Glandular-Haired Alfalfa Resistance to Potato Leafhopper (Homoptera: Cicadellidae) and Hopperburn: Development of Resistance Indices

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ABSTRACT Eight proprietary genotypes of glandular-haired alfalfa, *Medicago sativa* L., supplied by two different companies, were compared for the degree and types of resistance to the potato leafhopper, *Empoasca fabae* (Harris), and hopperburn. A tube cage no-choice bioassay was developed to test leafhopper mortality, feeding, settling preferences, severity of hopperburn symptoms (in this case, defined as both yellowing and stem growth reduction), and trichome density and type on feeding sites. Leafhopper mortality was both strongly and significantly associated with feeding and leaf trichome density; decreased hopperburn symptom severity was weakly, although significantly, associated with increased mortality. To quantify hopperburn in terms of both yellowing and stem growth reduction, we developed a ranking system that reduces overall hopperburn expression to a single number that considers the varying responses to both types of symptoms. Great variability in leafhopper settling, leafhopper mortality, and stem glandular trichome density was detected among alfalfa genotypes, suggesting that genotypic differences may be based on the concentration and/or chemical constituency of the trichome exudates. We postulate that, among variably resistant genotypes of glandular-haired alfalfa, differences among leafhopper responses and hopperburn severity are linked to forced movement from the stems to the leaves as refuge feeding sites. Principal component analysis was performed to reduce the 10 variables down to five biologically significant factors. Scores for these factors were then used to develop resistance indices for potato leafhopper resistance, hopperburn resistance, and an overall glandular-haired alfalfa resistance index.

KEY WORDS *Empoasca fabae*, *Medicago sativa*, glandular trichomes, host plant resistance, pubescence, principal components analysis

THE POTATO LEAFHOPPER, *Empoasca fabae* (Harris), is considered one of the most economically significant pests on alfalfa, *Medicago sativa* L., in the eastern half of the United States (Lamp et al. 1991, Elden and Elgin 1992) because it causes hopperburn. We define hopperburn as a noncontagious, disease-like condition characterized by both leaf yellowing and plant stunting. Severe cases of stunting (which is caused by reduced stem growth) can affect crop yield and limit the establishment and longevity of entire stands (Flinn and Hower 1984). Our past work has shown that hopperburn is initiated by the feeding of the potato leafhopper but then perpetuated by a plant physiological response cascade (Ecale and Backus 1995a, 1995b; Ecale Zhou and Backus 1999). Most conventional breeding programs seek to develop genotypes that are resistant to hopperburn. Early breeding efforts focused on resistance to yellowing, but breeders now include both yellowing and stunting in their standard tests for potato leafhopper resistance (NAAIC 1998).

The registration of the first glandular-haired alfalfa germplasm resistant to the potato leafhopper by Sorenson et al. (1985) initiated a new era in potato leafhopper control. Alfalfa breeding companies have spent the last 16 yr incorporating the glandular hair trait into their modern agronomic lines, but the trait has been unstable. Crosses between putatively resistant genotypes often led to variation in pubescence in the offspring (Elden et al. 1986, Hogg and McCaslin 1994, Elden and McCaslin 1997). The resistance is linked directly to the presence of glandular trichomes on the plant surface and/or some co-varying trait(s) carried through each generation. Yet, while a relationship between pubescence in the new, proprietary

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genotypes and resistance to the potato leafhopper is likely (Elden et al. 1986), laboratory and field tests have been largely unsuccessful in identifying the precise mechanism of the resistance (Hogg and McCaslin 1994, Elden and McCaslin 1997). Furthermore, many of these alfalfa genotypes still exhibit severe stunting and drastic reductions in yield and stand quality (Kindler et al. 1973, Hower and Flinn 1986).

Glandular trichomes have been shown to significantly reduce the level of infestation of homopteran pests, particularly aphids and leafhoppers, on a variety of agronomically significant crops such as tomato (Goffreda et al. 1988, 1989), potato (Tingey and Laubengayer 1981, 1986; LaPointe and Tingey 1984, 1986), and alfalfa (Brewer et al. 1986, Hogg and McCaslin 1994, Elden and McCaslin 1997). Variations in trichome structure, chemistry, and density may lead to differences in insect resistance levels among genotypes within a plant species.

Othman et al. (1981) noted the importance of trichome density in alfalfa, but could not use it to explain differences in resistance. Other researchers also have found variation in levels of resistance of some alfalfa genotypes that could not be explained by trichome density alone (Hogg and McCaslin 1994, Elden and McCaslin 1997, Lenssen et al. 1999). Glandular trichomes in species of *Medicago*, including alfalfa, have two distinct trichome morphologies: erect and procumbent. The erect glandular trichomes consist of a ball-like gland atop a multicellular stalk directly perpendicular to the plant surface. In contrast, the procumbent trichomes have glands atop a one- or two-cell stalk directly parallel to the plant surface (Ranger and Hower 2000a). Both types of trichomes have been shown to produce a sticky exudate that can be an effective mechanical and chemical deterrent to leafhopper nymphs. It has been hypothesized that trichomes also produce other compounds that act as a resistance mechanism against adult potato leafhoppers (Ranger and Hower 2000b). Hypothesized mechanisms have been both physical (entrapment) and chemical (antibiosis or antixenosis) (Hogg and McCaslin 1994, Elden and McCaslin 1997, Ranger and Hower 2000b).

Early studies on the original glandular-haired alfalfa genotypes demonstrated high levels of mortality against potato leafhopper nymphs, antixenosis against adult feeding as a result of physical interference in normal feeding behaviors, and decreased oviposition among adult females (Brewer et al. 1986). Nymphal mortality on trichomes was directly attributed to the presence of a sticky exudate that "glued" the first-instar nymphs to the leaf surface and led to death before the second-instar molt.

In this study, we compared leafhopper and hopperburn responses to plant characteristics among eight variably resistant, proprietary genotypes of glandular-haired alfalfa, from two alfalfa breeding companies. Our objectives were to: (1) provide insights into the possible mechanism(s) of resistance, and (2) to develop resistance indices, using multivariate principal component analysis (PCA), that would objectively and accurately represent resistance. Our results show that resistance to potato leafhopper is different from resistance to hopperburn, and most genotypes vary in their expression of the two types of resistance.

