Resistance of Apple Cultivars to *Dysaphis plantaginea* (Hemiptera: Aphididae): Role of Tree Phenology in Infestation Avoidance

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ABSTRACT To test the importance of flowering phenology in damage caused to apple cultivars by rosy apple aphid, *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphididae), susceptibility of 16 cultivars was compared in greenhouse conditions after infestation with aptera and in the field after natural tree colonization. Flowering phenology was recorded in the field for all the cultivars. In both greenhouse and field trials, there were differences among cultivars with respect to aphid infestation and damage. However, damage in the greenhouse and field was not significantly correlated. Flowering phenology was also different among cultivars. Flowering order among cultivars was significantly negatively correlated with aphid infestation and damage in field, i.e., early leafing cultivars showed higher infestation and damage than late-leafing cultivars. If egg hatching occurs before bud bursting, neonate larvae will suffer a high mortality because they cannot feed on these late cultivars. A later recolonization of these trees is hampered because (1) winged aphids cannot live on apple but only on the secondary host, (2) apterous forms have a limited dispersal capability, and (3) aphid predators progressively increase in the orchard. Therefore, synchronization between egg hatching and bud bursting is of critical importance in the success or failure of infestation.

KEY WORDS flowering phenology, *Malus* x *domestica*, pest avoidance, plant resistance, synchronization

Rosy apple aphid, *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphididae), is a key apple pest in Europe and North America (Hull and Starner 1983, Blommers 1994, Miñarro et al. 2005). *D. plantaginea* is holocyclic, because this species alternates parthenogenetic with sexual reproduction, and heteroecious, because this aphid spends different stages of the life cycle on different, unrelated plants. At the end of spring, winged *D. plantaginea* leave apple trees, their primary host, and migrate to their secondary host, plantain, *Plantago* spp. In autumn, *D. plantaginea* return to apple trees, where sexual reproduction takes place and overwintering eggs are laid. In spring, these eggs hatch and parthenogenetic generations occur. At this stage, *D. plantaginea* causes irreversible damage to apple trees. Injury affecting leaves and branches becomes visible in susceptible cultivars within 24 h (Forrest and Dixon 1975). After a strong attack, infested fruit remains small and deformed, losing economic value.

Compared with other apple aphids, *D. plantaginea* has a low developmental threshold: 4.5°C compared with 5.5°C for apple-grain aphid *Rhopalosiphum insertum* Walker or 5.9°C for green apple aphid *Aphis pomi* De Geer (Graf et al. 1985). Therefore, eggs hatch early in the spring when apple buds burst. However, cultivars show wide differences in flowering phenology (Mehlenbacher and Voordeckers 1991, Dapena 1996). Bonnemaison (1959) observed that some apple cultivars whose buds break late may have low *D. plantaginea* infestation. Briggs and Alston (1967) reported the same for this and other apple pests such as *R. insertum*, or the apple sucker, *Psylla mali* Schmid. Thus, synchronization between pest and host is significant in the resulting pest fitness (Dixon 1998). However, the lack of synchronization is known to protect plants from pest infestation. Traditional agriculture relies on this method of pest avoidance by adjusting the planting or harvesting time to keep the pest damage below economic thresholds (Abate et al. 2000).

In Asturias (Northwest Spain), there is a large apple varietal richness linked to the cider industry (Dapena 1996). A program to assess and select apple cultivars is being developed, and some cultivars have already been selected for their resistance or low susceptibility to fungal diseases (Dapena 1994, 1996, Dapena and Blázquez 1994). The flowering phenology of many of these cultivars has also been studied showing wide variability (Dapena 1996). However, little was known about the susceptibility to insect pests. The susceptibility to *D. plantaginea* of some of these local cultivars was evaluated to determine the role of flowering phenology in the avoidance of aphid damage.

