedure. Because the relationships between variables were not linear, the Spearman's rank correlation coefficient was used.

## 3 | RESULTS

### 3.1 | No-choice assays

No-choice bioassays were conducted to assess the concentrations of kaolin and saponin to be used in the field experiments. With the plant-derived crude saponin extract (0.4% saponins as α-hederin), there were no clear differences ($p = .30$) between different concentrations in the settling of the psyllids on treated leaves. However, the difference between dilutions of 1:10 and 1:5 was marginally significant ($p = .10$) (Table S1). In kaolin treatments without a surfactant, there were no significant differences in the settling of the psyllids ($p = .67$) on treated leaves. For kaolin treatments with a surfactant, there were marginally significant difference in the settling of the psyllids ($p = .10$) on the treated leaves. However, the difference between concentrations of 37.5 and 50 g L$^{-1}$ of kaolin with 0.01% surfactant was significant ($p = .02$) (Table S1). Based on these results, 37.5 g L$^{-1}$ of kaolin with 0.01% surfactant and a crude saponin extract in a 1:5 dilution was selected for reproduction and field experiments.

### 3.2 | Reproduction experiment

The effect of kaolin and saponin on reproduction of psyllids was studied in cage trials. In the reproduction experiment, the mean numbers of emerged females and males were 71.2 and 69.9 in the control, 15.4

**TABLE 3** The weather conditions (rain fall, mm and temperature, °C) during the experiments in 2016–2018 and the corresponding long-term average

| | 2016 | 2017 | 2018 | Mean (30 years) rainfall | 2016 | 2017 | 2018 | Mean (30 years) temperature |
|---|---|---|---|---|---|---|---|---|
| May | 29.3 | 20.1 | 18.8 | 37.0 | 12.7 | 8.7 | 14.6 | 9.7 |
| June | 49.6 | 74.5 | 45.3 | 64.2 | 15 | 13.2 | 14.3 | 14.2 |
| July | 31.4 | 31.8 | 49.1 | 69.4 | 17.3 | 15.4 | 20.7 | 16.5 |
| August | 81.5 | 80.7 | 39.8 | 78.8 | 15.1 | 15.1 | 17.1 | 14.9 |
| September | 62.4 | 32.7 | 95.1 | 57.7 | 11.8 | 10.8 | 12.4 | 10.0 |

**TABLE 4** Mean number of *Trioza apicalis* nymphs (±SE), and eggs (±SE) per plant after the control programs have been executed in 2016–2018

| | 2016 | | 2017 | | 2018 | |
|---|---|---|---|---|---|---|
| Treatment | Eggs | Nymphs | Eggs | Nymphs | Eggs | Nymphs |
| Control | 104.7 ± 31.0 b | 34.9 ± 11.0 a | 65 ± 15 a | 18.3 ± 4.2 a | 40.3 ± 10.8 b | 113.6 ± 33.4 a |
| Chemical control program | 57.3 ± 19.6 b | 3.5 ± 2.8 b | 66 ± 16 a | 3.3 ± 1.8 b | 87.1 ± 16.3 a | 51.9 ± 22.6 b |
| Insect net | 0.7 ± 2.2 d | 0.0 ± 0.3 b | 0 ± 0 b | 0.0 ± 0.0 c | 0.7 ± 1.3 c | 0.5 ± 2.1 c |
| Kaolin | 9.8 ± 8.0 c | 2.4 ± 2.3 b | 16 ± 9 b | 2.6 ± 1.6 b | 6.9 ± 4.4 c | 15.4 ± 12.3 c |
| Saponin/IPM | 221.7 ± 38.6 a | 36.7 ± 9.1 a | 50 ± 15 a | 3.2 ± 1.8 b | 5.8 ± 4.0 c | 4.5 ± 6.6 c |

*Note:* Means followed by the same letter do not differ significantly (analysis of variance, $p \geq .05$)

and 11.9 in kaolin treatment (with surfactant), and 95.2 and 102.0 in saponin treatment, respectively (Table S2). Although the mean numbers of emerged adult psyllids were considerably lower in the kaolin treatment than in the control or saponin treatments, the main effects of the treatment and sex as well as the treatment-by-sex interaction effect were all non-significant due to large variations between the cages.

