Physiological Response of Glandular-Haired Alfalfa to Potato Leafhopper (Hemiptera: Cicadellidae) Injury

W. O. LAMP,[1] L. C. ALEXANDER, AND M. NGUYEN

Department of Entomology, University of Maryland, College Park, MD 20742

Environ. Entomol. 36(1): 195–203 (2007)

ABSTRACT Plant tolerance to herbivory is a key approach for managing pests. In alfalfa, *Medicago sativa*, the potato leafhopper, *Empoasca fabae*, is a major pest as a result of the cascade of plant responses to piercing-sucking injury. To identify tolerance to its injury based on alfalfa physiology, experiments were conducted in the field and greenhouse. In our comparison of the response of field-grown alfalfa cultivars to standardized leafhopper densities, net photosynthesis and transpiration rates of 'Geneva' leaves were reduced by 18 and 21%, respectively, by leafhopper presence compared with a rate change of <1% of resistant 'EverGreen' leaves. Under greenhouse conditions, alfalfa clones varied in their level of gas exchange (net photosynthesis and transpiration) and stem elongation responses to leafhopper injury. For example, in the comparison of seven clones, net photosynthesis declined an average of 40.7% with leafhopper injury, although individual clones varied from 26.6 to 74.3% reduction. Internode elongation after 2 d was 60.3% less on injured stems compared with healthy stems, but again, the individual clones varied from 17.3 to 91.9%. In a time-course study of selected clones, clones varied in their level of injury just after and 3 d after insect removal. Gas exchange responses of all clones recovered by 7 d after cessation of injury. In a choice test, leafhoppers spent similar amounts of time on the susceptible clone and the most tolerant clone; however, their precise feeding behaviors were not measured. Thus, the variable response of clones to injury may be either true physiological tolerance or antixenosis from a change in feeding behavior. This study showed putative tolerance to leafhopper injury among alfalfa genotypes, suggesting that tolerance could be the basis for crop protection in alfalfa from potato leafhopper injury.

KEY WORDS *Empoasca fabae*, photosynthesis, sap-feeding, plant tolerance, host plant resistance

Plant tolerance to insect herbivory is recognized as one of the key sustainable approaches for managing pests in the future (National Research Council 1996). Tolerance is defined as the degree to which plant fitness is decreased by herbivore injury relative to fitness in the undamaged state (Strauss and Agrawal 1999). From the perspective of forage production, tolerance refers to the ability of a crop genotype to suffer little or no loss subsequent to pest-induced stress relative to the uninjured state. Historically one of the three main categories for host plant resistance to insect pests (Painter 1958), tolerance differs from other forms of resistance in that it does not have any selective impact on pest populations, providing for reduced pest-induced losses without pest adaptation (Smith 2005). Tolerance focuses on the plant response to injury instead of the population or behavioral response by the insect pest. Here, we seek to find a physiological basis for tolerance in a forage crop and its key piercing-sucking insect pest.

The potato leafhopper, *Empoasca fabae* (Harris), has been documented to feed and reproduce on >200 types of plants, including many eastern North American crops (Lamp et al. 1994). Native to the eastern half of the United States and Canada, potato leafhopper is considered a key pest of alfalfa, *Medicago sativa* L. (Sulc and Lamp 2006). Population characteristics, including its high vagility, polyphagy, and high rate of population increase, result in high densities during the summer months (Hogg and Hoffman 1989). In addition, potato leafhopper is a pest because of the plant response to feeding injury. The symptoms of hopperburn of alfalfa are the result of injury induced by its feeding (Granovsky 1928). The leafhopper feeds on alfalfa by rapid, repeated penetration of its stylets into the vascular tissue, from which plant material is ingested (Backus and Hunter 1989, Kabrick and Backus 1990). Through a combination of mechanical and salivary stimuli, potato leafhopper feeding enhances a wound response in alfalfa that changes the vascular tissue around the feeding site (Ecale and Backus 1995a, b). When this occurs, photoassimilates transported through the phloem build up around the injured site (Johnson 1934, Hibbs et al. 1964, Nielsen et

[1] Corresponding author: Department of Entomology, 4112 Plant Sciences Bldg., University of Maryland, College Park, MD 20742 (e-mail: lamp@umd.edu).

0046-225X/07/0195–0203$04.00/0

al. 1990, 1999), and rates of photosynthesis are reduced (Womack 1984, Flinn et al. 1990, Lamp et al. 2004). In addition, stomatal conductance and internode elongation is reduced (Lamp et al. 2004), resulting in the apparent stunting of stems. Thus, leafhopper feeding initiates a cascade of changes in alfalfa (Backus et al. 2005) that is ultimately expressed as hopperburn, a characteristic yellowing of leaves (Granovsky 1928), as well as delayed plant maturity, reduced nutritive components, stunted growth, and reduced yields (Kindler et al. 1973, Hower 1989, Hutchins and Pedigo 1989).

Commercial releases of resistant cultivars of alfalfa based on glandular trichomes began in 1997 (Elden and McCaslin 1997, Hansen et al. 2002). Antibiotic and antixenotic categories of resistance have been identified based on chemical (primarily) and physical characteristics of trichomes (Shockley et al. 2002, Ranger et al. 2005); however, tolerance has also been reported from field studies (Lefko et al. 2000a). More specifically, Lefko et al. (2000b) used the term "field tolerance" to describe mechanisms that operate at both the plant and population level within an alfalfa stand. Their research has led to recommendations for an increase in action thresholds for potato leafhopper in resistant alfalfa (Lefko et al. 2000b, Sulc et al. 2001).

