Bulletin of Insectology ***63*** *(2): 265-271, 2010*
ISSN 1721-8861

Probing behaviour of *Myzus persicae* on tomato plants containing *Mi* gene or BTH-treated evaluated by electrical penetration graph

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Abstract

The probing behaviour of the green peach aphid, *Myzus persicae* Sulzer (Rhynchota Aphididae) was evaluated by electrical penetration graph (EPG-DC) on the tomato (*Solanum lycopersicum* L. syn. *Lycopersicon esculentum* Mill.) cultivar Motelle, containing the *Mi* gene conferring resistance to the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Rhynchota Aphididae), to test the resistance degree of the cultivar to *M. persicae*. The aphid probing behaviour was also evaluated on a susceptible (*mi*) tomato cultivar, Moneymaker, after treatment by a chemical plant resistance elicitor, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (benzothiadiazole or BTH). Concerning a possible antixenotic effect due to physical and chemical barriers, no significant differences were found between the two cultivars in the probing and phloem phases. However, a difference was detected between the preinfested and non-preinfested susceptible cultivar in the total duration of phloem ingestion. The lack of significant differences in the entire process of host feeding between resistant and susceptible cultivars is probably due to the fact that the resistant cultivar identifies only the specific elicitors produced by *M. euphorbiae.* By contrast, the BTH treatment apparently makes the susceptible cultivar less palatable to a generalist aphid like *M. persicae*: the main component of this induced resistance is the reduced phloem ingestion.

Key words: *Myzus persicae*, *Solanum lycopersicum*, EPG-DC, insect plant resistance, *Mi* gene, chemical elicitor, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester, Motelle, resistance induction.

Introduction

The green peach aphid *Myzus persicae* Sulzer, the potato aphid *Macrosiphum euphorbiae* (Thomas), the melon aphid *Aphis gossypii* Glover and the black bean aphid *Aphis fabae* Scopoli (Rhynchota Aphididae) represent the most common aphid species infesting tomato crops *Solanum lycopersicum* L. syn. *Lycopersicon esculentum* Mill. (Solanaceae). All these species, except *M. euphorbiae*, are notably polyphagous. Usually settling in immediately after crop transplant, their colonies stunt plant growth and induce water stress that causes the wilting of leaves, flower buds and young fruits. The aphids ingest the phloem sap through piercing mouthparts and the resulting damage becomes serious only when pest density is high and leaves are covered by large aphid colonies that produce abundant honeydew on which sooty moulds rapidly develop (Blackman and Eastop, 1984). Aphid damage is mainly due to viral transmission, caused not only by the structure of mouthparts, but also by high aphid mobility associated with a typical feeding behaviour that involves random probing (Tjallingii and Hogen Esch, 1993, Powell *et al*., 2006).

The green peach aphid, *M. persicae*, is a Palearctic species now common in most world regions. It is characterized by high polymorphism and extremely high polyphagy (Blackman and Eastop, 1984). The aphid life cycle develops on one primary host, the peach tree, and many herbaceous plants, both cultivated and wild, as the secondary ones. There are about 440 of the latter herbaceous species belonging to about 40 families, including Solanaceae. *M. persicae* is a vector of about hundred viruses and its pathogenicity depends on season, attaining a peak during flights of winged virginoparae, which reach different herbaceous plants and increase the probability of viral infection (Van Emden *et al*., 1969).

Defence measures against the green peach aphid must therefore be timely distribution of specific aphicides when the first winged individuals appear. However, the aphicide treatments used to control aphids to avoid viruses are useful against "persistent" viruses but useless against "non-persistent" ones, among which the most relevant for tomato is CMV (Tomlinson, 1987; Francki *et al*., 1991; Ng and Falk, 2006). Current crop protection strategies include chemical approaches, that use pesticides which are toxic to beneficial insects, and genetic approaches that involve the incorporation of resistance genes into the plant germoplasm. Work on tomato aimed at inducing resistance to the root-knot nematodes *Meloidogyne* spp. introduced a single gene (*Mi*) in *S. lycopersicum* from the wild relative *Lycopersicon peruvianum* (L.) Mill.: the gene confers resistance to nematodes and also to the potato aphid, *M. euphorbiae* (Milligan *et al*., 1998; Rossi et al., 1998; Martinez de Ilarduya et al., 2003) and to the silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Rhynchota Aleyrodidae) (Nombela *et al*., 2003). It was previously thought that the resistance to the aphid was due to the presence of another gene, closely related to *Mi*, called *Meu 1*. The *Mi* gene was later cloned and the transformation of the tomato plant by this gene revealed that *Mi* and *Meu 1*

