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European Stone Fruit Yellows: A Mark, Release and Recapture Experiment Tracking the Dispersal of its Vector *Cacopsylla pruni* (Hemiptera: Psyllidae) in a Model Apricot Orchard and Epidemiological Studies in Lower Austria

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Abstract

During the last 15 years, European stone fruit yellows (ESFY) has become a major concern in Austrian fruit production. Therefore, presence and temporal dynamics of its vector *Cacopsylla pruni* were investigated using a beating tray method and yellow sticky traps on *Prunus armeniaca, Prunus domestica, Prunus spinosa* and *P. cerasifera nigra*. Infection rates of *C. pruni* and *Prunus* spp. trees were assessed by direct, nested and real-time PCR. Movement of remigrants in a model apricot orchard was tracked by aid of a mark, release and recapture study. Insects were marked by fluorescent dyes. Movement of the marked insects and presence of naturally occurring insects were monitored by yellow sticky traps. In 2011, remigration of *C. pruni* to *Prunus* spp. started in calendar week 10 (8th of March) and in 2012, in calendar week 12 (18th of March). Remigrants were observed until calendar week 20 (middle of May), significant numbers of the springtime generation adults were present until week 26 (end of June). The phytoplasma was ascertained in 0–11.5% of the remigrants and in 0–3.44% of the springtime generation insects. About 9.8–63.3% of the apricot samples, 20–40% of the plum samples and single blackthorn samples were infected. The mark, release and recapture study proved a fast and frequent tree-to-tree movement of remigrated *C. pruni* adults. Insects easily covered distances from row to row or even farther (ca. 13 m) within 24 h after release and were present in a large part of the model orchard after 8 days (up to 24 m from release point).

Introduction

European stone fruit yellows (ESFY) caused by '*Candidatus* Phytoplasma prunorum' is widespread throughout Europe except the North and most parts of Great Britain. Outside Europe, it is known to be present in large parts of Turkey (COST action FA 0807 2013). The phytoplasma primarily infects plants of the genus *Prunus*, causing severe damage particularly in apricots (*Prunus armeniaca*) and Japanese plums (*Prunus salicina*). In apricots, the disease is characterized by small-sized, conically rolled, chlorotic leaves, premature fruit fall, small tasteless fruits, decreased vitality of infected trees and eventually death of single branches or entire trees (Morvan 1977; Marcone et al. 2010).

Based on phylogenetic analyses of the 16 S rDNA, '*Ca*. P. prunorum' is placed in the apple proliferation

(16SrX) cluster together with other important phytoplasma diseases of fruit trees such as apple proliferation and pear decline (Seemüller and Schneider 2004).

European stone fruit yellows is transmitted by the plum psyllid *Cacopsylla pruni* (Scopoli) (Carraro et al. 1998). *Cacopsylla pruni* is univoltine, strictly oligophagous on *Prunus*, overwinters on conifers and remigrates to *Prunus* in early spring. On *Prunus,* the insects lay eggs and develop through five larval stages into adults which abandon their *Prunus* hosts in summer (Ossiannilsson 1992; Thébaud et al. 2009). Studies suggest that remigrated *C. pruni* returning to apricot orchards in spring are the most efficient disease vectors. Secondary spread within an orchard during a vegetation period seems to be marginal (Carraro et al. 2004; Thébaud et al. 2009; Poggi Pollini et al. 2010). Wild *Prunus* species such as *P. domestica* and *P. spinosa* are both frequently tolerant hosts of the phytoplasma and suitable hosts for *C. pruni*. These species could therefore play a significant role as sources of infectious vectors (Carraro et al. 2002). Proposed management approaches include the use of healthy propagation material, the removal of rootstock suckers, uprooting of infected trees and application of insecticides against both remigrants and springtime generation. Clearing strategies and insecticide treatments have to be designed according to the regional situation. In areas where the disease is present epidemically on wild *Prunus,* hosts uprooting of infected trees and control of immature vectors in orchards are likely to be insufficient or even inefficient (Thébaud et al. 2009). Insecticides against *C. pruni* are not authorized in many countries, and efficiency of insecticides both on the transmitting insect and on disease spread has yet to be evaluated (Marcone et al. 2010).

