Comparison of AC Electronic Monitoring and Field Data for Estimating Tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in Common Bean Genotypes

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ABSTRACT Two methods for estimating the tolerance of common bean genotypes to *Empoasca kraemeri* Ross & Moore were compared, using a yield trial carried out at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, versus stylet penetration tactics measured by AC electronic feeding monitors. A stylet penetration index was devised based on principal component scores of three penetration tactics identified (pulsing laceration, cell rupturing, and lancing sap ingestion), combined with knowledge of the hopperburn symptoms caused by each tactic. Tolerant genotypes, as classified by the CIAT yield index, showed significantly more unprotected yield and lower hopperburn scores than the susceptible control. They also induced performance of less pulsing laceration (the tactic considered most damaging to the plant), and more of the other two, mitigating tactics, especially cell rupturing. When index values were calculated for each genotype, stylet penetration index values matched those of the yield index for three out of five genotypes: two EMP-coded tolerant lines ('EMP 385' and 'EMP 392') and the susceptible control 'BAT 41'. Thus, for these three genotypes, all subsequent hopperburn symptoms are predictable by the type of feeding behavior performed on them. 'Porrillo Sintético' and 'EMP 84', considered borderline genotypes by the yield index, were overestimated and underestimated, respectively, by the stylet penetration index. We postulate that, for these two genotypes, plant physiological responses to feeding (either compensatory or heightened sensitivity, respectively) synergize with type of feeding performed to generate the overall hopperburn condition. This multivariate analysis of electronic monitoring data was successfully used to devise an index of resistance. The implications of using the stylet penetration index and the advantages of using electronic monitoring in a bean-breeding program are discussed.

KEY WORDS *Empoasca kraemeri*, plant resistance, resistance index, probing behavior, electropenetration graph

Empoasca kraemeri Ross & Moore is considered the number one pest of common bean, *Phaseolus vulgaris* L., in all of tropical America. Like its congener the potato leafhopper, *Empoasca fabae* (Harris), it causes hopperburn, a condition that reduces bean yields between 60 and 95% (DeLong 1938, Kornegay and Cardona 1991). Macroscopic symptoms of hopperburn in field beans include plant stunting and dwarfing, with tight downward curling of leaves. Chlorosis progresses from the leaf margins and tips toward the midribs. During heavy leafhopper infestations, these chlorotic leaves could become necrotic and, in extreme cases, abscise from the plant (Kornegay and Cardona 1991, Cardona and Kornegay 1999).

Empoasca-caused hopperburn probably develops as a consequence of the combined mechanical injury and salivary effects of leafhopper stylet penetration (also known as probing) on vascular (Kabrick and Backus 1990, Ecale and Backus 1995a) and nonvascular plant tissues (Njihia 1996, Serrano and Backus 1998). In vascular tissues of susceptible plants, leafhopper probing triggers a saliva-enhanced cascade of morphological responses that starts with necrosis of probed cells, leads to cambial cell hypertrophy and division, phloem blockage, xylem disruption, then eventually redifferentiation of phloem and xylem cells (Ecale and Backus 1995b, Serrano and Backus 1998, Ecale Zhou and Backus 1999). Systemic effects of phloem and xylem blockage evidently cause chlorosis, wilting, and stunting symptoms. In susceptible nonvascular tissues, such as leaf interveins, probing causes increased intercellular spaces in spongy parenchyma and disorganization of the palisade layer (Njihia 1996, Serrano and Backus 1998), leading to decreased photosynthesis and leaf necrosis, which further exacerbate systemic effects.

Scientists at the Centro Internacional de Agricultura Tropical (CIAT, located near Cali, Colombia) have bred common beans for resistance, based on two traits: tolerance and antixenosis for oviposition. CIAT breeders use a modified recurrent selection and in-

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termating program, with single pod descendant and bulk methods. Tolerance is selected for based upon higher yields under leafhopper attack and lower hopperburn scores than a susceptible control (Kornegay and Cardona 1990, 1991; Cardona and Kornegay 1999). The trait is quantitatively inherited, and has a significant environment by genotype interaction. This makes selections variable among seasons and localities (Galwey and Evans 1982a, 1982b; Kornegay and Temple 1986). In addition, the selection program relies exclusively on field trials. It takes ≈3 yr, assuming high leafhopper infestations and ideal field conditions, to advance materials to the F_6 generation when yield trials are carried out (Kornegay and Cardona 1991, Cardona and Kornegay 1999). The need exists for a method of selecting tolerant genotypes that can be as reliable as the field trials, yet at the same time, permits advancement of early generations faster than the field experiments.

