Scientia Horticulturae 185 (2015) 151–161

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Susceptibility of European pear germplasm to *Cacopsylla pyri* under Mediterranean climatic conditions

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ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 24 January 2015
Accepted 27 January 2015

Keywords:
Resistance
Breeding
Pyrus communis
Genotypes

ABSTRACT

The response of 160 European pear genotypes to the attacks of *Cacopsylla pyri* in natural conditions of infestation was studied in the *ex situ* germplasm collection of the IVALSA Institute – CNR located in Follonica (Grosseto, Italy) during three years. ANOVA showed that both the year and the period of survey have a significant effect upon pear infestation assessed by seven variables. The overall average values of the studied accessions for the variables taken into account were used for grouping the genotypes into 5 putative classes of susceptibility by cluster analysis. Four variables (number of colonies, small nymphs, large nymphs and length of the colonies) selected on the basis of discriminant analysis allowed us to classify 70% of the cultivars into the correct group of susceptibility by jackknifed classification matrix. Taking into account the mean values of the variables and their upper and lower limits all the genotypes were grouped into 5 different classes of susceptibility. On the whole, the number of highly tolerant or resistant cultivars were 8.1% of the investigate genotypes; part of them were ancient cultivars of Italian origin as 'Eletta Morettini', 'Fiorenza', 'Gentile', 'Moscatellina', 'Pera Volpina VP7', 'Precoce di Cassano' and 'Precoce di Masi', but there were also the French 'Notaire Lepin' and 'Président Loubet', the German 'Forelle' and the American 'Starking Delicious'. The remaining 91.9% of the germplasm collection showed different levels of susceptibility to psylla attack. The largest part of the genotypes (56.9%) was identified as slightly susceptible, often with minor infestation (that is some isolated larvae, few colonies and reduced honeydew) only in one or two out of three years. Several cultivars (46.3%) were scored as susceptible, suffering medium or high damage, including the widespread cultivated 'Conference', 'Doyenné du Comice', 'Highland' 'Curè', 'Etrusca', 'Kaiser', and 'William', these last 3 being classified as highly susceptible. The influence of the environment and growth condition of trees are discussed together with screening methods applied for the evaluation of pear susceptibility to psylla attack reported in world literature.

1. Introduction

European pear psylla, *Cacopsylla pyri* L. (Hemiptera, Psyllidae), is the most important insect pest of pear in all Italian pear-growing regions. All of the main cultivars of the European pear grown commercially ('Abbé Fétel', 'William', 'Conference', 'Doyenné du Comice', 'Kaiser', *etc.*) are susceptible to this arthropod pest (Bellini and Nin, 2002). Pear psylla may cause severe problems in orchards, reducing tree growth and productivity, producing plentiful honeydew that gives rise to black sooty mould on fruits, leaves and bark, inducing premature leaf drop. Finally, heavy fruit infestations result in a downgrading for fresh markets.

In the past growers have primarily based pear psylla control on the use of synthetic insecticides, as a consequence, also in Europe resistance cases to different active ingredients have been recorded and the evolution of *C. pyri* resistance has recently documented and discussed (Civolani, 2012). Therefore, control strategies should be based on a limited use of pesticides, possibly selective ones, in order to foster the development of the populations of the antagonist *Anthocoris nemoralis* F. (Hemiptera, Anthocoridae), which become

Abbreviations: IPM, integrated pest management; DCA-BO, Department of Fruit Tree and Woody Plant Sciences of the University of Bologna; ISF-Forlì, Experimental Institute for Fruit Tree – Section of Forlì now Fruit Growing Research Unit of Forlì (Fruit Tree Research Centre); DISPAA-UNIFI, Department of Agri-Food and Environmental Science of the Florence University; INRA, National Institute for Agricultural Research; RIFG – Pitesti-Maracineni, Research Institute for Fruit Growing, Pitesti, Maracineni, Romania.

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http://dx.doi.org/10.1016/j.scienta.2015.01.031

a key-factor to control the pest, preying on both eggs and nymphs of psylla. Presently, the recommended control strategy against *C. pyri* in Italy is based on integrated pest management (IPM), supported by natural control aimed at balancing the complex biological relationships of the field community (Civolani, 2012). In recent years new pesticides have been developed with generally low toxicity toward beneficial insects and other new control methods for psylla IPM might be available in the future by the discovery of new semiochemicals, as well as the recently identified sex attractant pheromone in *C. pyricola* winterform males (Guédot et al., 2009). Otherwise, breeding pear trees for resistance to *C. pyri* offers an interesting, preventative and valuable alternative to chemical control.

Since the 1980s the creation of new pear cultivars with resistance to psylla has been included as an important objective in some European pear breeding programs (Italy: DCA-BO, ISF-Forlì, DISPAA-FI; France: INRA; Romania: RIFG – Pitesti-Maracineni; see references for definition of abbreviations) (Rivalta and Dradi, 1998; Bellini et al., 2000; Musacchi et al., 2005; Lespinasse et al., 2008); unfortunately the rapid transfer of resistance into cultivars with *Pyrus communis* type fruit is limited by the negative fruit characteristics (small size and/or gritty and course texture) of both East Asian pear species (*P. ussuriensis*, *P. pyrifolia*, *P. longipes*, *P. serotina* or *P. betulaefolia*) and interspecific hybrids between *P. communis* and *P. ussuriensis*, although these last have shown high levels of resistance to *C. pyri* (Braniste et al., 1994; Berrada et al., 1995; Bellini and Nin, 2002; Robert et al., 2004; Nin et al., 2012). The transfer of resistance to *C. pyri* by interspecific crosses between the European pear species *P. communis* and Asian pear species such as *P. ussuriensis* and *P. serotina* followed by modified backcross to part of the progeny was demonstrated to be possible (Pasqualini et al., 2006), but it occurs gradually and it is probably controlled by several genes. Many seedlings and selections have been obtained by the European genetic pear breeding programs, nevertheless, up to now only one promising advanced selection derived from a cross between 'NY 10353' and 'Doyenné du Comice' is under evaluation in Italy at the DCA-BO (Musacchi et al., 2005) and two new resistant cultivars have been obtained in Romania: 'Euras' from the interspecific hybridization of *P. serotina* and 'Oliver de Serres' and backcross with 'Doyenné d'Hiver', and 'Haydeea' from the intraspecific hybridization of 'Beurré Hardy' and 'Buerré Six' (Braniste et al., 1994).

