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dynamics of orchard colonization

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Arthropods in Relation to Plant Disease

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**Population dynamics of *Cacopsylla melanoneura* (Hemiptera: Psyllidae) in Northeast
Italy and its role in the Apple proliferation epidemiology in apple orchards**

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Abstract

In the present study, incidence of ‘*Candidatus Phytoplasma mali*’ in an experimental apple orchard in Northeast Italy, in addition to abundance and phytoplasma infectivity of *Cacopsylla melanoneura* (Hemiptera: Psyllidae) were determined and the role of this psyllid as a vector of ‘*Ca. P. mali*’ in this region were reviewed. Insect samples collected in the orchard by the beating method indicated high abundance of *C. melanoneura*, (up to 7.92 specimens/branch); however the psyllid *C. picta* was not observed. Molecular analyses revealed presence of ‘*Ca. P. mali*’ in 6.25% of overwintered psyllids. This infection rate is quite high in comparison to other localities where *C. melanoneura* is known as the main vector of the phytoplasma. This finding supports the assumption that *C. melanoneura* also is paramount in the epidemiology of the apple proliferation disease also in Northeast Italy. Moreover, we correlated immigration dynamics to the temperatures registered in the apple orchard, and defined an immigration index to predict the progressive arrival of the overwintered adults from winter sites. Psyllids start to reach the apple orchards when either the average of the maximum temperature of the seven days is above 9.5°C or the immigration index has a positive value. This index will be a useful tool for the growers to prevent apple proliferation phytoplasma spread with well-timed insecticide treatments targeted against *C. melanoneura*. However, further research is needed to validate or adjust the index to other apple growing regions, which may affect more efficacious management of this disease and psyllid vector.

KEY WORDS: *psyllid vector*, ‘*Candidatus Phytoplasma mali*’, *immigration dynamics*, *temperature*, *apple orchards*, *overwintered adults*,

Introduction

Apple proliferation is a serious disease which has caused significant economic losses over the past ten years in all European apple-growing regions (Kunze 1989). Fruit weight is often reduced by 30-60%, the fruit color is unsatisfactory and the taste is poor, with the result that as much as 80% of the fruits is unmarketable.

The disease is induced by '*Candidatus Phytoplasma mali*', a phytoplasma that colonizes the phloem system of apple plants and is transmitted by insect vectors during their trophic activity.

The pathogen is acquired passively during feeding in the phloem of infected plants, moves through the intestine and pass intracellularly through the epithelial cells to enter the hemocoel.

Then the phytoplasma circulates in the hemolymph, replicate and finally penetrates specific cells of the salivary glands. With the next feeding, the insect can transmit the phytoplasma to a healthy plant. Affected plants lack vigor and fruits are markedly reduced in size with poor flavor and low sugar and acidity content (Osler and Loi 1986). Since the first incidence occurred in the Trentino region (northeast Italy) in the mid-1990s, many studies were carried out concerning the spread of the phytoplasma and the insect vectors. High percentages of symptomatic plants were first observed in several orchards of Trentino (Vindimian and Delaiti 1996, Vindimian et al. 2000) affecting several varieties, mainly Golden Delicious, Florina and Renetta del Canada. Studies on insect vectors revealed the important role of the Hemiptera, genus *Cacopsylla* in the transmission of this phytoplasma. In Trentino two species, *Cacopsylla melanoneura* (Förster) and *C. picta* (Förster), are regularly present in the orchards. Contrary to Northwestern Italy where *C. melanoneura* is the primary vector of 'Ca. P. mali' (Tedeschi et al. 2002, 2003; Tedeschi and Alma 2004), in Trentino low phytoplasma infection rate in this species and very low transmission efficiency by this psyllid were recorded (Mattedi et al. 2007, 2008). But in a recent

report elevated phytoplasma infection rates in *C. melanoneura* from Trentino region were correlated with the infection level in the plants (Malagnini et al. 2010). On the other hand, *C. picta* showed higher natural infection rate and transmission efficiency even at low density (Frisinghelli et al. 2000, Forno et al. 2002, Mattedi et al. 2007, 2008) in accordance also with German results (Jarausch et al. 2003, 2004, 2008, Mayer et al. 2009). Both these insects have a quite complex life cycle. The overwintered adults reach the apple orchards in winter, in the case of *C. melanoneura* (Tedeschi et al. 2002; Mattedi et al. 2007) and at the end of March in the case of *C. picta* (Mattedi et al. 2007). On this host plant they mate, lay eggs and develop. In late spring the newly emerged adults rapidly move to shelter plants, mainly conifers, for aestivation and overwintering.