Calderón and Backus (1992) used an AC electronic monitoring system to compare the stylet penetration behaviors of *E. fabae* and *E. kraemeri* on a susceptible ('Porrillo Sintético') and a tolerant ('EMP 84') genotype of *P. vulgaris*. They found that the average amount of time insects spent probing was not significantly different between the genotypes, but the type of probing performed differed significantly. In the tolerant genotype, there was a significant decrease in the overall duration and number of probes composed of the multiple-cell laceration (I_a) waveform, previously found to be most damaging to vascular tissues (Kabrick and Backus 1990). On the susceptible cultivar, both insects performed I_a 40% of the time, whereas on the tolerant line, *E. kraemeri* did it for only 28% and *E. fabae* for 21% of the time. Calderón and Backus (1992) proposed that tolerance might be the result of characteristics of the plant that cause a behavioral shift toward less-damaging styles of probing, the first time a "behavioral mechanism" is documented for tolerance to any herbivorous insect. Serrano and Backus (1998), referring to the Calderón and Backus (1992) findings, further proposed that tolerance could be caused by an interaction between the insect's probing behavior and the plant's response, i.e., the insect's behavioral switch toward less damaging behaviors combined with an enhanced vascular compensatory response. Serrano and Backus (1998) found that lower hopperburn scores of selected tolerant genotypes are caused, in part, by a vascular compensatory response that causes growth of more or larger xylem tracheary elements following leafhopper probing damage, compared with susceptible plants. Thus, electronic monitoring data plus a measure of plant physiological response could be combined with field data as part of the selection and breeding processes, to increase the levels of tolerance.

Our objective was to test whether electronic monitoring of *Empoasca* feeding could be used to predict tolerance or susceptibility in common bean genotypes, as well as hopperburn-caused reductions in yield. To ground-truth our data with relevant field data, we also replicated conventional yield experiment using tolerant, intermediate, and susceptible bean genotypes that previously have been tested extensively (Kornegay and Cardona 1991, Cardona and Kornegay 1999). We present herein a stylet penetration index of tolerance, based on principal component scores representing the stylet penetration tactics most commonly used by *E. kraemeri*. Our stylet penetration index values replicated those from a field yield index of resistance in three out of five genotypes, and explained the underlying mechanism of yield losses in the remaining two genotypes. This is the first time that multivariate analysis of electronic monitoring data has been used to successfully generate an index of resistance.

Materials and Methods

Field Experiment. A field trial was carried out at the CIAT headquarters located near Cali, Colombia (≈78° west longitude and 5° north latitude), from January to March 1995. These months coincide with a dry season and the highest natural populations of *E. kraemeri* (van Schoonhoven et al. 1978). CIAT scientists provided five bean genotypes that had been extensively tested in the past. Thus, it was felt that one season's worth of data would be sufficient for our comparative purposes. 'BAT 41' and EMP 84 susceptible and tolerant checks, respectively, Porrillo Sintético an intermediate or borderline cultivar, and 'EMP 385' and 'EMP 392' tolerant materials selected from their breeding cycle XVII (Cardona and Kornegay 1999).

Field plots occupied an area of 2,100 m^2. A split plot design with four replicates was used. One main plot was insecticide protected. The second was left under leafhopper attack. Five rows of corn were planted between the main plots to prevent insecticide drift from protected plots. Rows were 5 m long spaced 0.6 m apart. Each subplot (genotype) was planted in 14 rows, with one empty row to separate adjacent subplots. Seeds were hand sown every 0.1 m. The six central rows of each subplot were used for estimating crop yields, for nondestructive insect counts, and for visual hopperburn scores. Four additional rows on each side of the yield rows were used for a destructive counting of adults and nymphs (see below). Insecticide protection consisted of applications of 360 g (AI) $\cdot$ ha^{-1} of monocrotophos whenever leafhopper populations reached the economic threshold level of two to three nymphs per trifoliate leaf. Three applications were made at 12, 27, and 40 d after planting. Furrow irrigation and hand weeding were provided when necessary.