Several authors investigated European pear genotypes in the past 20 years, but only a small number of cultivars, namely 37, have been reported to be resistant or highly tolerant to *C. pyri* infestation and damage (Nin et al., 2012). Most of these studies were based on field observations (Benedek et al., 2010; Berrada et al., 1995; Bouvier et al., 2011; Braniste et al., 1994; Briolini et al., 1989; Quarta and Puggioni, 1985; Robert et al., 1999; Robert and Raimbault, 2005; Sestras et al., 2009; Stamenković et al., 1994; Szabó et al., 2010), while only few studies were made by the introduction of psylla nymphs or adults in a mesh covered tunnel containing the pear plants (Baldassarri et al., 1996; Pasqualini et al., 2006; Robert et al., 2004). On the contrary, nearly all studies performed in USA on *C. pyricola* resistance were carried out in the laboratory by rearing psylla nymphs on plant material (Bell, 1984, 1992, 2003, 2013; Bell and Stuart, 1990; Berrada et al., 1995; Butt et al., 1988, 1989; Puterka et al., 1993; Quamme, 1984). Except for 3 cultivars of French origin and the Italian 'Spina Carpi', most of these genotypes are ancient local varieties from Hungary, former Yugoslavia and Romania. All these genotypes are important sources of resistance within the primary gene pool available for improving *P. communis* cultivars, but unfortunately have often relatively poor fruit quality and some of them, like 'Spina Carpi' does not transfer the resistance to its progeny (Rivalta and Dradi, 1998). Recently, Bell (2013) recommended the Serbian cultivar 'Erabasma', with similar fruit quality to that of 'Spina Carpi', for the development of resistant cultivars as transmitting resistance to nymphal feeding to its progeny to a greater degree than 'Spina Carpi' and the resistant East European 'Batjarka', 'Ilinjaca', and 'Zelinka'.

In many countries *ex situ* pear collections have been established in some important pear growing areas including a great diversity of local, national and foreign cultivars, mainly for evaluation of resistance to major diseases and insects, to be used as potential parents in breeding (Quarta and Puggioni, 1985; Braniste et al., 1994; Braniste and Militaru, 2008; Benedek et al., 2010). The aim of this study was to classify, in the frame of AGER project 'INNOVAPERO: Management and crop innovations for high-quality pear production', European pear germplasm according to its tolerance or susceptibility to *C. pyri* using phenotypical screenings and allocate resistant individuals with acceptable fruit quality to be used in future breeding programs or exploited in organic farming.

2. Materials and methods

2.1. Plant material

European pear genotypes were evaluated for their response to natural infestation to pear psylla (*C. pyri*) under untreated conditions. The field investigation was focused on 160 commercial cultivars and old varieties of national and international origin, preserved in the *ex situ* germplasm collection of the IVALSA Institute – CNR located in Follonica (Grosseto, Italy) (4 meters above sea level; 42°56′19″ N and 10°46′29″ E). The trees were planted in 2001 and were grafted on quince rootstock. There were 3 replications of each cultivar. All along the experimental period no methods for pest control was applied. Plants received supplemental water supply and cutting regimes were used for weed control.

2.2. Survey and scoring

All the trees were evaluated for psylla infestation 3 times annually from the first decade of May until September during the three-year period 2011–2013. The assessment of the susceptibility was carried out taking into consideration seven different variables namely: number of eggs, isolated small nymphs (instars 1, 2 and 3), large nymphs (instars 4 and 5) and adults, number and development of the colonies, amount of honeydew. The quantity of honeydew was evaluated by ratio of the infested shoots using a 5 grade scale as follows: 0 = no infestation; 1 = ratio of infested shoots ≤20%; 2 = ratio of infested shoots 20–40%; 3 = ratio of infested shoots 40–60%; 4 = ratio of infested shoots 60–80%; 5 = ratio of infested shoots ≥80%. The observations were carried out on the shoots and growing twigs inserted on 6 two–three-year-old branches per tree. In this scoring greater emphasis was given to the presence of feeding nymphs since feeding antixenosis has been established as the key factor of resistance to pear psylla damage (Bell, 1992, 2003; Bell and Puterka, 2004; Bell and Stuart, 1990; Benedek et al., 2010). The phytosanitary state of the whole tree was evaluated based on the intensity of defoliation, the amount of honeydew and the intensity of sooty mould (data not reported). Additional observations on plant growth and development (*i.e.* tree vigor and tree habit) were recorded on each tree and for each survey; moreover, the presence of antagonists and/or infestation of other insect pests that might have influenced the pear psylla infestation level was noted.

2.3. Statistical analysis

All the scored data were subjected to the analysis of variance (ANOVA), considering year of observation, period of survey and genotype as independent factors; the means were separated by pairwise mean comparison by Bonferroni's test for $p = 0.01$. The overall average values of all accessions for the studied parameters were submitted to principal component analysis in order to calculate the component loadings. Unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis was applied to group the genotypes in relation to their putative class of susceptibility to the disease taking into account the variables with high loadings for the first three principal components. Finally discriminant analysis was performed to confirm or disconfirm the membership of each genotype to the predicted group. All the statistics were performed by Systat 11 statistical package (Systat Software Inc., Richmond, CA, USA). The maximum score for each survey and each plant was also taken into consideration.

3. Results

The results of ANOVA performed taking into consideration as sources of variation the year, the period of survey and the genotype are shown in Table 1; the result of pairwise mean comparison test post ANOVA is also presented when significant for the source. Genotype, year of observation and period of survey were significant for all the analyzed parameters, except for the number of eggs; also their interaction was found to be highly significant for most variables.

The infestation was more severe in 2012 and the means of 6 out of 7 variables were significantly affected by the year (Table 1). Also the period of survey played a significant role on psylla infestation, although not all the scored variables had an identical trend over the season. While the number of small nymphs, large nymphs and colonies was higher in the first sampling (May/June) influencing the length of the colonies and the quantity of honeydew, the number of eggs did not differ during the season and the number of adults was found to be higher in the second period of observation (July). These results confirm that both the period of sampling and the repetition of the field observations over time and years are very important.

ANOVA elaboration (Table 1) showed that the response of genotypes to psylla attack was deeply affected by both the period of survey along the season and the year, these two factors strongly influencing the level of determined susceptibility and resistance. For this reason, the mean separation of genotypes regardless of year and period of survey was used for allocating the evaluated cultivar into different classes of susceptibility. The result of the principal component analysis performed using the overall mean data of all the genotypes for the seven variables is shown in Table 2. The total variance explained by the first two principal components was 54,44%. The main effect on the first principal component was due to the variable "number" and "length of the colonies", while on the second principal component the major weight was associated to the "number of small and large nymphs".