Materials and Methods

Insects were reared on greenhouse-grown broad beans, *Vicia faba* L. variety Windsor, in a growth chamber and maintained under a 16:8 (L:D) h photoperiod at 25 ± 2°C. Before each bioassay, one cage of insects (≈300 adults) was sorted by sex, and adult females <10 d old were chosen and transferred to Ranger alfalfa for 2 d of conditioning experience.

Cal/West Seeds and Forage Genetics each provided four test genotypes of glandular-haired alfalfa (Cal/West: G98A, G98B, G98C, G98D; Forage Genetics: 1-27-1, 1-27-4, 1-27-7, 3-73-1). The genotypes selected were known to exhibit a spectrum of resistance to hopperburn. Alfalfa plants were vegetatively propagated and grown in growth chambers under a 16:8 (L:D) h photoperiod at 27°C during the day and 15°C at night, according to the methods of Kabrick and Backus (1990). Plants were cut once and allowed to regrow until they reached 10–12 internodes in height.

Tube Cage Studies. Two days before each test, tube cages were constructed in a modification of the design originally used by Backus et al. (1990). Each cage consisted of an overhead transparency film (21.59 by 27.94 cm) (Arkwright, Coventry, RI) rolled into a cylinder and secured with double-sided tape (Scotch brand). A 1.25-cm hole was then made in each cage 18.0 cm from the base using a heated cork borer. The top of the cylinder was sealed with organza cloth held in place by double-sided tape, and a foam bottom with a slit to accommodate the alfalfa stem was cut to fit each cage. This design allowed more precise quantification of excretion (see below), because each cage could be unrolled to produce a flat sheet. The cages were placed in a fume hood for an additional day to dissipate fumes from burning the access hole. The finished cage was then placed around each test plant and insects were added through the access hole.

Two stems from each of three plants were selected from each genotype per replicate, based on similar heights and similar number of internodes. Each stem (with attached leaves) was caged separately. Pots (each with two cages) were then randomly labeled and arranged under fluorescent lights on a metal shelf. On the day of the test, one tube cage on each plant was left uninfested as the control for stem elongation measurements. The other cage was infested with three adult female leafhoppers. In the first type of test, the four genotypes from each alfalfa breeding company were tested at one time, with three replicates (cages) for each genotype. This test was repeated twice; therefore, six cages were used for each genotype. In the second type of test, all eight genotypes, each with three replicates again, were tested at one time. Again, this type of test was repeated twice, for another six cages per genotype. Thus, a total of 12 cages were used for six out of eight genotypes. G98D (Cal/West Seeds)

Table 1. Rating scale for stunting comparing control stem elongation and infested stem elongation and establishing ranks based on hopperburn stunting

Score	Mean elongation difference (cm)[a]	% stem growth reduction
1	$-2.5 \leq \text{cm} < 0$	0–20
2	$0 \leq \text{cm} < 2.5$	20–40
3	$2.5 \leq \text{cm} < 5.0$	40–60
4	$5.0 \leq \text{cm} < 7.5$	60–80
5	$7.5 \leq \text{cm} \geq 10.0$	80–100

[a] Mean elongation differences less than 0 (Score 1) indicate more or equal growth compared with control.

and 3-73-1 (Forage Genetics) were highly susceptible genotypes that were difficult to rear and keep thrips- and mite-free. Consequently, a shortage of those plants meant that only three and nine cages, respectively, could be tested. Nonetheless, we felt that the comparison with susceptible genotypes was so important that we kept the data. Results showed no significant differences among repetitions (cages), so data were pooled for subsequent analyses.

Genotypes were tested for differences in parameters as follows: (1) leafhopper mortality, (2) leaf yellowing, (3) length of stems following constant leafhopper pressure, (4) mean number of excretory droplets per cage, (5) number of leafhoppers per plant part at each observation, and (6) trichome density and type on leaves and stems. Number of excretory droplets per cage adhering to the inner surface of the cage walls was manually counted to provide an approximate measure of feeding that occurred. Droplets appeared fairly uniform in size, so the number was a reasonable approximation for relative volume. Because it was desirable to maintain constant leafhopper pressure on the genotypes to initiate hopperburn symptoms, all dead insects were replaced at the daily observation time. No progeny appeared in the cages during the 5 d of the test. So, insects counted were only those put into the cage. Observations for leafhopper mortality, and number of insects on each plant part were recorded every 24 h for 5 d. Measurements of hopperburn severity (i.e., yellowing) and stem lengths (control versus infested) as well as trichome density and excretory droplets, were made only after the 5-d period had elapsed.

A rating scale for hopperburn was formulated by combining scores for yellowing and stem growth reduction (representing potential stunting). Yellowing scores were based on a 1–5 renumbering of the scoring system used by Elden and Elgin (1987), where 1 was 0–20% of leaves yellowed, 2 was 20–40%, 3 was 40–60%, 4 was 60–80%, and 5 was 80–100%. The stem growth reduction scores were derived by calculating the difference in the average amount of stem elongation that occurred between the infested stem and the control within each genotype. The actual growth reduction scores were derived by converting the percentage difference into one of five categories (Table 1), thus making the yellowing and growth reduction scales similar. In rare cases where the infested stem elongated more than the control stem, the "mean elongation difference" (Table 1) between the two stems was negative, and the stem was classified as having 0% growth reduction.

Trichome counts were performed for all test cages on 1-mm sections of the stem between the fifth and eighth internodes, and within 1-mm^2 sections of the leaves. These counts were aided by a window (1 by 1 mm) cut in a piece of wax filter paper. This "frame" was then placed over each of the count areas, and all trichomes contained within its area were counted under a dissecting microscope.

Statistical Analysis. Two analyses of variance (ANOVAs) were performed, using SAS (SAS Institute 1985) and a type I error of $\alpha = 0.05$. For mortality and feeding (i.e., excretory droplet) results, a simple, one-way analysis of variance (ANOVA) (SAS, PROC GLM) was used for testing differences among genotypes. For the second ANOVA (SAS, PROC GLM), the dependent variable, genotype, was analyzed in a split-plot, completely randomized design. The linear statistical model included the effects of genotype, plant number within genotype, plant part and the interaction of genotype with plant part. The effect of genotype represented the main plot, and was tested using plant number within genotype as the denominator of *F.* The subplot effects of plant part and the interaction of genotype × plant part were tested with the residual error as the denominator of *F.* All other effects used the residual error for the denominator of *F.* In cases of heterogeneous variance within genotype, the data were transformed using square roots or base 10 logarithms, depending on which transformation resulted in variance stabilization for each parameter. Mortality and trichome densities used square root; feeding (i.e., excretory droplets) used logarithms.

