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EVALUATION OF EXPERIMENTAL POPULATIONS AND GLANDULAR-HAIRED VARIETIES OF ALFALFA FOR ALFALFA BLOTCH LEAFMINER (DIPTERA: AGROMYZIDAE) FEEDING INJURY

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ABSTRACT

Following the spread of the alfalfa blotch leafminer, Agromyza frontella (Rondani) (Diptera: Agromyzidae), into Minnesota and Wisconsin U.S.A. during 1994-1997, three field trials were conducted in Minnesota to assess the potential for leafminer resistance among several sources of alfalfa (Medicago sativa), germplasm. In 1998, 86 entries were evaluated, most of which were experimental populations. In addition, six commercial varieties of alfalfa were evaluated. Of the six varieties, four had been bred for various levels of glandular-hair expression, specifically for resistance to the potato leafhopper, Empoasca fabae (Harris)(Homoptera: Cicadellidae). In two of three trials, we found no significant differences in leafmining injury to trifoliolates among the 86 entries, or among glandular-haired and traditional commercial varieties. At one loca-tion, 'Arrest,' 'Ameriguard 301,' and 'DK 121 HG' incurred significantly less pinhole injury than the glandular-haired variety '5347 LH' or the commercial standard, '5454.' However, after accounting for both pinhole and leafmining injury, only 'Arrest' and 'Ameriguard 301' had less injury than '5347 LH,' 'DK 121 HG,' or the standard '5454.' The low levels of resistance to A. frontella injury, among glandular-haired commercial alfalfa varieties and numerous experimental populations M. sativa, confirm the need for alternative A. frontella management strategies such as biological control and possible manipulation of harvest schedules.

Agromyza frontella (Rondani) (Diptera: Agromyzidae) is a pest of European origin that was first detected in alfalfa, Medicago sativa L., in North America in 1968 (Miller and Jensen 1970; Harcourt 1973). In the early- to mid-1970s, the insect was a significant pest of alfalfa in the northeastern United States and eastern Canada (e.g., Hendrickson and Day 1986; Harcourt et al. 1988). However, the economic impact of this insect on alfalfa yield and quality has been difficult to estimate. MacCollom (1980) observed no significant yield effect but reported losses of 0.7 to 2.4% in crude protein. Thompson (1981) found no significant impact by A. frontella on alfalfa yield, yet quantitative estimates of A. frontella density were not reported. Byers and Valley (1981) reported reductions in digestible dry matter of 11.04%, a 12.07% loss in crude protein, and a 22% reduction in chlorophyll for leaflets infested with mines when A. frontella densities were high. Summarizing several yield impact studies, within the context of a sustained A. frontella infestation in the northeastern U.S., Hendrickson and Plummer (1983) concluded that even small reductions in yield resulted in annual regional losses of ca. \$13 million.

Both the larvae and adult females of *A. frontella* cause injury (e.g., Guppy 1981). After emerging from eggs laid on the underside of alfalfa leaflets, larvae consume the mesoderm of the leaflet, and, in the process create a uniquely shaped mine or blotch, typically resembling a large comma (Bereza 1979). Adult

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females wound the plant by puncturing leaflets with their ovipositors. The females then imbibe the fluids. After some time, the injured tissues will senesce, leaving distinctive "pinholes." An individual leaflet may have as many as 1,000 pinholes (Bereza 1979).

Agromyza frontella was first detected in northern Minnesota in 1994 and rapidly spread to southern Minnesota and western Wisconsin by 1996 (Hutchison et al. 1997). The leafminer was subsequently detected in southern Wisconsin and northern Illinois in 1997 (Venette et al. 1999). By 1998-1999, we also confirmed infestations in North Dakota, South Dakota, and northwest Iowa (Venette et al. 1999). Lundgren et al. (1999) subsequently documented a westward movement of the leafminer across Manitoba, Canada; additional surveys confirmed the presence of A. frontella as far west as Saskatchewan, Canada (Soroka et al. 2000).