## 3.3 | Field experiments

In 2016–2018, field experiments were established on a commercial carrot farm to assess the efficacy of different control programs in *T. apicalis* control in the field conditions. Insect net, kaolin particle film and crude saponin extract represented the alternative control methods. The concentrations of kaolin and saponin used in the field experiments were determined in the pre-experiments in the greenhouse. In addition to the pyrethroids, thiacloprid and spirotetramat were added to the experimental programs in 2017, and 2018, respectively, because they were recently accepted to be used in psyllid control by Minor use permission. The variable weather conditions on each year (Table 3) affected both the carrot growth and psyllid flight which in turn were reflected to the timing and the number of sprayings conducted.

In 2016, there were significant differences between the treatments in the numbers of psyllid eggs ($p < .001$) and nymphs ($p < .001$) (Table 4) in the plants assessed after the treatments were done. The kaolin treatment significantly reduced the number of psyllid eggs, while the chemical control program did not differ, and the saponin treatment significantly increased the number off eggs compared to

the control. The insect net was superior for reducing the number of eggs as expected. In the chemical control treatment, the kaolin-treated plants or insect net-covered plants the number of nymphs significantly differed from the numbers of nymphs in the control and in the saponin treatment, but there were no significant differences between the numbers of psyllid nymphs in these three treatments. In other words, the reduction of psyllid nymphs by using the kaolin treatment was similar to the chemical control program and insect net. Significant differences between the treatments were also found in the root weight at harvest ($p = .02$) (Figure 1), the percentage of damaged leaves ($p < .001$) (Figure 1), and the number of discoloured leaves ($p < .001$, data not shown). The highest root weight was harvested under the insect net, followed by the chemical control program, kaolin treatment, control and saponin treatment. The leaf-curling percentage was highest in the saponin treatment and lowest under the insect net, and it negatively correlated with the root weight ($r = -0.71$). Furthermore, the shoot:root ratio significantly increased in treatments with a higher leaf-curling percentage ($p < .001$) (Table 5). The insect net was superior to other treatments to prevent CLso infections in the carrots. Only 13.6% of the net-covered plants were CLso infected, which significantly differed from the other treatments ($p < .001$). In the control, chemical control program, kaolin and saponin treatments the percentages of infected plants were 98.5, 92.4, 93.0, 99.7%, respectively. The CLso infection level was similar in the control and saponin treatments, but the levels significantly differed from the levels in the chemical control program ($P_c = 0.03$, $P_s = 0.01$) and in the kaolin treatments ($P_c = 0.04$, $P_s = 0.02$).