Our research goal was to identify physiological tolerance to leafhopper injury within glandular-haired alfalfa. We measured the effect of leafhopper injury on gas exchange rates and stem elongation among genetically distinct alfalfa clones because gas exchange rates (especially net photosynthesis and transpiration rates) are rapidly impacted by leafhopper injury (Lamp et al. 2004). This response leads to reduced stem elongation because of a lack of normal water flow within the plant.

Materials and Methods

Experiments were conducted to analyze the variation of physiological response of alfalfa subsequent to potato leafhopper injury. A field experiment was conducted to compare gas exchange rates subsequent to standardized leafhopper pressure. Greenhouse experiments were conducted to quantify variation in physiological responses to injury among susceptible and resistant clones. Clones were reproduced by cuttings to maintain the same genetic identity for replication. The clones included a known susceptible clone from 'Ranger' and a series of six clones provided by Forage Genetics, West Salem, WI (labeled here as 'FG-2', 'FG-3', 'FG-4', 'FG-8', 'FG-9', and 'FG-11'). While 'Ranger' had no glandular trichomes, all FG clones had glandular trichomes but varied in their level of resistance (as labeled by Forage Genetics). 'FG-2', 'FG-3', and 'FG-4' were labeled susceptible and 'FG-8', 'FG-9', and 'FG-11' were labeled resistant.

For greenhouse experiments, potato leafhoppers came from an annually renewed culture using fava bean as a host. Before experimentation, the leafhoppers were taken off the fava bean culture and were conditioned on susceptible 'WL323' alfalfa for at least 48 h.

Field Comparison of Leafhopper Injury. The experiment was conducted at the Western Maryland Research and Education Center, near Keedysville, MD. The locally adapted susceptible cultivar NK 'Geneva' and the Forage Genetics resistant cultivar 'EverGreen' were planted during 2000 in 24.4 by 24.4-m plots, arranged in a randomized complete block design with five blocks. During August 2001, the experiment was conducted to analyze the response of alfalfa cultivars under standardized leafhopper stress in a natural field setting. Just after harvest (day 0), two randomly located subplots within each plot were sprayed with a short-residual insecticide (1.1% rotenone, 0.8% pyrethrin) at the recommended rate and covered by lumite screen cages, 0.9 by 0.9 by 1.0 m high, for a total of 20 cages. Seven days later (day 7), 100 adult potato leafhoppers were placed in one of the two subplot cages within each plot. Adults had been collected within an adjacent alfalfa field using a D-Vac suction device.

After another 7 d (day 14), cages were removed, and gas exchange measurements were made on three randomly selected alfalfa stems within each of the 20 subplots using a LI-6400 Photosynthesis Measurement System (LI-COR, Lincoln, NE). The LI-6400 was set for standardized levels of light (1000 μmol/m^2/s) and CO_2 concentration (400 μmol CO_2/mol). On each stem, net photosynthesis and transpiration rates were measured on the uppermost fully expanded terminal leaf and the next leaf below it. Leaf area was determined using an image analysis system (CI-400; CID, Camas, WA), and gas exchange values were adjusted accordingly relative to leaf size. Data were analyzed using analysis of variance (ANOVA; Proc Mixed, SAS Institute 1997), with cultivar as a main plot treatment and leafhopper injury as a subplot treatment, whereas blocks and leaves within subplots were random effects.

Trichome Measurements. For comparison of trichome expression, measurements of the FG clones (except 'FG-8') were determined using an ocular micrometer fitted on a dissecting microscope. Length measurements were taken on 10 randomly selected erect trichomes from 10 stems from each clone. Trichome density (erect and procumbent) was also measured on three 1-mm sections from the internode between the second and third fully expanded leaf from the apex. Sections were split to aid in counting. Data were analyzed using ANOVA (Proc ANOVA), and means were separated using least significant difference (LSD) pairwise t-tests at $\alpha = 0.05$ (SAS Institute 1997).

All Clone Experiment in Greenhouse. An experiment was conducted in the greenhouse as a randomized complete block design with all clones to compare plant response to a standardized injury level. Clones were cut back a week before experimentation (day 0). After 1 wk of growth (day 7), five blocks containing two pots from each clone type of alfalfa were assembled. One main stem in each pot was selected as the

experimental unit for the experiment. Internode distance was measured between the first and the second fully expanded leaf from the apex using a Ultra-Cal II caliper (Fowler, Newton, MA). One screened snap-top plastic cage, 3 by 3 by 2 cm, was mounted on the internode between the second and third fully expanded leaf from the apex on all plants. Two fifth-stage laboratory-reared leafhopper nymphs were aspirated into the plastic cage of one of the two pots of each clone type within each block to serve as the injured treatment. The caged stem without leafhoppers served as the healthy control.

After 2 d of exposure to leafhoppers (day 9), cages and leafhoppers were removed, and internode measurements were repeated. The gas exchange rate of the second fully expanded leaf (just above the cage) was measured using the LI-6400 Photosynthesis Measurement System. Using the image analysis system, leaf area was determined and gas exchange values were adjusted relative to leaf size. Data were analyzed using ANOVA (Proc Mixed, SAS Institute 1997), with clone and leafhopper injury as fixed effects and block as a random effect. Means of gas exchange measurements for injured and healthy leaves were compared using LSD pairwise t-tests at $\alpha = 0.05$ (SAS Institute 1997).

Selected Clone Experiment in Greenhouse. An experiment was conducted in the greenhouse as a randomized complete block design with selected clones from the previous experiment. In contrast to the previous experiment, leafhoppers were caged over whole stems rather than on single internodes. Thus, each potted plant served as the experimental unit. Caged stems without leafhoppers served as controls. Six blocks were used to test clones 'Ranger', 'FG-4', 'FG-9', and 'FG-11'. All clones were cut back 13 d before experimentation (day 0). On day 12, two pots from each clone were assembled for each block. All stems but one were cut back to force leafhoppers to feed on one stem. The stem height was recorded, the internode distance was measured between the top two fully expanded leaves from the apex, and gas exchange measurements were taken on the same leaves before exposure to cages and leafhoppers.