The UPGMA cluster analysis performed using these four variables clustered the 160 genotypes into several groups, graphically expressed by a dendrogram produced on the base of similarity coefficient. On the base of these dendrogram five clusters usable as putative classes of susceptibility ranging from 13 to 91 genotypes per group were selected (Table 3). The subsequent discriminant analysis performed on the genotypes to validate their assignment to the predicted groups showed that in total 72% of the genotypes was identified as belonging to the right class, as reported in Table 4, where it can be noticed that for the putative class I (Highly tolerant) all 13 cases were confirmed. Thirty-three percent of the genotypes belonging to the slightly susceptible cluster (class II) was instead included in the class I.

The mean values of the variables calculated for the genotypes belonging to each class of susceptibility are shown in Table 5, together with the lower and upper limits of each class. Almost 92%

Table 1
Analysis of variance of pear psylla infestation level on European pear genotypes. Data represent the mean and its standard error calculated for 160 accessions (3 replicated plants) grown in the *ex situ* germplasm collection in Follonica (Grosseto, Italy) scored three times a year during the 2011–2013 period. Different letters within the same column indicate significant difference by Bonferroni's test ($p < 0.01$) performed after ANOVA.

Sources of variation	Number of eggs	Number of small nymphs	Number of large nymphs	Number of adults	Number of colonies	Length of colonies (cm)	Amount of honeydew
2011	0.010 ± 0.060	0.046 ± 0.011 a	0.381 ± 0.039 b	0.039 ± 0.009 a	0.146 ± 0.020 a	0.683 ± 0.022 a	0.111 ± 0.011 a
2012	0.019 ± 0.012	0.743 ± 0.176 b	1.622 ± 0.229 c	0.244 ± 0.024 b	0.377 ± 0.043 b	0.389 ± 0.051 b	0.028 ± 0.321 b
2013	0.012 ± 0.009	0.139 ± 0.025 a	0.027 ± 0.008 a	0.022 ± 0.006 a	0.140 ± 0.026 a	0.151 ± 0.032 a	0.306 ± 0.031 a
Year	*p = 0.842*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*
Spring (May/June)	0.011 ± 0.010	0.543 ± 0.170 c	1.396 ± 0.190 b	0.069 ± 0.011 a	0.422 ± 0.045 c	0.492 ± 0.056 c	0.274 ± 0.030 b
Summer (July)	0.017 ± 0.010	0.269 ± 0.053 b	0.247 ± 0.061 a	0.207 ± 0.023 b	0.188 ± 0.027 b	0.166 ± 0.029 b	0.048 ± 0.013 a
Late summer (September)	0.012 ± 0.010	0.117 ± 0.021 a	0.387 ± 0.121 a	0.026 ± 0.006 a	0.053 ± 0.011 a	0.027 ± 0.006 a	0.011 ± 0.003 a
Period of survey	*p = 0.903*	*p = 0.013*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*
Genotype	*p = 0.896*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*
Genotype × Year	*p = 0.070*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*
Genotype × Period	*p = 0.165*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*
Genotype × Year × Period	*p = 0.110*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*	*p = 0.000*

Table 2
Component loadings of each scored variable on the first three principal component and percent of total variance explained by each of the three components.

Component loadings	Principal component		
	1	2	3
Number of eggs	0.274	0.487	**0.667**
Number of colonies	**0.902**	0.149	0.036
Length of the colonies	**0.836**	0.078	0.015
Number of adults	0.118	−0.105	0.556
Number of small nymphs	0.479	−**0.726**	−0.550
Number of large nymphs	0.552	−**0.576**	−0.030
Amount of honeydew	0.765	0.156	−0.017
Percent of total variance explained	38.88	16.56	15.12

Table 3
Distribution of pear genotypes according to their susceptibility to pear psylla infestation and damage.

Resistance/susceptibility level	Number of genotypes	Percent ratio
Class I – Highly tolerant/resistant	13	8.1
Class II – Slightly susceptible	91	56.9
Class III – Medium susceptible	21	13.1
Class IV – Medium high susceptible	18	11.3
Class V – Highly susceptible	17	10.6

of the accessions showed a certain degree of susceptibility, while around 8% of them could be defined as highly tolerant/resistant to psylla attack.

Class I – Highly tolerant/resistant. As much as 13 genotypes (8.1% of the total) (Table 6) were falling into the category of highly tolerant/resistant. Genotypes showing no psylla infestation were ancient pear cultivars, such as 'Fiorenza' (Italy), 'Forelle' (Germany), 'Président Drouard' (France) and 'Starking Delicious' (USA). Among them, 'Président Drouard' is characterized by high productivity, large fruit size and high resistance to handling and transportation, equal to long well established cultivars largely spread on our territory. The other accessions belonging to this class were 'Eletta Morettini', 'Gentile', 'Moscatellina', 'Pera Volpina VP7', 'Precoce di Cassano' and 'Precoce di Masi', the French 'Notaire Lepin' and 'Président Loubet'. All these cultivars were free of psylla nymphs throughout the triennium of observation, even if not completely free of adults. Evidently, the presence of some adult insects on these cultivars is erratic and it does not mean that females can lay their eggs and that colonies can develop on the plant.

Class II – Slightly susceptible. The bulk of the investigated cultivars were classified as slightly susceptible. Minor infestation appeared only in one or two years out of three, with the presence of few isolated small nymphs and large nymphs and mean value of both number of colonies and amount of honeydew lower than 0.1 (Table 5). All together the number of slightly susceptible cultivars were as much as 91 of the 160 ones investigated (Table 7). Large part of them were ancient cultivars of Italian origin, among them it is worth mentioning the interesting early ripening 'Precoce di Altedo' and 'Precoce di Fiorano', the late ripening 'Bella Angevina', 'Spadona' and 'Ammazzacavallo', the Tuscan 'Giugnolina', abundantly producing small and very aromatic fruits, 'Spadoncina' largely cultivated in the past in Southern Italy and still appreciated for fruit quality on local markets, and the famous 'Spina Carpi' also listed as highly tolerant by Braniste et al. (1994), Quarta and Puggioni (1985), Robert and Raimbault (2005) and Sestras et al. (2009). This group also includes the French late ripening 'Passe Crassane' and the commercially grown 'Abbé Fétel', 'Coscia', and 'Beurré Hardy', which on the contrary have been described as highly susceptible by other authors (Quarta and Puggioni, 1985; Stamenković et al., 1994).