Preliminary evidence suggests that overwintered adults are already infected when they re-migrate into apple orchards (Jarausch et al. 2004; Mattedi et al. 2008), so well-timed treatments are very important to control the first individuals that reach the orchards. For this reason a phenology model will be a useful support for vector management decisions. Many forecasting models have been produced for other psyllid species, using means of driving variables, mainly temperature, and based on developmental thresholds (DT) and degree-days (DD) (Beránková and Kocourek 1994, Kapatos and Stratopoulou 1999, Kumral et al. 2008, Morgan and Solomon 1993; Schaub et al. 2005). These models refer to some phases of the life cycle and predict when a developmental stage (generally the most damaging stage) will appear. None of these models concerns the immigration period for psyllids that move between different host-plant species or exploit non-host plant species as overwintering sites.

In the present study we analyzed in detail the incidence of ‘Ca P. mali’ in an experimental orchards in Northeast Italy in addition to the abundance and phytoplasma infection of *C.*

melanoneura, with the aim to revise the role of this psyllid as a vector of ‘Ca P. mali’ in this region. Moreover, we propose an index based on the maximum temperatures registered in the apple orchard, to predict the arrival of the overwintered adults. This index will be a useful management tool for the growers to prevent phytoplasma spread thanks to well-timed insecticide treatments.

Materials and methods

Field samplings

The study was carried out over five growing seasons (2006-2010) in a conventionally treated 3 hectare apple orchard of the IASMA Research and Innovation Center, Fondazione E.Mach (Borgo Valsugana, 419 m a.s.l., Trento, Italy). Observations on the incidence of Apple proliferation disease and the population dynamics of *C. melanoneura* were performed in 4 untreated plots (area 1500 m²), which were randomly selected in the orchard. The main apple variety was Golden delicious, with 15-25 years old trees, 4.0±0.5 m high, and spaced 0,5-1.6 m within a row and 4.0-4.6 m between rows. The number of symptomatic trees was recorded at the end of September and a total number of twenty-one samples was randomly collected from symptomatic plants to be analyzed with molecular assays. During each season, insect monitoring started before the immigration in the orchard of the first overwintering adults (January/February) and it lasted till the emergence of the springtime generation (May/June). Adults were collected every 7 days, by means of beating method: for each replication plot, 25 branches (50±10 cm in length) were considered and every branch was shaken 2 times above a beating tray (diameter 7 cm, 60 x 40 cm of cloth). The collected adults were counted and identified in the laboratory by both morphological, examining female and male terminalia (Ossiannilsson, 1992) and molecular

tools (Tedeschi and Nardi 2010), then analyzed for phytoplasma presence. An in-depth study on the frequency of '*Ca. Phytoplasma mali*'-positive psyllids was carried out in 2008 and a total number of 194 batches, each of 5 specimens, was analyzed. The number of eggs and young stages was assessed by examining under the microscope 30 apical shoots (20-25 cm long) from each replication, randomly selected in the central rows of each plot. These controls were performed every 7 days, from the beginning of the oviposition until the emergence of the springtime generation.

Phytoplasma detection and species identification

Plant DNA was isolated from 100 mg (wet weight) of phloem tissue from symptomatic plants, previously ground with liquid nitrogen in a sterile mortar, using the QIAGEN's DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). DNA was eluted in 100 µl of elution buffer and kept at -20°C until used. Total DNA was extracted from batches of five adult psyllids following a protocol adapted from Marzachi et al. (1998) and previously applied to psyllids (Tedeschi et al. 2002). The final product was resuspended in 50 µl of TE.

Insect and plant DNAs were amplified firstly with the phytoplasma universal primer pair P1/P7 (Schneider et al. 1995) and then in nested PCR with the primers fO1/rO1 (Lorenz et al. 1995) specific for the AP-group phytoplasmas, after a 1:40 dilution. Reaction conditions were as in the original papers. Contamination by amplicons was avoided by using separate rooms and material as well as decontamination procedures (UV exposure and bleaching of materials and surfaces).

Moreover, for each amplification, a negative control containing Milli-Q water was included. Amplification products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized on a U.V. transilluminator. Specific '*Ca. Phytoplasma mali*'

profiles were obtained by RFLP analysis with endonuclease SspI, digesting seven microliters of the amplification product with 3U of SspI for 4.5 h at 37°C.