On day 13, stubble was covered with vermiculite to prevent access by leafhoppers. Each plant was covered with a cage, 7.5 cm diameter by 30 cm high plastic cylinder with screened windows for ventilation, and capped with organdy and a cut-out end cap. Unlike the previous experiment, leafhoppers had been collected as nymphs from a local alfalfa field 5 d earlier and placed in cages with greenhouse-grown 'WL323' alfalfa. Three adult females were added to one of each of the two pots of each clone within each block, with one pot representing the injury treatment and the other the healthy control.

After 2-d exposure (day 15), cages and leafhoppers were removed. On the day of cage removal (day 15), and again on 3 and 7 d after removal (days 18 and 22, respectively), the stem heights, internode distances, and gas exchange rates were remeasured as done on day 12. Data were analyzed using ANOVA (Proc Mixed, SAS Institute 1997), with clone and leafhopper injury as fixed effects, and block and leaf within stem as random effects. Means of gas exchange measurements, internode distances, and stem heights for injured and healthy leaves were compared using LSD pairwise t-tests at $\alpha = 0.05$ (SAS Institute 1997). If stems died or leaves fell off during the experiment, those observations were handled as missing data within SAS.

Table 1. ANOVA for gas exchange rates in the field study

Parameter	Source	df	F value	$P_r > F$
Net photosynthesis	Cultivar	1, 4	2.00	0.23
	Injury	1, 8	5.91	0.04
	Cultivar × injury	1, 8	5.25	0.05
Transpiration	Cultivar	1, 4	2.89	0.16
	Injury	1, 8	4.71	0.06
	Cultivar × injury	1, 8	4.25	0.07

Leafhopper Activity on Clones. An experiment was conducted to compare leafhopper settling behavior on 'Ranger' compared with 'FG-11'. 'FG-11' was chosen because this clone had displayed the highest tolerance level of all clones. Fifteen replications of the two clones were conducted. Each replication consisted of two cut stems from the same clone and pot. Both stems were placed within a 4 by 6 by 2-cm-deep Plexiglas cage, and openings below and above each stem were sealed using foam stoppers. The stems were supplied water using a plastic container for each stem. Two adult females were aspirated into the cage, and a video camera was used to record leafhopper activity for 2 h. Using the video recorder timer display, duration of

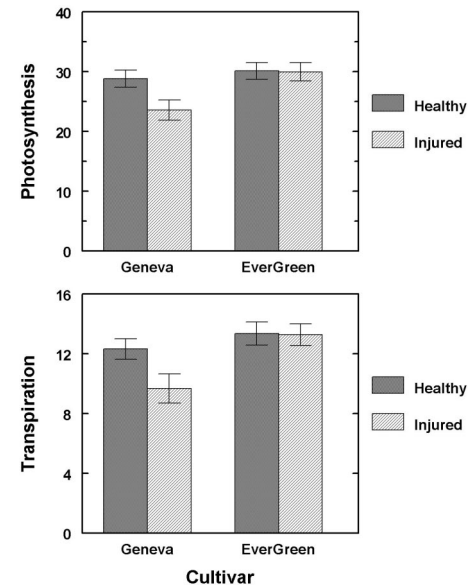

Fig. 1. Mean ± SE of net photosynthesis and transpiration rates of upper leaves from susceptible ('Geneva') and resistant ('EverGreen') alfalfa caged with or without potato leafhoppers. ANOVA given in Table 2. Units for net photosynthesis are μmol $CO_2/m^2/s$ and for transpiration are mol $H_2O/m^2/s$.

Table 2. Trichome lengths and densities (mean ± SE) for tested alfalfa clones

Clone	Category[a]	Trichome length (mm)[b]	Trichome density (no./mm of stem)[c]
'FG-2'	S	0.36 ± 0.01a	34.5 ± 0.7c
'FG-3'	S	0.32 ± 0.01b	33.1 ± 0.6c
'FG-4'	S	0.32 ± 0.01b	25.0 ± 0.4d
'FG-9'	R	0.36 ± 0.01a	77.1 ± 0.5a
'FG-11'	R	0.26 ± 0.01c	73.6 ± 0.5b

Column means followed by a different letter are significantly different (LSD test, $P < 0.05$).

[a] Categorization based on information provided by Forage Genetics. S, susceptible; R, resistant.

[b] Lengths are means of 10 trichomes on 10 stems per clone.

[c] Densities are means of 3 1-mm sections from 10 stems per clone.

time on stems was recorded whenever a leafhopper was seen on either of the two stems. Durations on each clone were compared using a *t*-test.

Results

Field Comparison of Leafhopper Injury. Rates of net photosynthesis and transpiration within field plots of susceptible ('Geneva') and resistant ('EverGreen') alfalfa responded with a significant interaction at the $\alpha = 0.05$ level for net photosynthesis and $\alpha = 0.07$ for transpiration to standardized levels of leafhopper injury (Table 1). While injury resulted in a reduction in net photosynthesis of 18% for 'Geneva', the reduction was <1% for 'EverGreen' (Fig. 1). Similarly, injury resulted in a reduction in transpiration of 21% for 'Geneva' but <1% for 'EverGreen'. Mortality within cages was not measured, but observations at the time of cage removal indicated that leafhopper density was similar in both cultivars.

