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RESEARCH ARTICLE

Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows

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Abstract

The efficacy and the instant effect of 13 insecticides and antifeedants towards *Cacopsylla pruni*, the vector of '*Candidatus* Phytoplasma prunorum' were examined in cage studies (no choice experiments with 10 field-collected overwintered adults per experiment) on potted apricot trees (budding trees under outdoor conditions in early spring and foliated seedlings kept at 21°C). Cypermethrin caused 100% insect mortality within 2–4 h, thiacloprid 90–100% mortality within 24 h both on foliated and on budding trees. On budding trees spinosad led to 70–90% mortality within 24 h, thixotropic white trunk paint to 90% mortality within 48 h. On foliated seedlings flonicamid gave 70–100% mortality within 1 day, abamectin, spinosad, acetamiprid and spirotetramat 70–100% within 72 h. Field studies monitoring the effects of thiacloprid on remigrants of *C. pruni* by yellow sticky traps were carried out in two apricot orchards. Additionally the influence of the insecticide on insect dispersal was examined by mark, release and recapture trials. As compared to the control thiacloprid significantly reduced the catches of naturally occurring and released insects, decreased the number of trees on which released insects were recaptured (by 25–100%) and shortened the migration distances of the released insects by more than half. Our results suggest that appropriate insecticide treatments both reduce *C. pruni* populations and have a direct effect on pathogen transmission. Application of Cypermethrin before bloom and thiacloprid after bloom seem best suited to achieve these objectives. Thixotropic white trunk paint could, a formulation for spray application provided, eventually be a sustainable alternative or complement for treatments before bloom.

Introduction

European Stone Fruit Yellows (ESFY) caused by '*Candidatus* Phytoplasma prunorum' is currently present in all European and Mediterranean regions where apricots are cultivated (Marcone *et al.*, 2010). The phytoplasma primarily infects plants of the genus *Prunus*, inducing severe damage in susceptible crops, such as apricots (*Prunus armeniaca*) and Japanese plums (*Prunus salicina*). ESFY is easily disseminated via propagation material (Marcone *et al.*, 2010; Riedle-Bauer *et al.*, 2012) and transmitted by the plum psyllid *Cacopsylla pruni* (Scopoli) (Carraro *et al.*, 1998). *C. pruni* is univoltine and migrates to *Prunus* spp. at the end of winter/in early spring. On *Prunus* spp. the insects lay eggs which develop through five immature stages into adults. The new generation adults abandon their *Prunus* hosts and spend the rest of the year on their overwintering shelter plants (conifers) (Ossiannilsson 1992; Jarausch & Jarausch, 2016).

C. pruni transmits the phytoplasma in a persistent manner. Minimum acquisition periods of 2–4 days, minimum latent periods of 2–3 weeks and minimum inoculation access periods (IAP) of 1–2 days were observed (Carraro *et al.*, 2001). Most new generation adults developing on infected plants complete their minimum latent period only after movement to their overwintering shelter plants. When they remigrate to their *Prunus* hosts in early spring they efficiently transmit the pathogen. In comparison pathogen spread by the springtime generation is regarded as negligible (Carraro *et al.*, 2001, 2004; Thébaud *et al.*, 2009).

Up to now management of *C. pruni* is not well studied, efficiency of insecticides both on the transmitting insect and on pathogen spread still have to be evaluated (Marcone *et al.*, 2010; Jarausch & Torres, 2014). The principal goal of insecticide application in orchards is to keep vector populations as low as possible and in consequence to minimise the pathogen spread. Remigrating *C. pruni*, however, move into the orchards from untreated host plants outside. Therefore the first tree(s) in an orchard reached by an infectious individual can only be protected by insecticides that not only reduce vector populations but also directly influence pathogen transmission. The crucial point in this context is the minimum IAP of 1–2 days. Insecticides with a 'knock down' or instant effect acting in less than 1 day would prevent successful inoculation of healthy trees. Rapid chemical-induced alterations of the vector feeding behaviour could also influence pathogen transmission as described for plant viruses (Perring *et al.*, 1999). In Austria overwintered *C. pruni* are present in apricot orchards from the beginning of March until the middle of May (Maier *et al.*, 2013). So far, however, the effects of insecticides sprayed at very early developmental stages of the trees and cool temperatures in spring are not well established. In addition the fact that the steadily remigrating adults can reinfest plants soon after application makes products with long lasting residues desirable.

Actual effects of insecticides on disease rates are difficult to ascertain in the field. Such experiments require a considerable amount of time to complete, as several years can elapse between the phytoplasma infection of a tree and a positive laboratory test (Riedle-Bauer *et al.*, 2012). For the same reason the true infection status of a future test orchard is difficult to evaluate. Initially undetected infections may in the end lead to false results in insecticide experiments. *C. pruni* management requires a strategy for the entire insect flight period which subsequently can be tested for its effect on disease rates. The effect of a single insecticide measure on the disease rate is hardly discernable.

Previous field experiments in Italy aiming to control ESFY by insecticides (chlorpyrifos, etofenprox, azadirachtin, malathion, phosalone, rotenone) showed inconclusive results. Impacts of treatments in April and May on disease rates were only detected in the minority of tests (Poggi Pollini *et al.*, 2007). In contrast, successful use of insecticides was reported for the management of apple proliferation phytoplasma ('*Candidatus* Phytoplasma mali'). Apple proliferation is transmitted by *Cacopsylla picta* and *Cacopsylla melanoneura*, two psyllid species hibernating on conifers and migrating to the orchards in late winter or spring (Tedeschi & Alma, 2004; Jarausch *et al.*, 2011; Jarausch & Jarausch, 2014). Semi-field experiments proved the effect of chlorpyrifos and etofenprox towards *C. melanoneura* (Baldessari *et al.*, 2010). In the field, insecticides (various active ingredients, up to three applications before, during and after flowering of the apple trees) significantly reduced the disease rate in the province of Bolzano (Italy) (Österreicher & Unterthurner, 2015).

The aim of the present study was to evaluate various insecticides with different modes of action for their effects on *C. pruni.* Extent and speed of insecticide action were examined in cage trials involving different developmental stages of test trees, different external conditions and insecticide residues of different ages. In field experiments the effects of thiacloprid were studied in two apricot orchards. Observations of insecticide effects towards *C. pruni* remigrants in treated and untreated plots were complemented by mark, release and recapture trials.