The proportion of infected insects was estimated by its maximum-likelihood estimator, $\hat{p}$, calculated according with Swallow (1985): $\hat{p} = 1 - H^{1/k}$, where H is the observed fraction of healthy groups and k is the number of insects per group, five in this case.

The psyllid identification by morphological analyses was confirmed by molecular analyses using the primer pairs MEL fw/MEL rev developed for *C. melanoneura* (Tedeschi and Nardi 2010).

Thirty-two randomly selected individuals were used to confirm morphological observations.

Reaction conditions were as in the original papers.

Population dynamics and index of immigration

The population trend of *C. melanoneura* in the apple orchards was based on data collected during the years 2006-2010 for adults and 2006-2008 for eggs and juveniles. In order to identify which environmental parameters in the orchards were possibly involved in *C. melanoneura* colonization we developed an original model according to the following procedure. Starting, from 2006 the maximum, and median daily and hourly temperatures were recorded at 2 meters above the ground from a meteorological station inside the apple orchard. For daily temperatures we also calculated the average of the seven days (T_{7n}) preceding any sampling date (a_n). Seven days was chosen since field samplings were conducted with such a periodicity. Temperature trends were associated with the insect dynamics, from the date of first detection (a_0) to the peak of captures (a_{max}). In the first instance, the absolute highest temperature among the 7 days preceding a_0 (T_{0max}) was taken and then the minimum across the years among the T_{0max} . This minimum was defined as hypothetical threshold temperature (**Tth**) of psylla orchard

immigration. The T_{th} , calculated both for median and maximal temperatures was checked whether it had been previously passed without eliciting the psyllid immigration and we selected only those that were not passed. The same procedure was done for the T_{7n} where a relative hypothetical threshold (T_{7th}) was defined.

The average of degree days were counted with temperature above the threshold for any T_{7n} (dd_n).

The index of immigration was defined as:

$$I_i = [(T_{7n} - T_{7th}) + dd_n]$$

The immigrated population (I_p) as:

$$(p_n - p_{n-1})$$

where $(p_n - p_{n-1})$ is the difference of sampled adults (expressed as % referred to the population peak) between two consecutive samplings.

A simple regression analysis was performed between I_i and I_p , from the a_0 to the a_{max} of each year (2006-2010). If the regression was significant then the previsions were verified concerning the detection of any a_0 by calculating I_i related to any sampling from 2006 to 2010 *a posteriori*.

The first positive value of I_i was assessed whether it was associated to the first psylla detection in the field. The same procedure was followed for historical data taken from the same orchard in the period 2002-2004. Eventually, all data were associated to the apple phenology, in particular bud breaking and first flowering.

Results

Apple proliferation incidence

The symptoms observed in the orchards were predominantly witches' brooms, reddening of the leaves, enlarged stipulae, abnormally long flower stalks, flowering out of season and small fruits. Basing on the percentage of symptomatic trees, the incidence of apple proliferation disease ranged from 60 to 100%, according to the plot, with a mean annual increase of 5%.

Psyllid identification and phytoplasma detection

Following the morphological observations, almost all the psyllids collected in the experimental plots were identified as *C. melanoneura*. *C. mali* was sporadically observed; whereas, *C. picta* was never found. Molecular analyses confirmed the identifications of *C. melanoneura*.

The PCR analyses performed with primer pair fO1/rO1 gave AP-group specific amplicons of the expected size (1050 bp) with both plant and insect DNA. No amplification products were obtained from the negative controls. All 21 samples of symptomatic plants were positive to AP-group phytoplasmas and the RFLP with *SspI* restriction enzyme confirmed the presence of 'Ca. Phytoplasma mali'. The results of PCR detection in the insects are shown in table 1. One hundred and seventy-four samples of overwintered adults, 5 samples of nymphs and 15 samples of newly emerged adults were tested. Forty-eight out of 174 batches of overwintered psyllids were positive for 'Ca. Phytoplasma mali', with an estimation of 6.25% of infected *C. melanoneura*. No amplification was obtained from nymphs and newly emerged adults.

Population dynamics and index of immigration

The population trend of *C. melanoneura* overwintered adults was extremely variable during the years. The immigrant adults were recorded starting from the beginning of February in 2008,

while in 2006 they were delayed to mid-March. The peak of overwintered adults was reported between the 10th and the 13th week, depending of the year, but it was mostly concentrated in the second half of March. Eggs of *C. melanoneura* were observed on apple branches starting from the beginning of March in 2008 and the last ten days of the month in 2006 and 2007. The oviposition lasted until mid-April in 2007 and the beginning of May in 2006 and 2008. Young stages were recorded from the beginning of April in 2007 and 2008 and the end of the same month in 2006. Nymphs remained in the orchards until the beginning of May in 2007 and the end of May in 2006 and 2008 (Fig. 1).