Class III – Medium susceptible. The number of cultivars that suffered medium damage were 21 (Table 8); all of them showed rather profuse larvae, especially isolated nymphs, on shoots (overall average value of 1.7), honeydew on less than 40% of shoots and twigs and a small amount of black sooty mould on leaves and bark. The important English cultivar 'Conference', the French 'Doyenné du Comice', the American 'Highland' belonged to this class, together with old international varieties from Belgium, France and Italy. Maximum scores related to the number of isolated nymphs found on trees of genotypes belonging to this group were rather high, ranging from 10 to 43, while maximum values of the number of colonies varied from 2 to 6.

Class IV – Medium strong attack. Cultivars mainly infested by a high number of colonies of larvae (overall average value of 0.4) and with honeydew on large part of their shoots and twigs (mostly more than 40%) were 18 (Table 9). 'Butirra Rosata Morettini' and 'S. Maria Morettini', both Italian cultivars known for their good

Table 4
Jackknifed classification matrix of the 160 genotypes into the 5 putative classes of susceptibility.

Class of susceptibility	Putative class of susceptibility					
	I	II	III	IV	V	% Correct
Class I – Highly tolerant/resistant	13	0	0	0	0	100
Class II – Slightly susceptible	30	59	2	0	0	65
Class III – Medium susceptible	0	2	17	2	0	81
Class IV – Medium high susceptible	0	1	3	14	0	78
Class V – Highly susceptible	0	0	1	4	12	67
Total	43	62	23	18	12	72

Table 5
Average values of the seven variables used for psylla infestation assessment on 160 pear genotypes in relation to the class of susceptibility calculated by discriminant analysis. The values are the overall average mean of three plants per accession, three surveys per year and three years.

Cluster class of susceptibility	Number of eggs	Number of isolated small nymphs	Number of isolated large nymphs	Number of adults	Number of colonies	Length of colonies (cm)	Amount of honeydew
I – Highly tolerant	0	0	0	0.071 ± 0.030	0	0	0
II – Slightly susceptible	0.008 ± 0.006	0.122 ± 0.016	0.274 ± 0.025	0.100 ± 0.012	0.085 ± 0.009	0.080 ± 0.011	0.065 ± 0.011
III – Medium susceptible	0.015 ± 0.003	0.392 ± 0.068	1.664 ± 0.118	0.111 ± 0.042	0.278 ± 0.033	0.236 ± 0.042	0.161 ± 0.028
IV – Medium High susceptible	0.031 ± 0.003	0.694 ± 0.140	0.470 ± 0.074	0.120 ± 0.027	0.415 ± 0.045	0.492 ± 0.068	0.191 ± 0.046
V – Highly susceptible	0.037 ± 0.004	1.039 ± 0.046	2.426 ± 0.554	0.099 ± 0.053	0.841 ± 0.110	0.899 ± 0.135	0.301 ± 0.041
Genotype's average	0.014 ± 0.007	0.310 ± 0.006	0.676 ± 0.088	0.102 ± 0.009	0.221 ± 0.024	0.228 ± 0.028	0.111 ± 0.012

Table 6
List of the 13 pear accessions belonging to Class I – Highly tolerant/resistant to psylla infestation and damage during 2011–2013 (IVALSA-CNR, Follonica, Grosseto, Italy).

Cultivar	Country of origin and year of release	Notes
Buerré Bonamour	Unknown	
Eletta Morettini	Italy, 1963	Very susceptible according to Quarta and Puggioni (1985)
Fiorenza	Italy, 1974	Susceptible according to Quarta and Puggioni (1985)
Forelle	Germany, 1800	Susceptible according to Quarta and Puggioni (1985)
Gentile	Italy, very ancient	Soft fruit
Moscatellina	Italy, very ancient	
Notaire Lepin	France, 1860	
Pera Volpina VP7	Italy, local	Local germplasm from Tuscany
Precoce di Cassano	Italy, ancient	
Precoce di Masi	Italy, ancient	For local markets
Président Drouard	France, 1876	
Président Loubet	Unknown	
Starking Delicious	USA, 1953	Tolerant according to Quarta and Puggioni (1985)

Table 7
List of the 91 pear accessions belonging to Class II – Slightly susceptible to psylla infestation and damage during 2011–2013 (IVALSA-CNR, Follonica, Grosseto, Italy).

Cultivar	Country of origin and year of release	Notes
Abate Fétel Rugginoso	Italy, unknown	
Abbé Fétel	France, 1876	Very susceptible according to Quarta and Puggioni (1985)
Alexandrine Douillard	France, 1852	
Ammazzacavallo	Italy, ancient	For local markets
Passe Colmar	Belgium, 1758	
Angelica	Italy, 1839	
Baldwin	Unknown	
Bella Angevina	France, very ancient	Susceptible according to Quarta and Puggioni (1985)
Bergamote Crassane	France, very ancient	
Beurré d'Anjou	Belgium, 1819	For niche markets Very susceptible according to Quarta and Puggioni (1985)
Beurré de Naghin	Belgium, 1840	
Beurré Diel	Belgium, 1805	
Beurré Hardy	France, 1840	Very susceptible or susceptible according to Quarta and Puggioni (1985) and to Sestras et al. (2009)
Beurré Pammion	Unknown	
Beurré Sterckmans	Belgium, 1820	
Blanca de Aranjuez	Spain, ancient	Slightly susceptible according to Braniste et al. (1994)
Bon Chrétien d'Hiver	France, very ancient	
Bonne Louise d'Avranches	France, 1780	For niche market Very susceptible according to Quarta and Puggioni (1985)
Buerré d'Amanlis	France, 1862	Susceptible according to Quarta and Puggioni (1985)
Buerré d'Angleterre	Great Britain, very ancient	
Buerré de l'Assomption	France, 1865	Very tolerant according to Braniste et al. (1994)
Buerré Le Brun	France, 1863	
Buerré Six	Belgium, 1845	
Buona Luisa (Lucca)	Italy, ancient	
Butirra Precoce F4	Italy, 1960–70	Susceptible according to Sestras et al. (2009)
Butirra Precoce F7	Italy, 1960–70	
Campana (Lucca)	Italy, very ancient	
Catillac	France, 1675	
Cayuga	USA, 1920	
Cedrata Romana	Italy, 1876	
Coccitoia	Italy, very ancient	
Colette	USA, 1953	
Colmar d'Aremberg	Belgium, 1821	Slightly susceptible according to Quarta and Puggioni (1985)
Colorée de Juillet	France, 1857	
Coscia	Italy, very ancient	Very susceptible according to Quarta and Puggioni (1985)
Coscia Precoce di Modena	Italy, 1910	
Coscia Tardiva	Italy, 1910	
Dea	Italy, 1956	
Directeur Hardy	France, 1893	
Doyenné de Juillet	Belgium, very ancient	
Doyenné d'Alençon	France, 1810	
Duchesse Bérerd	France, 1905	Susceptible according to Quarta and Puggioni (1985)
Duchesse d'Angoulême	France, 1809	Very susceptible according to Quarta and Puggioni (1985)
Ercole d'Este	Italy, 1988	Susceptible according to Quarta and Puggioni (1985)
Fico di Treviso	Italy, ancient	
Fico di Udine	Italy, ancient	
Fragrante	Italy, 1956	Susceptible according to Quarta and Puggioni (1985)
Giugnolina	Italy, very ancient	
Goodale	USA, 1914	
Ladè Butterbirne	Germany, 1893	Slightly susceptible according to Braniste et al. (1994)
Laxton' Superb	Great Britain, 1913	