In 2017, the numbers of psyllid eggs ($p < .001$) and nymphs ($p < .01$) in the plants assessed after applying the control treatments significantly differed between the treatments (Table 4). Significantly
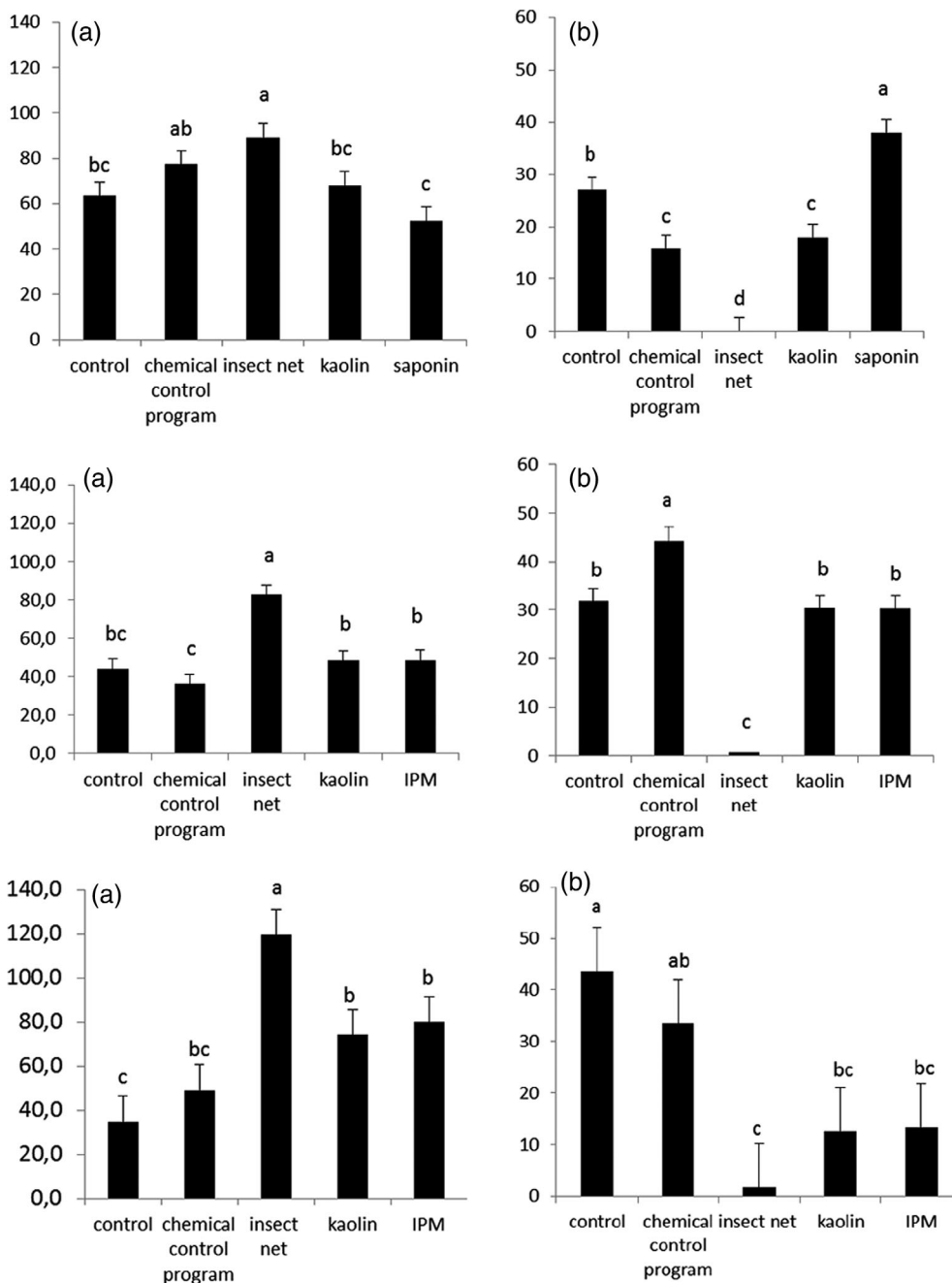
**FIGURE 1** Mean root weight (±SE) (a) and mean leaf damage percentage (±SE) (b) in different treatments in the field experiments in 2016 (up), in 2017 (middle) and in 2018 (down). Means followed by the same letter do not differ significantly (analysis of variance, $p \geq .05$)

**TABLE 5** Mean shoot:root ratio (±SE) in different treatments in 2016–2018

| | 2016 | 2017 | 2018 |
|---|---|---|---|
| Treatment | Estimate | Estimate | Estimate |
| Control | 0.27 ± 0.012 b | 0.34 ± 0.017 a | 1.18 ± 0.366 a |
| Chemical control program | 0.18 ± 0.008 d | 0.39 ± 0.020 a | 0.75 ± 0.231 ab |
| Insect net | 0.16 ± 0.007 d | 0.14 ± 0.007 c | 0.26 ± 0.070 c |
| Kaolin | 0.22 ± 0.010 c | 0.36 ± 0.018 a | 0.33 ± 0.101 bc |
| Saponin/IPM | 0.33 ± 0.014 a | 0.29 ± 0.015 b | 0.27 ± 0.082 c |