Combining the population dynamics of *C. melanoneura* with the apple phenology (Table 2), a good correspondence between overwintered adult peak – first egg detection and apple bud breaking was found, with the peak of egg always preceding the first flowering.

Values and indexes referred to the psylla migration analysis are reported in table 3. The **T_{th}** calculated from maximum (12.8°C) and median (3.3°C) temperatures did not explain the first appearance of the psylla in the orchard, and no correlation was found with the insect migration into the orchard. Similarly, the **T_{7th}** taken from median temperatures (2.0°C) did not represent an effective threshold value, because it has been passed several times in the years without triggering the appearance of psylla in the orchard. Instead, the **T_{7th}** calculated from maximum values (9.5°C) was found to be a candidate for a possible migration threshold. The regression analysis between **I_p** and **I_i** (Fig.2) was highly significant ($F = 24.2$; $df = 1,26$; $p < 0.001$).

Positive values of **I_i** corresponded with the first appearance of the psylla in the orchard in 2006 and 2008-10. Instead, in 2007 values slightly above zero (1.5 and 0.9) in the first five weeks did not correspond to the first presence. However, when the **I_i** reached for a more relevant value

(6.5), individuals were eventually found. Positive **Ii** fitted in verifying all the **a₀**, also for historical data of 2002-2004.

Discussion

The present research provides important and new information on the epidemiology of apple proliferation in northeast Italy and develops an index of immigration that enables well-timed control measures against the insect vectors. The role of *C. melanoneura* as vector of ‘*Ca. Phytoplasma mali*’ has been revised in this area confirming the results obtained by Malagnini et al. (2010) on psyllid infectivity. Previous reports concerning Trentino region pointed out the predominance of *C. picta* in Val d’Adige and *C. melanoneura* in Val di Sole and Val di Non (up to 2 overwintered adults/branch) (Mattedi et al. 2007, 2008). Transmission trials and phytoplasma quantification in insect bodies revealed a low transmission efficiency for *C. melanoneura* while an important increase in phytoplasma concentration was observed only in *C. picta*, indicating an efficient phytoplasma multiplication in this species (Pedrazzoli et al. 2007). For this reason it is now popularly held belief that *C. picta* is the most important vector of ‘*Ca. Phytoplasma mali*’ in northeast Italy, as well as in Germany (Jarausch et al. 2003, 2004, 2008; Mayer et al. 2009), while in northwestern Italy, *C. melanoneura* represents the only psyllid vector of this phytoplasma (due also to the absence of *C. picta*). Evidence supports the role of *C. melanoneura* as a vector in Valsugana, a valley in the south east of the Trentino region with a long tradition in apple production. This area is characterized by an high incidence of the apple proliferation disease and the only important psyllid species which was found in the repeated collections trough a five year period was *C. melanoneura*; *C. picta* was never recorded. The molecular analyses revealed the presence of ‘*Ca. Phytoplasma mali*’ (as the

Swallow's estimated proportion $p^{\wedge}$) in 6.25% of overwintered psyllids, confirming roughly the results obtained by Malagnini et al. (2010) with specimens coming from the same area. This discrepancy may be due to the different number of psyllids analyzed. During the peak presence there were up to 7.92 overwintered adults/branch in 2007, and from the beginning until the end of March in all the three years the number of recorded *C. melanoneura* was almost always higher than 2 psyllids/branch. Thus, in this valley, *C. melanoneura* should be considered the only significant vector associated with the rapid spread of this disease.

The infection rate of the overwintered adults tested high in comparison to other localities where *C. melanoneura* is already known as the main vector of 'Ca. phytoplasma mali' (an estimation of 6.25% vs 3.6% of 'Ca. Phytoplasma mali'-positive insects) (Tedeschi et al. 2003). This fact strengthens the evidence that *C. melanoneura* has an important role in the epidemiology of the apple proliferation disease also in northeast Italy, where its control is already regulated by insecticide treatments (Baldessari et al. 2007, 2009).