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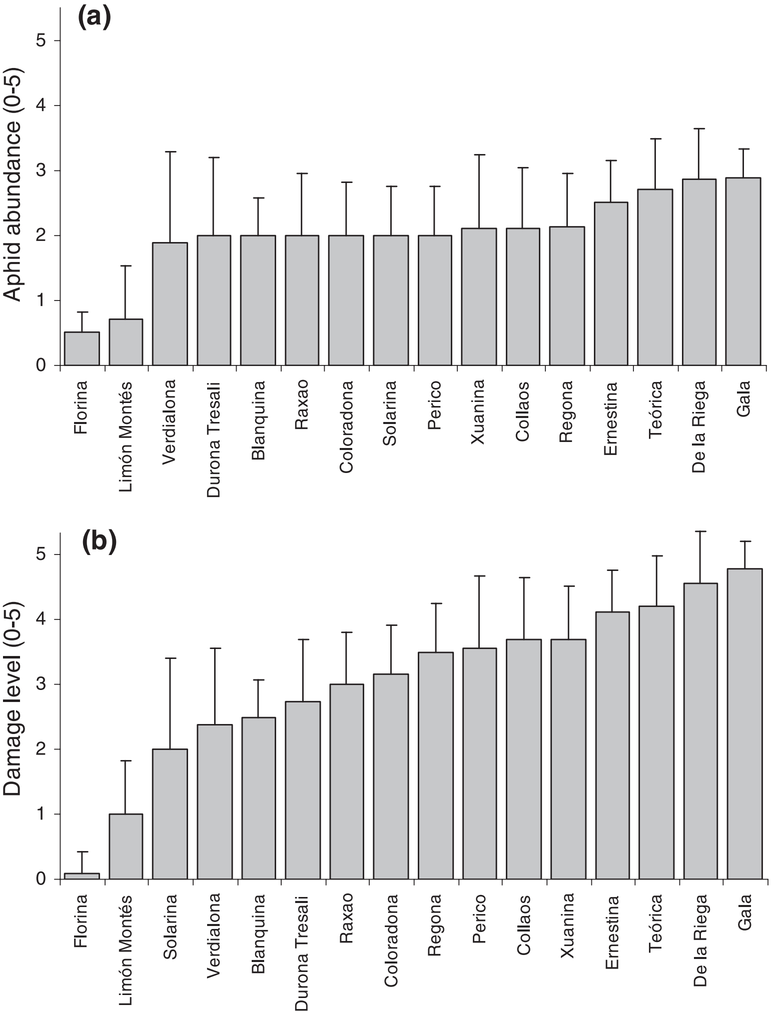

Fig. 1. Average level of aphid abundance (a) and damage (b) exhibited by the cultivars 21 d after infestation in greenhouse. Error bars are SDs. Shoot damage was coded from 0 to 5: 0 = no damage; 1 = leaf slightly curled at the edge; 2 = leaf curled longitudinally; 3 = typical *D. plantaginea* leaf rolling; 4 = 2–5 typically rolled leaves; and 5 = >5 typically rolled leaves. Aphid abundance was coded from 0 to 3: 0 = no aphids; 1 = 1–5 aphids; 2 = 6–25 aphids; and 3 = >25 aphids.

Materials and Methods

Plants were evaluated under greenhouse conditions after infestation with apterae to determine the intrinsic susceptibility of each cultivar. The same cultivars were also evaluated in the field under natural infestations.

Greenhouse Conditions

Plant Material. The susceptibility to *D. plantaginea* of 16 cultivars was studied in 1998: 14 local cider apple cultivars and 'Florina' and 'Gala', which were used as resistant and susceptible controls, respectively. Twenty plants of each cultivar were grafted on Pajam two rootstocks and kept outdoors in 4-liter pots. Plants were fertilized with 8 g of Osmocote plus. When new shoots were ≈25 cm, plants were introduced into the greenhouse. The number of replicates for each cultivar ranged between 4 and 10. The plants were randomly distributed in the greenhouse and irrigated three times a week. Aphid movements from one plant to another were prevented by putting the pots in dishes filled with water. Secondary shoots were periodically pruned to keep the aphids on the principal stem. There were no overlapping branches.