Twenty days after planting and at 10-d intervals thereafter, visual hopperburn scores were taken. A 1–9 scale was used, where a score of 1 was assigned to healthy plants and 9 to severely hopperburned plants (stunted, with most leaves curled downward and necrotic; Kornegay and Cardona 1991). In addition, the number of nymphs on 10 randomly selected trifoliates was counted. The day after these counts were made, a "destructive" sample was taken in the lateral rows. A plastic bag (60 by 80 cm) was inflated by waving it in

the air and, carefully but rapidly, used to cover a randomly selected plant, to capture as many as possible of the insects present on it. Each bagged plant was removed from the soil, brought to the laboratory, and kept refrigerated at −5°C until evaluated, usually within 1 or 2 d. All adult leafhoppers captured were counted, and all leaves were carefully examined to count nymphs (data not shown). Ten plants per subplot were sampled in this fashion. Four samplings were made at 20, 30, 40, and 50 d after planting.

Crop Growth Rates. After all insects were counted, the plants were laid on paper bags and placed in an oven at 80°C for 4 d, then weighed. Ten plants were also collected from the protected plots during the sampling dates, and were treated in the same way (except for the counting of the insects) to obtain their dry weights. Dry weight data were used to calculate instantaneous crop growth rates when vegetative growth approached its maximum value, between 20 and 50 d after planting, according to the formula from Gardner et al. (1985) for both protected and unprotected subplots. The coefficient of partitioning was calculated as the ratio of economic yield ($g \cdot m^{-2}$) to crop growth rates (Gardner et al. 1985). This coefficient expresses the efficiency of a genotype in conversion of photoassimilate to economic yield.

Bean Yields and Yield Components. At harvest, 0.5 m on each end of the yield rows were left on the ground to avoid border effect. The effective yield plot was 14.4 m^2. All plants were manually harvested and counted. Yield components measured included number of pods in 10 plants, number of seeds in 30 pods, and the weight of 100 seeds. Seeds were sun-dried to 12–14% humidity and weighed. Yield data were used either as $g \cdot plot^{-1}$ or converted to $kg \cdot ha^{-1}$ for statistical purposes.

Statistical analysis of yield data included analysis of variance (ANOVA) (SAS Institute 1988) and the Ryan–Einot–Gabriel–Welsch multiple F test for separation of means. Values for the yield index were calculated for all genotypes according to the method of Kornegay and Cardona (1991) using SAS (SAS Institute 1988) code provided by CIAT. The yield index (or susceptibility index, Cardona and Kornegay 1999) is a proportion of the yield of a particular genotype to the yield of all genotypes tested during a yield trial. This makes it comparable among experiments, by standardizing the differences that can be obtained in different years or localities. Index values lie between 0 and 2.0, with a decision line at 1.0. Therefore, for a genotype to be designated as tolerant, its yield index should be below 1.0.

Electronic Monitoring. *E. kraemeri* females, 3–4 d old, were used for electronic monitoring experiments. They were obtained from age-specific colonies maintained in an environmental room at CIAT at 25 ± 2°C, 70–80% RH, and a photoperiod of 12:12 (L:D) h on *P. vulgaris* 'ICA-PIJAO'. Females were removed from the colony cages when they were 1–2 d old and placed in separate acclimation cages for 48 h with plants of the genotype on which they would be tested. After the acclimation period, individual females were tethered by holding them in place on a Plexiglas stand with a gentle vacuum. Under the microscope a small drop of electrically conducting silver paint (Ladd Research Industries, Burlington, VT) was used to attach a 12.7-μm, 99.99% gold wire (Sigmund Cohn, Mount Vernon, NY) to the female's pronotum. The other extreme of the 3–4 cm long gold wire was glued with silver paint to a copper stub. Tethered females were allowed access to plants for ≈1 h for acclimation to the wired condition. They were then starved for 30–40 min before the electronic monitoring session began.