Trichome Measurements. The five alfalfa clones in which trichome measurements were taken significantly varied both in length of trichomes ($F = 26.3$; df = 4,45; $P < 0.0001$) and especially in the density of trichomes on the stem ($F = 2086$; df = 4,45; $P < 0.0001$). Mean values for length varied from 0.26 mm on 'FG-11' to a maximum of 0.36 mm on both 'FG-2' and 'FG-9' (Table 2). Mean trichome density ranged from 25.0/mm of stem on 'FG-4' to 77.1/mm on 'FG-9'. The values for density, but not length, corresponded to expected patterns of susceptibility and resistance, with resistance-rated 'FG-9' and 'FG-11' with higher densities of trichomes than the susceptible-rated 'FG' clones. In contrast to the 'FG' clones, the 'Ranger' clone had no glandular trichomes.

All Clone Experiment in Greenhouse. ANOVAs for both net photosynthesis and transpiration rates indicated a highly significant leafhopper injury impact without an effect of either clone or the interaction of clone and injury (Table 3). While the ANOVA for the elongation of the internode above the site of injury during the 7 d after cage removal followed the same pattern, elongation during the 2 d after cage removal had a significant interaction term.

Rates of net photosynthesis for all healthy clones averaged 27.2 μmol $CO_2/m^2/s$, whereas rates for injured clones averaged 13.6 μmol $CO_2/m^2/s$, or an average of 49.3% reduction (Table 4). Certain clones were less affected by injury. Differences of least squares means of net photosynthesis were not significant for 'FG-4', 'FG-8', and 'FG-11', with reductions of 26.6, 35.9, and 27.0%, respectively. In contrast, 'Ranger', 'FG-2', 'FG-3', and 'FG-9' had significant reductions of 60.4, 62.4, 58.5, and 74.3%, respectively. Similar patterns were observed for transpiration rate, which averaged 9.2 mol $H_2O/m^2/s$ for all healthy clones and 3.0 mol $H_2O/m^2/s$ for injured clones, with an average of 66.9% reduction. As with net photosynthesis, difference least squares means of transpiration were not significant for 'FG-4', 'FG-8', and 'FG-11', with reductions of 40.5, 60.6, and 39.8%, respectively. In contrast, 'Ranger', 'FG-2', 'FG-3', and 'FG-9' had significant reductions of 83.9, 75.5, 70.2, and 97.6%, respectively.

Internode elongation patterns across clones were dissimilar to the patterns for gas exchange rates; however, all clones had reduced elongation subsequent to injury (Table 4). Elongation after 2 d averaged 16.3 mm for healthy internodes and 5.4 mm for injured internodes, or an average of 66.9% reduction caused by injury. Differences of least squares means of elongation after 2 d were not significant for 'FG-3' and 'FG-8', with reductions of 24.8 and 17.3%, respectively. In contrast, 'Ranger', 'FG-2', 'FG-4', 'FG-9', and 'FG-11' had significant reductions of 71.5, 80.1, 72.3, 92.4, and 72.9%, respectively. After 7 d, elongation averaged 49.3 mm for healthy internodes and 20.9 mm for injured internodes, or an average of 57.6% reduction. Differ-

Table 3. ANOVA for gas exchange and internode elongation rates for all alfalfa clones

Parameter	Source	*F* value	df	$P_r > F$
Net photosynthesis	Clone	1.60	6, 52	0.17
	Injury	64.70	1, 52	<0.0001
	Clone × injury	1.62	6, 52	0.16
Transpiration	Clone	1.54	6, 52	0.18
	Injury	64.24	1, 52	<0.0001
	Clone × injury	1.43	6, 52	0.22
Internode (2 d)	Clone	1.01	6, 56	0.43
	Injury	55.02	1, 56	<0.0001
	Clone × injury	2.53	6, 56	0.03
Internode (7 d)	Clone	1.48	6, 56	0.20
	Injury	30.47	1, 56	<0.0001
	Clone × injury	0.38	6, 56	0.89

Table 4. Comparison of mean gas exchange and internode elongation rates subsequent to cage removal for healthy and injured stems for all alfalfa clones

Clone	Net photosynthesis (μmol/m^2/s)			Transpiration (mol/m^2/s)			Internode elongation (mm, 2 d)			Internode elongation (mm, 7 d)		
	Healthy	Injured	$P > t$	Healthy	Injured	$P > t$	Healthy	Injured	$P > t$	Healthy	Injured	$P > t$
'Ranger'	29.8	11.8	0.0002	11.8	1.9	<0.0001	20.0	5.7	0.0005	63.2	21.3	0.003
'FG-2'	29.8	11.2	0.0001	9.8	2.4	0.0007	14.1	2.8	0.005	49.6	18.1	0.02
'FG-3'	28.2	11.7	0.0005	9.4	2.8	0.002	11.7	8.8	0.46	48.4	23.8	0.08
'FG-4'	26.3	19.3	0.12	8.4	5.0	0.10	17.3	4.8	0.002	56.6	35.9	0.14
'FG-8'	22.0	14.1	0.08	6.6	2.6	0.06	10.4	8.6	0.65	44.7	26.5	0.19
'FG-9'	25.7	6.6	<0.0001	8.3	0.2	0.0002	18.5	1.4	0.0001	37.9	2.5	0.01
'FG-11'	28.5	20.8	0.09	9.8	5.9	0.06	22.1	6.0	0.0001	45.0	18.1	0.05

ences of least squares means were not significant for 'FG-3', 'FG-4', and 'FG-8', with reductions of 50.8, 36.6, and 40.7%, respectively. In contrast, 'Ranger', 'FG-2', 'FG-9', and 'FG-11' had significant reductions of 66.3, 63.5, 93.4, and 59.8%, respectively.