*Note:* Means followed by the same letter do not differ significantly (analysis of variance, $p \geq .05$)

less psyllid eggs were found under the insect net and in the kaolin treatment than in other treatments. The number of the psyllid nymphs was lowest under the net, whereas in the kaolin, chemical control program and IPM treatments it was at the same level, which significantly differed from both the insect net and from the water control. Significant differences between the treatments were also found in the

root weight at harvest ($p < .001$) and the percentage of damaged leaves ($p < .001$) (Figure 1). The highest root weight was harvested under the insect net, followed by the kaolin treatment and the IPM control program, which, however, were not significantly higher than the control treatment. Also in this experiment, the leaf-curling percentage negatively correlated with the root weight ($r = -0.66$) and the shoot:root ratio significantly increased in treatments with a higher leaf-curling percentage ($p < .001$) (Table 5). Only the insect net significantly reduced the CLso infection level (8.7%, $p < .001$) compared to the other treatments. The percentages of CLso infected plants were 95.0, 95.1, 95.0, 95.4% in the control, chemical control program, kaolin and IPM treatments, respectively.

In 2018, the number of psyllids eggs ($p < .001$) and nymphs ($p < .001$) was significantly different between the treatments (Table 4). In the control, a significantly higher number of nymphs were observed than in the chemical control program ($p = .01$) and in the other treatments ($p < .001$). Again, the carrots protected by the insect net produced the highest root weight. The root weight in the kaolin and IPM treatments was significantly lower ($p = .03$, $p = .05$) than under insect net, but higher than in the control ($p < .001$, $p < .01$) (Figure 1). However, the difference was non-significant compared to the root weight in the chemical control program, which was low despite of the intensive spraying program. As in the previous years, the leaf-curling percentage negatively correlated with the root weight ($r = -0.59$) and the shoot:root ratio significantly increased with a higher leaf-curling percentage ($p = .02$) (Table 5). Again, the insect net significantly reduced the CLso infection rate (4.0%, $p < .001$) compared to the other treatments. The percentages of CLso infected plants were 99.5, 95.6, 71.7, 63.4% in the control, chemical control program, kaolin and IPM treatments, respectively. The CLso infection level in the kaolin and IPM treatments was significantly lower than in the control ($p = .04$, $p = .03$), whereas the CLso infection level in the chemical control program did not differ from that in the control.

# 4 | DISCUSSION

## 4.1 | Psyllid feeding

The results clearly show that the higher the leaf-curling percentage the lower the root yield is. This is also supported by the increasing shoot:root ratio in treatments with higher leaf-curling percentages, indicating biomass allocation to the leaves as a consequence of the psyllid feeding damage. In the case of *T. apicalis*, the adults cause the most severe damage (Markkula et al., 1976) and the damage occurs very quickly (Markkula et al., 1976; Nissinen et al., 2007, 2012, 2014). Further, Nissinen et al. (2014) showed statistically that the leaf-curling was affected by psyllid feeding, but not by the CLso bacterial titre in the plants. In this study, the leaf-curling percentages exceeding 10–15% led to root yield declines, which is in line with an earlier greenhouse study by Nissinen et al. (2007: figures 1 and 2). In the case of *T. apicalis*, the

psyllid feeding seems to be more destructive to the root yield than the CLso infection.