During the last 15 years, the importance of ESFY in Austria has significantly increased (Richter 1999; Laimer Da Câmara Machado et al. 2001; Riedle-Bauer et al. 2012). Currently, severe disease outbreaks occur in the Wachau, one of the most important apricot growing areas of Austria, located on both sides of the Danube river in Lower Austria. The deep and narrow river valley is characterized by apricot orchards in the plain near the river and forests consisting of deciduous trees and conifers at higher altitude. The apricots produced there are designed as 'Wachauer Qualitätsmarille', a product with protected designation of origin (PDO, Commission of the European Community Regulation EC 1107/96). Fruits are produced mainly of the local cultivar 'Klosterneuburger Marille' (a clone of 'Hungarian Best').

Due to the frequent cultivation of the highly susceptible cv. 'Klosterneuburger Marille', small-scale structured apricot orchards and the close vicinity of conifers as putative overwintering hosts for the vector *C. pruni* disease incidence are high. The development of a well-adapted pest management is an urgent need. Therefore, presence and temporal dynamics of *C. pruni* on apricots, plums, blackthorns and cherry plums were investigated. ESFY infection rates of *C. pruni* and their *Prunus* host plants were also determined. To observe extent and speed of vector movement and to estimate the size of the natural vector population in a model apricot orchard a mark, release and recapture study with remigrants was carried out.

Material and Methods

Insect and plant sampling

Location and description of the experimental sites included in the study are illustrated in Table 1. Experimental sites 1–7 are located in the Wachau area. For comparison purposes, two sites (N° 8 and 9) situated apart from commercial apricot growing areas were also included in this study.

Adult *C. pruni* were captured from end of February until beginning of July 2011 and 2012 on *Prunus armeniaca, P. domestica*, *P. spinosa and P. cerasifera nigra.* In 2011, captures were made at test sites 1, 3, 5, 6, 7, 8 and 9, and in 2012, presence of insects was surveyed at sites 2, 8 and 9. Remigrants were collected once or twice a week, and springtime generation adults were collected every week or every 2 weeks using a beating tray method with a white plastic tray (30 × 40 cm). Two branches per tree were chosen for insect collection and sampled by ten hits, respectively. Numbers of sampled branches per tree were kept to this low level to prevent unreliable data due to escape of insects. The number of probed trees per experimental site ranged from 2 to 5 for *P. spinosa*, 2 for *P. domestica* and 5 to 13 for *P. armeniaca*. In the case of *P. cerasifera nigra,* only one tree was present at the experimental site. Trapped psyllids were identified (according to Ossiannilsson 1992; Burckhardt and Jarausch 2007), counted and frozen at −18°C. Additionally, yellow sticky traps (Rebell® amarillo, Andermatt Biocontrol AG, Grossdietwil, Switzerland) were used in apricot orchards to record the return of the remigrated *C. pruni* in early spring and to proof their presence in orchards with low population densities. In each orchard, 4 yellow sticky traps were mounted and changed weekly.

Leaf samples were taken in late summer 2011 and 2012 to analyse the presence of the phytoplasma at all

Table 1 Description of test sites included in the study

Test site	Location	Number of *Prunus armeniaca* trees	Tree age in years	Rootstock/cultivar	Other *Prunus* species
1	Rohrendorf	~1420	~10	'Brompton'/'Klosterneuburger Marille'	*P. spinosa* hedgerow
2	Krems	~100	5–10	'Brompton'/'Klosterneuburger Marille'	*P. domestica* and *P. cerasifera nigra* in hedgerow
3	Krustetten	~140	8–60	'*P. domestica* ssp. *insititia*'/'Klosterneuburger Marille' or unknown/unknown	25 *P. domestica* trees in orchard
4	Baumgarten	~40	6	–	*P. spinosa* hedgerow
5	Rührsdorf	28	2–60	'Brompton'/'Klosterneuburger Marille' (or unknown/unknown)	–
6	Aggsbach-Dorf	~850	8–10	'Brompton'/'Klosterneuburger Marille'	Single *P. spinosa* plants in vicinity
7	Aggsbach-Markt	33	8–10	'Brompton'/'Klosterneuburger Marille'	–
8	Langenzersdorf	0	–	–	Special area of conservation according to European Union's Habitats Directive (92/43/EEC), *P. spinosa* widespread
9	Hagenbrunn	0	–	–	Vineyard with vast *P. spinosa* hedgerows, distance to site 8: 3 km

test sites in all four *Prunus* species. At this time of the year, symptom bearing trees were present in all apricot orchards included in this study. Observed symptoms were rolling and yellowing of leaves, reduced vigour and death of entire trees or single branches. No symptoms were observed on *P. domestica*, *P. cerasifera nigra* and *P. spinosa*.

To obtain an overview of infection rates, sampled trees were randomly selected independently whether they showed symptoms or not. Numbers of sampled trees per orchard are presented in Table 2.