Table 7 (*Continued*)

Cultivar	Country of origin and year of release	Notes
Leopardo Morettini	Italy, 1967	
Madame Verté	Belgium, 1860	
Mellina	Italy, 1956	Susceptible according to Quarta and Puggioni (1985)
Mercedes	Italy, 1956	Susceptible according to Quarta and Puggioni (1985)
Merton Pride	Great Britain, 1959	Medium susceptible according to Quarta and Puggioni (1985)
Mirandino Rosso	Italy, 1700	
Moltke	Germany, 1875	
Monchallard	France, 1818	Susceptible according to Quarta and Puggioni (1985)
Mora	Italy, 1956	
Morettini 113	Italy, 1961	
Moscato San Pietro	Italy, ancient	For local markets
Muslo de Dama	Spain, unknown	
New York 8760	USA, 1960–64	Susceptible according to Quarta and Puggioni (1985)
Passe Crassane	France, 1845	Slightly susceptible according to Stamenković et al. (1994)
Patten	USA, 1922	
Pera dell'Orto	Italy, 1958	
Pera Volpina VP6	Italy, 2004	Local germplasm from Tuscany
Pero Codatorta	Italy, ancient	
Pirovano 202	Italy, 1956	
Precoce Baldassarri	Italy, unknown	
Precoce di Altedo	Italy, very ancient	
Precoce di Fiorano	Italy, 1955	Very susceptible according to Quarta and Puggioni (1985)
Prince Napoléon	France, 1865	
Rosina Tessaro	Italy, unknown	
S. Germano d'Inverno	Italy, very ancient	
S. Giovanni	Italy, very ancient	
S. Lazzaro a Grappoli	Italy, very ancient	
San Pietro	Italy, ancient	
Soldat Laboureur	Belgium, 1820	
Spadona	Italy, very ancient	Slightly susceptible according to Braniste et al. (1994)
Spadona di C. Madama	Italy, unknown	For niche market
Spadoncina	Italy, very ancient	For niche market
Spina Carpi	Italy, very ancient	For niche markets Slightly susceptible according to Quarta and Puggioni (1985), to Braniste et al. (1994) and to Sestras et al. (2009)
Starkrimson	USA, 1956	
Sucrée Rosée	Unknown	
Tarda	Italy, 1956	
Triomphe de Jodoigne	Belgium, 1843	Slightly susceptible according to Braniste et al. (1994) and to Sestras et al. (2009)
Ucciardona	Italy, 1889	
William d'Hiver	France, 1868	
Wintercitrone	Germany, unknown	

Table 8
List of the 21 pear accessions belonging to Class III – Medium susceptible to psylla infestation and damage during 2011–2013 (IVALSA-CNR, Follonica, Grosseto, Italy).

Cultivar	Country of origin and year of release	Notes
Alessandro Terzo	Unknown	
Allora	Italy, very ancient	
Beurré Durondeau	Belgium, 1825	Susceptible according to Quarta and Puggioni (1985)
Beurré Giffard	France, 1825	Slightly susceptible according to Braniste et al. (1994)
Beurré Liegel	Belgium, 1788	Slightly susceptible according to Braniste et al. (1994)
Campana	Italy, 1839	For niche market
Conference	Great Britain, 1885	Susceptible according to Quarta and Puggioni (1985) and to Sestras et al. (2009)
Coscia Precoce	Italy, 1910	
Doyenné du Comice	France, 1849	Very susceptible according to Quarta and Puggioni (1985) and to Stamenković et al. (1994)
Doyenné d'Hiver	Belgium, 1828	Slightly susceptible according to Braniste et al. (1994) For niche market Very susceptible according to Quarta and Puggioni (1985)
Eva	Italy, 1958	
Highland	USA, 1974	Susceptible according to Stamenković et al. (1994)
Impériale a Feuilles de Chêne	France, 1752	
Magness	USA, 1960	Resistant or moderately resistant according to Braniste et al. (1994) and to Stamenković et al. (1994)
Marguerite Marillat	France, 1874	
Martin Secco	Italy, very ancient	For niche market
New York 2480	USA, 1964	Susceptible according to Quarta and Puggioni (1985)
Pera Volpina VP1	Italy, 2004	Local germplasm from Tuscany region
Pirele	Unknown	
Spadona d'Inverno	Italy, 1894	
William Duchesse	Great Britain, 1841	

Table 9
List of the 18 pear accessions belonging to Class IV – Medium-high susceptible to psylla infestation and damage during 2011–2013 (IVALSA-CNR, Follonica, Grosseto, Italy).

Cultivar	Country of origin and year of release	Notes
Bella di Giugno	Italy, ancient	Susceptible according to Quarta and Puggioni (1985)
Butirra Rosata Morettini	Italy, 1960	For niche market
Capucci 17	Italy, 1948	
Charles Ernest	France, 1889	
Dirce	Italy, 1956	
Directeur Varenne	France, 1897	Very susceptible according to Quarta and Puggioni (1985)
Eva Baltet	France, ancient	
Clapp's Favorite	USA, 1860	
Fondante de Bois	Unknown	Highly susceptible according to Quarta and Puggioni (1985)
Gentilona	Italy, 1900	
Pero Ruggine	Italy, ancient	
Piero di Romagna	Italy, ancient	
Precoce di Luglio	Italy, ancient	
Rocha Portuguesa	Portugal, unknown	Resistant according to Braniste et al. (1994)
Saint Michel Archange	France, 1700	
Santa Maria Morettini	Italy, 1951	Very susceptible according to Quarta and Puggioni (1985)
Spino	Italy, very ancient	
Triomphe de Tournai	Belgium, 1868	

commercial characteristics, fell in this group, as well as the American 'Clapp's Favorite' and the important Portuguese cultivar 'Rocha Portuguesa', which has been conversely listed as resistant by Braniste et al. (1994).