Selected Clone Experiment in Greenhouse. The ANOVA for gas exchange measurements showed no significance before cages were placed over the stems (Table 5). Subsequently, net photosynthesis rates were affected by both clone and injury treatments, but not their interaction, on 0 and 3 d after cages and leafhoppers were removed. By 7 d, the injury treatment was not significant, although a significant clone effect remained. Transpiration rates followed a similar pattern; however, the ANOVA just after cage removal showed a significant (at $\alpha = 0.07$) interaction between clone and injury. Injury was no longer significant at 7 d after cage removal.

After cage and leafhopper removal, gas exchange measurements were reduced on leafhopper exposed stems (injured) compared with healthy stems, although this response varied among clones (Table 6). Just after cage removal, least squares means for net photosynthesis were significantly reduced on leaves when exposed to leafhoppers for 'Ranger', 'FG-4', and 'FG-9' clones, with 29.3, 44.9, and 32.2% reductions, respectively. In contrast, the reduction of 16.9% for 'FG-11' was not significant. Three days after cage removal, least squares means were still significantly reduced for 'Ranger' and 'FG-4', with 31.8 and 37.0% reductions, respectively, whereas the nonsignficant reduction for 'FG-9' and 'FG-11' were 23.1 and 8.4%, respectively. By 7 d after cage removal, no differences in means between healthy and injured stems were significant for net photosynthesis.

For transpiration, differences between healthy and injured stems were more pronounced than with net photosynthesis (Table 6). Just after cage removal, least squares means for transpiration were significantly reduced when exposed to leafhoppers for 'Ranger', 'FG-4', and 'FG-9' clones, with 37.5, 47.8, and 49.6% reductions, respectively. In contrast, the reduction of 5.8% by 'FG-11' was not significant. By 3 d after cage removal, least squares means were still significantly reduced for the three clones, with 42.5, 44.6, and 41.3% reductions for 'Ranger', 'FG-4', and 'FG-9', respectively. 'FG-11' had a nonsignificant 10.9% reduction. By 7 d after cage removal, no differences in

Table 5. ANOVA for gas exchange and rates for the selected alfalfa clone experiments

Parameter	Days after cage removal	Source	F value	df	$P_r > F$
Net photosynthesis	Before caging	Clone	0.49	3, 83	0.69
		Injury	1.31	1, 83	0.26
		Clone × injury	0.43	3, 83	0.73
	0	Clone	8.79	3, 83	<0.0001
		Injury	24.21	1, 83	<0.0001
		Clone × injury	0.47	3, 83	0.71
	3	Clone	13.60	3, 80.4	<0.0001
		Injury	17.10	1, 80.5	<0.0001
		Clone × injury	0.84	3, 80.4	0.48
	7	Clone	4.92	3, 71.2	0.004
		Injury	0.08	1, 71.3	0.78
		Clone × injury	0.53	3, 71.2	0.66
Transpiration	Before caging	Clone	0.83	3, 83	0.48
		Injury	1.39	1, 83	0.24
		Clone × injury	1.26	3, 83	0.29
	0	Clone	6.00	3, 83	0.0009
		Injury	31.48	1, 83	<0.0001
		Clone × injury	2.43	3, 83	0.07
	3	Clone	8.50	3, 80	<0.0001
		Injury	31.83	1, 80.1	<0.0001
		Clone × injury	1.52	3, 80	0.22
	7	Clone	6.68	3, 71.3	0.0005
		Injury	0.28	1, 71.6	0.60
		Clone × injury	0.98	3, 71.5	0.41

Table 6. Comparison of mean gas exchange rates for healthy and injured stems for the selected alfalfa clone experments

Parameter	Clone	0 d after cage removal			3 d after cage removal			7 d after cage removal		
		Healthy	Injured	$P > t$	Healthy	Injured	$P > t$	Healthy	Injured	$P > t$
Net photosynthesis (μmol $CO_2/m^2/s$)	'Ranger'	16.4	11.6	0.02	19.2	13.1	0.005	19.9	18.9	0.63
	'FG-4'	14.7	8.1	0.001	15.4	9.7	0.008	18.1	15.9	0.34
	'FG-9'	14.3	9.7	0.02	16.9	13.0	0.09	14.1	15.1	0.66
	'FG-11'	19.5	16.2	0.10	22.6	20.7	0.36	19.3	20.4	0.59
Transpiration (mol $H_2O/m^2/s$)	'Ranger'	12.8	8.0	0.003	13.4	7.7	0.0003	16.3	14.5	0.37
	'FG-4'	11.5	6.0	0.001	11.2	6.2	0.002	15.6	13.0	0.23
	'FG-9'	13.1	6.6	<0.0001	12.6	7.4	0.002	9.3	11.2	0.36
	'FG-11'	13.7	12.9	0.58	14.7	13.1	0.30	15.8	16.2	0.83

means between healthy and injured stems were significant for transpiration.

While gas exchange measurements are instantaneous measures of plant health, internode elongation and change in stem heights during the experiment provide an integrated response of the plant over time. The ANOVA for internode elongation showed a significant interaction effect based on the change in internode length from before caging to both 3 and 7 d after cage removal (Table 7). The ANOVA for stem height change over the same periods also showed a significant interaction effect.

The reduction in internode elongation caused by leafhoppers varied among clones (Table 8). Least squares means of internode elongation, measured from cage removal to 3 d later, was significantly different between healthy and injured stems for 'Ranger' and 'FG-4', which had a 59.3 and 51.0% reduction, respectively. Similarly, elongation measured from cage removal to 7 d later was significantly different between healthy and injured stems for 'Ranger' and 'FG-4', with 52.4 and 56.1% reductions, respectively. For 'FG-9' and 'FG-11', differences between healthy and injured stems were not significant.