Table 2 Infections of *Prunus* plants with '*Ca.* P. prunorum'

Test site	No. of tested trees/No. of positive trees			
	Prunus armeniaca	*Prunus domestica*	*Prunus spinosa*	*Prunus cerasifera nigra*
1	41/4	–	8/1	–
2	16/5	5/2	1/0	1/0
3	11/7	5/1	–	–
4	40/15	–	4/2	–
5	6/1	–	–	–
6	23/2	–	2/0	–
7	6/2	–	–	–
8	–	–	5/0	–
9	–	–	5/0	–

Detection of '*Ca.* P. prunorum'

DNA extraction from plant and insect samples was performed as described by Maixner et al. (1995). Each plant or insect was processed individually.

Insect and plant DNA (except *P. spinosa* DNA) were amplified in a direct PCR procedure using the primer pair f01/r01 (Lorenz et al. 1995). A 20 μl of reaction volume contained 1–5 μl template preparation, 0.5 μM of each primer, 200 μM of each dNTP, 0.5U Taq DNA polymerase (Qiagen, Erlangen, Germany) and 1× reaction buffer (Qiagen). PCR was performed in an Eppendorf Mastercycler (Hamburg, Germany). The reaction mixtures were subjected to 40 cycles with 45 s denaturation at 94°C, 45 s annealing at 45°C and 60 s extension at 72°C. All samples yielding the expected PCR fragment were analysed by restriction fragment length polymorphism (RFLP) to differentiate '*Ca.* P. prunorum' from other 16SrX phytoplasmas and to exclude unspecific PCR products. 10 μl of amplicon was digested with 5U of RsaI (Promega, Madison, WI, USA) at 37°C for 4 h (Seemüller and Schneider 2004). PCR and RFLP products were stained with Midori Green (Nippon Genetics Europe, Dueren, Germany), separated on a 2% agarose gel and visualized under UV light. *P. spinosa* samples were analysed by nested PCR in 2011 and by qualitative real-time PCR in 2012. For nested PCR, the primers P1/P7 (Deng and Hiruki 1991; Smart et al. 1996) and f01/r01 were used. Incubation parameters were identical as described for direct PCRs except for the annealing temperature in the first amplification which was 50°C. For qualitative real-time PCR, the Sensifast NoRox kit (Bioline, London, UK) based on SYBR Green technology was used. Reaction preparations comprised 0.5 μl of template DNA, 0.2 μM of primers ESFYf and ESFYr (Yvon et al. 2009), 10 μl 2× Sensifast mix in a volume of 20 μl.

Real-time PCR was carried out in a Rotor Gene 2.0 (Corbett, Mortlake, Australia) thermocycler with a denaturation step at 94°C for 3 min and 40 PCR cycles with 5 s denaturation at 94°C, 10 s annealing at 65° and 10 s extension at 72°C.

Mark, release and recapture study

Remigrated *C. pruni* adults were collected on *P. spinosa* at test site 9 by beating tray method. Marking was essentially performed as previously described (Hagler and Jackson 2001; Nakata 2008); 50 specimens at a time were transferred into 50-ml centrifuge tubes and 5 mg of a pink luminescent powder pigment (Karmin Tagesleucht pigment, Artmaxx, Berlin, Germany) or 10 mg of a light blue luminescent powder pigment (Guardi, Prussian blue UV active, Boesner, Vienna, Austria) were added to each tube. Insects were stained by gentle rolling and turning of the tube. Pigment-coated psyllids showed a pink or blue colour under daylight and strong luminescence in the dark under UV light.

Stability of the dyes in the field was investigated by transferring 10 dead, stained specimens per dye to a yellow sticky trap exposed to outdoor conditions. Visibility of the stain in daylight and UV light was examined weekly.

Effects of the pigment on insect survival were examined by a laboratory experiment. 10 individuals at a time (pigment coated or untreated) were caged in pot cages (20 cm long, 15 cm wide) on apricot seedlings. Insects were kept under laboratory conditions (L16: D8, 21°C). Numbers of dead and surviving individuals were recorded daily for 8 days. The experiment was repeated four times. Statistical analysis (Kruskal–Wallis H Test) was performed by aid of the statistics programs SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