In addition, two linear regression models were generated using SAS (SAS Institute 1985). One model used mortality as the dependent variable and the parameters feeding, stem trichome density, and leaf trichome density served as the independent variables. The other model used hopperburn severity as the dependent variable and the parameters mortality, feeding, stem trichome density, and leaf trichome density served as the independent variables. For all regressions, P values are listed to indicate significance of relationships, and r^2 values are listed to indicate the strength of those relationships (i.e., the percentage of the variation in the dependent variable explained by the independent variable).

Principal Components Analysis. We performed principal component analysis (PCA) to combine and reduce variables, with a goal of distilling all of our findings into a single value that could be used as a resistance index. PCA (SAS Institute 1985) was carried out on the covariance matrix of the mean-corrected values for 10 variables. The eight leafhopper resistance measures (i.e., mortality, feeding [i.e., excretory droplets], settling on stem, leaf, petiole or off the plant, and density of trichomes on stems or leaves) and the two hopperburn measures (stem growth reduction and yellowing) were analyzed both together

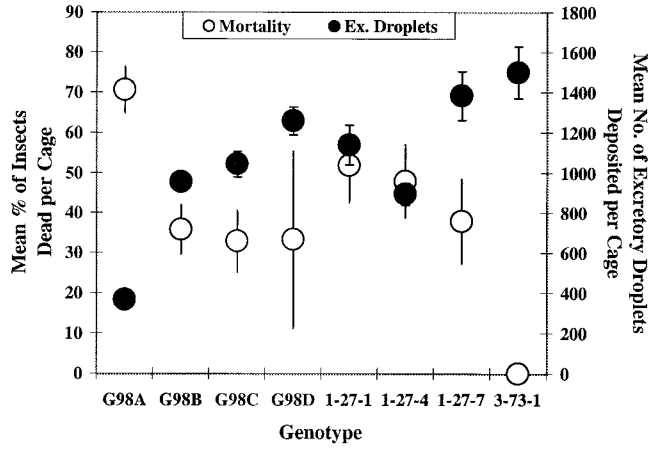

Fig. 1. Mean mortality versus mean number of excretory droplets per cage. Lines represent standard errors of the mean.

(all 10 variables) and separately (two tests, with eight and two variables, respectively). PCA results best matched the experimental findings when two separate tests were used. Four of the principal components (PCs or 'factors') for potato leafhopper resistance, and one for hopperburn resistance, were retained for further analysis. A linear combination of the PCA scores for these five factors was used to generate two resistance indices, one for potato leafhopper resistance and the other for hopperburn resistance; the two were then averaged for a combined resistance index. Index values were then calculated for each genotype and were compared by univariate ANOVA and least significant differences (LSD) (SAS Institute 1985), with $\alpha = 0.05$.

Results

Leafhopper Mortality and Feeding. Mortality data were not significantly different among repetitions (cages), so were pooled for all four replicates. High variation among genotypes suggested varying levels of resistance to the potato leafhopper (defined as induction of mortality) (Fig. 1). Genotypes G98A and 1-27-1 demonstrated more leafhopper mortality than all other genotypes over the course of the 5-d study. In contrast, 3-73-1 induced no mortality. The remaining genotypes induced ≈30–50% mortality per cage.

There was a highly significant ($P = 0.0001$) inverse association between feeding (i.e., excretory droplets) and mortality across the genotypes (Fig. 1) using linear regression, notwithstanding a low strength to the association ($r^2 = 0.184$). Therefore, in those genotypes with high mortality, less feeding occurred, and in those genotypes with low mortality, high feeding occurred. Such was especially the case for the most lethal (G98A) and least lethal (3-73-1) genotypes.

Hopperburn Symptoms and Severity. Expression of hopperburn symptoms, based on plant appearance by the final observation period, greatly differed among genotypes. Some, such as G98A, exhibited little yellowing but severe stem growth reduction. Others (i.e., 3-73-1) were strongly yellowed, but showed near-normal growth. Mean yellowing and stem growth reduction scores were averaged to create an overall hopperburn severity score for each genotype; scores were then ranked for overall resistance to hopperburn (Table 2).

Table 2. Mean yellowing and stunting scores (±SE) for all tested glandular-haired alfalfa genotypes on a scale of 1 to 5, 1 being least and 5 being most severe symptoms

Genotype	Mean yellowing scores	Mean growth reduction scores	Mean hopperburn scores	Hopperburn resistance rank	Leafhopper resistance rank
G98A	1.33 ± 0.14	2.92 ± 0.36	2.13	4	1
G98B	2.75 ± 0.37	2.42 ± 0.26	2.59	6	5
G98C	4.50 ± 0.19	2.58 ± 0.31	3.54	8	6
G98D	3.67 ± 0.67	3.33 ± 0.67	3.50	7	7
1-27-1	1.42 ± 0.19	2.00 ± 0.33	1.71	1	2
1-27-4	1.83 ± 0.30	1.75 ± 0.25	1.79	2	3
1-27-7	2.00 ± 0.33	1.92 ± 0.19	1.96	3	4
3-73-1	2.67 ± 0.41	1.78 ± 0.22	2.23	5	8

Includes average hopperburn severity score and rankings based on overall resistance to hopperburn, 1 being most resistant and 8 being most susceptible. For comparison, the leafhopper resistance rank is also shown.