Research in Minnesota indicated that currently registered alfalfa insecticides, including the pyrethroids, provided inconsistent control of *A. frontella* (Burkness et al. 1999a,b). In addition, although several parasitoids have been shown to attack the leafminer in the eastern U.S. and in Canada (Drea and Hendrickson 1986; Harcourt et al. 1988), a relatively low incidence of parasitism has been reported thus far in Minnesota (Hutchison et al. 1997).

Given the need for consistent management strategies for A. frontella, we initiated studies to assess the potential for varietal resistance to the leafminer, using multiple sources of *Medicago sativa* germplasm and selected glandularhaired commercial varieties. Glandular-haired experimental populations, with activity against the potato leafhopper, Empoasca fabae (Harris)(Homoptera: Cicadellidae), were first developed in the 1970s (e.g., Shade et al. 1979); commercial varieties were first released in the late 1990s (Ranger and Hower 2001; Sulc et al. 2001). Although several glandular-haired varieties confer resistance to E. fabae (Sulc et al. 2001), the mechanisms of resistance are poorly understood. For A. frontella, the trichomes may provide a physical barrier to adults seeking oviposition or feeding sites (MacLean and Byers 1983), or they may produce substrates that repel the insects when searching the leaf surface. If either mechanism were true, it is possible that the glandular hairs that affect E. *fabae* could also confer some level of resistance to A. *frontella*. The purpose of this study was to assess the potential of several glandular-haired varieties and recent *M. sativa* germplasm for tolerance to *A. frontella*.

MATERIALS AND METHODS

In 1998, we sampled three established alfalfa variety trials in Minnesota for plant damage caused by *A. frontella*. The trials were located at the Rosemount Agricultural Experiment Station (Dakota Co.), University of Minnesota, and on a commercial farm near St. Martin, in central Minnesota (Stearns Co.). All trials were designed to evaluate cultivars for agronomic characteristics and forage quality following established protocols (Sheaffer et al. 1998).

The general design of each trial (designated by its location and original purpose) and the specific parameters we measured are described below. The number of parameters was adjusted by the size of the trial and the size of the plants. Sample dates for all locations were selected to coincide with periods of pinhole and leafmining activity in July-August (e.g., Venette et al. 1999). In all variety trials, 20 stems were collected at random from each plot. Stems were cut at the soil surface. Samples were cooled to 16°C and brought to the laboratory for processing. Each trifoliolate on a stem was examined for pinhole or mining), a measure of the incidence of plant injury, was noted. Percentages were arcsine (sqrt)-transformed and analyzed using PROC GLM to compensate for missing observations (SAS 1998); means separation was done using the Ryan-Einot-Welsch multiple range test (SAS 1998). All remaining variables for each

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location also were analyzed by PROC GLM and the Ryan-Einot-Welsch multiple range test (SAS 1998).

Rosemount, Multiple Entries. This trial consisted of 86 entries, which were planted 3 May 1995, in a randomized-complete-block design. 'Vernal' and '5454' were included as common, non-glandular-haired, commercial varieties for comparison. Each entry was replicated four times, of which we sampled the first three replicates. In total, 5,160 stems were examined. Individual entry plots within a replicate were 0.9m x 6.0m. Within a block, no border separated the length of each plot. Blocks were separated by 3-m borders of alfalfa. Plots were sampled 10 July 1998, just before the second cutting. None of the plots were treated with insecticides.

Rosemount, Glandular-haired Varieties. Data for commercial varieties were collected from a variety x insecticide study designed to assess the impact of glandular-haired varieties and insecticidal control on E. fabae. However, based on preliminary data obtained from insecticide treated plots (RCV, unpublished) showing minimal impact of permethrin (Pounce 3.2EC, FMC Corp.) on A. frontella, we only collected A. frontella data from the untreated alfalfa plots. The trial consisted of six entries, including four glandular-haired varieties ('5347', 'Ameriguard 301', 'Arrest', and 'DK121 HG') and two standard varieties ('5454' and 'Vernal'). Each variety was planted 12 May 1997, using standard production practices for Minnesota (Sulc et al. 2001). A 3-m fallow border separated the untreated block of varieties from the insecticide-treated block. Each complete block was replicated four times for a total of 48 plots, each 1.8m x 6m. Blocks were separated by a 3 m fallow border. Plots were sampled 16 July 1998, just before the second cutting. In addition to measuring the incidence of injury caused by A. frontella, we also measured the fresh and dry weights of each 20-stem sample. Samples were oven dried for 48 h before weighing.