For the field experiments, the standard tree apricot orchard at test site 2 (Table 1) was chosen. In this, orchard within a row tree spacing ranged from 4–5 m, while between rows distance was 6 m. A hedgerow with hosts for *C. pruni (P. domestica* and *P. cerasifera nigra*) was located at a distance of approximately 100–130 m. Numbers and arrangement of trees included in the experiments are illustrated in Figs 4 and 5. The apricot orchard was surrounded by an apple orchard in the South, a sports ground in the North, a hedgerow with hosts for *C. pruni* in North-West and an apricot orchard in the East. The model orchard breadthways only comprised five rows. To ensure conditions that can normally be found in bigger orchards, the release tree was not chosen in the middle of the apricot orchard but closer to the apple orchard than to the sports ground. Wind and temperature measurements were taken by a weather station (Adcon, Klosterneuburg, Austria) present in close vicinity to the apricot orchard. Measurements were taken three times a day. The insect release was conducted twice (start 19th and 26th of April 2012). In the first release, insects were stained with the pink fluorescent dye, for the second release, the blue dye was used. 600 *C. pruni* per release were trapped on *P. spinosa*, starved for 2 h, stained and freed on one single tree in the apricot orchard. At the beginning of the experiment, insects were caged on four single branches using transparent plastic tubes (20 cm long, 15 cm wide) with gauze houses on both ends (50 individuals per cage). After the insects had settled and started to feed, the plastic tubes were removed (c. after 90 min). Movement of the stained insects and presence of naturally occurring insects within the orchard were monitored by yellow sticky traps (2 traps per tree including the release tree) from 19th of April (release of the first stained insects) until 3rd of May (8 days after the second insect release). Positions of the sticky traps are illustrated in Figs 4 and 5. Trees within the model orchard were allocated to sectors according to their distance to the release tree (Figs 4 and 5). The approximate distances to the release tree were 4–7 m for sector 1, 8–14 m for sector 2, 12–21 m for sector 3 and 16–24 m for sector 4. During the experiment, sticky traps were controlled at night using a UV tube (portable bank note tester, Conrad, Vienna, Austria). After the end of the experiment, all sticky traps were transferred to the laboratory and inspected under a binocular microscope and a standard UV transilluminator (Vilber Lourmat, Marne-la-Vallée, France).

Results

Temporal dynamics of *Cacopsylla pruni*

The results of the insect captures on *P. armeniaca, P. domestica, P. spinosa* and *P. cerasifera nigra* using the beating tray method are as shown in Figs 1–3. In 2011, remigration of *C. pruni* to *Prunus* spp. started in calendar week 10 (8th of March) and in 2012, in calendar week 12 (18th of March). Remigrants were observed until calendar week 20 (middle of May), significant numbers of springtime generation adults were present on *Prunus* until week 26 (end of June). Insect captures in apricot orchards were low. By the beating tray method, on average, significantly less than one specimen per sampling (10 hits per branch) was trapped. On test sites, 1, 6 and 7 no *C. pruni* were collected by this method. Yellow sticky traps, how-

ever, allowed capture of low insect numbers in all investigated apricot orchards in both years (data not shown). In contrast, high insect numbers were trapped by beating tray method on *P. spinosa* in both years and on *P. cerasifera nigra* in 2012. By far, the highest numbers of *C. pruni* with average insect numbers exceeding 25 per sampling were found on *P. spinosa* on test sites 8 and 9.

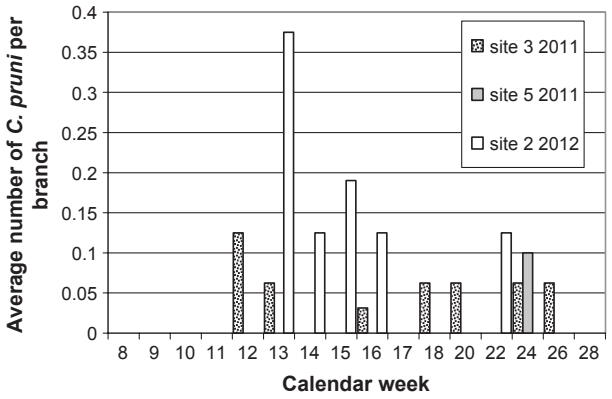

Fig. 1 Catches of *Cacopsylla pruni* adults in apricot orchards in 2011 and 2012 by the beating method.

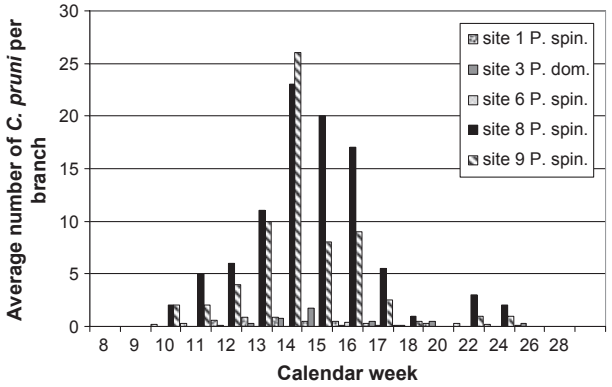

Fig. 2 Catches of *Cacopsylla pruni* adults by the beating method: Temporal dynamics of density on *Prunus spinosa* and *Prunus domestica* in 2011.