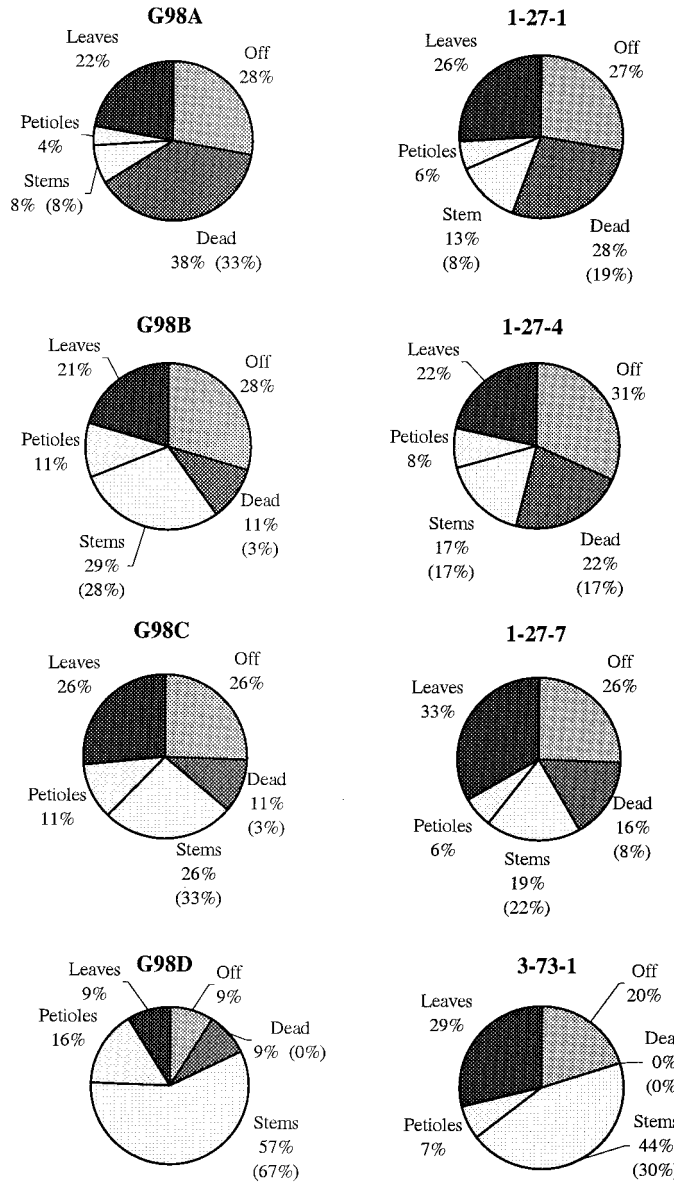

Fig. 2. Settling preferences of potato leafhopper on glandular-haired alfalfa genotypes. Percentages represent the total number of observations (across replicates) on each plant part, off of the plant, and dead per genotype, over the 5 d of the experiment. Values in parentheses are data after 1 d of the experiment.

Settling Preference on Glandular-Haired Alfalfa. Gruenhagen and Backus (1999) showed that ≈85% of adult female potato leafhoppers, after 24 h on a susceptible glabrous alfalfa ('Ranger'), preferred to settle on the stem. As shown graphically in Fig. 2, even the glandular-haired genotypes least lethal to leafhoppers that we tested (e.g., G98D and 3-73-1; Table 2) reduced settling on the stem (compare the 5-d percentages with 1-d percentages in parentheses), compared with Ranger. For the genotypes that appeared to be intermediate in potato leafhopper-lethality (e.g., G98B, G98C, 1-27-4, and 1-27-7), the settling preference shifted from 'stems' to 'off' or 'leaves' (depending on genotype). Some insects died as well. On the genotypes most highly lethal to leafhoppers (i.e., G98A and 1-27-1), those insects that survived shifted their distribution to 'off' and 'leaves'. The petioles acted as intermediate settling sites in ways dependent upon the genotype and which alfalfa breeding company produced it. For G98A, G98B, G98C, and G98D (Cal/West genotypes), the percentages of insects on the petioles were quite variable (4–16%). But they were directly proportional to those on the stems. For 1-27-1, 1-27-4, 1-27-7, and 3-73-1 (Forage Genetics genotypes), percentages of insects on petiole were essentially the same for all genotypes, averaging 6.75%.

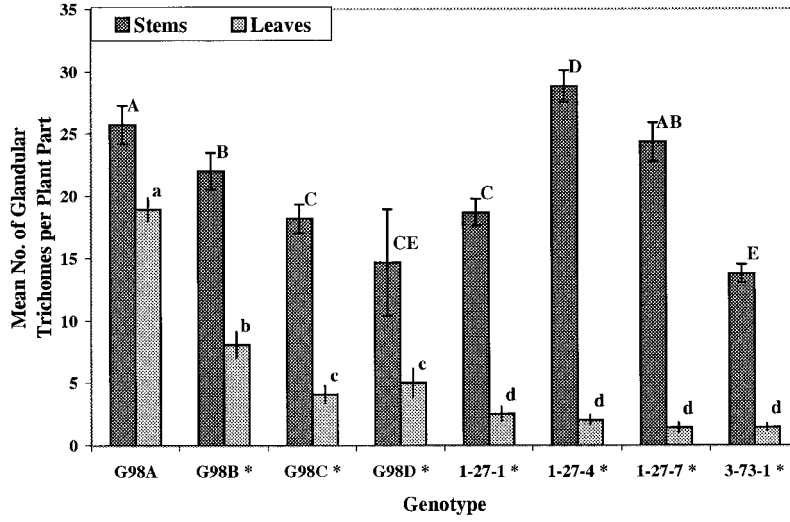

Fig. 3. Mean number of glandular trichomes per 1 mm^2 section of stems and leaves of glandular-haired alfalfa genotypes. Genotypes with identical capital letters (stems) or identical small letters (leaves) are not significantly different ($\alpha = 0.05$). *, Denotes significant differences between stems and leaves within each genotype.

This probably reflects the differences in parental genotypes between the companies.

Stem and Leaf Glandular Trichome Density. Variations in glandular trichome density were found among genotypes, as well as between stems and leaves within each genotype (Fig. 3). All genotypes had significantly fewer glandular trichomes on the leaves than on stems, with the exception of G98A. In the latter genotype, LSD tests confirmed that, although variance appeared to be heterogeneous, the trichome density on stems and leaves was not significantly different. Thus, nonoverlapping standard errors for stem and leaf hairs of G98A were the result of calculating the standard errors within each plant part, not within each genotype.