are the same gene (Milligan *et al*., 1998; Rossi *et al*., 1998; Goggin *et al*., 2001). *Mi* belongs to one of the major families of resistance genes encoding proteins containing nucleotide binding sites and leucine-rich repeats (NBS-LRR) conferring plant resistance to pathogens such as bacteria, fungi and viruses (Hammond-Kosack and Jones, 1996; Milligan *et al*., 1998). The *Mi* gene confers resistance to nematodes and other pathogens through a hypersensitivity reaction (HR) at the point of penetration. The resistance to *M. euphorbiae* is characterized by reduced longevity and fertility and causes rapid death 24 hours after exposure to resistant plants: literature data suggest that the resistance involves modifications of the feeding behaviour of the aphid. The probing behaviour of *M. euphorbiae* on tomato containing the *Mi* gene was evaluated by alternating current electrical penetration graph (EPG-AC) (Kaloshian *et al*., 2000) and direct current electrical penetration graph (EPG-DC) (Palliparambil *et al*., 2007): in the first case only a marked reduction of phloem ingestion was observed, while in the second one an antixenotic effect in peripheral tissues such as epidermis and mesophyll was also detected.

A number of researchers have proposed the use of plant resistance elicitors ("signals") to control arthropod pests and diseases in agriculture (Karban and Baldwin, 1997; Inbar *et al*., 1998; Thaler *et al*., 1999). The approach involving chemical analogues to plant signals was successful because these compounds were more effective in inducing resistance and had low toxicity (Karban, 1999). Among these compounds, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (benzothiadiazole or BTH), a synthetic analogue of salicylic acid (SA), produces no direct effect against pests but induces resistance in the target plants. Application of BTH on *Arabidopsis thaliana* (L.) Heynh. led to a decrease in *M. persicae* reproduction (Moran and Thompson, 2001). BTH and other elicitors were employed on tomato (Cooper *et al*., 2004; Boughton *et al*., 2006): BTH reduced the growth of *M. euphorbiae* and *M. persicae* populations in comparison to untreated controls, apparently because of reduced aphid fertility.

Our study aims to evaluate the effect of *Mi* gene on *M. persicae*, by examining the probing behaviour of the aphid by EPG-DC on the Motelle cultivar (*Mi*), in comparison to Moneymaker (*mi*), susceptible to *M. persicae* and *M. euphorbiae*. Differences between these two cultivars were also evaluated by preinfestation of both cultivars with *M. persicae* 96 h before the trial. The induction of resistance to *M. persicae* after BTH treatment was also evaluated by EPG (EPG-DC) on Moneymaker.

Materials and methods

Plants

Three-week plants of tomato of the susceptible cultivar Moneymaker (*mi*) and of the cultivar Motelle (*Mi*), resistant to *M. euphorbiae* were grown under greenhouse conditions (22-24 °C) in pots containing a mixed soil, watered daily and fertilized each week (Fito Universale®, Guaber, Bologna, Italy).

Collection and preparation of insects

Individuals of the apterous form of *M. persicae* were reared in environmental growth chamber 21 ± 1 °C (L16:D8 photoperiod) on aphid-susceptible tomato cultivar Moneymaker at the Department of Agroenvironmental Sciences and Technologies, University of Bologna (Italy). At the beginning of each trial, the aphids were carefully collected from infested tomato leaves with a thin brush and gently immobilized by a small vacuum air sucker.

Plant preinfestation

Aphid parameters were investigated also on plants that had been previously infested (preinfested) or not by *M. persicae*. Preinfestation was performed by individually placing about 20 apterous adults on ten different leaflets of a single plant and removing them and their progeny 96 h later, about 1 h before EPG-DC recording. No other individuals were added during preinfestation.