Presence of '*Ca.* P. prunorum' in plants and insects

Significant numbers of infected apricot and plum trees were detected in all investigated orchards. Between 9.8 and 63.3% of the apricot samples and 20–40% of the plum samples were infected. The pathogen was also detected in *P. spinosa* at test sites 1 and 4 but not in plants at sites 2, 6, 8 and 9 (Table 2). Infection rates of remigrated *C. pruni* individuals ranged from 0 to 11.5%. 0 to 3.44% of the springtime generation adults were also infected (Table 3).

Mark, release and recapture study

After exposure of marked insects to outdoor conditions on a yellow sticky trap for 5 weeks (precipitation during this period 20.2 mm), the pink dye was still clearly visible both in daylight and in UV light. All stained specimens were identified. The light blue dye was equally visible in UV light. In daylight with the unaided eye, however, discrimination between blue dye and dust on the sticky traps turned out difficult already 1 week after the beginning of the experiment.

Effects of the pigments on insect survival were examined under laboratory conditions. Kruskal–Wallis H test revealed no statistically significant differences between survival of unstained and stained insects and between survival of insects coated with the different pigments.

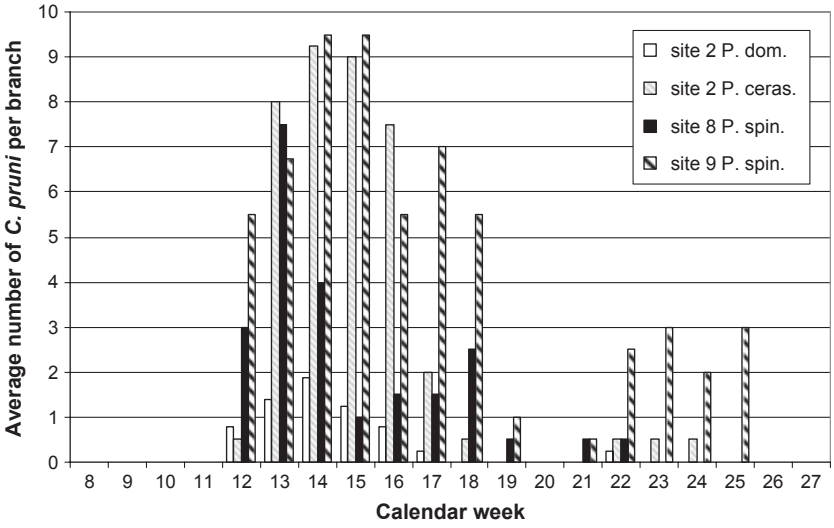

Fig. 3 Catches of *Cacopsylla pruni* adults by beating method: Temporal dynamics of density on *Prunus domestica*, *P. spinosa* and *P. cerasifera nigra* in 2012.

Table 3 Presence of '*Ca.* P. prunorum' in *Cacopsylla pruni* (PCR amplification with primers f01/r01 and RFLP analysis)

	No. of tested insects/No. of positive insects			
	Sampling in 2011		Sampling in 2012	
Test site	Overwintering adults	Springtime generation	Overwintering adults	Springtime generation
1	80/2	29/1	–	–
2	–	–	81/7	7/0
3	26/3	2/0	–	–
6	6/0	–	–	–
8	32/2	–	–	–
9	38/2	–	68/7	79/0

The results of the two release experiments are illustrated in Figs 4 and 5. Six hundred marked insects per experiment were released. In the first trial, 20 specimens (3.3%) were recaptured within 15 days (19 within the orchard, 1 in the *P. cerasifera nigra* hedgerow), in the second trial, 18 (3%) were trapped in the orchard within 8 days. In both experiments, insect spread occurred rapidly. In the first trial, marked insects were observed on trees in sectors one and two (on trees adjacent to the release point and also in the next row of trees) within 1 day after release (covered distance up to 10 m). In the second trial, marked specimens were ascertained on trees in sector one and three within 24 h (up to 13 m from release tree). After 8 days, marked insects were present on traps in sector 4 (up to 24 m from release tree). Table 4 illustrates numbers of marked and unmarked psyllids on the sticky traps in each sector of the orchard at the end of the experiment. Neither strong winds nor a prominent wind direction was present during the survey. The mornings were usually windless and wind direction often changed during the day.