Hopperburn Severity in Relation to Mortality, Feeding, and Trichome Density. Mortality and hopperburn scores were plotted with the stem and leaf glandular trichome densities, across the genotypes (Fig. 4). Mean hopperburn scores were significantly ($P = 0.0295$) and inversely associated with mortality; however, the association was very weak ($r^2 = 0.056$). Some genotypes, especially 1-27-1, strongly demonstrated this direct relationship. However, especially among genotypes with intermediate levels of resistance, low mortality did not necessarily predict high hopperburn scores, and vice versa. For example, 1-27-7 and G98B caused little mortality but were still relatively resistant to hopperburn. Among G98B, G98C, and G98D, very small differences in mortality

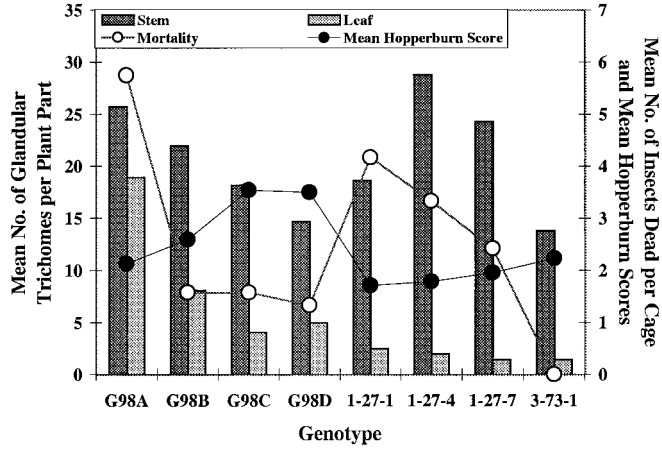

Fig. 4. Mean number of glandular trichomes on stems and leaves plotted with mean mortality and mean hopperburn scores. Lines drawn between genotypes for mortality and hopperburn scores are not intended to imply interrelatedness between genotypes but only to make comparison of this data with the trichome density data more manageable.

Table 3. Loadings for the first four principal components formed with variables related to potato leafhopper resistance

Variable	Loadings			
	R	S	R	S
	Fac. A	Fac. B	Fac. C	Fac. D
Feeding (i.e., excretory droplets)	−0.81*	0.18	−0.15	−0.22
Mortality	0.74*	−0.21	−0.06	−0.58*
Settling off of plant	0.47*	0.33	−0.63*	0.50*
Settling on stems	−0.76*	−0.30	0.08	−0.01
Settling on petioles	−0.41	−0.52*	0.22	0.45*
Settling on leaves	−0.20	0.77*	0.48*	−0.01
Stem trichome density	0.48*	0.26	0.53*	0.21
Leaf trichome density	0.61*	−0.45*	0.34	0.14
% variance explained (cum.)	35.14	52.71	66.59	77.95
% variance explained (propor.)	35.14	17.57	13.88	11.36

*, Variables that constitute the principal component. Only four out of a total of seven principal components were retained. R = resistance factor, S = susceptibility factor.

occurred with relatively large differences in hopperburn scores. In contrast, among 1-27-1, 1-27-4 and 1-27-7, large differences in mortality occurred with almost no change in hopperburn scores. Hopperburn severity was not associated with feeding (i.e., excretory droplets) ($P = 0.4405$, $r^2 = 0.007$).

Glandular trichome densities of both leaves and stems were not significantly associated with hopperburn ($P = 0.5565$ for leaves, $P = 0.0743$ for stems) (Fig. 4). Mortality was significantly, positively associated with leaf trichome density ($P = 0.0017$), but again the association was weak ($r^2 = 0.113$). Stem trichome density was almost significantly associated with mortality ($P = 0.0571$), although again weakly ($r^2 = 0.043$). This weak association was probably caused by those genotypes that had relatively high stem trichome density, low leaf trichome density, yet high mortality (i.e., 1-27-1 and 1-27-4).

Principal Components Analysis. The findings described above strongly suggest that potato leafhopper resistance is not directly related to hopperburn resistance. We chose to test this hypothesis, as well as to more completely analyze the data, using PCA methods outlined in more detail in Serrano et al. (2000). The hypothesis was supported by an initial PCA of all 10 variables, which loaded yellowing and stem growth reduction to widely different factors, and in directly opposite sign of those for leafhopper-affecting variables. In contrast, when two separate PCA tests were performed, results matched and explained well the biological inferences described above. Thus, we separated the overall GH-alfalfa resistance into potato leafhopper resistance and hopperburn resistance.

PCA successfully combined and reduced the variables, providing scores that were directly comparable to one another, unlike the values for the original variables. PCA on the two hopperburn-related variables, yellowing and stem growth reduction, reduced them to a single factor that equally loaded the contributions for both variables (loading = 0.74, % variance explained = 54.04). PCA reduced the eight leafhopper resistance-related variables to four factors that together explained nearly 78% of the variance in the data. In turn, these factors explained the proportional contribution of each variable to leafhopper resistance or susceptibility (Table 3). We usually used any loading of 0.45 or greater to ascertain biological meaning of the factors, although greater emphasis was given to higher values.

Of the potato leafhopper resistance factors, factors A and C represent resistance to the potato leafhopper. Factor A's largest loadings (of nearly the same value) were for mortality (positive) and feeding (negative). This confirms that feeding and mortality are inversely correlated. Settling on the stem also had a large, but negative, loading value. Lower, but still important, loadings were for leaf trichomes, stem trichomes, settling off of plant, and settling on the petiole (all positive). Therefore, *factor A represents primarily the plant's physiological ability to kill the insect.* It also represents (although to a much lesser extent) the degree to which the stem and leaf trichomes are driving the insects from the stem and onto the petiole or off of the plant entirely. Thus, we considered factor A to be a strong resistance (both antibiosis and antixenosis) factor. Not surprisingly, the mortality contained in factor A accounted for the highest percentage of the experimental variance (>35%). Factor C had positive loadings for leaf settling and stem trichomes, but negative loading for settling off of the plant. Also, feeding, though small and not significant, was negative. Thus, *factor C represents the physiological ability of the stem trichomes to strongly repel leafhoppers off of the stems and onto leaves.* Overall, we considered factor C mostly a resistance (antixenosis) factor.