Materials and methods

Cage studies

The effect of various insecticides and putative antifeedants was studied in no choice tests on potted apricot trees. Concentrations and manufacturers of the products evaluated in this study are indicated in Table 1. Test concentrations were calculated on basis of the amounts of formulated ingredients per hectare and the spray volume recommended by the manufactures. Out of the tested compounds only one 'classical insecticide', the neonicotinoid thiacloprid is currently registered for plum sucker treatment in Austria (AGES, 2015). In addition the compound kaolin is allowed as plant strengthening agent. All other compounds are either currently registered or have recently been registered for other pests of apricots, for other fruit crops, or are intended for other purposes in fruit production. The test plants were sprayed to run off with the tested products using a 2 L hand sprayer. Treatments were applied starting at the uppermost leaves and working towards the base of the plant, ensuring that the entire surface was sprayed. Plants were allowed to air dry before the placement of insects for approximately 2 h, in case of aged residue tests for the specified

duration. Control plants received water treatment only. All experiments were carried out with *C. pruni* remigrants. Insects were field collected by beating tray method on *Prunus spinosa* in March and April immediately before (1–2 h) release on the test trees.

The experiments included test plants in different developmental stages and different temperature conditions. For the first group of experiments aiming to simulate orchard conditions before flowering one-year-old apricot seedlings obtained from a nursery (size approximately 50 cm) were potted and cultivated until BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) stage 03–09 (end of bud swelling/development of green tips, the BBCH scale has been used according to Meier *et al.*, 1994). The experiments were conducted in March and at the beginning of April under outdoor conditions, but protected from rain. Ten insects per experiment were introduced into transparent cylindrical cages (diameter 3 cm, length 10 cm) attached to the individual plants. Insect mortality was assessed daily for 4–5 days.

For the second group aiming to simulate apricot trees with developing leaves after flowering at already warmer outdoor conditions apricot seeds were harvested in an apricot orchard (cv. 'Klosterneuburger Marille'), grown in our lab and cultivated for approximately 3 months until they had 8–10 fully expanded leaves. Single plants were entirely covered with transparent cylindrical cages (diameter 9 cm, height 25 cm). Ten insects were released into each cage. Insect survival was recorded daily for 6 days. Before and during the insecticide experiments the test plants were maintained in a growth chamber at 21°C.

The effect of residue age on insecticide activity was studied on test trees in BBCH stage 10 (BBCH scale: Meier *et al.*, 1994; the already mentioned potted, one year old apricot seedlings were allowed to develop leaves). After insecticide treatment the test trees were kept under outdoor conditions unprotected from rain for 2, 7 and 14 days before the beginning of the experiments. Cage experiments were carried out as described above and numbers of surviving insects were determined after 1 day.

The instant or 'knock down' effects of thiacloprid and cypermethrin were also investigated. Test plants and conditions were the same as described above (one-year-old apricot seedlings at BBCH stage 03–09 under outdoor conditions and foliated 3 months old seedlings at 21°C), but insect survival was recorded at more frequent intervals (starting 30–60 min. after insect release). The assessment of instant effects included fresh (2 h old) residues of thiacloprid and cypermethrin and also 4 days old residues of thiacloprid.

All tests were repeated at least four times (four independent experiments each including one test tree and 10 insects caged on it – in total 40 insects and four trees per compound/developmental stage of the tree/age of the residue).

Field studies

The field experiments were carried out in two apricot orchards in Lower Austria. On basis of our cage results and due to the fact that thiacloprid is registered for plum sucker treatment in Austria this compound was selected.

Description of the experimental orchards and spraying conditions:

1. Krems: The orchard comprises five standard tree rows with 23 approximately 15 years old trees per row, within row tree spacing ranges from 4 to 5 m, while between row distance is 6 m. Random sampling of trees and PCR assays (2012) revealed a phytoplasma infection rate of 31.25% (Maier *et al.*, 2013). Application of insecticide: Mitterer trailer sprayer (1000 L series) (Mitterer, Terlan, Italy), cross flow blower equipped with 14 Albuz ATR hollow cone spray nozzles, spray pressure 10 bar, driving speed 5 km h^{-1}, volume of spray 500 L ha^{-1}, 96.2 g of thiacloprid (active ingredient) ha^{-1} in water, no spreader or other addition. Each trial covered 10 trees in 5 rows. In 2013 one control and one insecticide trial were performed successively (control 18–25.4, stain green, thiacloprid: 25.4–2.5, stain pink), in 2014 simultaneously (7.4–14.4, control plot stain pink, thiacloprid plot stain white).
2. Klosterneuburg: The orchard includes nine rows with 34 trees per row, within row spacing is 2 m, the distance between rows is 4 m, the spindle-shaped trees are approximately 15 years old. The overall phytoplasma infection rate is not known, presence of the pathogen on five symptomatic trees was confirmed by PCR assays only (unpublished). Application of insecticide: Wanner DA 32 axial cross flow blower (Wanner, Wengen im Allgäu, Germany) equipped with 16 Albuz ATR hollow cone spray nozzles, spray pressure 9 bar, driving speed 4.5 km h^{-1}, volume of spray 500 L ha^{-1}, 96.2 g of thiacloprid (active ingredient) ha^{-1} in water, no spreader or other addition. Each experiment comprised 15 trees in nine rows. In 2013, one control and one insecticide trial were performed successively (control 23–29.4, stain pink, thiacloprid: 29.4–6.5, stain green), in 2014 simultaneously (7.4–17.4, control plot stain pink, thiacloprid plot stain green).

The insecticide was applied to the entire thiacloprid plots. Control plots remained untreated (no water spraying).