Discussion

Our data, namely the numbers of *C. pruni* on wild *Prunus*, the percentages of PCR-positive insects and the infections of blackthorn bushes are signs of an endemic disease spread in the investigated areas. Reports from Italy (Carraro et al. 2002) pointed out that the diffuse presence of the phytoplasma in wild *Prunus* spp. such as blackthorn allows an ESFY transmission cycle independent from cultivated stone fruit trees. Occasionally, transmissions from blackthorn (or other wild *Prunus* species) to cultivated stone fruit trees are likely to occur. We therefore conclude that generally recommended management strategies such as use of healthy planting material, orchard management and eradication of infected fruit trees (Marcone et al. 2010) alone will not suffice to manage ESFY in the Wachau area.

In our study, however, infection rates of blackthorns were lower than we expected from insect

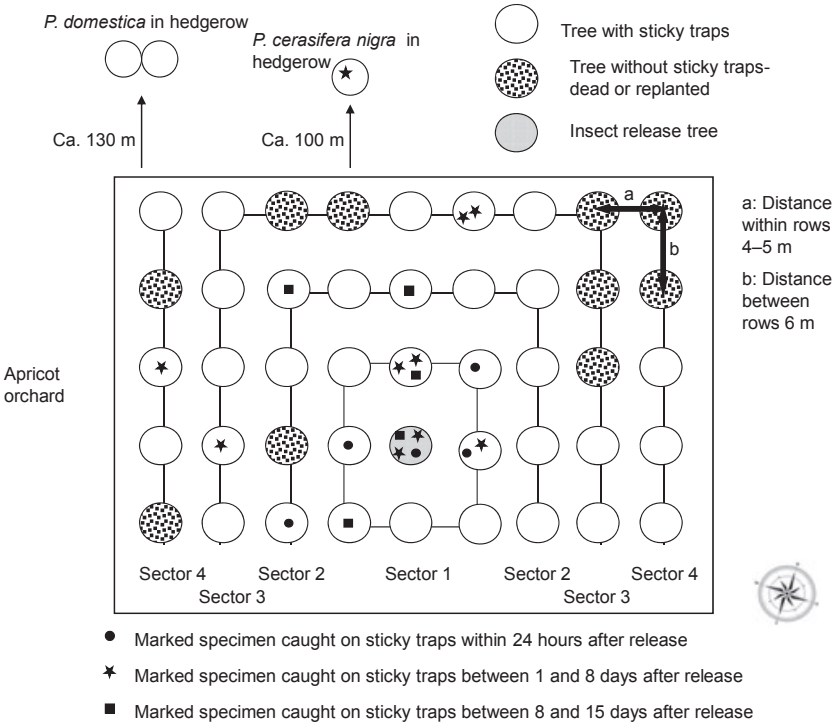

Fig. 4 Mark and recapture experiment 1 (Release 19th of April, end of the experiment 3rd of May; pink fluorescent dye): Size and shape of the model orchard, allocation of trees to sectors, position of insect release tree, position of yellow sticky traps and number and position of marked insects recaptured on sticky traps.