St. Martin, Glandular-haired Varieties. This trial was designed much like the Rosemount experiment including insecticidal control for *E. fabae*. The same varieties were used in the same-sized plots. However, the varieties were planted 13 May 1998, with *A. frontella* data recorded during the first establishment year. Plots were sampled on 19 August 1998, before any cuttings were taken. Only one-half of the experiment was sampled because no insecticides had yet been applied to the plots. Severity of *A. frontella* injury was assessed by determining the percentage of trifoliolates with pinholes or mines. A trifoliolate with both pinholes and mines was simply classified as mined. Additionally, because the plants were relatively small, we also used a 0-9 maturity. Due to the small size of the plants, weights were not recorded.

RESULTS

Rosemount, Multiple Entries. Nearly all entries incurred over 90% injury, with at least one trifoliolate per stem injured by *A. frontella* (Table 1); no significant differences in injury were observed (F = 0.79; total df = 256, P = 0.90). In addition, all stems of '3452 ML', '5454', 'BPR 365', 'C/W 4206', 'ICI 620', 'PGI 3512', and 'ZC 9339' were injured by *A. frontella* (Table 1).

Rosemount, Glandular-haired Varieties. The proportion of stems with at least one trifoliolate injured per stem, ranged from 83.8 to 100%, with no significant differences observed among glandular-haired and traditional alfalfa varieties (Table 2; P > 0.05). There were also no significant differences in fresh or dry weights among glandular-haired and traditional varieties (P > 0.05).

St. Martin, Glandular-haired Varieties. The glandular-haired varieties 'Arrest,' 'Ameriguard 301,' and 'DK 121 HG' had significantly fewer trifoliolates with pinhole injury than did the glandular-haired variety '5347 LH' or the traditional variety '5454' (Table 3; P < 0.05). However, only 'Arrest' had

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Table 1. Percentage of stems (mean \pm SEM) with at least one trifoliolate injured by Agromyza frontella among alfalfa experimental populations and varieties, 1998, Rosemount, Minnesota.

Entry	%	Entry	%	Entry	%	Entry	%
	Injured		Injured		Injured		Injured
F010			0.5	A DI GOOG	0		00.00
5312	85 ± 1	ABT 405	95 ± 3	ABI 9220	97 ± 3	ABI 923DD	98 ± 2
WL 252H	88 ± 2	BPR 371	95 ± 3	ABI 9222	97 ± 3	BPR 373	98 ± 2
WI 95-1	92 ± 2	C/W 3231	95 ± 3	ABT 205	97 ± 3	BPR 374	98 ± 2
ZC 9346	92 ± 2	C/W 4308	95 ± 3	AlfaStar	97 ± 3	C/W 3408	98 ± 2
C/W 3414	92 ± 3	C/W 4319	95 ± 3	C/W 3322	97 ± 3	Demand	98 ± 2
ZM 9431	92 ± 3	DS 907	95 ± 3	C/W 3327	97 ± 3	Dividend	98 ± 2
Viking 1	93 ± 2	$DS \ 9502$	95 ± 3	C/W 4324	97 ± 3	DK 127	98 ± 2
3B21	93 ± 3	Guardian	95 ± 3	C/W 4413	97 ± 3	DS 9503	98 ± 2
C/W 3307	93 ± 3	ICI 645	95 ± 3	Ciba 2888	97 ± 3	GH 767	98 ± 2
$DS \ 9501$	93 ± 3	Oneida	95 ± 5	Defiant	97 ± 3	Lightning	98 ± 2
Enhancer	93 ± 3	PGI 3522	95 ± 5	GA-APGC	97 ± 3	PGI 2512	98 ± 2
W110	93 ± 3	3L18	97 ± 2	MP 2000	97 ± 3	Ultraleaf 8	98 ± 2
C/W 3416	93 ± 4	4T63	97 ± 2	Sterling	97 ± 3	ZC 9335	98 ± 2
GH 787	93 ± 4	5262	97 ± 2	TMF Gene	97 ± 3	$3452 \ \mathrm{ML}$	100 ± 0
ZC 9348	93 ± 4	93-39	97 ± 2	ZN 93DD	97 ± 3	5454	100 ± 0
3T26	95 ± 0	BPR 372	97 ± 2	Oneida VR	97 ± 4	BPR 365	100 ± 0
C/W 4409	95 ± 0	DS 9504	97 ± 2	$2555 \ \mathrm{ML}$	98 ± 2	C/W 4206	100 ± 0
3B19	95 ± 1	$DS \ 9505$	97 ± 2	5246	98 ± 2	ICI 620	100 ± 0
3B12	95 ± 3	Vernal	97 ± 2	631	98 ± 2	PGI 3512	100 ± 0
3L48	95 ± 3	ZC 9445	97 ± 2	92-31	98 ± 2	ZC 9339	100 ± 0
4L65	95 ± 3	ZN 93AA	97 ± 2	94GG	98 ± 2		
ABI 9134	95 ± 3	3T44	97 ± 3	ABI 9220	98 ± 2		