Once ascertained that *C. melanoneura* represents an important risk for apple growers, a new approach to the management of this vector needs to be developed. Data collected during the years concerning the population dynamics of this species, the apple phenology and the weather parameters allowed us to define an index to predict the re-migration of overwintered adults into apple orchards. The aim was to provide to the growers an easy to use tool which would predict the time of arrival of *C. melanoneura*. in order to refine the pest management decisions. The efficiency of an insecticide treatment depends on the product and on the timing of application in order to affect the most sensitive or harmful stage on the crop. In the case of *C. melanoneura*, overwintered adults showed, in comparison with all the other stages, the highest population density, the highest percentage of 'Ca. Phytoplasma mali'-positive specimens, and the longest

time spent in apple orchards. All these characteristics suggest that this is the crucial role of this stage in vectoring AP-phytoplasma (Tedeschi et al. 2002, 2003). For this reason the treatments should be focused on the overwintered adults as soon as they start to colonize apple orchards. As a consequence, we established a index of immigration related to the immigrant psylla adults. Similar studies were built in the past to forecast the occurrence of a particular life stage of a pest with the aim to improve its control (Beránková and Kocourek 1994, Kapatos and Stratopoulou 1999, Kumral et al. 2008, Morgan and Solomon 1993, Schaub et al. 2005), but never to predict the adult immigration in the orchards. Here we propose an empirical model which cannot be supported by laboratory trials due to the impossibility of reproducing the entire biological cycle of *C. melanoneura* in controlled conditions. This correlation is based on the temperatures recorded in the apple orchards, a variable that can be easily monitored by the growers or at least by the phytosanitary services.

It is known that temperature influences apple phenology and that the migration could also be influenced by certain phenological stages of the trees. Immigration into orchards take place during budding, which may be detectable by the overwintering psylla, thereby attracting them to the stimuli associated to budding.

However, the distances occurring between psyllid host plants and shelter plants can be considerable (Čermák and Lauterer 2008, Thebaud et al. 2009), thus it is improbable and temperature is most likely the critical factor. There was no correlation between immigration dynamics and apple phenology demonstrated, however oviposition occurs at bud burst while egg peak and hatchings are always before the first flowering. This confirmed a good degree of synchrony between *C. melanoneura* and host-plant growth, being linked with temperatures as

stated for psyllids in general by Hodkinson (2009). These data are likewise useful for the growers for possible further treatments during the season.

In the present study, the hypothetical threshold calculated as an average of the maximum temperatures of the seven days (**T_{7th}**) proved to be a good tool to forecast immigration, by calculating a proper Index of immigration (**Ii**). Psyllids started to reach the apple orchards when the **T_{7th}** was 9.5°C. Also including in the model single episodes, meant as hours above this threshold, we could individuate with extremely precision the time of first occurrence and the following immigration trend for a period of 9 years. It is feasible that any **Ii** over 0 can elicit psyllid migration, with direct correlation between **Ii** value and number of migrant specimens, and our failure in detecting individuals in correspondence of **Ii** < 2 depended likely on the extremely low number occurring in the field in those circumstances.

On the other hand, there are still some relevant limits that we need to point out. The calculated threshold fits well for apple orchards located in the Valsugana valley, but not necessarily for other locations, where the correlation needs to be validated by adjusting the threshold value, either according to historical collection data or by programming periodical field collections. Other geographical factors associated with the winter sites location (e.g. the regional orography, the main air streams and distance from apple orchards) may differently affect the psylla migration process and influence its presence/absence, both in terms of time and quantity, in a given apple orchard.

A straight correlation between maximum temperature and adult mobility was found in addition to a, temperature threshold that, independently on the period in the winter, favors the psylla to abandon overwintering sites. However, more research is required to set up a proper forecasting model, which could be applied in different apple-growing regions.

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423 **Table 1 - Estimated proportion ($p^{\wedge}$) of ‘*Candidatus Phytoplasma mali*’-infected *Cacopsylla***
 424 ***melanoneura* (Förster) collected by beat tray samplings in 2008 in the experimental plots**
 425

	Date	AP+/tot	$p^{\wedge}$		AP+/tot	$p^{\wedge}$
overwintered	1 February	0/3	0	}	48/174	6.25
	8 February	5/10	12.94			
	15 February	6/8	24.2			
	22 February	1/8	2.63			
	28 February	5/30	3.58			
	7 March	11/30	8.73			
	13 March	8/30	6.01			
	21 March	5/30	3.58			
	28 March	6/20	6.88			
	4 April	1/5	4.36			
nymphs	15 May	0/15	0	}	0/15	0
offspring	16 April	0/1	0	}	0/5	0
	15 May	0/4	0			

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Table 2 - Number of weeks when an event of psylla or apple phenology was recorded for each year of investigation (2006-2010). For the historical data (2002-2004) only the first detection and the peak of captures of psylla were available.