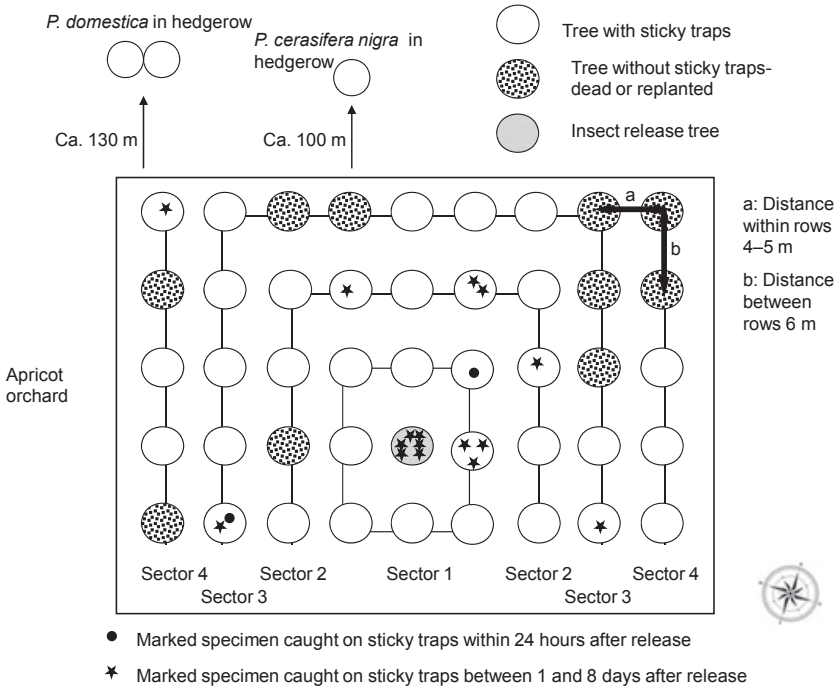

Fig. 5 Mark and recapture experiment 2 (Release 26th of April, end of the experiment 3rd of May; blue fluorescent dye): Size and shape of the model orchard, allocation of trees to sectors, position of insect release tree, position of yellow sticky traps and number and position of marked insects recaptured on sticky traps.

Table 4 Mark and recapture experiments: catches of marked and unmarked psyllids on the sticky traps in each sector of the model orchard

	Recaptured insects released in experiment 1 between 19th of April and 3rd of May	Recaptured insects released in experiment 2 between 26th of April and 3rd of May	Unmarked insects trapped between 19th of April and 3rd of May
Sector 1	12	10	20
Sector 2	3	4	17
Sector 3	3	3	12
Sector 4	1	1	4
Total	19	18	53

catches on the same plants. Virtually, all *P. spinosa* bushes were colonized by high numbers of infected insects in 2011 and 2012, and this had probably also been the case in previous years. Consequently, the question arises whether blackthorn is indeed a favourable host of *C. pruni* but not very susceptible to ESFY. The quick epidemic disease spread in Austria might therefore rather be pushed by infected cultivated *Prunus* trees allowing the development of numerous infectious *C. pruni* than by blackthorn.

The data on *C. pruni* population dynamics are similar to investigations in other European countries with comparable climatic conditions (Labonne and Lichou 2004; Jarausch et al. 2007; Thébaud et al. 2009; Ermacora et al. 2011). In 2011, remigration of the overwintering adults in Lower Austria started 2 weeks earlier than in 2012. Comparisons with weather data gathered at test site 2 (not shown) suggest that the start of remigration was influenced by the temperature profile. Hibernal temperatures in the first half of March 2012 could have delayed remigration. In both years, we detected the first specimens on *Prunus* more or less simultaneously at all test sites. Up to now, overwintering sites in the investigated areas of Austria were not known. Further work should therefore focus on the identification of overwintering sites. Recording both temperature profiles at these sites and population densities on *Prunus* hosts might perhaps allow conclusions on the influence of temperature on migratory activity.

Our PCR analyses showed considerable infection rates of remigrated *C. pruni*. These findings confirm the prominent role of the remigrants for disease spread which has already been proposed by other authors (Carraro et al. 2004; Jarausch et al. 2008; Thébaud et al. 2009; Poggi Pollini et al. 2010). Moreover, it shows the urgent need for effective control strategies against the remigrants. In contrast, only low

numbers of infected springtime generation insects were observed. The majority of springtime generation insects analysed in this work were collected on blackthorn. In most of these host plants, the phytoplasma was not detected, and consequently, our data do not allow conclusions on the role of springtime generation psyllids on disease transmission. Further studies with more springtime generation adults collected in diseased apricot orchards are required.

The incidence of new ESFY infections in an apricot orchard depends on the number of vectors present, on the infection rates of the insects as well as on insect mobility and numbers of trees that are visited by one individual. To estimate the number of remigrants in an apricot orchard and to track their behaviour, the mark release and recapture experiment was carried out. Our tests showed that fluorescent dyes provide an easy and effective marking tool for field studies on psyllid movement. The selected pigments were stable for several weeks under outdoor conditions including rain. The pink dye, however, was more suitable for visual detection by bare eye than the light blue one. There was no detectable effect of the dyes on insect survival. Moreover, in our field experiment, marked individuals were able to survive for more than 8 days and to fly >10 m within 24 h of their release. These results coincide with previous works on the effect of a pink fluorescent powder on the Asian citrus psyllid (*Diaphorina citri*, Hemiptera: Psyllidae). The dye neither influenced survival nor significantly affected flight behaviour (Nakata 2008). Similar results were obtained by Coviella et al. (2006) who used several fluorescent dusts for tracking the movement of the glassy winged sharpshooter (*Homalodisca coagulata*, Hemiptera: Cicadellidae). Marking experiments on mountain pine beetles (*Dentroctonus ponderosae*, Coleoptera: Cucurlionidae), however, showed that fluorescent dyes did not reduce the viability of the beetles but they influenced their physical condition. The authors presumed, therefore, that fluorescent powders might affect optimal dispersal behaviour (Reid and Reid 2008).