The reduction in stem height caused by leafhoppers also varied among clones in a similar way (Table 8). Least squares means of the change in stem height from cage removal to 3 d later was significantly different between healthy and injured stems for 'Ranger', 'FG-4', and 'FG-9', with 68.8, 71.6, and 40.7% reductions, respectively. Change in stem height from cage removal to 7 d later was significantly different for 'Ranger' and 'FG-4' only, with 64.4 and 67.3% reductions, respectively. Differences between healthy and injured stems for 'FG-11' were not significant.

Table 7. ANOVA for internode elongation and stem growth rates for the selected alfalfa clone experiments

Parameter	Days after cage removal	Source	F value	df	$P_r > F$
Internode	3	Clone	5.14	3, 35	0.005
		Injury	10.67	1, 35	0.002
		Clone × injury	4.42	3, 35	0.01
	7	Clone	5.21	3, 31.1	0.005
		Injury	10.51	1, 31.7	0.003
		Clone × injury	4.16	3, 31.5	0.01
Stem height	3	Clone	4.42	3, 40	0.009
		Injury	32.72	1, 40	<0.0001
		Clone × injury	3.68	3, 40	0.02
	7	Clone	2.86	3, 37	0.05
		Injury	27.06	1, 37	<0.0001
		Clone × injury	2.96	3, 37	0.04

Leafhopper Activity on Clones. Observations of the average time a leafhopper settled on 'Ranger' stems were not significantly different from the time settled on 'FG-11' stems (t-test; $t = 1.81$; df = 58; $P > 0.05$). Average times (means ± SE) were 23.0 ± 3.2 min on 'Ranger' and 27.0 ± 3.1 min on 'FG-11'. We did not compare specific feeding behaviors (e.g., stylet penetration tactics measured by AC electronic feeding monitor; Serrano et al. 2000) of the leafhopper on the two clones.

Discussion

Tolerance, as a category of host plant resistance, is shown when the plant response to the same herbivory differs among different genetic lines within a species (Painter 1958). Here, we provide evidence for tolerance by comparing the gas exchange response of two field-grown alfalfa cultivars at a standardized level of leafhopper density. The significant interaction term (at $\alpha = 0.10$) for both net photosynthesis and transpiration rates implies a different physiological response of the two cultivars to injury, such that 'Geneva' was susceptible to injury, whereas 'EverGreen' was tolerant. Similar differences were observed among clones expressing glandular trichomes under greenhouse conditions. Although the interaction term was not always significant, the leafhopper-induced reductions in gas exchange measurements and stem elongation rates varied among clones.

Although we provided similar levels of leafhoppers to cultivar/clone treatments within experiments, we do not know if the specific feeding behavior of leafhoppers differed between treatments (e.g., as described in Backus et al. 2005). Only the use of an electrical penetration graph (EPG) monitor can detect such a difference (Serrano et al. 2000). However, we did not observe any difference in the amount of mortality within experiments in relation to treatments. In addition, we did not observe a significant difference in the time that leafhoppers spent on 'Ranger' versus the tolerant 'FG-11' stems. Thus, feeding behavior differences may be responsible for the variable response of cultivar/clone treatments to standardized levels on leafhopper exposure, in which case anti-

Table 8. Comparison of mean change in internode elongation (mm) and stem height (cm) for healthy and injured stems for the selected alfalfa clone experments

Parameter	Clone	To 3 d after cage removal[a]			To 7 d after cage removal		
		Healthy	Injured	$P_r > t$	Healthy	Injured	$P_r > t$
Internode	'Ranger'	39.8	16.2	<0.0001	39.1	18.6	0.0004
	'FG-4'	21.0	10.3	0.05	28.5	12.5	0.02
	'FG-9'	14.0	15.7	0.76	13.5	15.8	0.66
	'FG-11'	19.7	17.5	0.68	19.8	17.9	0.71
Stem	'Ranger'	13.8	4.3	<0.0001	20.8	7.4	<0.0001
	'FG-4'	8.8	2.5	0.002	14.7	4.8	0.005
	'FG-9'	10.8	6.4	0.02	17.4	12.4	0.09
	'FG-11'	10.7	9.7	0.59	16.9	14.5	0.40

[a] Change calculated from measurements before caging until 3 or 7 d after cage removal.

xenosis, and not tolerance, is the category of host plant resistance (Painter 1958).

Indeed, EPG research of the closely related *Empoasca kraemeri* on genotypes of common bean, *Phaseolus vulgaris,* suggests that apparent tolerance of one bean genotype ('EMP 84') is caused by a shift from pulsing laceration to less injurious feeding tactics (Serrano et al. 2000). Their research found no significant difference in total feeding time among the bean genotypes, so the switch in feeding tactics was associated with less yield loss caused by the leafhopper. However, a second genotype, 'Porrillo Sintético', incurred the highly injurious feeding tactic but was able to compensate physiologically for the injury through true tolerance. In related research, Serrano and Backus (1998) found that the number and cross-sectional area of xylem vessels can change after feeding by *E. kraemeri,* resulting in a change in relative flow rates of xylem vessels. Such a change was not found in 'Porrillo Sintético', suggesting that functional compensation for feeding injury may have been one of several possible mechanisms underlying true tolerance.