Aphids. Aphids were field-collected from 10 different apple cultivars to capture some of the natural variability. Individuals from each cultivar were reared separately on 'Golden delicious' apple plants. Thus, 10 distinct populations of *D. plantaginea* were maintained in the laboratory. When there were enough aphids for the infestation, four apterous adults or fourth-instar larvae were carefully placed with a small paintbrush on the youngest leaf of each plant. Each of the aphids on a plant came from a

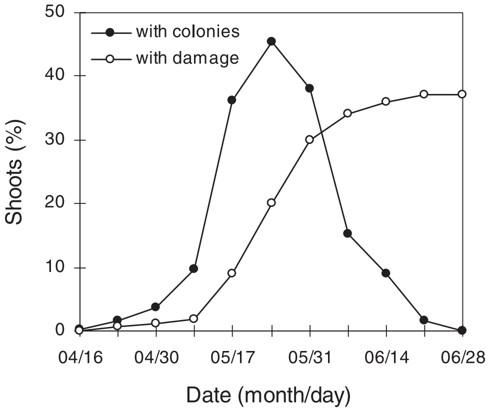

Fig. 2. Aphid occurrence and shoot damage in the field. Data from all cultivars are pooled.

different population. When necessary, reinfestations were performed during the first week of the experiment to make sure that the initial number of aphids was four per plant.

Damage Assessment. Observations were made three times a week from the day after the infestation to the end of the experiment, 21 d later. Shoot damage was coded from 0 to 5 based on Rat-Morris (1993): 0 = no damage; 1 = leaf slightly curled at the edge; 2 = leaf curled longitudinally; 3 = typical *D. plantaginea* leaf rolling; 4 = 2–5 typically rolled leaves; and 5 = >5 typically rolled leaves. Aphid abundance was described by four values: 0 = no aphids; 1 = 1–5 aphids; 2 = 6–25 aphids; and 3 = >25 aphids.

Orchard Studies

Studies were carried out in 1999 in a 0.3-ha experimental orchard at Villaviciosa, Asturias, Northwest Spain (43°30′ N, 5°30′ W), consisting of 5-yr-old apple trees growing on Pajam-2 rootstock. There were 16 apple cultivars randomly distributed into three blocks: the same 14 local cultivars tested in the greenhouse, as well as 'Golden Delicious' and 'Florina', which were used, respectively, as susceptible and resistant controls.

Four trees of each cultivar were selected before bud burst, and five buds on each tree were randomly chosen and marked with colored plastic strips. The presence of *D. plantaginea* colonies on the selected shoots was recorded every week from mid-April to the end of June. Aphid damage was recorded as in the greenhouse experiment. Tree phenology was recorded weekly following Fleckinger (1964), as it has habitually been recorded (Dapena 1996). Stage A of Fleckinger corresponds approximately with the dormant stage; B with silver tip stage; C_3 with green tip; D with half-inch green; D_3 with tight cluster; E with first pink; E_2 with full pink; F with first bloom; F_2 with full bloom; H with petal fall; and I with fruit set .

Neither insecticide, acaricide, nor fungicide was ever sprayed in this orchard to allow the development of natural infestation of the trees by the aphids.

Statistical Analysis

Cultivar differences in aphid abundance and plant damage were analyzed by Kruskal-Wallis tests. Mann–Whitney *U* tests were used to establish groups of cultivars using the Bonferroni adjustment for multiple tests (α = 0.05/number of pairwise comparisons). Spearman correlation coefficients were calculated to test the existence of statistical association between (1) damage in greenhouse and field, (2) flowering order and field infestation, and (3) flowering order and field damage. Because 'Florina' is resistant to *D. plantaginea* (Rat-Morris 1993), it was not included in the correlations. The susceptible control was not used in the correlations either because it was different in greenhouse ('Gala') and field ('Golden Delicious') conditions.