Table 1 Compounds tested in this study

Product Name	Active Ingredient/Mode of Action[a]	Amount of Active Ingredient in Formulated Product/ Tested Concentration (formulated product in water)	Distributor
Calypso	Thiacloprid/nerve action, nicotinic acetyl-choline receptor competitive modulator	480.8 g L^{-1}/0.02% (v/v)	Bayer Crop Science, Monheim, Germany
CutiSan	Kaolin (aluminium silicate)/repellent or deterrent effect of particle film	95% kaolin/5% (w/v)	Biohelp, Vienna, Austria
Cymbigon	Cypermethrin/nerve action, sodium channel modulator	100 g L^{-1}/0.02% (v/v)	Kwizda Agro, Vienna, Austria
Knoblauch Power	Garlic extract/repellent or deterrent effect?	0.5% (v/v)	Schacht, Braunschweig, Germany
Movento 100 SC	Spirotetramat/lipid synthesis, inhibitor of acetyl CoA carboxylase	100 g L^{-1}/0.22% (v/v)	Bayer Crop Science, Monheim, Germany
Mospilan 20SC	Acetamiprid/nerve action, nicotinic acetylcholine receptor competitive modulator	200 g kg^{-1}/0.05% (w/v)	Kwizda Agro, Vienna, Austria
NeemAzal TS	Azadirachtin A/uncertain action	3–4% azadirachtin/0.3% (v/v)	Biohelp, Vienna, Austria
Spin Tor	Spinosad/nerve action, nicotinic acetylcholine receptor allosteric modulator	480 g L^{-1}/0.016% (v/v)	Kwizda Agro, Vienna, Austria
Promanal	Mineral oil (paraffin oil)/repellent or deterrent effect of residual film	546 g L^{-1}/2% (v/v)	Biohelp, Vienna, Austria
Teppeki	Flonicamid/nerve action, chordotonal organ modulator	500 g kg^{-1}/0.021% (w/v)	Belchim, Burgdorf, Germany
Vertimec	Abamectin/nerve and muscle action, glutamate-gated chloride channel allosteric modulator	18 g L^{-1}/0.075% (v/v)	Syngenta, Dielsdorf, Germany
Baumwachs-Sprühverband	Natural waxes and resins intended for treatment of pruning wounds/repellent or deterrent effect of residual film?	Undiluted	Schacht, Braunschweig, Germany
Weißanstrich	Thixotropic white paint to prevent frost damage of trunks/ repellent or deterrent effect of particle film?	5% (w/v)	Schacht, Braunschweig, Germany

[a]Buteler & Stadler (2011), Puterka *et al.* (2005), Insecticide Resistance Action Committee (2016).

The survey on naturally occurring *C. pruni* in the experimental plots was enlarged by mark, release and recapture experiments. Remigrant *C. pruni* were collected on *P. spinosa* by beating tray method. Marking of insects with fluorescent pigments was performed as previously described (Nakata 2008; Maier *et al.*, 2013). The following stains were used: Pink (Karmin Tagesleuchtpigment, Artmaxx, Berlin, Germany), white and green (UV Elements, Germany, http://www.uv-elements.de/tagesleuchtpigment-p-37.html). Effects of the pigment on insect survival and stability of the stains under outdoor conditions were examined as previously described (Maier *et al.*, 2013). Insect release was carried out approximately 4 h after insecticide application (dry spray deposit). Collection of insects, staining and release happened in no more than 6 h. In each experiment 600 stained *C. pruni* were freed on one single tree (in case of thiacloprid plots an insecticide treated tree) in the middle of the experimental plot. At the start of each experiment the insects were caged on the branches of the release tree in transparent plastic tubes with gaze houses on both ends for approximately 90 min. (Maier *et al.*, 2013). This procedure allowed us to check insect survival during and after transfer to the field and ensured that the insects settled on the release tree and started to feed.

Both naturally occurring and artificially released specimens in the orchards were monitored by yellow sticky traps for 1–2 weeks (63–74 traps per experiment, size of traps 15 cm × 21 cm). The traps were fixed in the middle of the trees at 1.5–2 m height and remained in the orchard for the duration of the experiments (8–14 days). During the experiments the traps were examined with free eye in regular intervals. Every single *C. pruni* detected on the traps (without discrimination between stained or unstained individuals) was immediately labelled by a self-adhesive sticker (diameter 3 mm glued onto the traps right beside each individual, different colours of stickers according to days after begin of the experiment). At the end of the trials the traps were transferred to the laboratory and inspected under a binocular microscope. All *C. pruni* were recorded and assigned to the capture period (days after begin of the experiment) by aid of the coloured stickers. Marked and unmarked insects were differentiated by means of UV light (standard UV transilluminator Vilbert Lormat, Marne la Valee, France).

Statistical analyses

All statistical analyses were performed by aid of the statistics program SPSS 22.0 (SPSS, Chicago, IL, USA).

Cage studies

Insect survival rates in the experiments were displayed as boxplots. The boxes represent the interquartile (IQ) range which contains the middle 50% of the records and show the median. Outliers (values between 1.5 and three times the IQ range) are identified with an O. Extreme values (more than three times the IQ range) are marked with a ♦.

The normality of the variables was evaluated by the Kolmogorov-Smirnov test. The values of insect survival were not normally distributed, even after transformation. Therefore, data were analysed by the nonparametric Kruskal Wallis test. For this test the sum of the ranks for each group and then the test statistic H was computed. H was compared to the critical value of a chi square distribution. When significant, post hoc analyses by pairwise comparisons (Mann–Whitney test for each pair of groups using a Bonferroni multiple comparison procedure) were performed.

Field experiments

For analysis of insect presence in the thiacloprid and control plots numbers of individuals on each sticky trap were compared. Each experiment (Krems and Klosterneuburg, 2013 and 2014) was analysed individually. Data were not normally distributed, thus comparisons were carried out by aid of Mann Whitney *U*-test (on the basis of the ranks for each group the test statistic *U* is computed and compared to critical values).

The numbers of trees visited by the marked specimen in the mark, release and recapture experiments were also compared by Mann Whitney *U*-test. For the analysis data from all experiments (Krems and Klosterneuburg, 2013 and 2014) were pulled together.

Results

Cage experiments on budding seedlings (BBCH stage 03–09) under outdoor conditions

On budding test trees kept at outdoor conditions application of cypermethrin resulted in 100% insect mortality, application of spinosad and thiacloprid in 70–90% mortality within 24 h. Spraying of white trunk paint caused around 50% mortality within 24 h and 90% mortality within 2 days. On plants sprayed with flonicamid, kaolin, paraffin oil or wax 30–40% of the insects were still alive after 4 days. Comparisons of insect survival 1, 2 and 4 days after release on the test plants revealed significant differences between treatments (Kruskal Wallis test, $P \leq 0.001$, d.f. = 9, $H_{day1} = 38.3$; $H_{day2} = 41.78$; $H_{day4} = 35.4$). Pairwise comparisons of survival after 1 day validated a significant effect ($P \leq 0.05$) of cypermethrin, thiacloprid and spinosad as compared to the untreated control. A significant effect of white trunk paint on insect mortality was verified after 2 days ($P \leq 0.05$). Applications of flonicamid, paraffin oil, kaolin and wax did not have any statistically validated effect on insect survival in comparison to the control (Fig. 1).