BTH applications

A commercial solution of BTH (Bion® 50 WG; Syngenta Crop Protection, Milan, Italy) was dissolved in distilled water. The concentration applied was 125 mg l^{-1}. Plants of susceptible cultivar Moneymaker were randomly assigned to treatment and removed from the greenhouse prior to solution applications in open field by hand atomizers. Plants were sprayed until runoff of leaves, left to dry for 1 h and then returned to the greenhouse for 4 days before EPG-DC recording.

EPG-DC recording

EPG-DCs of aphids on aphid-susceptible Moneymaker and aphid-resistant Motelle cultivars, both preinfested and non-preinfested with *M. persicae*, along with further tests on BTH only on susceptible Moneymaker, were performed in spring (from March to April) in the laboratory at 21 ± 1 °C and artificial fluorescent HF light (4000 Lux) with a L16:D8 photoperiod. Aphids were recorded for 12 hours. The insects were individually placed on the lower surface of terminal plant leaflets at pre-bloom, i.e. when plants were about 25 cm tall with at least ten leaves. Before each experiment, test aphids were carefully brushed from the infested tomato leaves on which *M. persicae* was reared. On the dorsum of each insect, gently immobilized by a vacuum device, a small drop of electrically conductive glue was applied and a thin (20 μm) gold-wire electrode about 2 cm long was attached. All these steps were performed under a stereomicroscope.

The EPG device used was a Giga-4 model (Wageningen Agricultural University, the Netherlands) with an input resistance of 1G Ω (Tjallingii, 1985a; 1985b). After A/D conversion at 100 Hz (Di710 USB, Dataq, Akron, Ohio, USA), the EPG signals were stored on a computer hard disk, data acquisition was mediated by PROBE 3 software (for Windows; Wageningen Agricultural University, the Netherlands) and signals were analysed using the same software. The variables measured were the same as in Tjallingii (1978) and accordingly indicated.

Data analysis

EPG-DC features were split in non-probing variables, probing variables, and phloem variables. Means and standard errors of the mean (SEM) of variables were calculated on each treatment and on each individual tested, and differences were analysed by the non-parametric Mann-Whitney *U*-test (software STATISTICA 6, StatSoft, Tulsa, Oklahoma, USA). Fisher's exact test was applied to analyse the number of aphids showing phloem ingestion (STATISTICA 6).

Results

Comparison of preinfested and non-preinfested cultivars

After preinfestation, besides the first apterous adults, also *M. persicae* offspring was observed on both Moneymaker and Motelle cultivars, uniformly distributed on all plant leaves.

The non-probing variables over 12 hrs of EPG-DC recording of *M. persicae*, on preinfested and non-preinfested Moneymaker and Motelle, are shown in table 1. No significant differences were detected between cultivars throughout the total duration of non-probing (np, variable n 1). Significant differences were detected between the non-preinfested Moneymaker and the same preinfested cultivar in the number of non-probings (variable n 2; 36.41 ± 2.63 *vs* 55.81 ± 6.41, respectively; P = 0.01). The mean np duration, i.e. the ratio between total duration expressed in seconds and the detected frequency, showed a statistically significant difference between preinfested Moneymaker and preinfested Motelle (variable n 3; 127 ± 26s *vs* 157 ± 10s, respectively; P = 0.003). No significant differences were detected between the two cultivars in duration of first (1st np) and second (2nd np) non-probing (variable n 4 and 5, respectively). No significant differences were detected in the duration of the first non-probing after first E2 between the susceptible and resistant cultivars, whether preinfested or non-preinfested (variable n 6).

Table 2 shows a comparison of probing variables over 12 hours of EPG recording of *M. persicae* on preinfested and non-preinfested cultivars. No significant differences were detected between the susceptible and resistant cultivars, whether preinfested or non-preinfested, for the total duration of stylet intercellular penetration across non-phloem tissues (ABC, variable n 8) for the total duration of potential drops (pd, variable n 18), the total duration of xylem ingestion (G, variable n 15) or the total duration of derailed stylet mechanics (F, variable n 12). By contrast, statistically significant differences were detected between the susceptible and resistant cultivars in duration of the first probing (ABC + pd + E), the duration being much longer in the susceptible than in the resistant cultivar (variable n 11; 3854 ± 1544s *vs* 194 ± 61s; P = 0.01).