Potato leafhopper induced damage to alfalfa depends on the response of the plant to feeding injury. If a leafhopper feeds on alfalfa stem tissue, all leaves distal to that point show a decline in gas exchange rates (Lamp et al. 2004). In addition, starch accumulates in all tissues distal to the site of feeding injury (Pirone et al. 2005). Although past researchers have suggested that the cause of this plant physiological response is feedback inhibition of photoassimilates building up in phloem tissue (Hibbs et al. 1964), the lack of an effect of leafhopper injury on assimilation rates at various internal CO_2 concentrations (ACi curves) suggests that leafhopper injury primarily impacts stomatal behavior and xylem function (W. Lamp and L. Alexander, unpublished data). The decline in xylem function associated with injury fits the observations reported here of a greater effect of injury on transpiration than net photosynthesis (Tables 1 and 5), as well as the effect of injury on internode elongation and short-term stem growth (Tables 3 and 7). Physiological tolerance among alfalfa plants of varying genetic background was represented by the ability of certain plant genotypes (especially 'FG-11') to continue near healthy levels of transpiration (Table 6) and of internode elongation and stem height (Table 8) subsequent to injury.

Injured stems had significant reductions of both net photosynthesis and transpiration rates after 2 d of exposure to leafhoppers in the greenhouse. Seven days after cage and leafhopper removal (or 9 d after initiation of feeding injury), we observed the return of injured stems to healthy levels of gas exchange rates for all clones. This observation fits with other reports on the return of physiological function of alfalfa subsequent to injury (Lamp et al. 2004). In addition, in a time-course study of anatomical changes within alfalfa stems subsequent to injury, Ecale Zhou and Backus (1999) reported that plants were able to repair cell structure in 8 d after injury. As a point of comparison, the yellowing symptom of hopperburn requires ≈5 d for development after injury (Granovsky 1928). Thus, symptoms of injury develop several days after a significant decline in gas exchange rates, and several days before the plants are able to repair cell structure. The cascade of events leading to the hopperburn discoloration remains elusive.

While potato leafhopper is native to eastern North America, alfalfa is derived from plants found in west Asia (Lamp et al. 1994). As of the late 1970s, all germplasm used in the development of North American cultivars was traced back to nine sources of alfalfa (Barnes et al. 1988). Thus, the herbivory of alfalfa by potato leafhopper is not a coevolved interaction.

We know that the unique hopperburn cascade exhibited by alfalfa is a result of both mechanical and salivary constituents during potato leafhopper feeding (Ecale and Backus 1995b). Although studies to date are lacking for this plant–insect interaction, general understanding of plant responses suggests that it is likely that the foreign injury caused by potato leafhopper is associated with specific signaling pathways and gene expression at the molecular level and that alfalfa genotype will influence the response when viewed at molecular, physiological, or whole plant levels (Taylor et al. 2004). The goal of breeding programs should be for genetic lines that do not result in the disruptive physiological response caused by leafhopper injury while maintaining acceptable agronomic characteristics. Such an endeavor would be enhanced by screening for physiological tolerance

and by application of the knowledge of the molecular mechanisms that result in tolerance.

Acknowledgments

We thank T. Ellis and resources for field research at Western Maryland Research and Education Center. In addition, we thank L. Hellman for assistance with greenhouse studies and E. Backus, C. Ranger, J. Reese, B. Smith, and L. Culler for reviews of the manuscript. Portions of the research were conducted as part of an undergraduate honors thesis from the University of Maryland's Department of Entomology Honors Program. Support for this research was provided in part by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Education Program and by USDA-CSREES Project MD-H-176 (NC-193) and USDA NE Regional IPM Project 93-34103-8418.

References Cited

Backus, E. A., and W. B. Hunter. 1989. Comparison of feeding behavior of the potato leafhopper *Empoasca fabae* (Homoptera: Cicadellidae) on alfalfa and broad bean leaves. Environ. Entomol. 18: 473–480.

Backus, E. A., M. S. Serrano, and C. M. Ranger. 2005. Mechanisms of hopperburn: an overview of insect taxonomy, behavior, and physiology. Annu. Rev. Entomol. 50: 125–151.

Barnes, D. K., B. P. Goplen, and J. E. Baylor. 1988. Highlights in the USA and Canada, pp. 1–24. *In* A. A. Hanson, D. K. Barnes, and R. R. Hill, Jr. (eds.), Alfalfa and alfalfa improvement. ASA-CSSA-SSSA, Madison, WI.

Ecale, C. L., and E. A. Backus. 1995a. Time course of anatomical changes to stem vascular tissue of alfalfa, *Medicago sativa*, from probing injury by the potato leafhopper, *Empoasca fabae.* Can. J. Bot. 73: 288–298.

Ecale, C. L., and E. A. Backus. 1995b. Mechanical and salivary aspects of potato leafhopper probing in alfalfa stems. Entomol. Exp. Appl. 77: 121–132.

Ecale Zhou, C. L., and E. A. Backus. 1999. Phloem injury and repair following potato leafhopper feeding on alfalfa stems. Can. J. Bot. 77: 537–547.

Elden, T. C., and M. McCaslin. 1997. Potato leafhopper (Homoptera: Cicadellidae) resistance in perennial glandular-haired alfalfa clones. J. Econ. Entomol. 90: 842–847.

Flinn, P. W., A. A. Hower, and D. P. Knievel. 1990. Physiological response of alfalfa to injury by *Empoasca fabae* (Homoptera: Cicadellidae). Environ. Entomol. 19: 176–181.

Granovsky, A. A. 1928. Alfalfa "yellow top" and leafhoppers. J. Econ. Entomol. 21: 261–267.

Hansen, J. L., J. E. Miller-Garvin, J. K. Waldron, and D. R. Viands. 2002. Comparison of potato leafhopper-resistant and susceptible alfalfa in New York. Crop Sci. 42: 1155–1163.

Hibbs, E. T., D. L. Dahlman, and R. L. Rice. 1964. Potato foliage sugar concentration in relation to infestation by the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. 57: 517–521.