Cage experiments on foliated, 3 months old apricot seedlings kept at 21°C

Cypermethrin sprayed on foliated test plants caused 100% insect mortality within 24 h. Applications of thiacloprid led to 90–100% mortality, applications of flonicamid to 70–100% mortality within 1 day. Insect mortality on plants sprayed with abamectin and spinosad varied considerably after the first 24 h, but mortality rates rose to 70–100% within 72 h. Insect mortality for applications of acetamiprid and spirotetramat was only 10–40% after 24 h, rising steadily to approximately 80% after 3 days and to 100% by 6 days (Fig. 2). Statistical analysis of insect survival 1, 3 and 6 days after insect release proved significant differences between treatments (Kruskal Wallis test, $P \leq 0.001$, d.f. = 9, $H_{day1} = 40{,}9$; $H_{day3} = 29{,}8$; $H_{day6} = 40{,}4$). Pairwise comparisons of insect survival validated a significant effect ($P \leq 0.05$) of cypermethrin, thiacloprid and flonicamid after 1 day as compared to the untreated control. Three days after release insect mortality on plants sprayed with abamectin and spinosad varied significantly from the control treatment ($P \leq 0.05$). A statistically significant effect of acetamiprid and spirotetramat in comparison to the water control was verified after 6 days only ($P \leq 0.05$). Application of garlic extract and azadirachtin showed no statistically significant effect on insect survival.

Cage experiments with aged insecticide residues

Residues of thiacloprid and cypermethrin on trees in BBCH stage 10 remained effective for more than 1 week. Two and 7 days old residues of both compounds gave insect mortality rates of up to 100% within 24 h. Effects of both compounds in comparison to the water treated control were statistically validated (Kruskal Wallis analysis $P \leq 0.001$, d.f. = 6; H = 35.5; pairwise comparisons $P \leq 0.05$). After 14 days cypermethrin still induced insect mortality rates between 80% and 100%, thiacloprid rates between 50% and 80%. At this point of time, however, with our test procedure insect survival on treated plants and control plants was not statistically discernible any more (Fig. 3).

Cage studies on instant effects

Cypermethrin sprayed on seedlings with fully developed leaves kept at 21°C caused 100% insect mortality within

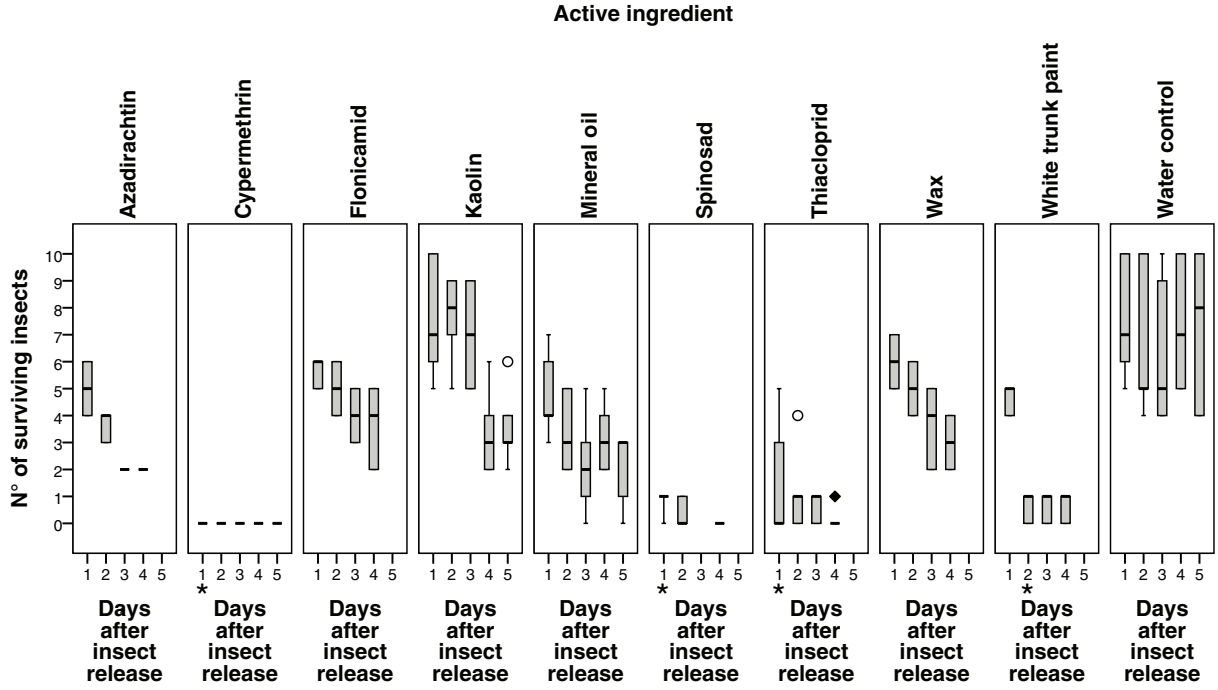

Figure 1 Effects of test compounds towards survival of *C. pruni* remigrant adults on budding apricots seedlings (BBCH stage 03–09) kept under outdoor conditions (March and April). Boxplots are showing the median, box boundaries mark the 25th and 75th percentiles of each distribution. Outliers (values between 1.5 and three times the interquartile range) are identified with an O. Extreme values (more than three times the interquartile range) are marked with a ♦. Kruskal Wallis test followed by pairwise comparisons was carried out for insect survival data on a day by day basis. * Statistically significant difference in comparison to the water treated control ($p \leq 0.05$) from the indicated day onwards.

2 h. Fresh (2 h old) thiacloprid killed 80–90% of the insects within 4 h. On test plants with 4 days old thiacloprid residues more than half of the insects were still alive after 4 h (Fig. 4). Cypermethrin acted faster than thiacloprid. Already after 30 min. significantly fewer insects were alive on cypermethrin trees than on trees with 2 h old thiacloprid residues (Mann Whitney $U = 4.03$, $P \leq 0.001$). After 60 min insect mortality on cypermethrin trees varied significantly from mortality on trees with 4 day old thiacloprid residues (Kruskal Wallis analysis $P \leq 0.001$, d.f. = 6; H = 35.5; pairwise comparisons $P \leq 0.05$).