The frequency of the different waveforms over 12 hours of recording shows significant differences between the preinfested susceptible cultivar and the same non-preinfested in the number of xylem ingestions (G, variable n 16; 2.25 ± 0.30 and 3.71 ± 0.29, respectively, P = 0.01). Other statistically significant differences were found between the preinfested susceptible and resistant cultivar in xylem ingestions (G), which were lower in Moneymaker than in Motelle (variable n 16; 2.25 ± 0.30 *vs*. 2.94 ± 0.27; P = 0.02).

Table 3 shows a comparison of the phloem phase over 12 hrs of EPG-DC recording of *M. persicae* on preinfested and non-preinfested cultivars. The only significant difference in total duration was detected in phloem ingestion (E2) between the preinfested Moneymaker and the same cultivar non-preinfested: E2 was longer in preinfested than in non-preinfested plants (variable n 22; 3320 ± 887s *vs* 1554 ± 337s, respectively; P = 0.04). Concerning the frequency of the different waveforms over 12 hours of recording, we found significant differences in the number of E1 (variable n 23; 18.35 ± 2.38 and 16.00 ± 2.54; P = 0.007) and E2 (variable n 24; 2.71 ± 0.63 and 8.06 ± 1.80; P = 0.002). No significant differences were detected in time to 1st E from the beginning of that probe (variable n 27), the number of preceding 1st E1 (variable n 28), and the duration of the 1st E1. However, significant differences were detected between Moneymaker and Motelle in the duration of the 1st E2, which was higher in the former than in the latter (variable n 31; 625 ± 131s *vs* 264 ± 59s, respectively; P = 0.04).

No significant differences were detected in such variables as the time to 1st E2 from start penetration (variable n 29) and the number of penetrations preceding 1st E2 (variable n 30). The percentage of aphids with phloem ingestion (E2) (variable n 32) was higher on preinfested (100%) than in non-preinfested Moneymaker (82.35%). The same rate difference in Motelle was not significant between preinfested (84.20%) and non-preinfested plants (81.25%). Notably, preinfested Moneymaker was the only one in which all tested aphids ingested sap from the phloem during the trials.

Comparison between BTH-treated and untreated Moneymaker

Table 1 shows a comparison of data of the non-probing phase over 12 hrs of EPG-DC recording of *M. persicae* on untreated Moneymaker and the same BTH-treated cultivar. The duration of the first non-probing (1st np, variable n 4) and the second non-probing period (2nd np, variable n 5) were not significantly different between untreated and BTH-treated cultivar (P = 0.06). However, a significantly higher number of non-probing (np, variable n 2; P < 0.001) was detected in BTH-treated Moneymaker in comparison to untreated cultivar.

Table 2 shows a comparison of data of the probing phase over 12 hrs of EPG-DC recording of *M. persicae* on untreated Moneymaker and the BTH-treated cultivar. Significant differences were found in the total number of probes (ABC, variable n 10; P = 0.001), in the total number of pd (variable n 19; P = 0.002) and in the duration of individual pd (variable n 20; P < 0.001) between untreated and BTH-treated cultivar.

Table 3 shows a comparison of the phloem phase over 12 hrs of EPG-DC recording of *M. persicae* on untreated and BTH-treated Moneymaker. Significant differences between untreated and BTH-treated plants oc-

Table 1. Comparison of non-probing (np) variables (mean ± SEM) measured by EPG-DC (12 h of recording) of *M. persicae* on tomato. Time in seconds; n = number of EPG-DC replicates.