Plants were reared in a greenhouse at 25 ± 5°C, 60–80% RH, and a photoperiod of 12:12 (L:D) h. Plantings in 10-cm-diameter pots were made as frequently as needed to maintain a constant supply of 15-d-old plants with two fully expanded primary leaves. For electronic monitoring, one primary leaf of each plant was placed on a 100-cm^2 plastic stand, and held in place with stretched strips of Parafilm M (American National Can, Greenwich, CT), with the abaxial surface facing up. Tethered females were placed on the input electrode of the electronic monitor, and recorded continuously for 10,000 s (≈2 h 47 min). Thirty leafhoppers were monitored per genotype, for 150 insects total, and ≈420 h of observation time and data acquisition.

Two AC electronic insect feeding monitors (Backus and Bennett 1992; Missouri Monitor type 2.0; Electronic Instruments, University of Missouri, Columbia, MO) were used. All electronic monitoring was done between 1000 and 1300 hours, with a few exceptions in the afternoon, between 1300 and 1600 hours. Five insects were monitored daily, one on each of the same genotypes used in the field experiment, and randomly assigned to five of the eight possible channels available from the electronic monitors. New plants and newly tethered insects were used, and new channel assignments were made whenever a recording session was started. The monitors were calibrated, standardized, and set to output a 100-mV signal at 500 Hz to potted bean plants by inserting the output electrodes into the wet potting substrate.

Waveform output from the electronic monitors was digitized using a DAS-16 Analog to Digital I/O board (Keithley Instruments, Taunton, MA) with 12 bit resolution, and 100-Hz sampling rate. Acquisition, real time display and storage of these waveforms were achieved using Viewdac software version 2.1 (Keithley Instruments, Taunton, MA), running on a Gateway 2000 486 DX-33V computer (Gateway 2000, North Sioux City, SD). Data from each monitoring session were stored in a table format with channels in separate columns, and data points (samples) in rows.

PostAcquisition Measurements. Data from individual channels were read from binary Viewdac files into Windaq/EX version 2.1 (Data Instruments, Akron, OH). Waveforms were displayed on a time scale, so that probes and waveform events (see definitions in Serrano 1997, Serrano et al. 2000) could be visually identified, coded, and their terminations marked by the researcher. Windaq then provided measurements, in seconds, of each waveform event. Data were stored

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as 2-column lists of codes representing each type of waveform and respective duration for each waveform event. These text files were then read into a Microsoft Excel (Microsoft, Redmonton, WA) workbook (van Giessen and Jackson 1998) to count the number of events and probes, and to calculate the duration of stylet penetration by adding the duration of events in each probe. These data were output with added identification variables (e.g., date, genotype identification) for statistical analysis. This final data set, with 20,120 observations at the event level for 150 insects on five genotypes, constituted the full behavioral record.

Waveforms Measured and Variables Generated. All waveforms identified have been described previously (Backus and Hunter 1989, Calderón and Backus 1992, Ni 1993, Njihia 1996), but not all in one article. A summary of the most frequently found waveforms is as follows.

I_a *Multiple-cell laceration,* meaning continuous intracellular channel-cutting through columns of many cells by the stylets, with simultaneous or rapidly alternating ingestion and salivation, especially of watery saliva.

I_b *Single-cell puncturing,* meaning insertion of stylets into large, primarily mesophyll cells, and ingestion of their contents with little detectable salivation.

I_c *Still-stylet ingestion,* meaning ingestion with the stylets motionless. If an event of this waveform is longer than 1–3 min, it is likely to be ingestion from a phloem sieve element (Kabrick and Backus 1990, Njihia 1996). Otherwise, especially in short duration, it probably represents ingestion of lacerated cell contents.

Four other "minor" waveforms were also measured, including I_f, I_g (Ni 1993) an unknown waveform "U" not yet associated with a specific behavior (Njihia 1996) and waveform "D" correlated with shallow stylet contact with the plant (Backus and Hunter 1989). During subsequent reclassification of probes (see below), these were associated with the "major" waveforms as behaviorally similar "families" of waveforms.

Quantification included counting and measuring the number of and the duration of each waveform event that occurred during each probe. Probes were then counted and the number and duration of events were averaged per probe or per insect (e.g., duration of I_a per probe, or I_a per insect). These combinations of duration and frequencies at the per event, per probe, and per insect levels produced 37 variables (or parameters) that described the probing behavior of each insect during the recording period (see Serrano 1997 for detailed explanation). Univariate analysis for these variables included ANOVA, and mean separation by Ryan–Einot–Gabriel–Welsch multiple F test (SAS Institute 1988).