Class V – Highly susceptible. The number of highly susceptible cultivars was 17 (Table 10), all of them suffering severe or very heavy damage by the pear psylla in almost all years, with many colonies formed by small and large nymphs, abundant honeydew (mostly on more than 60% of shoots) and black sooty mould on some fruits, leaves and bark, premature leaf drop. Overall average values for this class of susceptibility were the highest for all considered variable, except for the number of adults. The widespread cultivated 'William', 'Kaiser', and 'Curè' fell in this group, as well as the Italian 'Etrusca'. Peaks of infestation were generally high both for number of both small and large nymphs and number of colonies. For instance, 'William' showed the highest amount of small nymphs (more than 150) and the presence of 15 colonies; the same number of colonies was found on 'Summerking'. The highest quantity of nymphs was detected on 'Comte Lelieur', while 'Grata' had colonies developing for 21 cm in length.

4. Discussion

4.1. Effect of year of observation and period of survey

Pear psylla infestation was rather variable during the three years of study, with the heavier damage occurring in 2012. Other authors also reported that infestation level and damage can be greatly different in consecutive years (Bell, 2009; Benedek et al., 2010; Kocsisné et al., 2005; Quarta and Puggioni, 1985), but no correlations between the intensity of psylla attack and weather conditions were reported.

Although the evaluated germplasm collection is under organic management, with natural enemies of the psyllid potentially able to reduce the damage of this pest, the climate is probably the factor that largely affected the dynamic of populations of the insect. Among climatic factors, the temperature is usually considered the most important parameter affecting pear psylla development and adaptation and it is therefore used as driving variable for providing phenological models based on biological mechanism. Though moderate temperatures shorten the psylla developmental time and increase the number of generations, excessive summer temperatures and low humidity conditions cause severe nymphal mortality (Bonnemaison and Missonnier, 1956; Marshall, 1959; Wilde and Watson, 1962; Madsen et al., 1963; Savanelli, 1979). Slower nymphal development at the same temperature probably indicates less favorable plant conditions (*i.e.* quality or diseases) (Schaub et al., 2005). High temperatures (over 30 °C) cause a substantial reduction in fecundity and oviposition (McMullen and Jong, 1977). Estimates of egg-laying potentials for winterforms of *C. pyri*, nymphal development times and thermal thresholds are available in Bonnemaison and Missonnier (1956), Lyoussoufi et al. (1988) and Kapatos and Stratopoulou (1999). Under conditions of high temperature and low humidity, the honeydew may crystallize and entrap the young nymphs (Marshall, 1959; Wilde and Watson, 1962; Madsen et al., 1963). Moreover, large numbers of psylla nymphs may be washed from the pear leaves in areas that receive heavy amounts of rainfall, in fact installation of over-tree sprinklers to provide spring frost protection and summer irrigation recently has become popular in Italy as an alternative for pear psylla control (Civolani, 2012).

In this regard, it is likely that the high average temperatures recorded during the period of late winter – early spring of 2012 (Fig. 1) have foster a greater proliferation of psylla nymphs, also causing a lengthening of its periods of development, determining a marked scaling of all the ontogenetic stages and phases that partly overlapped each other in the months of May and June. The decreasing trend of infestations with the advancing of the summer was then essentially due to the high temperatures that characterized the summer months, especially July and August, with peaks higher than 35 °C, causing water stress to the plants, with tree growth reduction, light leaf browning, and significantly slowing the development of psylla and devitalizing most of the laid eggs. Particularly in 2012, there have been numerous heat waves; the most intense and lasting was detected in the period 16 June–15 July. The increase of maximum summer temperatures caused a sharp decline in soil moisture, due to the prolonged absence of rainfall from late May through August. In contrast, spring and summer seasons in 2013 were characterized by abundant rainfalls and temperatures often below the seasonal average until late June; these factors have lowered the density of the first generations of psylla and severely limited the following ones. In fact, the initial springtime pear psylla infestation is a benchmark for the subsequent population level but it can be mediated by biotic (*e.g.* natural enemies and disease) or abiotic factors like temperature and rainfall (McMullan and Jong, 2012), of increasing importance due to the earth climate change. Although the overall level of infestation scored in 2013 was very low, it is noteworthy that the presence of the insect was found on cultivars which were more infested in the previous biennium (2011–2012). In this contest relative humidity seemed not to play a determinant effect on infestation levels since monthly average values of RH showed a similar trend during the

Table 10
List of the 17 pear accessions belonging to Class V – Highly susceptible to psylla infestation and damage during 2011–2013 (IVALSA-CNR, Follonica, Grosseto, Italy).

Cultivar	Country of origin and year of release	Notes
André Desportes	France, 1854	
Beurré Dumont	Belgium, 1831	
Comte Lelieur	France, 1765	
Curé	France, 1760	For niche market
Etrusca	Italy, 1987	For niche market
Général Leclerc	France, 1950	Very susceptible according to Stamenković et al. (1994)
Grata	Italy, 1956	Very susceptible according to Quarta and Puggioni (1985)
Kaiser (Beurré Bosc)	France, 1830	Very susceptible according to Quarta and Puggioni (1985)
Le Lectier	France, 1889	
Olivier de Serres	France, 1851	Slightly susceptible according to Quarta and Puggioni (1985)
Pera Lauro	Italy, very ancient	
Président Mas	France, 1867	
Prof. Molon	Italy, 1956	Medium susceptible according to Quarta and Puggioni (1985)
Reimer Red	USA, 1961	Susceptible according to Quarta and Puggioni (1985)
Sierra	USA, 1969	Slightly susceptible according to Braniste et al. (1994)
Summerking	USA, unknown	
William	Great Britain, 1790	Very susceptible according to Quarta and Puggioni (1985), to Stamenković et al. (1994) and to Sestras et al. (2009)

three-year period of observation, typically ranging from near 40% to about 93% during May-September. Maximum humidity conditions also reached almost 100% during these months because of frequent rains. The months of July and August were the hottest and driest and average RH was usually slightly lower (respectively 37.5–87% RH and 34.5–82% RH) than in May, June and September, though small fluctuations were registered in some months, decreasing in May 2011 and June 2012 while increasing in July and August 2011.