Variable number	EPG-DC variable	Cultivar	Moneymaker		Preinfested Moneymaker		Motelle		Preinfested Motelle		Moneymaker + BTH		Moneymaker vs preinfested Moneymaker	Moneymaker vs Motelle	Motelle vs preinfested Motelle	Preinfested Moneymaker vs preinfested Motelle	Moneymaker vs Moneymaker + BTH
			(n = 17)		(n = 16)		(n = 19)		(n = 16)		(n = 12)						
			mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	P level	P level	P level	P level	P level
	Non-probing phase																
1	total duration of np		5381	578	6201	1124	6921	1060	6312	487	6303	794	0.18	0.47	0.50	0.11	0.32
2	total number of np		36.41	2.63	55.81	6.41	41.74	4.41	42.44	4.06	58.08	5.04	0.01*	0.48	0.64	0.12	<0.001***
3	mean duration of np		156	18	127	26	219	57	157	10	109	11	0.08	0.98	0.32	0.003**	0.09
4	duration of 1st np period		204	32	360	108	347	135	209	41	132	42	0.66	0.86	0.81	0.55	0.06
5	duration of 2nd np period		109	26	49	8	1803	824	99	23	157	58	0.06	0.11	0.11	0.06	0.06
6	duration of np after 1st E2		187	53	133	30	116	16	152	44	151	38	0.63	0.69	0.85	0.86	0.96

*P<0.05, **P<0.01, ***P<0.001, Mann–Whitney U-test.

Table 2. Comparison of probing variables (mean ± SEM) measured by EPG-DC (12 h of recording) of *M. persicae* on tomato. Time in seconds; n = number of EPG-DC replicates.

Variable number	EPG-DC variable	Cultivar	Moneymaker		Preinfested Moneymaker		Motelle		Preinfested Motelle		Moneymaker + BTH		Moneymaker vs preinfested Moneymaker	Moneymaker vs Motelle	Motelle vs preinfested Motelle	Preinfested Moneymaker vs preinfested Motelle	Moneymaker vs Moneymaker + BTH
			(n = 17)		(n = 16)		(n = 19)		(n = 16)		(n = 12)						
			mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	P level	P level	P level	P level	P level
	Probing phase																
7	total probing duration (ABC + pd + E)		21826	1489	26111	1460	23342	1040	24353	700	23640	1433	0.07	0.41	0.52	0.40	0.75
8	total probing duration (ABC)		15481	990	17912	858	16846	869	17872	685	18392	718	0.80	0.98	0.50	0.77	0.02*
9	mean probing duration (ABC)		43	1	46	2	47	2	47	1	35	1	0.85	0.10	0.17	0.37	0.001**
10	total number of probe		366.71	28.32	400.63	18.86	367.47	25.35	375.94	13.26	532.25	30.56	0.51	0.76	0.44	0.43	0.001**
11	duration of 1st probe (ABC + pd + E)		3854	1544	1378	545	194	61	403	249	743	401	0.17	0.01*	0.84	0.58	0.82
	F phase																
12	total duration of F		6995	1174	5435	1222	6325	1225	6692	998	6013	1908	0.13	0.45	0.27	0.98	0.45
13	total number of F		2.18	0.33	1.13	0.24	1.74	0.34	2.13	0.34	3.33	1.32	0.47	0.88	0.50	0.41	0.14
14	mean duration of F		3337	686	4411	1018	3229	746	3766	704	1998	669	0.29	0.17	0.61	0.66	0.006**
	G phase																
15	total duration of G		8965	1279	5422	964	6591	945	5608	536	7242	1719	0.33	0.73	0.67	0.38	0.37
16	total number of G		3.71	0.29	2.25	0.30	3.26	0.33	2.94	0.27	5.16	1.00	0.01*	0.32	0.42	0.02*	0.62
17	mean duration of G		2439	290	2547	522	1923	238	1988	158	1214	297	0.69	0.91	0.38	0.96	0.12
	Potential drop (pd)																
18	total duration of pd		1232	111	1284	59	1270	88	1282	63	1287	73	0.51	0.96	0.74	0.22	0.53
19	total number of pd		309.65	27.06	331.88	15.57	309.47	21.52	317.88	10.53	452.42	26.05	0.36	0.21	0.97	0.90	0.002**
20	mean duration of pd		3.99	0.11	3.88	0.04	4.16	0.12	4.01	0.09	2.85	0.05	1.00	0.33	0.76	0.29	<0.001***

*P < 0.05, **P < 0.01, ***P < 0.001, Mann-Whitney U-test.

Table 3. Comparison of phloem phase variables (mean ± SEM) measured by EPG-DC (12 h of recording) of *M. persicae* on tomato. Time in seconds; n = number of EPG-DC replicates.