In contrast, factors B and D represent potato leafhopper susceptibility. Factor B had a very strong positive loading for settling on the leaf, and negative loadings for petiole settling and leaf trichomes. Although low in value, mortality was also negative. Therefore, *factor B represents the degree to which the insects can survive by shifting their behavioral choice from the petioles onto the leaves,* primarily if the number of leaf trichomes is low. Factor D had positive loadings for off of the plant and on to the petiole, but a fairly strong negative loading for mortality. Thus, *factor D represents the degree to which the insects' be-*

Table 4. Mean scores (± SEM) for four potato leafhopper PCA factors and one hopperburn resistant factor, generated by principal component analysis of 10 leafhopper and hopperburn-related variables

Genotype	Potato leafhopper				
	A: Resist.	B: Suscept.	C: Resist.	D: Suscept.	Hopperburn
G98A	1.62 ± 0.14a	−0.68 ± 0.23ac	0.81 ± 0.21a	0.08 ± 0.23a	−0.08 ± 0.28a
G98B	−0.05 ± 0.16bd	−0.45 ± 0.33a	−0.01 ± 0.36b	0.67 ± 0.25b	0.28 ± 0.21a
G98C	−0.39 ± 0.18b	−0.08 ± 0.31ab	−0.14 ± 0.26b	0.23 ± 0.19ab	1.24 ± 0.21b
G98D	−1.27 ± 0.33c	−1.74 ± 0.22c	−0.10 ± 0.37ab	−0.41 ± 0.38c	1.33 ± 0.66b
1-27-1	0.06 ± 0.19bd	0.28 ± 0.21bd	−0.54 ± 0.28c	−0.61 ± 0.34c	−0.64 ± 0.25c
1-27-4	0.39 ± 0.14d	0.37 ± 0.20bd	0.05 ± 0.27ab	0.31 ± 0.44ab	−0.60 ± 0.21c
1-27-7	−0.32 ± 0.21b	0.84 ± 0.17d	0.15 ± 0.29ab	−0.25 ± 0.20ac	−0.41 ± 0.19ac
3-73-1	−1.33 ± 0.18c	0.20 ± 0.26bd	−0.40 ± 0.29bc	−0.44 ± 0.16c	−0.18 ± 0.21ac

Means followed with the same letter within a column are not significantly different ($\alpha = 0.05$).

havioral choice is to return to the plant to feed on the petioles (to prevent starvation), even though they are initially being repelled from the plant. Both of these factors are susceptibility measures (i.e., representing the ability of the insects to overcome antixenosis factors by the plant).

Thus, PCA successfully reduced the number of variables by half, and in so doing, provided stronger, more objective evidence for the same conclusions inferred from our univariate examination of the data. It especially supported the hypothesis that, for certain genotypes, the insects can partially compensate for stem trichomes by shifting to the leaves.

Mean scores for the PCA factors for each genotype are shown in Table 4. In principal component terms, a '0' level for a score means that the resistance or susceptibility components of that factor matched the overall average of those components on all eight genotypes. Therefore, the more positive the principal component score for any of the five factors, the more the plant stimulated its outcome; the more negative the score, the less the plant stimulated that factor's outcome. The highly potato leafhopper-lethal genotype G98A stands out with a very high, positive score for factor A; genotypes with intermediate-level lethality (e.g., G98B, G98C, 1-27-1, and 1-27-4) had scores nearer to 0, and the nonlethal genotypes (G98D and 3-73-1) have strongly negative scores. The scores show significant differences among genotypes, related to leafhopper-lethality or hopperburn-severity levels (Table 4).

Development of Resistance Indices. We followed the general formula described in more detail in Serrano et al. (2000), which in turn was developed from a yield-based resistance index formulated by bean breeders at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Our only modification (compared with Serrano et al. 2000) is that we weighted each PCA factor score for importance in the overall index. Thus, first each genotype's mean score was multiplied by the percentage of the experiment's overall variance explained by that factor. Second, the weighted factor scores were subtracted or added together, depending on whether the factor was considered to represent resistance (subtracted) or susceptibility (added). Finally, all values were subtracted from 1.0, to set 1.0 as a threshold level separating resistant from susceptible genotypes. By these conventions, the formula for the potato leafhopper resistance index is:

$$\text{Potato leafhopper index} = 1 - [(B \times 0.18) - (C \times 0.14) + (D \times 0.11) - (A \times 0.35)],$$

where A is factor A score (resistance), B is factor B score (susceptibility), C is factor C score (resistance), and D is factor D score (susceptibility).

Index values for all eight genotypes are graphed in Fig. 5A, which clearly shows that both strong potato leafhopper-resistance and strong potato leafhopper-susceptibility are rare among the genotypes we tested. Five of the eight genotypes fell into a "borderline" area on the chart, defined as index values between 0.90 and 1.10 (i.e., the 1.0 decision threshold ± 10%; these genotypes cannot be classified as clearly resistant or susceptible to potato leafhopper). All genotypes within this area were not significantly different from one another. This index confirmed that G98A was the most potato leafhopper-resistant and 3-73-1 was the most potato leafhopper-susceptible of the genotypes. The Cal/West genotypes generally were more leafhopper-resistant than the Forage Genetics genotypes; Cal/West's most susceptible genotypes were in the "borderline" area. In contrast, the Forage Genetics genotypes generally were more leafhopper-susceptible; their most resistant genotypes were in the "borderline" area.

The formula for the hopperburn resistance index is

$$\text{Hopperburn index} = 1 - (E \times 0.54),$$

where E is the hopperburn score.

In contrast to the potato leafhopper index values, hopperburn index values varied widely among genotypes. The biological validity of the PCA approach for a resistance index is shown by the almost identical (although inverted) appearance of the hopperburn lines in Figs. 4 and 5. Also, the ranking of genotypes was the same regardless of method used (compare Table 2 and Fig. 5B).