On test trees in BBCH stage 03–09 under outdoor conditions cypermethrin killed all insects within 4 h. Fresh (2 h old) thiacloprid residues caused 90–100% mortality after 24 h, 4 days old thiacloprid residues 50–90% mortality within 24 h (Fig. 4). Our statistical test procedure, however, did not indicate any significant difference between the treatments.

Effects of thiacloprid under field conditions

In all test replications (Krems and Klosterneuburg, 2013 and 2014) application of thiacloprid sharply reduced the numbers of unmarked, naturally occurring *C. pruni* on the yellow sticky traps in comparison to the untreated control plots. This effect, however, was not noticed within the first 24 h after spraying (Fig. 5 and Appendix S1). Accordingly, statistically significant differences between the treatments within the first 24 h were not detected in any of the replications. After 72 h numbers on individuals on the traps were significantly lower in treated plots Krems 2014: Mann Whitney $U = 3.15$, $P \leq 0.001$; Klosterneuburg 2013: Mann Whitney $U = 2.03$, $P \leq 0.001$; Klosterneuburg 2014: Mann Whitney $U = 4.03$, $P \leq 0.001$), except in Krems 2013, where no statistically significant insecticide effect was verified. Seventy-two hours to 8 days after spraying the insecticide significantly reduced the number of individuals per trap in all test replications, Krems 2013: Mann Whitney $U = 2.47$, $P \leq 0.001$; Krems 2014: Mann Whitney $U = 3.15$, $P \leq 0.001$; Klosterneuburg 2013: Mann Whitney $U = 2.77$ $P \leq 0.001$; Klosterneuburg 2014: Mann Whitney $U = 3.80$, $P \leq 0.001$). In Klosterneuburg 2014 a significant insecticide effect was still verified 8–14 days after spraying (Mann Whitney $U = 4.01$, $P \leq 0.001$) (Fig. 5 and Appendix S1).

Mark, release and recapture experiments

The applied dyes were stable under outdoor conditions. After exposure of insects (dead, on yellow sticky traps) to

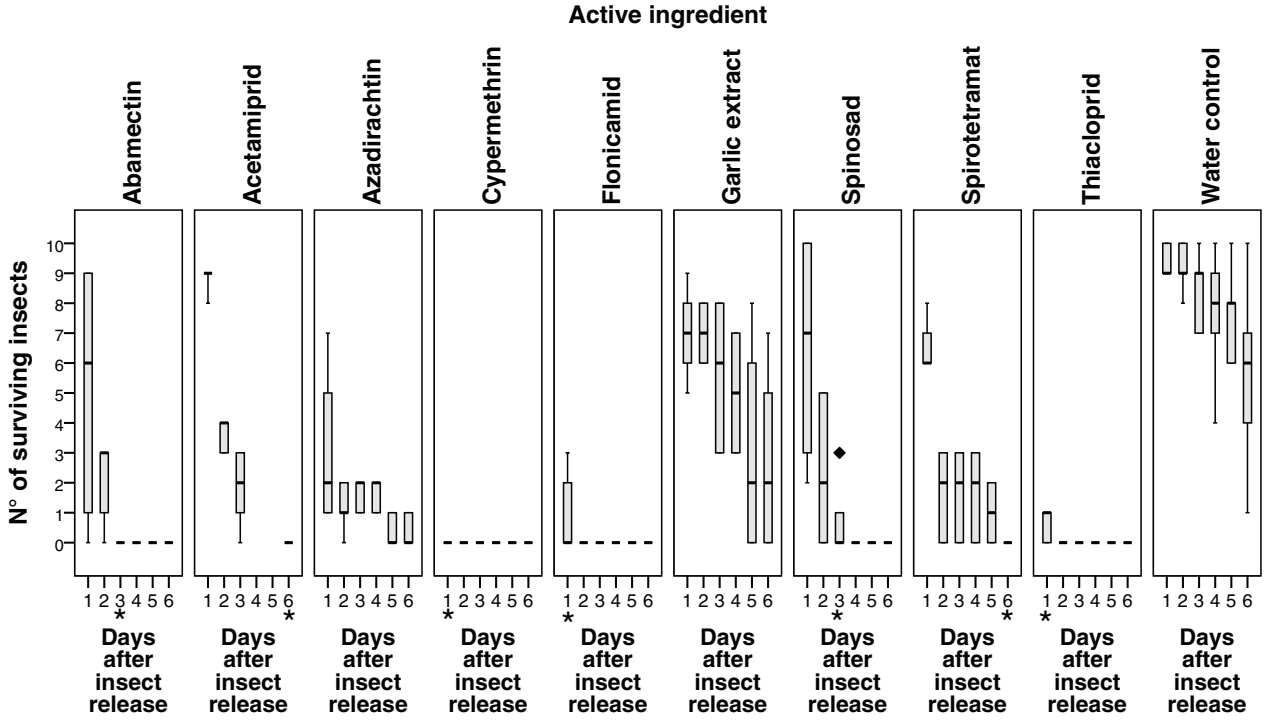

Figure 2 Evaluation of insecticide effects towards *C. pruni* remigrant adults on foliated apricot seedlings kept at 21°C in a plant growth chamber. Boxplots are showing the median, box boundaries mark the 25th and 75th percentiles of each distribution. Extreme values (more than 3 times the interquartile range) are marked with an ♦. Kruskal Wallis test followed by pairwise comparisons was carried out for insect survival data on a day by day basis. * Statistically significant difference in comparison to the water treated control ($P \leq 0.05$) from the indicated day onwards.

outdoor conditions for 5 weeks the marked insects were clearly discernible from unmarked specimens by means of UV light. Adverse effects of the stains on insect survival were not observed. Survival rates of stained insects 1, 3 and 7 days after beginning of the experiments were comparable to unstained insects (Kruskal Wallis analysis: d.f. = 3, day 1: $P = 0.85$, H = 0.79; day 3: $P = 0.63$, H = 1.72; day 7: $P = 0.86$, H = 0.734) (Appendix S2).

Application of thiacloprid reduced the number of trees on which marked insects were recaptured by 25–100% in comparison to the untreated control (Fig. 6, Appendices S3–S6). Pulling data from all experiments (Krems and Klosterneuburg, 2013 and 2014) together this reduction was statistically significant (Mann Whitney $U = 15.5$, $P = 0.029$).