Classification of Probes by Stylet Penetration Tactic. The behavioral and univariate statistical analyses of Serrano (1997) yielded no straightforward means of classifying and predicting tolerance among the bean genotypes. We therefore reclassified each probe in the behavioral record as belonging to one of three stereotypical, sequential combinations of waveforms termed "stylet penetration tactics." A summary of the three tactics is as follows.

Pulsing Laceration. Composed of bouts of short probes (≈1 min) containing primarily single events of I_a. Normally, the interprobe intervals (the amount of time between two probes) were very short, between 0.3 and 3 s, which gave these sequences the characteristic appearance of tight bouts of short probes.

Cell Rupturing. Composed of few, long-duration probes with primarily waveform I_b, but also short duration events of I_a, usually at the beginning of these long probes.

Lancing Sap Ingestion. Long-duration probes composed almost exclusively of long (>1 min) to very long events of 'spiky' I_c. Sometimes short events of I_a occurred at the beginning of these probes, but they were less frequent than those observed during the cell-rupturing tactic.

When the entire duration of a tactic was established for each insect, the number of probes during it was counted. These were the frequency variables for the respective tactic. Most probes were short and unambiguously assignable to only one tactic. However, some long probes contained waveforms representing two tactics; in such cases, the duration of each was measured separately (for duration measures) and the probe was counted twice (for frequency measures).

Principal Component Analysis. This reclassification process generated six variables, i.e., the frequency and duration of each of the three tactics. Although this reduced the number of variables to analyze from 37 to 6, there were still too many to generate a simplified index that could be used to segregate genotypes as tolerant or susceptible. Therefore, we performed principal component analysis to further reduce variables. We included four other variables considered by us to be behaviorally important, i.e., probing duration by insect, number of waveform events by insect, duration of waveform events, and total number of probes by insect were incorporated into the new data set. These variables are defined in detail in Serrano (1997). Thus, the final data set analyzed contained 10 variables and 150 observations. Principal component analysis (SAS Institute 1988) was carried out on the covariance matrix of the mean-corrected values for these 10 variables. Varimax rotation was used for interpretation of the principal components. Duration variables were log transformed to maintain normality (Jollife 1986, Sharma 1996). Scores generated by the three retained principal components were treated as new variables and analyzed by univariate ANOVA and Ryan–Einot–Gabriel–Welsch multiple F test (SAS Institute 1988). A linear combination of these scores was used to generate a simplified stylet Penetration index.

Results

Leafhoppers and Hopperburn in the Field. High leafhopper populations were observed during this crop season. Thirty days after planting, nymphs in unprotected plots were in excess of the economic

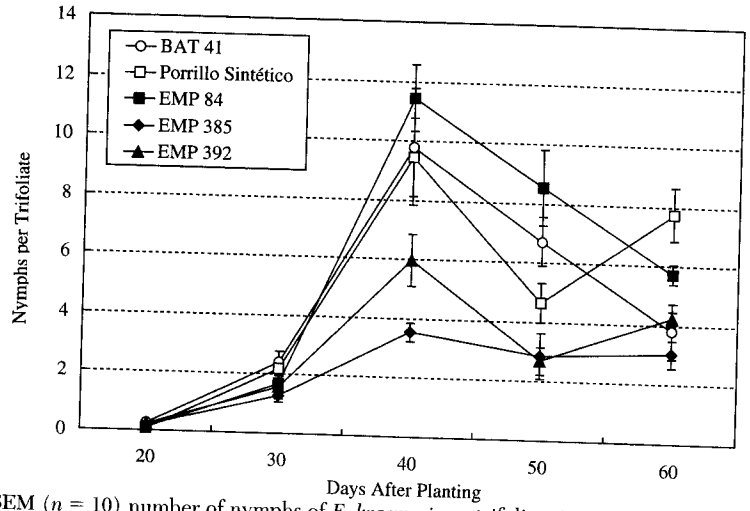

Fig. 1. Mean ± SEM (n = 10) number of nymphs of *E. kraemeri* per trifoliate in unprotected common bean genotypes at 10-d intervals.