Hogg, D. B., and G. D. Hoffman. 1989. Potato leafhopper population dynamics, pp. 26–34. *In* E. J. Armbrust and W. O. Lamp (eds.), History and perspectives of potato leafhopper (Homoptera: Cicadellidae) research.

Hower, A. A. 1989. Potato leafhopper as a plant stress factor on alfalfa, pp. 35–39. *In* E. J. Armbrust and W. O. Lamp (eds.), History and perspectives of potato leafhopper (Homoptera: Cicadellidae) research.

Hutchins, S. H., and L. P. Pedigo. 1989. Potato leafhopper-induced injury on growth and development of alfalfa. Crop Sci. 29: 1005–1011.

Johnson, H. W. 1934. Nature of injury to forage legumes by the potato leaf hopper. J. Agric. Res. 49: 379–406.

Kabrick, L. R., and E. A. Backus. 1990. Salivary deposits and plant damage associated with specific probing behaviors of the potato leafhopper, *Empoasca fabae*, on alfalfa stems. Entomol. Exp. Appl. 56: 287–304.

Kindler, S. D., W. R. Kehr, R. L. Ogden, and J. M. Shalk. 1973. Effect of potato leafhopper injury on yield and quality of resistant and susceptible alfalfa clones. J. Econ. Entomol. 66: 1298–1302.

Lamp, W. O., G. R. Nielsen, and S. D. Danielson. 1994. Patterns among host plants of potato leafhopper, Empoasca fabae (Homoptera: Cicadellidae). J. Kans. Entomol. Soc. 67: 354–368.

Lamp, W. O., G. R. Nielsen, C. B. Fuentes, and B. Quebedeaux. 2004. Feeding site preference of potato leafhopper (Homoptera: Cicadellidae) on alfalfa and its effect on photosynthesis. J. Agric. Urban Entomol. 21: 25–38.

Lefko, S. A., L. P. Pedigo, and M. E. Rice. 2000a. Symptoms and growth of potato leafhopper-tolerant alfalfa in response to potato leafhopper feeding. Agronomy J. 92: 721–725.

Lefko, S. A., L. P. Pedigo, and M. E. Rice. 2000b. Alfalfa stand tolerance to potato leafhopper and its effect on the economic injury level. Agronomy J. 92: 726–732.

National Research Council. 1996. Ecologically based pest management: new solutions for a new century. National Academy Press, Washington, DC.

Nielsen, G. R., W. O. Lamp, and G. W. Stutte. 1990. Potato leafhopper (Homoptera: Cicadellidae) feeding disruption of phloem translocation in alfalfa. J. Econ. Entomol. 83: 807–813.

Nielsen, G. R., C. Fuentes, B. Quebedeux, Z. Wang, and W. O. Lamp. 1999. Alfalfa physiological response to potato leafhopper injury depends on leafhopper and alfalfa developmental stage. Entomol. Exp. Appl. 90: 247–255.

Painter, R. H. 1958. Resistance of plants to insects. Annu. Rev. Entomol. 3: 267–290.

Pirone, C. L., L. C. Alexander, and W. O. Lamp. 2005. Patterns of starch accumulation in alfalfa subsequent to potato leafhopper (Homoptera: Cicadellidae) injury. Environ. Entomol. 34: 199–204.

Ranger, C. M., R.E.K. Winter, E. A. Backus, G. E. Rottinghaus, M. R. Ellersieck, and D. W. Johnson. 2005. Discrimination by the potato leafhopper (Hemiptera: Cicadellidae) of host volatiles from resistant and susceptible alfalfa, *Medicago sativa* L. Environ. Entomol. 34: 271–280.

SAS Institute. 1997. SAS/STAT software: changes and enhancements through release 6.12. SAS Institute, Cary, NC.

Serrano, M. S., and E. A. Backus. 1998. Differences in cellular abnormalities induced by the probing behaviors of *Empoasca kraemeri* (Homoptera: Cicadellidae) on tolerant and susceptible common beans. J. Econ. Entomol. 91: 1481–1491.

Serrano, M. S., E. A. Backus, and C. Cardona. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796–1809.

Shockley, F. W., E. A. Backus, M. R. Ellersieck, D. W. Johnson, and M. McCaslin. 2002. Glandular-haired alfalfa resistance to potato leafhopper (Homoptera: Cicadellidae)

and hopperburn: development of resistance indices. J. Econ. Entomol. 95: 437–447.

Smith, C. M. 2005. Plant resistance to arthropods. Springer, Dordrecht, The Netherlands.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. TREE. 14: 179–185.

Sulc, M., and W. O. Lamp. 2007. Insect pest management. *In* R. F. Barnes, D. A. Miller, and C. J. Nelson (eds.), Forages: the science of grassl. agriculture, 6th ed., vol. II. Iowa State University Press, Ames, IA (in press).

Sulc, M., R. E. van Santen, K. D. Johnson, C. C. Sheaffer, D. J. Undersander, L. W. Bledsoe, D. B. Hogg, and H. R. Willson. 2001. Glandular-haired cultivars reduce potato leafhopper damage in alfalfa. Agronomy J. 93: 1287–1296.

Taylor, J. E., P. E. Hatcher, and N. D. Paul. 2004. Crosstalk between plant responses to pathogens and herbivores: a view from the outside in. J. Exp. Bot. 55: 159–168.

Womack, C. L. 1984. Reduction in photosynthetic and transpiration rates of alfalfa caused by the potato leafhopper (Homoptera: Cicadellidae) infestations. J. Econ. Entomol. 77: 508–513.

Received for publication 30 May 2006; accepted 1 November 2006.