In untreated control plots between 2.6% and 7.5% of the released specimens were recaptured, in insecticide plots between 0% and 1.8% (Fig. 6, Appendices S3–S6). At the end of the experiments (after 8 and 14 days, respectively) a significant influence of thiacloprid on the overall number of marked and recaptured specimens per trap was ascertained in Klosterneuburg in both experimental years (2013: Mann Whitney $U = 2.52$, $P \leq 0.001$; 2014: Mann Whitney $U = 3.29$, $P \leq 0.001$) and in Krems in 2014 (Mann Whitney $U = 2.81$, $P = 0.033$). Within 24 h of insect release, however, recaptures in treated and control plots were not statistically different in any of the trials.

In all untreated control experiments marked specimen migrated to the farthest traps and even beyond (into the neighbouring insecticide plot) within 24 h (in Krems 2014 maximum distance around 40 m, in all other experiments around 20 m). In the thiacloprid plots insects were predominantly recaptured in the vicinity of the release tree (maximum distance covered around 12 m in Krems 2014, in all other experiments maximum distance around 8 m) (Fig.6, Appendices S3–S6).

Discussion

The first objective of *C. pruni* management in apricot orchards is to keep populations as low as possible and in consequence to minimise the potential for pathogen spread. Our data indicate that especially cypermethrin and thiacloprid cause high insect mortality and will therefore contribute to achieve this goal. In addition our results show that both products effectively control *C. pruni* in orchards for more than 1 week. In course of our

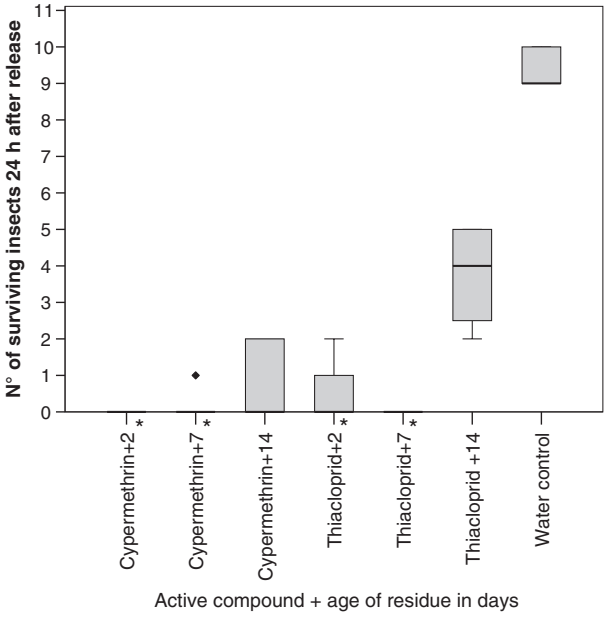

Figure 3 Effects of aged cypermethrin and thiacloprid residues on survival of *C. pruni* adults 24 h after insect release. The experiments were carried out on one-year-old apricots seedlings at BBCH stage 10. Extreme values (more than three times the interquartile range) are marked with an ◆. Boxplots are showing the median, box boundaries mark the 25th and 75th percentiles of each distribution. * Difference significant in comparison to the water treated control at $P \leq 0.05$.

experiments on budding trees we observed interesting effects of Weissanstrich (white trunk paint) on insect mortality. In principle this product is used to avoid frost damage of stems during winter. We included it in our tests because presumably it produces a stable particle film. Previous experiments have demonstrated that particle films reduced settling and oviposition of adult *C. pyricola* and that the insects had difficulty grasping particle film-treated leaves (Puterka *et al.*, 2005). In addition our study indicated that application of spinosad before and after bloom and flonicamid, abamectin, acetamiprid and spirotetramat after bloom could decrease vector populations and thus also lower the risk of pathogen spread.

The benefit of the tested insecticides in terms of actually preventing pathogen inoculation, however, requires a differentiated assessment. Insecticides protecting trees from becoming infected must disrupt the insect feeding behaviours in a shorter time than the minimum IAP. In line with expectations our experiments proved a rapid knock down effect of the pyrethroid cypermethrin in a considerably shorter period of time (2–4 h) than the reported minimum IAP. These results indicate that cypermethrin effectively prevents phytoplasma inoculation before and after blossom. Comparable to our results high knock down rates exceeding 90% within 1 day were observed for the pyrethroid etofenprox towards overwintered stages of the psyllid *C. melanoneura* (Baldessari *et al.*, 2010).

High mortality rates within 24 h were also assessed for thiacloprid on foliated seedlings indicating that fresh spraying deposits of thiacloprid provide at least some direct protection against infections of trees after bloom. On budding trees in contrast, the compound was less effective. In all experiments ageing of thiacloprid residues for 4 days seemed to delay the insecticide effect although differences between the two thiacloprid variants were not significant. Thus the ability of thiacloprid to actually prevent pathogen inoculation in case of dormant trees, cold temperature and aged residues appears probable but it is not completely clear. However, literature data point out that neonicotinoids could quickly change the feeding behaviour of insects. The neonicotinoid imidacloprid disrupted the feeding behaviour of the Asian citrus psyllid (*Diaphorina citri*), the vector of '*Candidatus* Liberibacter spp.', and the authors concluded that the compound greatly reduced the likelihood of pathogen transmission (Serikawa *et al.*, 2010, 2012). Imidacloprid also significantly prevented the transmission of chrysanthemum yellows phytoplasma by the leafhopper *Macrosteles quadripunctulatus* (Saracco *et al.*, 2008). Wheat aphids (*Sitobion avenae*) exposed to sublethal doses of thiacloprid performed longer no-probing phases and shorter phloem-sap-ingestion phases (Miao *et al.*, 2014). In Italy thiacloprid is successfully implemented in strategies against apple proliferation (Österreicher & Unterthurner, 2015).

In the present study a significant insecticide action within 24 h was also ascertained for flonicamid on foliated trees. Flonicamid acts systemically against hemipterous pests. In case of aphids this compound inhibited the feeding behaviour within 0.5 h of treatment and this antifeeding activity was not recoverable until death (Morita *et al.*, 2007). Provided an equivalent effect on *C. pruni* flonicamid could reduce phytoplasma transmission. Our experiments also proved a rapid action of spinosad on budding trees in BBCH stage 03–09. Spinosad, however, has the disadvantage of quick degradation (Belien *et al.*, 2013).