Table 1. Flowering phenology (Fleckinger 1964) in the field

Cultivar	16 April	23 April	30 April	7 May	17 May	24 May	Flowering order
Coloradona	F_1F_2	G	HI	IJ	—	—	1 (earliest)
Florina	E_2F_1	F_2	HI	IJ	—	—	2
Golden	E_2F_1	F_2	HI	IJ	—	—	3
De la Riega	E_1	F_1	G	I	J	—	4
Blanquina	D_3E_1	F_1	F_2G	HI	J	—	5
Ernestina	D_3E_1	F_1	F_2G	HI	IJ	—	6
Solarina	D_3	E_2	F_1F_2	GH	J	—	7
Teórica	D_3	E_2	F_1F_2	GH	IJ	—	8
Verdialona	D_3	E_2	F_1	G	I	—	9
Collaos	C_3D	E_1E_2	E_2F_1	F_2	I	—	10
Durona Tresali	C_3	D_3	E_2F_1	F_1F_2	I	—	11
Xuanina	CC_3	D_3E_1	E_2F_1	F_2	I	—	12
Perico	BC	D_3	E_2	F_1F_2	HI	IJ	13
Raxao	B	D	E_1E_2	F_1F_2	HI	IJ	14
Regona	—	C_3	D_3E_1	F_1F_2	GH	HI	15
Limón Montés	—	C	D_3	E_2	GH	HI	16 (latest)

Date on which the first aphid colony was observed on each cultivar is represented in gray. Last column reports the flowering order, from the earliest to the latest cultivar. (Stage B corresponds approximately with the silver tip stage; C_3 with green tip; D with half-inch green; D_3 with tight cluster; E with first pink; E_2 with full pink; F with first bloom; F_2 with full bloom; H with petal fall; and I with fruit set).