Obviously, when the infestation pressure was low, smaller differences were detected among genotypes compared to when the infestation reached high levels. In agreement with other authors' observations (Benedek et al., 2010; Quarta and Puggioni, 1985), it

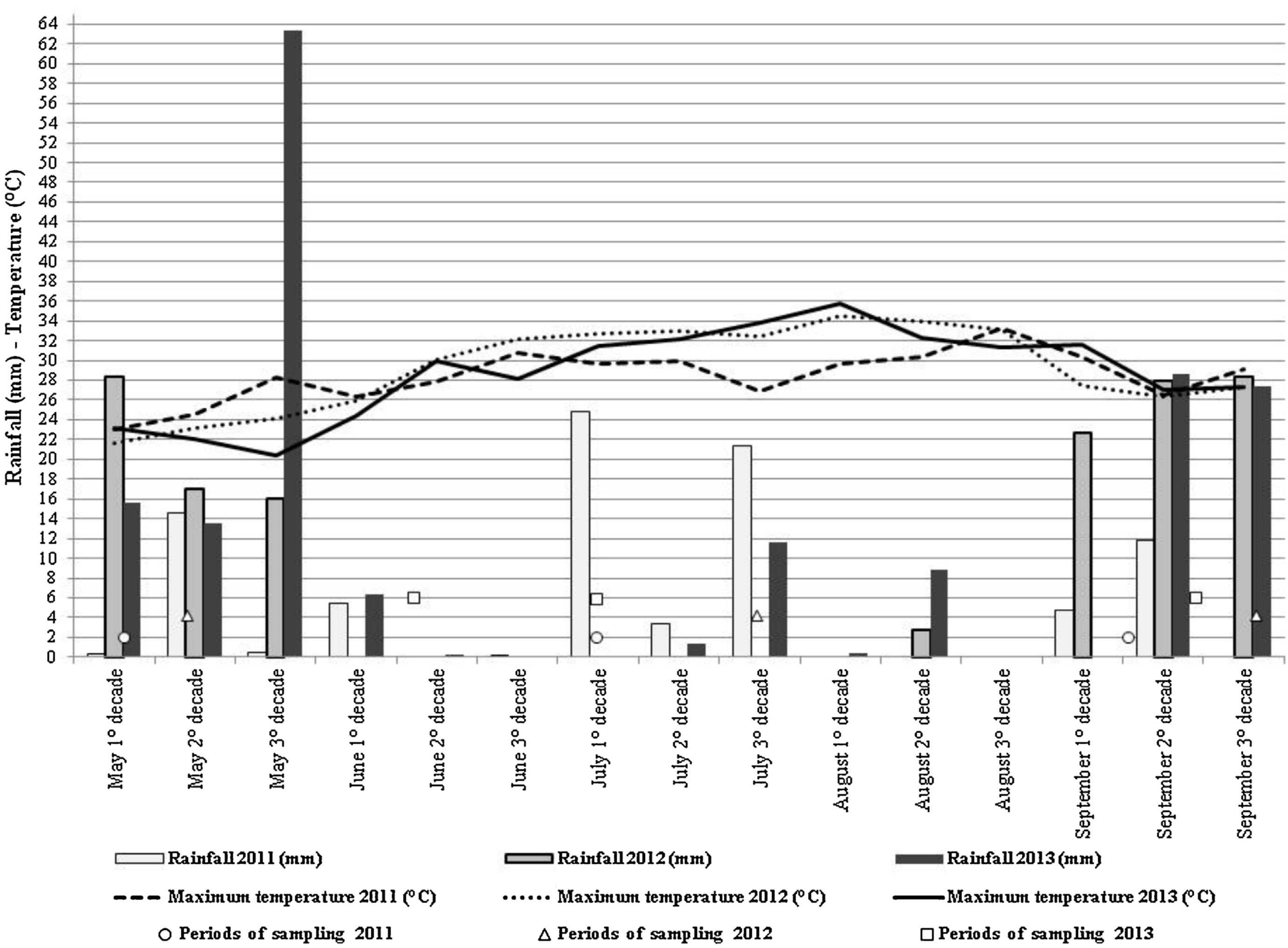

Fig. 1. Maximum temperature and rainfall recorded in the decades from May to September 2011–2013 in relation to the periods of survey on pear collection in Follonica (Grosseto, Italy).

was found that resistant genotypes were free of pear psylla infestation in the orchard even when other cultivars suffered medium or heavy damage, especially in 2012, year of heavy infestations. In years with slight or weak infestations, on the other hand, both resistant and susceptible genotypes can evenly be free of damage.

In site survey in the orchard can give reliable results when the investigation period is long enough to cover different years even with slight and/or heavy infestations (Benedek et al., 2010). On the other hand, low scores inevitably affects and reduces the final overall average records of genotype susceptibility, even in those cultivars that show peaks of infestation in some periods of survey. It is worth mention that in the present research, plants which were severely infested and suffered heavy damage in 2012 (data not shown), had a poorer growth during the following spring and a limited infestation, since slow growth offers at least initially an unfavorable environment to the insect, that might erroneously suggest they are resistant.

4.2. Genotype effect

On the whole, the number of resistant or highly tolerant cultivars made up the 8.1% of the investigated types, in agreement with other statements that no more than a minor portion of European pear genotypes show resistance or high tolerance to pear psylla damage (Benedek et al., 2010; Quarta and Puggioni, 1985; Sestras et al., 2009). The amount of slightly susceptible cultivars was 56.9% of the germplasm collection, while 24.4% of them were susceptible genotypes suffering medium or medium–high damage and 10.6% were highly susceptible cultivars that suffered heaviest damage. This pattern of distribution into the five classes of susceptibility showed that this character was poorly taken into consideration in the selection of cultivated European pears.

Quarta and Puggioni (1985) found no more than 12% of the inspected genotypes (cultivar and selection) of medium–low susceptibility and no one of them was immune. Additionally, no more than 3.5% of the 486 Hungarian cultivars investigate by Benedek et al. (2010) proved to be resistant and only 2.8% of them resulted highly tolerant to pear psylla damage; Sestras et al. (2009) found more than 80% of total analyzed cultivars and selections to be susceptible to psylla.

No more than 2 widely grown cultivars were slightly susceptible, 'Abbé Fétel' and 'Coscia', but these results are in contradiction with those of Quarta and Puggioni (1985) who listed the above-mentioned cultivars in the higher class of susceptibility. These authors also found to be highly susceptible other well know cultivars like 'Beurré Hardy', 'Belle Angevine', 'Buerré d'Anjou', 'Bonne Louise d'Avranches', 'Ercole d'Este', 'Eletta Morettini' and other 10 minor cultivars that resulted rather or highly tolerant in this study, besides 'Fiorenza' and 'Forelle' that were found to be immune to psylla damage in the present field condition. Furthermore, the same Authors listed as slightly susceptible 'Olivier de Serres' that resulted in the group of the very highly susceptible cultivars in this research. Very similar results were reported for other 14 cultivars, all listed as medium or highly susceptible (Tables 8–10).