Several of the insecticides included in the current study partly or predominantly act via systemic translocation in the plant and ingestion by herbivorous insects. Their effect might start after some days only. Thus our test set up with insect release right after insecticide application could have led to an underestimation of their knock down effect. For example on foliated trees effects of spirotetramat and acetamiprid were only observed after some days. An adapted test procedure including some days between insecticide application and insect release might be additionally required to judge their speed of action.

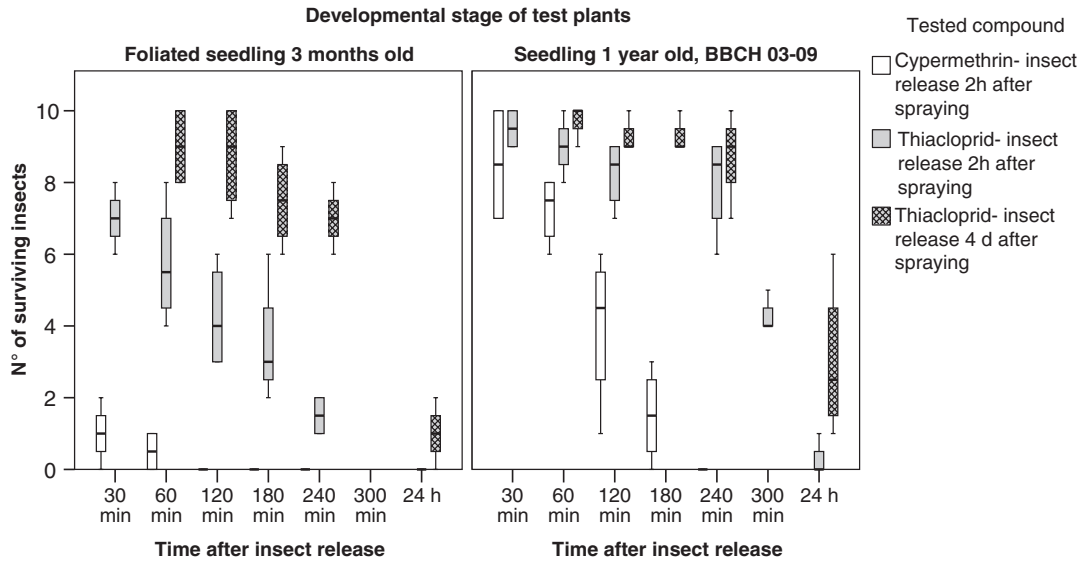

Figure 4 Evaluation of instant effects (knock down effects) of cypermethrin, thiacloprid and 4 days old thiacloprid residues on *C. pruni* adults. The experiments were carried out on foliated (left) and budding seedlings (BBCH stage 03–09) (right). Boxplots are showing the median, box boundaries mark the 25th and 75th percentiles of each distribution.

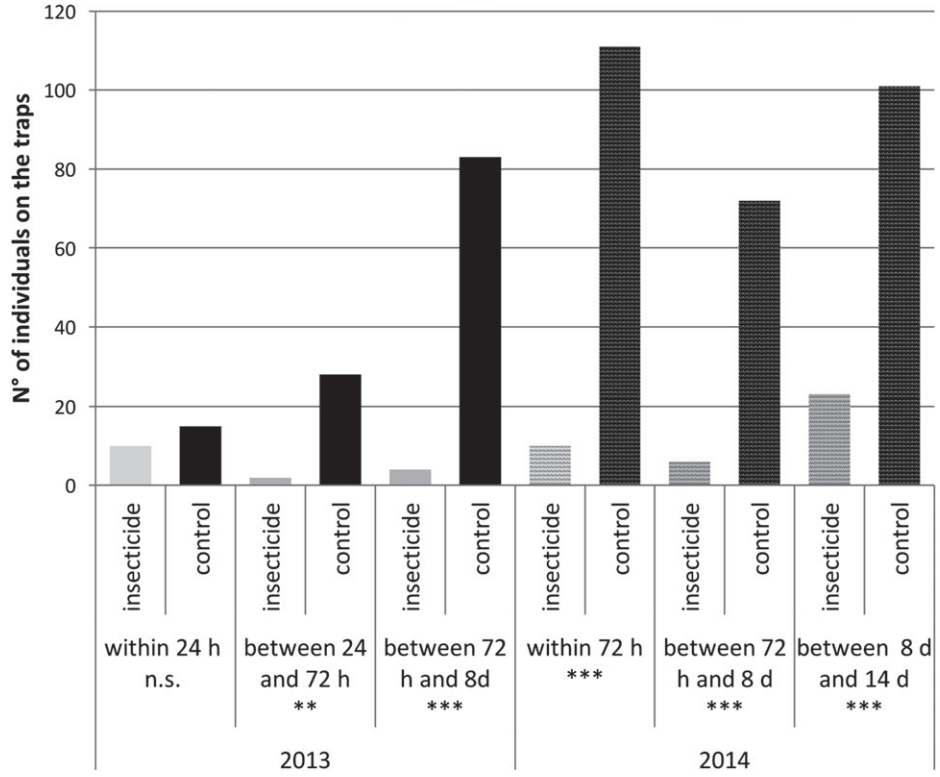

Figure 5 Total numbers of *C. pruni* (unmarked, naturally present) captured on yellow sticky traps in untreated and thiacloprid treated plots in the orchard Klosterneuburg. Insect numbers were compared by Mann Whitney *U* test. n.s., difference not significant at $P \leq 0.05$; **, difference significant at $P \leq 0.01$; ***, difference significant at $P \leq 0.001$. Dates of experiments: 2013: Untreated control: 23.4–29.4, thiacloprid experiment: 29.4–6.5; 2014: Both variants 4.4–17.4.