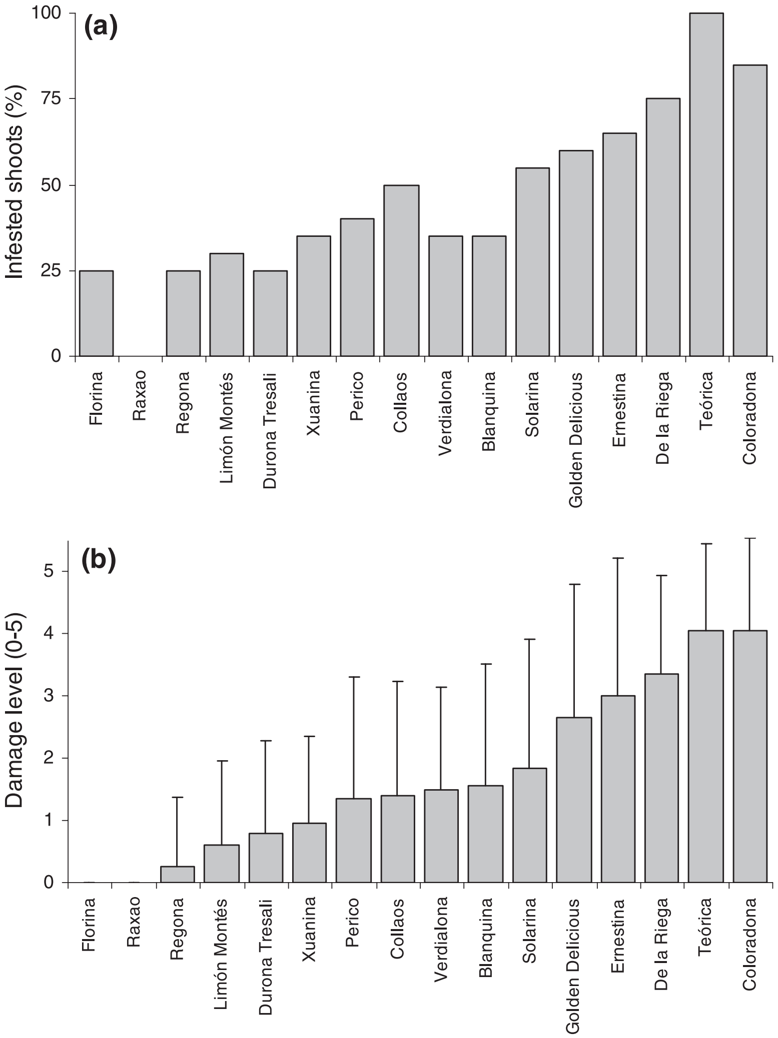

Fig. 3. Maximum percentage of shoots with aphid colonies (a) and damage (b) exhibited by the cultivars in the field. Cultivars are ordered by the flowering time, from the earliest to the latest. Error bars are SDs.

Results

Greenhouse Conditions

Aphid abundance ($\chi^2 = 79.43$; df = 15; $P < 0.001$) and damage level ($\chi^2 = 51.33$; df = 15; $P < 0.001$) at the end of the experiment revealed significant differences in susceptibility to *D. plantaginea* among cider apple cultivars (Fig. 1). However, none of the local cultivars were considered tolerant because all of them showed typical leaf-rolls. Groups of cultivars could not be statistically established using the Bonferroni adjusted α value of 0.0004 (0.05/120 pairwise comparisons). The response in terms of damage symptoms and the abundance of aphids of the control cultivars 'Florina' and 'Gala' confirmed the efficiency of the screening technique (Fig. 1).

Dysaphis plantaginea settled and developed well on plants; the first larvae were observed the day after the infestation and the first adults 10 d later. Damage induction by *D. plantaginea* was quick; the typical foliar deformation was already observed 4 d after the infestation on cultivars that would become the most susceptible at the end of the experiment.

Orchard Studies

Dysaphis plantaginea colonies occurred on the trees from mid April to late June (Fig. 2). Population peaks occurred in late May when 45% of the observed shoots in the orchard were infested by *D. plantaginea.* Field trials showed differences in the flowering phenology among cultivars as well as in the date at which the first colony was observed; *D. plantaginea* colonies developed earlier on early-leafing cultivars than in late-leafing cultivars (Table 1). Aphid infestation and damage varied greatly among cultivars (Fig. 3). Damage depended significantly on cultivars ($\chi^2 = 134.63$; df = 15; $P < 0.001$). However, no groups could be consid-

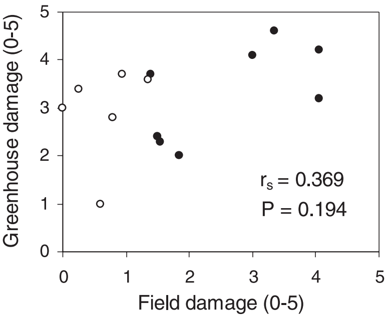

Fig. 4. Correlation between damage in greenhouse and field (open circles indicate the latest-leafing cultivars).

ered after the Bonferroni adjustment. Some of the local cultivars showed a damage response as low as the resistant control 'Florina,' whereas other cultivars suffered higher damage than the susceptible control 'Golden Delicious.'

Damage in greenhouse and field were not significantly correlated ($r_s = 0.369$; $n = 14$; $P = 0.139$; Fig. 4). However, there were significant negative correlations between flowering order and aphid infestation ($r_s = -0.758$; $n = 14$; $P < 0.01$) and between flowering order and field damage ($r_s = -0.899$; $n = 14$; $P < 0.001$; Fig. 5).

Discussion

Differences in infestation and damage in greenhouse conditions indicate that Asturian local cultivars are not equally susceptible to *D. plantaginea.* Chemical composition of leaves may affect herbivore performance on plants (Slosser et al. 2004, Pontius et al. 2006). In the case of *D. plantaginea,* foliar phenolic composition may play a role in the resistance of 'Florina' (Rat-Morris 1994). Quantitative and qualitative differences in foliar polyphenols composition have been observed in Asturian cultivars (Piccinelli et al. 1995). However, sap composition is likely more important in aphid resistance mechanisms than leaf composition (Dixon 1998). Sap comparisons in cultivars with different level of resistance may help to understand the resistance mechanism to *D. plantaginea* exhibited by some cultivars (Rat-Morris 1993, Dapena and Miñarro 2001, Miñarro and Dapena 2004).