threshold level of two to three nymphs per trifoliate leaf (Fig. 1) in BAT 41 and Porrillo Sintético. The highest number of nymphs occurred at 40 d after planting with populations ranging from 3.6 ± 0.3 (mean ± SEM) nymphs per trifoliate in EMP 385 to 11.5 ± 1.1 in EMP 84. BAT 41 and Porrillo Sintético also had high nymphal infestations at this stage. EMP 385 and EMP 392 maintained consistently fewer nymphs ($P < 0.05$) throughout the experiment, with the lowest (EMP 385) never reaching above four nymphs per trifoliate. A drop in the number of nymphs per trifoliate was seen for all genotypes at 50 d, however, in Porrillo Sintético there was a significant increase at 60 d. The number of adults per plant increased continuously for all genotypes during the sample period (Fig. 2). Significant differences in the number of adults per plant could not be detected among genotypes at any sampling date with the plastic bag sampling method.

Twenty days after planting the susceptible control, BAT 41 had the highest hopperburn scores ($P < 0.05$) and remained the most damaged genotype for the rest of the experiment (Table 1). In general, tolerant lines were less damaged than BAT 41 or Porrillo Sintético. EMP 84, the tolerant control, showed the highest hopperburn scores of the tolerant genotypes, in particular toward the end of the experiment. At the end of the evaluation period, the susceptible BAT 41 and the intermediate Porrillo Sintético were highly damaged (visual rating score 7.0 or greater), whereas the tolerant lines ranged between 4 and 6.5. EMP 385 maintained more-or-less uniform scores throughout the experiment, ranging between 4.5 and 6.8, but tended to show more hopperburn than EMP 392 (Table 1).

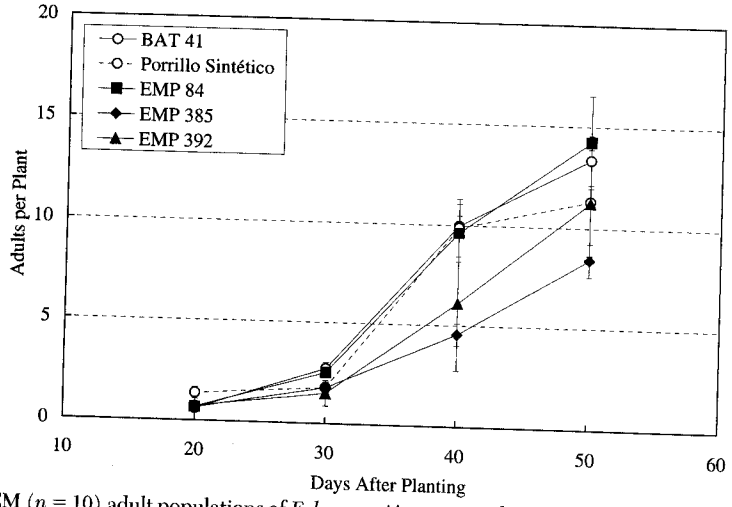

Fig. 2. Mean ± SEM (n = 10) adult populations of *E. kraemeri* in common bean genotypes at 10-d intervals. Samples were whole plants collected using the plastic bag method (see text for description).

Table 1. H genotypes of *P.*

Genotype
BAT 41
Porrillo Sintético
EMP 84
EMP 385
EMP 392

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Table 1. Hopperburn scores for susceptible and tolerant genotypes of *P. vulgaris* under leafhopper attack

Genotype	Days after planting				
	20	30	40	50	60
BAT 41	9.0a	9.0a	9.0a	9.0a	9.0a
Porrillo Sintético	6.8b	7.0a	7.3a	7.5b	7.0a
EMP 84	6.0b	5.5b	5.3b	6.5b	6.5a
EMP 385	5.3b	5.3b	4.5c	6.8b	5.3b
EMP 392	4.3c	4.0c	4.5c	5.3c	4.0c

Scores were taken every 10 d using a scale where 1 = no damage and 9 = severely hopperburned plants (stunted and chlorotic plants with downward curled leaves).

[a] Within a column, means followed by the same letter do not differ significantly at $\alpha = 0.05$ Ryan–Einot–Gabriel–Welsch multiple F test (ANOVA $P < 0.05$ [SAS Institute 1988]).