Graphing the hopperburn index reveals the resistance spectrum across the genotypes more clearly (Fig. 5B). Again, the two alfalfa breeding companies' genotypes differed by group. Generally speaking, the Cal/West genotypes were more susceptible to

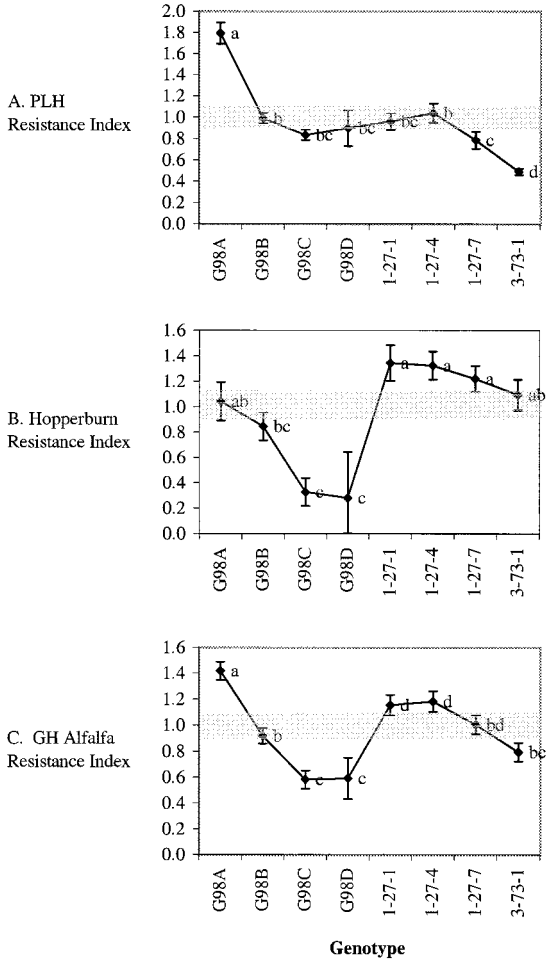

Fig. 5. Resistance indices for glandular-haired alfalfa. A. Potato leafhopper resistance index. B. Hopperburn resistance index. C. Glandular-haired alfalfa resistance index. Lines drawn between genotypes on all indices are not intended to imply interrelatedness between genotypes. Genotypes with identical letters on all indices are not significantly different ($\alpha = 0.05$).

hopperburn than the Forage Genetics genotypes. The two most potato leafhopper-resistant Cal/West genotypes were in the "borderline" area of the hopperburn index. In contrast, three out of four Forage Genetics genotypes were strongly hopperburn-resistant, with only the most potato leafhopper-susceptible one in the "borderline" area for hopperburn resistance. Thus, results of the potato leafhopper and hopperburn indices support our postulate that the genotypes vary independently for the two types of resistance.

The potato leafhopper index (Fig. 5A) and the hopperburn index (Fig. 5B) were combined into a single GH alfalfa resistance index (Fig. 5C), by averaging the two indices.

Discussion

Potato Leafhopper Versus Hopperburn Resistance. Results of this study show that resistance to potato leafhopper (i.e., mortality [antibiosis] or decreased feeding [antixenosis]) does not necessarily lead directly to resistance to hopperburn (i.e., decrease in hopperburn severity). This is because, even though leafhopper mortality is associated with decreased feeding and with hopperburn, the effect of mortality on hopperburn is minor (explaining only 5.6% of the variability in hopperburn) and not predictive of ultimate symptom severity.

Mean hopperburn scores were not associated with total amount of feeding. This may not be surprising, because the only known case of a strong association between hopperburn and feeding occurs when the plant induces the leafhopper to shift its type (not total amount) of feeding (i.e., from a more to a less damaging feeding tactic), while still feeding for the same duration (Serrano et al. 2000). Determination of this type of antixenosis can currently be made only via electronic monitoring of leafhopper feeding. Nonetheless, this study shows that even a relatively simple bioassay without electronic monitoring can yield insights into the underlying complexity of the alfalfa-potato leafhopper-hopperburn interaction.

Glandular trichome density was associated with both insect mortality and hopperburn, but very tenuously. It was initially hypothesized (Shockley and Backus 1999) that, on genotypes with high stem trichome densities, leafhoppers would move to the leaves to feed. Genotypes with high densities on both stems and leaves would force the insect to leave the plant, resulting in high mortality due to starvation. This leaf-refuge hypothesis was supported by the settling preference data for some, but not all, of the glandular-haired genotypes. Two genotypes (1-27-4 and 1-27-7) had relatively high stem trichome densities, low leaf trichome densities, yet relatively high mortality. Therefore, in those cases, the increased mortality must be due to an unknown (possibly chemical) factor located in the trichomes or associated internally with the plant tissues. The more complete analysis (compared with Shockley and Backus 1999) described herein suggests that the efficacy of glandular trichomes is a combination of leaf and stem trichomes. Stem trichome density (and/or chemistry) must be sufficiently high to impede feeding on that site; additional density (and/or chemistry) of leaf trichomes then becomes the pivotal factor for survival. On those genotypes, only insects that can tolerate leaf trichomes and feed on that alternative site will survive.

Resistance Indices. Principal component analysis (PCA) reduced the number of variables being examined and placed them into PCA factors (scores) that were biologically definable. We then developed equations using these scores, to determine the impact of potato leafhopper resistance and/or hopperburn resistance on the total resistance of all genotypes. Although averaging the potato leafhopper and hopperburn indices together to give the overall GH-alfalfa

resistance index could imply that the impacts of the two are equivalent, in actuality we cannot assign relative impact to each. Averaging was performed as an approximation. Nonetheless, this approximation provided clearer information about overall resistance than any previous measure, especially compared with the univariate analyses. It is also an improvement over simple ranking of genotypes, because the index provides an objective decision threshold to identify resistance or susceptibility.

The potato leafhopper resistance index was particularly useful in that it distills results from several different measures into a single value. This provides a comparison and ranking of genotypes not available by any other means. The potato leafhopper index clearly showed that most of the genotypes tested fall into a "borderline" area that is neither strongly resistant nor susceptible. Thus, it is likely that the effects of trichomes on leafhoppers are so indirect that differences among genotypes in trichome numbers and/or chemistries must be very large to cause significant differences in resistance.

A comparison and combination of the potato leafhopper and hopperburn indices shows that a certain genotype can have borderline leafhopper resistance, but strong hopperburn resistance, or vice versa. Plant characteristics more internal than trichomes probably control hopperburn resistance. These could be characteristics that cause the insect to switch to a less or more damaging type of feeding, or physiological tolerance/sensitivity to damaging types of feeding, such that the hopperburn response cascade is affected.