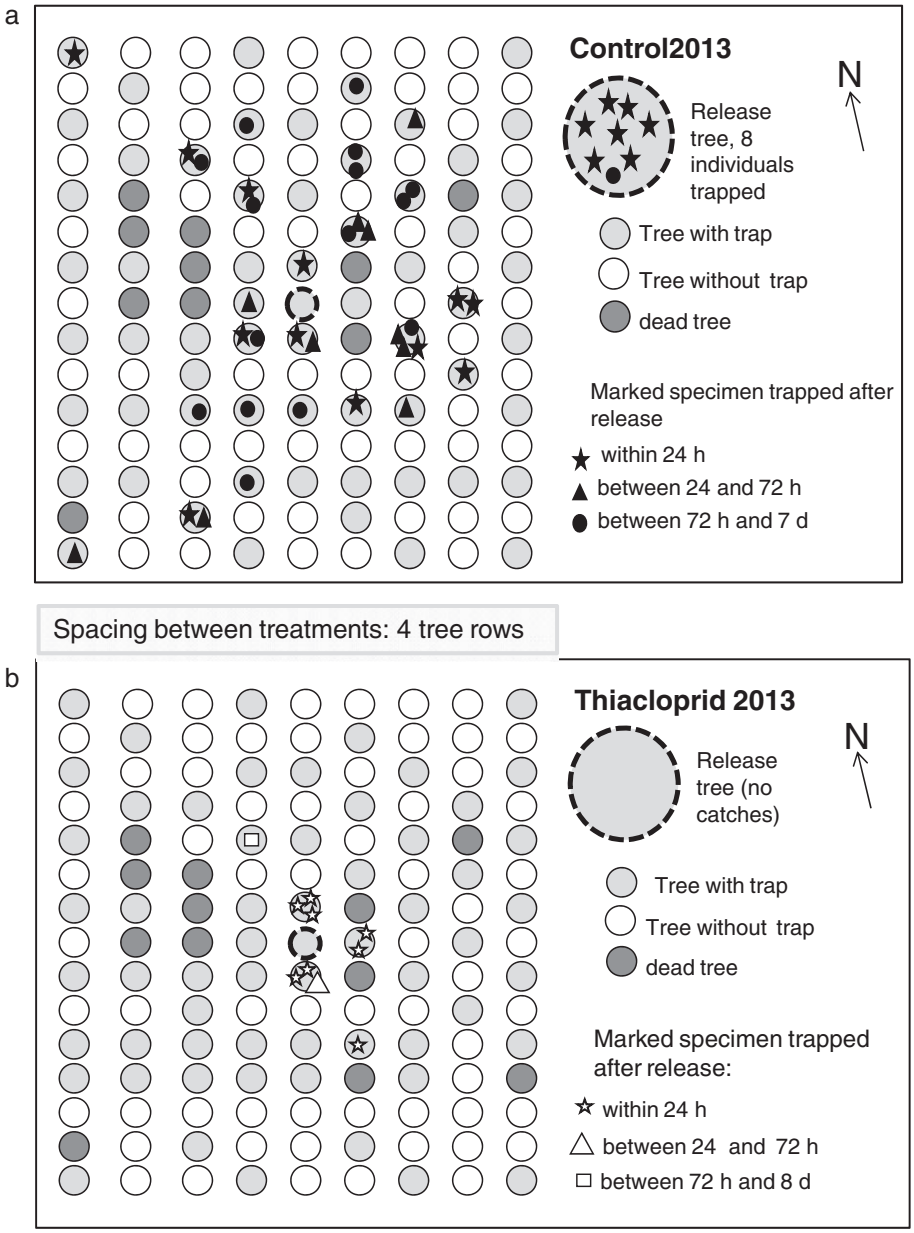

Figure 6 Field experiment in Klosterneuburg 2013: The size and shape of the control plot (a) and the thiacloprid plot (b) and the number and position of the yellow sticky traps used for insect monitoring are presented. In addition the position of the release tree in the mark-, release- and recapture experiment and the recaptures of marked insects in both plots are illustrated. The control experiment was carried out from 23.4 to 29.4, the thiacloprid experiment from 29.4 to 6.5.

The data of our cage studies indicate that some insecticides, for example cypermethrin and thiacloprid provide at the least some direct protection against infections of trees. For unequivocal conclusions, however, the actual effects of these and all other insecticidal compounds must additionally be assessed by transmission experiments.

At the beginning of the experiments we feared that insecticide effects on potted seedlings might, to some degree, differ from field tests, for example due to altered application efficiency or plant age and size. As it turned out, however, our field experiments yielded largely the same results in terms of effectiveness, speed of action and durability of thiacloprid. In addition the mark, release and recapture experiments enabled us to track the influence of thiacloprid on spatial dispersal of *C. pruni*. In treated plots significantly fewer marked insects were recaptured on significantly fewer trees. The distances covered by the insects were reduced by more than half.

These observations suggest that thiacloprid also lowers the risk of pathogen spread by influencing the spatial movement of the (incoming) vectors. The congruence between cage and field results allows the conclusion that cage studies are a valuable tool for pre selection of insecticides.

On basis of our data an application of thiacloprid right after flowering can be recommended for ESFY vector management. Recent studies suggest that the agent adversely influences the navigation memory of honey bees (Fischer *et al.*, 2014), thus an application shortly before or during flowering could give rise to unwanted bee problems. Our present results indicate a very good effect of cypermethrin both on trees before and after bloom. Pyrethroids, however, are non-selective, target also beneficial insects and have adverse effects on honeybees (Civolani, 2012; Palmquist *et al.*, 2012; Belien *et al.*, 2013). An authorisation provided an application of cypermethrin for phytoplasma vector management seems most reasonable on budding trees at the beginning of insect remigration. Also in South Tyrol (Italy) a pyrethroid (etofenprox) is recommended for management of *Caco-psylla picta* at the beginning of insect remigration (Österreicher & Unterthurner, 2015). Weissanstrich (white trunk paint) could be an environmentally friendly alternative or complement to 'classical insecticides' in orchards at early developmental stages. Before use in practice, however, an adapted formulation of the active ingredients and extensive field tests would be required. Beyond the current considerations intensified research efforts are necessary to test additional insecticides, antifeedants and repellents for their potential to be included in sustainable management concepts.

All in all the present study permits an assessment of the tested insecticides with regard to their effect on vector populations and their direct effect on pathogen inoculation. The current and similar experiments are suitable as a basis for management concepts. In the end, however, it must be borne in mind that the effect of any *C. pruni* management strategy on the disease rates must be proven by long standing field observations.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.

Appendix S2. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.

Appendix S3. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.

Appendix S4. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.

Appendix S5. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.

Appendix S6. Cage and field experiments as basis for the development of control strategies against *Cacopsylla pruni*, the vector of European Stone Fruit Yellows.