## 4.2 | Chemical control program

During summer of 2016, which was quite normal in terms of weather, the chemical control program was the most effective for the control of carrot psyllid damages after the insect net. The spraying program consisted of pyrethroids, but the efficacy was still quite good, that is, reduction of psyllid nymphs was at the same level as under insect net and kaolin treatments. This result does not support the previous suspicion of pyrethroid resistance in *T. apicalis* (Meadow, 2010). However, in the following years the success of chemical control programs turned out to be highly dependent on the environmental conditions. Still, the reduction of psyllid nymphs was significant compared to the control, but the leaf curling percentage was high—in 2017 significantly higher than in the control—indicating failure of controlling the adult psyllids. In the case of *T. apicalis*, the efficacy should be reached immediately after application to prevent the feeding damage caused by the adults. In case the nymphs are the most damaging phase of the psyllid life cycle, for example, *B. cockerelli* (Munyaneza, Crosslin, & Upton, 2007), an active ingredient such as spirotetramat, which reaches the highest efficacy within 14–23 days after application, is still effective (Brück et al., 2009).

In the presence of climatic stress factors—either cold and wet or hot and dry—the root weight reached under the chemical control programs did not significantly differ from the control. The summer of 2017 in Finland was one of the coldest in 30 years, the temperatures during May–July were approximately one degree lower than the long-term mean (Table 3). As a consequence, the carrots grew very slowly and remained at the vulnerable stages for carrot psyllid feeding (from cotyledon to the 4-leaf stage Nissinen et al., 2007, 2012) longer than they usually do. At the same time, the carrot psyllid flight lasted longer than in the previous summer and numerous sudden rain showers especially in June complicated the timing of the treatment applications. On the other hand, the summer in 2018 was very dry in western Finland; such a low rainfall occurs less frequently than once in 30 years (Finnish Meteorological Institute, 2019). The drought retarded the carrot germination and growth. Simultaneously, the psyllid flight started early, but the flight peaked late. In 2018, the carrot psyllids' flight peaked on 11 July when trap catches reached 147 specimens per trap. Normally, psyllid flight is over or finishing by that time, but in 2018 flight still continued for 15 days. In the greenhouse, the development of a new generation of *T. apicalis* takes 7 weeks (A. Nissinen personal observation), but in the field the peak was reached 6 weeks after the psyllid flight started. This suggests that the high temperatures, especially in July, accelerated the development of the nymphs and the flight of the new generation adults started while the old generation was still on flight. The carrots were still exceptionally small at the time of the flight peak, and therefore several extra sprayings were required. Also, the late flight peak complicated the timing of the applications of the different active ingredients. For

example, the poor efficacy of spirotetramat against adult psyllids is known (Brück et al., 2009). Therefore, spirotetramat application should be timed just after the flight peak to match the highest efficacy to the developing nymphal stages, but the timing of these sprayings was non-optimal in 2018. The lack of carrot psyllid flight model further complicates the timing the sprayings because the flight peak cannot be predicted yet.

The plants were subjected to the stress of the gall-forming process, which redirects key metabolic products (Raman, 2011), simultaneously to climatic stress factors. The increasing shoot:root ratio in treatments with higher leaf-curling percentages indicated biomass allocation to the leaves as a consequence of the psyllid feeding damage, which was even pronounced under the drought stress in 2018. On the other hand, drought stress has been shown to increase the fraction of root biomass in relation to the leaf and stem biomass (Eziz et al., 2017). Our results suggest that psyllid damage can overrule the resource allocation pattern caused by drought stress. Furthermore, insecticide treatments may also increase the plant stress. Ruckert (2017) observed that a neonicotinoid exacerbated the effect of water-stress on maize. Previously, resistance of *T. apicalis* against pyrethroids has been suspected (Meadow, 2010), however, the effect of climatic conditions on the success of control measures has not been addressed. Our results show that the combination of different stressors can be destructive to the carrot seedlings despite of control measures as observed by the poor root weights both in 2017 and in 2018.