The findings of this study are mostly in accordance with those reported by Sestras et al. (2009) for 5 out of 7 cultivars listed in different scale of susceptibility, while they were in great part in contrast with those indicated by Braniste et al. (1994) who described 'Doyenné du Comice', 'Sierra', 'Buerré Giffard', 'Buerré Liegel', and 'Blanca de Aranjuez' as slightly attacked by psylla and 'Magness' and 'Rocha Portuguesa' as not attacked by *Psylla* spp. Also Stamenković et al. (1994) classified the cultivar 'Magness' as moderately resistant, but found 'William', 'Doyenné du Comice', 'Général Leclerc' and 'Highland' to be very susceptible and 'Passe Crassane', a late ripening cultivar largely cultivated in Italy in the past, to be slightly susceptible in accordance with our results. On the other hand, 'Sierra' has been reported to be resistant by Quamme (1984), but his observation referred to *C. pyricola*, which has been only assumed to have a similar host-related biology to the closely associated *C. pyri* (Bell and Stuart, 1990).

Differences in cultivar susceptibility to pear psylla have been reported in previous studies by other authors. Kocsisné et al. (2005) classified as resistant many genotypes that Benedek et al. (2010) found to be at least slightly susceptible or susceptible during long-term investigation. Sestras et al. (2009) described 'Imperiale' as slightly susceptible and 'Butirra Precoce Morettini' as being highly susceptible, while Braniste et al. (1994) reported opposite results. Contradictory results have been reported for 'Dr. Guyot', which has been classified as poorly, medium and highly attacked by pear psylla by various authors (Braniste et al., 1994; Quarta and Puggioni, 1985; Stamenković et al., 1994). Differences may depend upon period and time of investigation, climate and environment, age of the tree and also the method of scoring the level of infestation. Size of the pear genotype collection also plays an important role, since psylla may have no possibility to exert a preference choice when only few palatable genotypes are present in the orchard (Bell and Puterka, 2004).

Moreover, pest population growth is strongly affected by plant vigor and quality (Daugherty et al., 2007). Nymphs on older leaves or on leaves injured by previous infestations may be unsuited for development; practices such as irrigation and fertilization influence tree growth with a remarkable effect on psylla population densities too (Westigard and Zwick, 1972). Overuse of nitrogen causes excess tree vigor, and highly vigourous trees are very suitable for growth and reproduction of pear psylla (McMullen and Jong, 1972; Pfeiffer and Burts, 1983).

Generally, the greater is the number of investigated cultivars, the less accurate are the field observations made on each single genotype. Mostly scoring/rating psylla infestation is based on subjective deliberations, while laboratory tests are usually performed by objective counts of eggs and nymphs. Some authors used a simple 0–5 scale for scoring, but without giving any information about number of eggs, nymphs, adults and honeydew (Braniste et al., 1994; Sestras et al., 2009). Other researchers counted hatched eggs and nymphs per leaf and expressed the degree of susceptibility directly *via* nymph/egg ratio and used a subjective scale from 0 to 5 for rating the amount of honeydew, sooty mould and defoliation for evaluating the degree of damage (Briolini et al., 1989; Stamenković et al., 1994). Benedek et al. (2010) counted the ratio of infested shoots using a 5 grade scale; similarly Quarta and Puggioni (1985) used a scoring based on the visual estimate of percentage of damaged shoots, but their 0–5 scale was very different to the previous one and they took into consideration also the presence of eggs, nymphs and adults. Finally, Bouvier et al. (2011) used a 0–5 grade scale according to the abundance of honeydew and quantity of nymphs present on the plant, expressed as a percentage of total tree. Robert et al. (2004) compared basically two different screening methods: the first one by scoring nymph presence (on leaves, shoots and upper parts of the shoots) and ranking the number of nymphs in four classes; the second one by recording symptoms of psylla damage (sooty mould quantities and shoot status) and ranking from 1 (no apparent injury) to 5 (one or several dead shoots). They demonstrated that the method based on injury symptoms resulted in a lower variability within pear genotypes and therefore was considered as the best indicator of the infestation to discriminate among accessions, at least from a commercial point of view. Although subjective ratings made in consecutive years have been reported to be correlated fairly well to objective counts (Bell, 2009), the method of screening pear genotypes adopted in this research was based on objective counts for four different variables such as number of both small and large nymphs, number and length of the colonies; moreover the classes of resistance/susceptibility were defined according to mean group separation while they were usually arbitrarily designated in other similar studies reported in world literature.

5. Conclusions

In this research a field investigation was carried out on 160 European pear genotypes, 110 of which have been never evaluated for their susceptibility to pear psylla in previous studies, while 50 of them were already screened for psylla resistance by different authors (Braniste et al., 1994; Quarta and Puggioni, 1985; Sestras et al., 2009; Stamenković et al., 1994). The results here reported are partially in agreement for approximately 52% of these cultivars, while the remaining 48% were listed according to the present study in different classes of susceptibility. In contrast to the description of 'Fiorenza' and 'Forelle' by Quarta and Puggioni (1985), our data indicate that these genotypes are not susceptible to pear psylla. Other two cultivars, 'Président Drouard' and 'Starking Delicious' did not suffer psylla attack in our field condition; 'Président Drouard' have been not screened before, while 'Starking Delicious' has been reported to be slightly susceptible by Quarta and Puggioni (1985). We have identified other 8 *P. communis* cultivars with high tolerance to pear psylla attack, falling into class I together with the above mentioned, but one of them ('Eletta Morettini') was considered as highly susceptible in previous screening (Quarta and Puggioni, 1985).

Unfortunately, the different environmental and tree growing conditions as well as the various methodologies applied for screening pear germplasm for resistance to psylla hardly reduce the comparability with previous studies. Indeed, feeding antixenosis has been established as the key factor of resistance (because it is associated with increased nymphal mortality and delayed nymphal development) and methods of rapid nymphal feeding bioassays have been developed to screen pear germplasm under controlled condition, but the development of a common standard protocol for evaluating field host resistance is still necessary.

On the other hand, it has been reported that cultivars which were not attractive to oviposition or considered 'resistant' under field conditions hosted high densities of both eggs and nymphs in cage-tests with psylla adults (Harris, 1971, 1973, 1975; Westigard et al., 1970). Therefore, additional observations of the cultivars in greenhouse studies with artificial infestation and under controlled conditions are needed to more completely characterize the resistance of these genotypes. Some of these cultivars might be used in breeding programs for resistance to psylla by crossing if our field results will be supported by observations from greenhouse experiments.

Acknowledgement

Supported by Progetto AGER, grant no. 2010–2107.

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