Variable number	EPG-DC variable	Cultivar	Moneymaker		Preinfested Moneymaker		Motelle		Preinfested Motelle		Moneymaker + BTH		Moneymaker vs preinfested Moneymaker	Moneymaker vs Motelle	Motelle vs preinfested Motelle	Preinfested Moneymaker vs preinfested Motelle	Moneymaker vs Moneymaker + BTH
			(n = 17)		(n = 16)		(n = 19)		(n = 16)		(n = 12)						
			mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	P level	P level	P level	P level	P level
							P h l o e m		*p h a s e*								
21	total duration of E1		3557	710	3594	983	3206	430	3603	622	3882	856	0.11	0.79	0.77	0.18	0.65
22	total duration of E2		1554	337	3320	887	2019	487	1594	360	77	50	0.04 *	0.21	0.61	0.55	<0.001***
23	total number of E1		18.35	2.38	16.00	2.54	14.16	1.24	13.75	1.10	18	2.24	0.007**	0.18	0.32	0.01*	0.82
24	total number of E2		2.71	0.63	8.06	1.80	4.00	0.74	2.88	0.60	0.5	0.26	0.002**	0.20	0.37	0.12	0.003**
25	mean duration of E1		183	21	197	35	235	30	289	66	189	39	0.34	0.24	0.67	0.85	0.65
26	mean duration of E2		570	113	606	236	452	110	511	112	64	47	0.38	0.14	0.55	0.95	0.001**
27	time to 1st E from start penetration		2157	822	936	250	2334	993	1762	679	701	194	0.88	0.76	0.61	0.58	0.50
28	n. of penetration preceding 1stE1		5.24	1.52	10.38	2.40	5.00	1.00	11.25	3.13	5.42	0.93	0.16	0.72	0.09	0.93	0.37
29	time to 1st E2 from start penetration		943	197	1219	409	1561	576	1209	430	438	82	0.19	0.92	0.98	0.32	0.07
30	n. of penetration preceding 1st E2		17.85	2.71	17.69	5.31	9.53	2.86	14.50	3.16	15.75	2.43	0.81	0.10	0.20	0.86	0.79
31	duration of 1st E2		625	131	700	266	264	59	309	95	64	47.77	0.91	0.04*	0.85	0.42	0.001**
32	% aphids with phloem ingestion (E2)[1]		82.35		100		84.20		81.25		33.33		ns	ns	ns	ns	0.006**

*P < 0.05, **P < 0.01, ***P < 0.001, Mann-Whitney U-test.
[1] Fisher's exact test.

curred in total time of phloem ingestion (E2). In BTH-treated plants a decrease of total duration (variable n 22; 1554 ± 337s *vs* 77 ± 50s; P < 0.001) and of number of phloem ingestions (E2) (variable n 24; 2.71 ± 0.63 *vs* 0.5 ± 0.26; P = 0.003) was detected. Therefore, the mean of individual phloem ingestions (E2) was reduced about 10-fold (variable n 26; P = 0.001). The BTH treatment also induced an almost 10-fold reduction of the duration of the first phloem ingestion (1st E2, variable n 31; P = 0.001).

No difference was detected between the BTH-treated and the untreated susceptible cultivar concerning salivation within the phloem (E1) (variable n 21 and 23). The parameters of salivation during the first phloem probe (E) show no significant differences in penetration time from the beginning of the first "successful" penetration to the 1st E1 pattern (variable n 27), and in the number of penetrations preceding 1st E1 (variable n 28). The most interesting finding is probably the lower percentage of aphids that succeeded in ingesting phloem in the untreated in comparison to the BTH-treated cultivar (variable n 32; 82.35% *vs* 33.33%; P = 0.006).