Analysis of variance indicated no significant differences among means (F = 0.79; total df = 256, P = 0.90).

Table 2. Severity of injury (mean \pm SEM) caused by Agromyza frontella among and glandular-haired and traditional alfalfa varieties, 1998, Rosemount, Minnesota.

Variety ¹	% Stems with injured trifoliolates	Fresh weight (g)	Dry weight (g)
5347 LH*	$93.8 \pm 3.8a$	$106.7 \pm 6.2a$	$25.0 \pm 1.7a$
5454	$84.9 \pm 10.1a$	$90.4 \pm 6.9a$	$21.0 \pm 1.7a$
Ameriguard 3	01^* 88.8 ± 5.5a	$106.8 \pm 2.4a$	$25.8 \pm 1.0a$
Arrest*	$85.0 \pm 6.1a$	$110.1 \pm 11.7a$	$25.6 \pm 3.4a$
DK121 HG*	$83.8 \pm 13.1a$	$94.5 \pm 8.0a$	$21.9 \pm 1.7a$
Vernal	$100 \pm 0.0a$	$82.8 \pm 5.2a$	$20.0\pm1.5a$

Means followed by the same letter are not significantly different (P > 0.05); ANOVA and Ryan-Einot-Welsch multiple range test (SAS 1998).

¹Entries marked with an * refer to glandular-haired, commercial varieties.

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significantly less pinholing injury than the traditional variety 'Vernal' (P < 0.05). After accounting for both pinhole and mining injury, only 'Ameriguard 301' and 'Arrest' incurred significantly less injury than '5347 LH,' DK 121 HG,' and '5454.' However, the reduction in total trifoliolate injury in 'Arrest' and 'Ameriguard 301' was not significantly different from that of 'Vernal' (P > 0.05). 'Ameriguard 301' and '5347 LH' were significantly taller than most of the other varieties, but only 'Ameriguard 301' had a higher maturity rating than all other varieties (Table 3).

DISCUSSION

Although glandular-haired varieties have been investigated during the past 30 years for other alfalfa pests, such as *E. fabae* (e.g., Shade et al. 1979; Sulc et al. 2001) and the alfalfa weevil, *Hypera postica* (Gyllenhal) (Johnson et al. 1980), very little work has been published regarding the potential impact of glandular-haired germplasm or traditional alfalfa varieties on *A. frontella* (Hill and Byers 1979; MacLean and Byers 1983). Our results with more recent germplasm and glandular-haired commercial varieties are similar to those of Hill and Byers (1979), who found low levels of leaf mining resistance in six commercial varieties commonly grown in the 1970s ('Cherokee,' 'Saranac,' 'Culver,' 'Team,' 'Lahontan,' 'Vernal'). Of all varieties tested over two years, only 'Cherokee' showed a moderate level of resistance on one of four sample dates (Hill and Byers 1979). Moreover, leaf mining injury among the commercial varieties was similar to the progeny mean injury level of 54 polycross families of *M. sativa*. These authors found that heritability of resistance to *A. frontella*, among the 54 families, was low (4.2%).