## 4.3 | Crude saponin extract

A deterrent effect of saponins on *T. apicalis* was not observed. In the cage experiment, the two psyllid females produced more descendants on saponin-treated plants than in the other treatments. The number of emerged adults in cages was not significantly higher, but still 39.7% higher than in the control. In line with this, the number of eggs was significantly higher than in the control in the field experiment in 2016. Thus, rather than having a deterrent effect on *T. apicalis*, the crude saponin extract seemed to stimulate the egg-laying or alternatively attract more psyllids to the plants. Indeed, there was also a higher leaf damage percentage in the saponin treatment than in the control treatment, which may indicate the attractive effect of saponins. Previously, saponins have been shown to have deterrent effects or negative effects on herbivore performance on several arthropods including aphids (Christensen et al., 2018; De Geyter et al., 2012; Gołaawska, 2007), which have a similar mode of feeding to psyllids. In De Geyter et al. (2012), a toxic effect against pea aphids was obtained by adding saponins to an artificial diet. *T. apicalis* feeds from the phloem and thereby it may avoid the toxic effect of saponins sprayed on carrot leaves, however, this does not explain the lack of deterrent effects, which have been observed in aphids.

Previously, Nissinen, Ibrahim, Kainulainen, Tiilikkala, and Holopainen (2005) showed that limonene sprayed on carrot leaves increased *T. apicalis* oviposition marginally significantly within 24 hr in

one of the two cultivars studied, and it also induced a concentration of (Z)-β-ocimene in the leaves of both cultivars. To our knowledge, there are no studies about the effect of exogenous saponin treatments on plant volatile emissions. Besides saponins, the crude extract also contained various flavonoid sugar conjugates and ecdysteroids based on an UHPLC-QTOF MS analysis (data not shown). Quinoa saponins consist of a complex mixture of sugar conjugates of serjanic, oleanolic, phytolaccagenic and 3β,23,30-trihydroxy-olean-12-en-28-oic acids as well as hederagenin, and their ability to serve as phytoalexins on carrots are unknown.

## 4.4 | Kaolin

Kaolin in turn, significantly reduced the number of *T. apicalis* eggs and nymphs on the plants compared to the control in all years. The effect of the kaolin particle film against several psyllid species has also been shown in previous studies. Peng et al. (2011) showed that kaolin reduced the number of *B. cockerelli* eggs and adults on tomatoes both in laboratory and field experiments. Sheibani, Shojaii, Shojaaddini, Imani, and Hassani (2016) observed that kaolin significantly reduced the oviposition of the common pistachio psyllid, *Agonoscena pistaciae*. Similarly, kaolin treatment both delayed egg-laying of overwintered *Cacopsylla pyri* females and suppressed oviposition significantly compared to water control (Erler & Cetin, 2007). Further, Daniel, Pfammatter, Kehrli, and Wyss (2005) showed that the population density of *C. pyri* remained lower in a kaolin-treated area than in an IPM-treated plot. In northern Italy, kaolin showed a very good efficacy against *C. pyri* in comparison to a mineral oil and untreated control (Pasqualini, Civolani, & Corelli Grappadelli, 2002). Pasqualini et al. (2002) observed that kaolin treatment prevented host plant acceptance by the psyllids and hindered the anchoring of the eggs into the leaf tissue. The mechanism of kaolin is probably similar against *T. apicalis* since the number of eggs was lower as well as the number of curled leaves, which suggests that fewer psyllids landed or started to feed on the carrot leaves. In addition, kaolin was among the pesticides that consistently appeared to be most compatible with *Tamarixia radiata*, which is a parasitoid of the Asian citrus psyllid (Hall & Nguyen, 2010). In 2017 and 2018, the shoot:root ratio in the IPM control programs was nearest to the normal ratio reached under the insect net. In 2018, allocation of resources to leaves (shoot:root ratio) in the chemical control program was at the similar level as in the control treatment indicating that the chemical control program was not able to prevent the psyllid-damage driven resource allocation to leaves under the drought. This may suggest that kaolin treatment reduced the climatic stresses while chemical control measures in the IPM program further reduced the psyllid feeding damage. On the other hand, the shoot:root ratio in the chemical control program does not differ from the one reached under the insect net in 2016. In other words, chemical control program seems to prevent the psyllid-damage driven resource allocation to leaves under normal weather conditions but not under the drought. Previously, kaolin treatment has

been shown to improve the water potential, and increase the stomatal conductance, net $CO_2$ assimilation rate, intrinsic water use efficiency as well as indole-3-acetic acid for grapevines under summer stress (Dinis et al., 2018). The compatibility with natural enemies and ability to mitigate climatic stresses further highlights the suitability of kaolin for IPM control strategies.