	2002	2003	2004	2006	2007	2008	2009	2010
First Psylla	5	8	6	11	7	4	8	9
Psylla Peak	10	10	11	13	11	10	12	12
First Egg	-	-	-	13	11	10	12	12
Egg Peak	-	-	-	14	13	14	14	13
Bud break	-	-	-	13	10	11	12	12
Flowering	-	-	-	16	14	14	15	15

Table 3 - Absolute (T) and average of 7 days (T7) median (med) and maximal (max) temperature (°C) associated with the first psylla detection (suffix a₀) and with periods before the first detection (suffix a_{-n}) in the years 2002-2004 and 2006-2010 . Ii is the Immigration index. In bold are indicated the minimal a₀ and maximal a_{-n} values across the years for each parameter.

	2002	2003	2004	2006	2007	2008	2009	2010
<i>Ta_{0med}</i>	-	-	-	4.9	7.5	12.9	3.3	5.4
<i>Ta_{-nmed}</i>	-	-	-	4.4	8.5	4.2	4.5	4.4
<i>Ta_{0max}</i>	-	-	-	12.8	16.4	20.3	12.5	13.0
<i>Ta_{-nmax}</i>	-	-	-	11.8	21.8	11.4	9.6	9.8
<i>T7a_{0 med}</i>	-	-	-	3.5	5.1	5.3	2,0	4.5
<i>T7a_{-n med}</i>	-	-	-	2.3	3.7	3.1	3.7	3.0
<i>T7a_{0 max}</i>	9.8	10.2	10.2	10.1	12.1	11.2	9.5	10.4
<i>T7a_{-nmax}</i>	5.8	6.6	7.7	8.8	9.4	7.0	8.0	7.7

Table 4. Calculated Index of immigration (Ii) and Immigrated population (Ip) from the first week of January until the population peak, in the periods 2002-2004 and 2006-2010. Samplings (s) were conducted weekly. In gray cells, in bold, are highlighted values of first positive Ii and first Psylla detection in the orchard.

s	2002		2003		2004		2006		2007		2008		2009		2010	
	Ii	Ip	Ii	Ip	Ii	Ip	Ii	Ip	Ii	Ip	Ii	Ip	Ii	Ip	Ii	Ip
1	-8.0	0	-7.0	0	-7.5	0	-7.0	0	-3.2	0	-7.9	0	-9.5	0	-6.7	0
2	-6.4	0	-9.0	0	-5.2	0	-8.3	0	-0.4	0	-4.8	0	-6.3	0	-6.0	0
3	-6.1	0	-7.0	0	-6.0	0	-8.2	0	1.5	0	-2.1	0	-5.6	0	-6.3	0
4	-3.6	0	-2.9	0	-8.2	0	-7.0	0	-3.9	0	6.1	0.03	-3.8	0	-7.5	0
5	2.2	0.04	-4.9	0	-1.2	0	-1.7	0	0.9	0	0.0	0.05	-0.9	0	-6.6	0
6	4.3	0.09	-5.0	0	2.3	0.07	-5.1	0	-1.2	0	1.4	-0.02	-2.6	0	-5.3	0
7	-1.3	-0.09	-1.7	0	2.3	0.11	-2.8	0	6.5	0.01	-0.2	0.00	-2.6	0	-3.3	0
8	4.0	0.27	3.4	0.05	-5.7	-0.04	-3.4	0	7.8	0.01	9.3	0.50	1.5	0.04	-1.5	0
9	5.1	0.08	6.9	0.50	-0.3	-0.07	-2.5	0	15.3	0.50	12.2	0.26	2.3	0.15	2.8	0.01
10	14.6	0.61	11.3	0.45	3.2	0.07	-0.1	0	12.2	0.13	4.1	0.19	6.9	0.22	-1.6	0.02
11					6.1	0.85	3.2	0.41	9.2	0.35			11.4	0.60	6.9	0.17
12							7.8	0.50							15.2	0.81
13							10.8	0.10								

Figure Captions

Fig. 1. Presence of eggs and nymphal stages of *Cacopsylla melanoneura* (Förster) in the orchard in 2006-2008.

Fig. 2. Simple Regression analysis ($F = 24.2$; $df = 1,26$; $p < 0.001$) between Immigration Index (**Ii**) and Immigrated population (**Ip**) calculated from the average of maximal temperature of the 7 days preceding any adult sampling of the period 2002-2010.

Fig. 1

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469 Fig. 2