Although we observed no significant differences in larval mining or adult pinhole injury within the 86-entry test, in the St. Martin study, a few of the glandular-haired varieties incurred less adult pinhole injury, and subsequently reduced mining injury at one location (Table 3). For example, significant reductions in pinholing were observed for 'Arrest,' 'Ameriguard 301'and 'DK 121 HG.' The lower incidence of adult pinholing in some of the glandular-haired varieties is in agreement with the results of MacLean and Byers (1983). In their no-choice

Variety ¹	% Trifoliolates with pinholes	% Trifoliolates with mines	Total % trifoliolates with injury	Stem height (cm)	Maturity rating ²
5347 LH*	$42.8 \pm 2.5a$	12.4 ± 1.1ab	$55.2 \pm 2.7a$	21.7 ± 0.6 ab	0.9 ± 0.04 b
5454	$48.3 \pm 2.5a$	$13.8 \pm 1.5 ab$	$62.1 \pm 2.7a$	$19.7 \pm 0.6c$	$0.8 \pm 0.05 \mathrm{b}$
Ameriguard 301	* 29.3 ± 1.6 bc	11.3 ± 1.2 abc	$40.6\pm2.0\mathrm{b}$	$23.3 \pm 0.6a$	$1.0\pm0.05a$
Arrest*	$28.0 \pm 1.9c$	$9.9 \pm 1.1 bc$	$37.9 \pm 2.3b$	$20.1 \pm 0.5 bc$	$0.8 \pm 0.04 b$
DK121 HG*	$29.7 \pm 2.4 bc$	$16.1 \pm 1.5a$	$56.6 \pm 3.6a$	$19.5 \pm 0.6c$	0.7 ± 0.06 b
Vernal	$33.7\pm1.9\mathrm{b}$	$9.1\pm1.4c$	$42.7\pm2.4\mathrm{b}$	$18.4\pm0.4\mathrm{c}$	$0.7\pm0.05\mathrm{b}$

Table	3.	Severit	y of	injury	(me	an ± S	EM)	caused	d by Ag	romy	za fron	tella or	ı
gland	ular	-haired	and	traditio	onal	alfalfa	ı vari	eties,	during	the	establis	hment	year,
1998,	St.	Martin,	Min	nesota.									

Means followed by the same letter are not significantly different (P > 0.05); ANOVA and Ryan-Einot-Welsch multiple range test (SAS 1998).

¹Entries marked with an * refer to glandular-haired, commercial varieties.

²Maturity Rating System (0-9 scale; where 0 = early vegetative, 1 = mid-vegetative, and 2 = late vegetative; Fick and Mueller 1989).

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laboratory test for oviposition preference, they found a significant correlation between reduced pinholing and an increase in glandular hairs among *Medicago* spp. germplasm. In addition, Sawyer and Fick (1987) concluded that adult pinhole feeding, rather than larval mining, was more likely to contribute to yield losses over a wide range of environmental conditions. In summary, each of the studies evaluating pinhole injury suggest that additional breeding work to improve alfalfa resistance to *A. frontella* should focus on mechanisms that will inhibit adult feeding or oviposition.

Given the results of our study showing few differences in alfalfa resistance to A. frontella, as well as previous research confirming inconsistent insecticidal control (Burkness et al., 1999a,b), we agree with Hendrickson and Day (1986) that additional work with beneficial parasitoids may be the most promising long-term management strategy. Establishment of *Dacnusa dryas* (Nixon) and other parasitoids have been very effective in the eastern U.S. (Drea and Hendrickson 1986) and in Canada (Heimpel and Meloche 2001). In addition to biological control, the simulation study of Sawyer and Fick (1987) indicates that early-harvest could be used to reduce yield loss, before larval mining becomes excessive. Finally, insecticide use for A. frontella should be limited to outbreak situations, which would minimize disruptions to effective biological control of the alfalfa weevil in Minnesota (Flanders and Radcliffe 2000).

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