Monitoring of *C. pruni* by the beating tray method only allowed capture of few specimens, and in some investigated orchards, insects were not ascertained. Use of yellow sticky traps proved the presence of the insect species at all investigated sites, but still numbers of captured specimen were low. In our mark and capture study, numbers of unmarked psyllids on the sticky traps between 19th of April and 3rd of May were a multiple of the marked specimens in all sectors of the model orchard. Numbers of marked insects per release and per dye amounted to 600. Presuming that unmarked and marked insects had the same chance to be caught on the sticky traps, it can be concluded that at least more than thousand *C. pruni* individuals were naturally occurring in the model orchard. One can easily imagine that in this orchard (test site 2), the number of ascertained insects together with their observed mobility and the rate of 8.64% PCR-positive remigrants account for the high rate of infected trees.

Before the experiment started, we noticed that a simple transfer of insects to the release tree led to a high number of insects dropping to the ground. To accelerate settling and feeding of the marked insects on the release tree and to minimize losses, insects were starved for 2 h before the trial. Moreover, at the beginning of the experiment, insects were caged on the release tree in transparent plastic tubes. The tubes were not removed until the insects had started to feed. With this procedure, we were able to keep virtually all specimens on the tree.

In our study, captures of marked *C. pruni* on the sticky traps proved a fast and frequent tree-to-tree movement. From our experiments, it cannot be excluded that the dyes affected the condition of the psyllids similarly to the mountain pine beetles. In this case, however, dispersal of unmarked insects would be even faster than observed for the stained ones. All in all, it seems likely that one infectious individual present in an orchard visits and infects several apricot trees.

The numbers of insects in our mark and recapture experiments were relatively small as compared with other release experiments with psyllids. Van den Berg and Deacon (1988), for example, released a total of 25.000 *Trioza erytreae* in two experiments. The authors studied insect dispersal without host plants in ploughed land of 3 × 2.5 km. Kobori et al. (2011) carried out two experiments with 11.000 marked *Diaphorina citri* that were released in an experimental plot with 1089 potted citrus trees arranged at 2.5 m intervals. Both studies were carried out with newly hatched adults. *T. erytreae* and *D. citri* are multivoltine, complete their whole life cycle on *Citrus* spp. or other Rutaceae and produce high numbers of progeny. Insects can readily be reared or obtained in the field in high numbers (Annecke and Cilliers 1963; Van den Berg and Deacon 1988; Hall et al. 2012). In contrast, *Cacopsylla pruni* is a univoltine species hibernating on conifers. In our laboratory-rearing experiments, field-captured *C. pruni* produced high numbers of eggs which developed into numerous springtime generation adults. Migration to conifers and hibernation under laboratory conditions, however, proved difficult (data not shown). Rearing of significant numbers of

remigrants seemed hardly possible. As already stated, the remigrants are considered as crucial disease vectors. Therefore, we studied their dispersal behaviour in the mark and recapture experiments. Consequently, insects were captured in field where their occurrence was limited. Compared with the citrus experiments mentioned above, our experiments not only comprised lower number of released insects, they were also carried out in a smaller test plot. So the recapture rate of approximately 3% of the marked *C. pruni* enabled us to prove a frequent tree-to-tree movement and to estimate the natural population size in the model orchard although also in our experiments a higher number of released insects would have been desirable. In the release experiments with *T. erytreae* and *D. citri*, the authors observed a prominent role of prevailing winds on psyllid dispersal (Van den Berg and Deacon 1988; Kobori et al. 2011). In our study, neither strong winds nor a prominent wind direction was present during the survey. Nevertheless, it can be presumed that stronger winds would have enhanced dispersal of *C. pruni* in a similar manner to *T. erytreae* and *D. citri*.

ESFY transmission experiments in the greenhouse revealed a minimum inoculation period of 1–2 days (Carraro et al. 2001). Our data show a considerable tree-to-tree movement of the remigrants. In theory, a fast acting, enduring insecticide should therefore decrease inoculation of the trees, reduce insect numbers in orchards, downsize movement of the vectors from tree to tree and in the end diminish rates of new infections. Practical experiments with insecticide applications, however, showed inconclusive results. Effects of the treatments on disease spread were only detected in the minority of tests (Poggi Pollini et al. 2007). Mark and recapture experiments might allow observation of the effects of possible insecticide applications on psyllid survival and movement. In this way, they could contribute to the development of appropriate management strategies.

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