## 4.5 | CLso infection

In all years, insect net was superior to other treatments to prevent the CLso infection in carrots. This is quite expected since CLso is a phloem limited bacterium transmitted by *T. apicalis* (Nissinen et al., 2014). The net effectively prevents the psyllid feeding as seen in the low leaf-curling percentages in the insect net protected plots. Some CLso positive plants, however, were also found in the insect net covered plots. This suggests that a low number of psyllids still in flight after the removal of the net, were capable of transmitting the bacterium onto the carrots. Total prevention of CLso transmission would require insect net cover for even longer than 2 months. This, in turn, might advance weed growth and increase humidity, which may enhance the development of other plant diseases.

In the 2018 experiment, kaolin and IPM treatments significantly reduced the level of CLso infection in the carrots. However, 63–72% is still a high infection rate and it is not known whether this reduction of the infection rate would affect the epidemiology of CLso in the long run. The control program was very intensive in 2018, due to high number of psyllids counted in the traps. The leaf-curling percentage in the treatments consisting of kaolin sprays was lower, which indicates that less psyllid feeding occurred. The intensive spraying program together with very little rain fall in 2018, may also have facilitated the persistence of kaolin on the carrot leaves and enhanced the feeding deterrent effect of the kaolin.

## 4.6 | Insect net

The use of insect nets is an effective control method (this study; Meadow, 2010), but it is not considered an economically sustainable method (Munyaneza, Sengoda, Sundheim, & Meadow, 2014). Lefebvre, Langrell, and Gomez-y-Paloma (2015) considered that one constraint in the adoption of IPM is that costs have been calculated for single strategic parts of IPM toolbox and the calculations do not take into account the potential variability in the results according to crops and agro-climatic conditions. This study shows that climatic conditions significantly affect the efficacy of the control measures. In severe drought, the carrot root weight was 33–59% higher under the insect net than in the other control treatments. In addition to the yield increase, the use of an insect net helps to avoid the quality losses due to psyllid feeding and the associated CLso infection (Nissinen et al., 2012; Seljåsen et al., 2013). Furthermore, an insect net also prevents damage by *Lygus* bugs as well as from carrot rust fly larvae (*Psila rosae*) which also require control measures in the open field. Ensuring a harvest with marketable yields, insect nets could pay off the purchasing cost in 1 year, if the comparative scenario is that no marketable yield would be obtained using other control measures under climatic stressors, for example, drought.

## 5 | CONCLUSIONS

In all the years, insect nets were superior to the other treatments to prevent psyllid feeding damage as well as CLso infection in carrots. Insect nets are an effective control method, but the high purchasing and labour costs will limit their use in wider areas. In addition, the use of nets ensures the yield production under extreme weather conditions, which may pay off the investment within a short time. However, insect nets are not suitable in areas where moose and deer live; their movement through the fields will easily destroy a new net. Therefore, other control measures in addition to insect nets are still needed. Kaolin treatment effectively reduced psyllid reproduction: both the numbers of eggs and nymphs. This in turn may be affected by lower numbers of adult psyllids landing on the leaves because the leaf damage percent was also reduced in kaolin treatments. In addition, kaolin was effective as a part of the IPM control programs where half of the chemical treatments were substituted with kaolin suggesting that kaolin was able to mitigate climatic stresses. This is very important knowledge as the number of active ingredients registered against *T. apicalis* will further decline in 2020 when several pyrethroids will leave the market, and extreme weather conditions are predicted to increase as a consequence of the climate change.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.