However, although there were also significant differences in infestation and damage among cultivars in the field, results did not coincide with those of the greenhouse. On one hand, damage was significantly higher in the greenhouse than in the field, where only one cultivar showed more intense symptoms. This might be because the initial infestation in the greenhouse was high, because greenhouse-grown leaves could be thinner and more delicate or because predation or meteorology could interfere with the colony development in the field. However, field susceptibility was negatively correlated with the flowering time. Thus, late-leafing cultivars showed significantly lower infestation and damage than earlier cultivars.

Apple trees have evolved in part to regulate their development with the climatic conditions. However, there are strong differences in tree phenology among cultivars, including the chilling and heat requirements to budbreak (Mehlenbacher and Voordeckers 1991, Labuschagné et al. 2002). In this study, there is nearly 3 wk of difference in the flowering phenology and almost all the cultivars burst after 'Golden Delicious'. Dapena (1996) found that 86.5% of Asturian local cultivars burst later than 'Golden Delicious'. This may be adaptive in Asturian winters with alternating cold and mild periods (Dapena 1996). High chilling requirements avoid bud breaking during mild periods and the consequent damage to young tissues caused by following cold spells. Similarly, *D. plantaginea* evolved to synchronize its life cycle with the seasonal rhythm of its host. If egg hatching occurs before bud bursting, the neonate larvae will not be able to feed on that cultivar and probably will die. Bonnemaison (1959), Hull and Starner (1983), Rat-Morris (1994), and Graf et al. (2006) have reported that *D. plantaginea* eggs hatch early in the season and over a short period of time. Therefore, if a cultivar bursts bud after the egg hatching period, it will avoid pest infestation. As in this study, Bonnemaison (1959) and Briggs and Alston (1967) also observed that earlier cultivars were more infested than cultivars with late bud development. *D. plantaginea* is a dioecic species that needs winged forms to move from apple to the secondary host. However, those winged forms cannot feed nor reproduce on apple trees, and thus, dispersal to other shoots or trees is restricted to apterous forms, which have a limited dispersion capability. Moreover, as the pres-

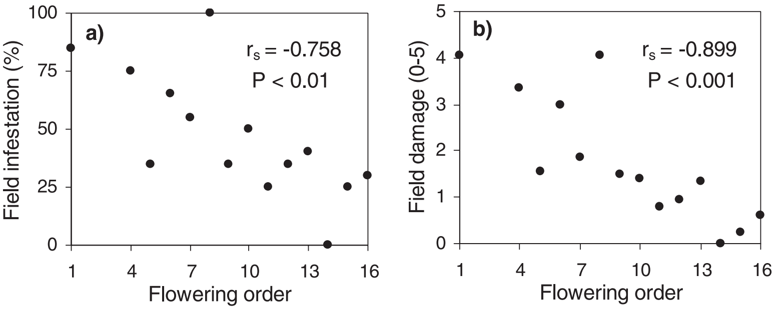

Fig. 5. Correlations between flowering order and the percentage of infested shoots (a) and field damage (b).

ence of aphid natural enemies increases progressively in the orchard (Miñarro et al. 2005), the viability of those apterous forms would be also progressively reduced and the recolonization of late-leafing cultivars more difficult. Thus, late-leafing cultivars suffer lower aphid infestation and damages.

This research showed how some characteristics such as plant phenology may be as important in the plant response to pests as the intrinsic level of susceptibility. In summary, late-leafing apple cultivars are less vulnerable to *D. plantaginea* in Asturias, Spain. Thus, apple growers could reduce pesticide use against this apple pest through the cultivation of late-leafing cultivars.

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