The combined GH alfalfa resistance index enables a direct, numerical comparison of the two types of resistance for the first time. The total resistance of a particular genotype can be differentiated into one of three specific categories: resistant, intermediate (i.e., in the "borderline" area on the chart), and susceptible. Our data strongly suggest that the companies are selecting for different forms of resistance (Cal/West for potato leafhopper resistance, Forage Genetics for hopperburn resistance). However, this may be an artifact of the small number of genotypes chosen for our study by each company. The indices presented herein will aid those efforts by providing a technique for more quantitative and rapid assessment of genotypes, thus increasing the likelihood of developing new lines that are resistant to both the leafhopper and hopperburn.

The results of this work demonstrate that trichomes (or associated co-varying traits) influence the level of resistance to potato leafhopper in some genotypes of glandular-haired alfalfa. It seems likely that trichomes are directly affecting the establishment of a physicochemical defensive perimeter. However, trichomes and potato leafhopper resistance are only indirectly linked to hopperburn resistance. The separable mechanisms of potato leafhopper- and hopperburn-resistance imply that hopperburn severity can be only partially predicted by trichome-induced insect mortality. Therefore, other factors in the plant physiological response that causes hopperburn are also likely to be involved. Determining any possible differences in trichome chemistry among genotypes will now be critical to understanding their role in the resistance mechanisms of alfalfa, to both the potato leafhopper and hopperburn.

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References Cited

Backus, E. A., N. M. Gruenhagen, and S. A. Becker. 1990. The potato leafhopper (Homoptera: Cicadellidae) exhibits different settling distributions on alfalfa and broad bean. J. Econ. Entomol. 83: 814–818.

Brewer, G. J., E. Horber, and E. L. Sorenson. 1986. Potato leafhopper (Homoptera: Cicadellidae) antixenosis and antibiosis in *Medicago* species. J. Econ. Entomol. 79: 421–425.

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

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

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

Elden, T. C., and J. H. Elgin, Jr. 1987. Recurrent seedling and individual-plant selection for potato leafhopper (Homoptera: Cicadellidae) resistance in alfalfa. J. Econ. Entomol. 80: 690–695.

Elden, T. C., and J. H. Elgin, Jr. 1992. Mechanisms of resistance to the potato leafhopper (Homoptera: Cicadellidae) in selected alfalfa clones. J. Econ. Entomol. 85: 576–582.

Elden, T. C., J. H. Elgin, Jr., and J. F. Soper. 1986. Inheritance of pubescence in selected clones from two alfalfa populations and relationship to potato leafhopper resistance. Crop Sci. 26: 1143–1146.

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., and A. A. Hower. 1984. Effects of density, stage and sex of the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae) on seedling alfalfa growth. Can. Entomol. 116: 1543–1548.

Goffreda, J. C., M. A. Mutschler, and W. M. Tingey. 1988. Feeding behavior of potato aphid affected by glandular trichomes of wild tomato. Entomol. Exp. Appl. 48: 101–107.

Goffreda, J. C., M. A. Mutschler, D. A. Ave, W. M. Tingey, and J. C. Steffens. 1989. Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii.* J. Chem. Ecol. 15: 2135–2147.

Gruenhagen, N. M., and E. A. Backus. 1999. Diel distribution patterns of male and female potato leafhoppers (Homoptera: Cicadellidae) on alfalfa. Environ. Entomol. 92: 1321–1328.

Hogg, D. B., and M. McCaslin. 1994. Performance of potato leafhopper nymphs and adults on selected clones of glandular-haired alfalfa. Proceedings, 34th North American Alfalfa Improvement Conference, 10–14 July 1994, Guelph, Ontario.

Hower, A. A., and P. W. Flinn. 1986. Effects of feeding by potato leafhopper nymphs (Homoptera: Cicadellidae) on growth and quality of established stand alfalfa. J. Econ. Entomol. 79: 779–784.

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. Schalk. 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. Nielson, and G. P. Dively. 1991. Insect pest-induced losses in alfalfa: patterns in Maryland and implication for management. J. Econ. Entomol. 84: 610–618.

LaPointe, S. L., and W. M. Tingey. 1984. Feeding response of the green peach aphid (Homoptera: Aphididae) to potato glandular trichomes. J. Econ. Entomol. 77: 386–389.

LaPointe, S. L., and W. M. Tingey. 1986. Glandular trichomes of *Solanum neocardenasii* confer resistance to green peach aphid (Homoptera: Aphididae). J. Econ. Entomol. 79: 1264–1268.

Lenssen, A. W., S. L. Blodgett, S. D. Cash, and R. Ditterline. 1999. Variation for erect glandular hair density in perennial and annual *Medicago.* Proceedings, 36th North American Alfalfa Improvement Conference, 2–6 August 1998, Bozeman, MT.

(NAAIC) North American Alfalfa Improvement Conference. 1999. Standard tests to characterize pest resistance in alfalfa cultivars (http://www.naaic.org/stdtsts/potatolh.htm on August 20, 2001).

Othman, R. B., E. L. Sorensen, G. H. Liang, and E. K. Horber. 1981. Density and distribution of erect glandular hairs on annual *Medicago* species. Bot. Gaz. 142: 237–241.

Ranger, C. M., and A. A. Hower. 2000a. Glandular-haired alfalfa: trichome morphologies and their roles in resistance to the potato leafhopper. Proceedings, 37th North American Alfalfa Improvement Conference, 16–19 July 2000.

Ranger, C. M., and A. A. Hower. 2000b. Biology and behavior of the potato leafhopper on resistant glandular-haired alfalfa. Proceedings, 37th North American Alfalfa Improvement Conference, 16–19 July 2000.

SAS Institute. 1985. SAS user's guide: statistics, 1985 ed. SAS Institute, Cary, NC.

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., and E. A. Backus. 1999. Insights into the mechanism of resistance to the potato leafhopper by glandular-haired alfalfa. Proceedings, 36th North American Alfalfa Improvement Conference, 2–6 August 1998, Bozeman, MT.

Sorensen, E. L., E. K. Horber, and D. L. Stuteville. 1985. Registration of KS108GH5 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 25: 1132.

Tingey, W. M., and J. E. Laubengayer. 1981. Defense against the green peach aphid and potato leafhopper by glandular trichomes of *Solanum berthaultii.* J. Econ. Entomol. 74: 721–725.

Tingey, W. M., and J. E. Laubengayer. 1986. Glandular trichomes of a resistant hybrid potato alter feeding behavior of the potato leafhopper (Homoptera: Cicadellidae). J. Econ. Entomol. 79: 1230–1234.

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