Discussion

Our EPG-DC data on feeding behaviour of the green peach aphid *M. persicae* on tomato cultivar resistant to the potato aphid *M. euphorbiae* support the hypothesis that the cultivar Motelle (*Mi*) is non-resistant to *M. persicae* (Goggin *et al*., 2001). The only differences detected during the first 12 hours of recording between the non-preinfested susceptible cultivar Moneymaker and the same cultivar preinfested 4 days before the beginning of recording is that the latter became even more susceptible. This is a common occurrence and can be ascribed to the fact that the saliva injected by the aphids probably either prevents wound response or enhances the ingestion of phloem sap, as suggested by Prado and Tjallingii (1997). The same researchers advanced the hypothesis that the increased phloem ingestion and the reduction in salivation by the black bean aphid *A. fabae*, observed on susceptible preinfested bean plants, could be responsible for improving phloem sap quality or increasing food elicitors. These events were also observed in *M. persicae*: the aphid was able to increase susceptibility in GF305, a susceptible peach cultivar (Sauge *et al*., 2002), and in Desirée, a susceptible potato cultivar (Dugravot *et al*., 2007).

The total duration of phloem ingestion increased only in the preinfested susceptible cultivar, with all individuals succeeding in feeding on this tissue. The lack of significant differences between Moneymaker and Motelle was probably due to the lack of detection or absence of elicitors provided by *M. persicae* on Motelle, since this cultivar normally recognizes the elicitors produced by *M. euphorbiae.*

By contrast, BTH treatment of the susceptible Moneymaker apparently makes the cultivar less acceptable for *M. persicae*, inducing an increase of number of pd, a lower duration of pd, and especially a sharp decrease of total duration and of number of phloem ingestions

(already detectable in the first ingestion of the tissue). These results are in agreement with changes in gene expression observed on tomato and *A. thaliana* after BTH treatment, which cause a production of final defence chemicals (Thompson and Goggin, 2006).

No reduction in number of pd responsible for non-persistent virus transmission was actually observed, although the data did not concern the winged aphid forms, transmitting virus from an affected plant to a healthy one during flight. Moreover, the experimental insects (apterous forms) were forced to remain on the same plant longer than they would remain in the wild (Powell *et al*., 2006). The lower number of aphids which succeeded in feeding on phloem on BTH treated plants could be due to the induction by BTH of defence chemicals. This effect could also limit the transmission of persistent viruses that need to reach the phloem.

The BTH treatment was analyzed also in other homopteran and lepidopteran pests. No significant effects on silverleaf whitefly *B. tabaci* populations were detected by BTH treatment on tomato plants, but in the same conditions some effects were detected on the leaf miner *Liriomyza trifolii* (Burgess) (Inbar *et al*., 1998). The BTH treatments on tomato plants against the moths *Helicoverpa zea* (Boddie) and *Spodoptera exigua* (Hubner) failed to induce resistance against these two leaf-eaters (Stout *et al*., 1999). More recently, in the same conditions a significant reduction in *B. tabaci* population was reported by Nombela *et al*. (2005). Bressan and Purcell (2005) observed that the survival of the leafhopper *Colladonus montanus* (Van Duzee) was significantly reduced on BTH-treated *A. thaliana* in comparison to untreated plants.

Based on all the above results, the plant defensive system could be used to enhance plant resistance to pests. Elicitors could be employed to activate plant defensive systems, but they should be applied as a preventive measure, before a large increase of pest populations (Karban, 1999). However, elicitors will probably not be effective in all plants, given the variety of plant defensive systems; moreover, we can not expect them to be active against all insects, given their abilities to overcome plant defensive systems. More research is required to finely tune the use of plant defence responses in insect control strategies.

According to our results, the level of control of *M. persicae* populations on BTH-treated tomato plants attained in this study would not be acceptable for farmers. Nevertheless, the induction of resistance by BTH or other elicitors can be a valuable tool within integrated pest management programs, interfering with pest outbreaks, enhancing the effectiveness of other management strategies and reducing doses and costs of pesticide application.

Acknowledgements

The authors wish to thank Syngenta Crop Protection (Milan, Italy) for kindly supplying the active ingredients employed in this study. Mention of a proprietary product does not constitute an endorsement or a recommendation by Università di Ferrara (Dipartimento di Biologia ed Evoluzione) and Università di Bologna (DiSTA - Entomologia) for its use. This research was funded by Consorzio Ferrara Ricerche (Ferrara Research Consortium) project n. 18-2008.

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Received June 16, 2010. Accepted October 28, 2010.