Crop Growth Rates. Instantaneous crop growth rates were calculated from dry weight data. BAT 41 had the lowest protected crop growth rates of all genotypes (Fig. 3), and also lost ≈12% under leafhopper attack. In contrast, Porrillo Sintético had the highest protected crop growth rates of all genotypes, but it showed the highest reduction under leafhopper attack, ≈40%. Under leafhopper attack, EMP 84 had a significant reduction of 15% in its crop growth rates, although this reduced yield was still significantly higher than unprotected yields of BAT 41 and Porrillo Sintético, thus giving it the highest crop growth rates of the EMP-coded genotypes. The highest loss of seed yield caused by leafhoppers in a tolerant genotype occurred in EMP 385; its 30% loss was significantly more than EMP 84 and EMP 392. Interestingly, the lowest unprotected crop growth rates (yet the smallest loss-only 10%) of the tolerant genotypes under protected conditions was produced by EMP 392, the most advanced selection.

The highest coefficients of partitioning under insecticide protection (Fig. 4) were found in the tolerant genotypes (especially EMP 392 and EMP 385) and BAT 41; Porrillo Sintético had the lowest. Under leafhopper attack, BAT 41 and EMP 84 were reduced 66 and 51%, respectively, whereas reductions for Porrillo Sintético, EMP 385, and EMP 392 ranged from 33 to 39%.

Bean Yield and Yield Components. Under leafhopper attack, all the tolerant, EMP-coded lines yielded significantly ($P < 0.05$) more than BAT 41 (Table 2). Porrillo Sintético and EMP 84 had intermediate unprotected yields, but nonetheless yielded ≈200–300 kg · ha^{-1} more than BAT 41 (40–50% more). The highest yielding genotypes were EMP 392 and EMP 385. Without leafhoppers, no significant differences ($P > 0.05$) were detected in the yields of any genotype. It is striking, however, that even the best yielding lines lost ≈50% of their yield potential because of the high intensity of leafhopper attack during this experiment. The yield index values for EMP 385 and EMP 392 were the only ones below the decision line of 1.0 (Table 2), indicating that both were truly tolerant and superior to the susceptible, and even the tolerant, controls BAT 41 and EMP 84, respectively.

When protected from leafhoppers, these genotypes showed very few significant differences for most yield components measured (Table 3). Differences found seem to reflect inherent genotypic characteristics. In contrast, three of the four yield components in unprotected genotypes were reduced by leafhoppers. Only the number of plants per plot was not affected, which is characteristic of the damage caused by *E. kraemeri* and adds confidence to the data by not having this as a co-variable in the analyses. For the highly susceptible and intermediate genotypes (BAT 41 and Porrillo Sintético, respectively), all three yield components were strongly decreased by effects of leafhopper feeding. However, the EMP-coded genotypes showed smaller reductions in yield, but varying by specific component. For example, in EMP 84 and EMP 385, the main reduction was in seeds per pod (23 and 20%, respectively), whereas in EMP 392, the highest yielding genotype, lower losses in seeds per pod, and

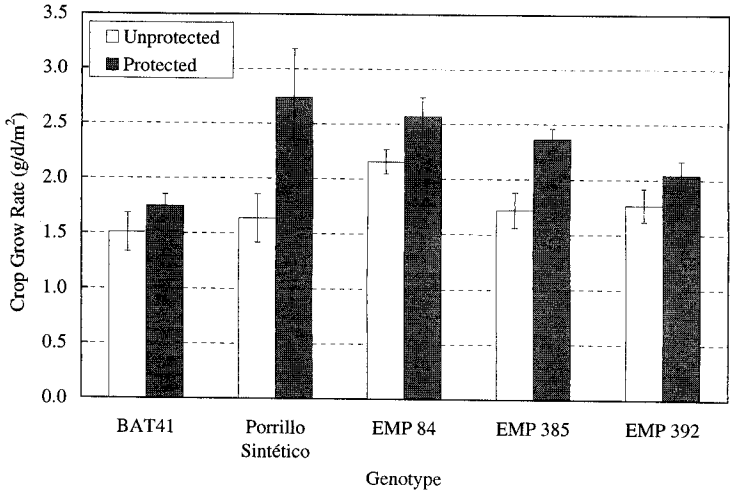

Fig. 3. Mean ± SEM ($n = 4$) crop growth rates for five genotypes of *P. vulgaris* under leafhopper attack and insecticide protection. Crop growth rates were calculated between 20 and 50 d after planting.

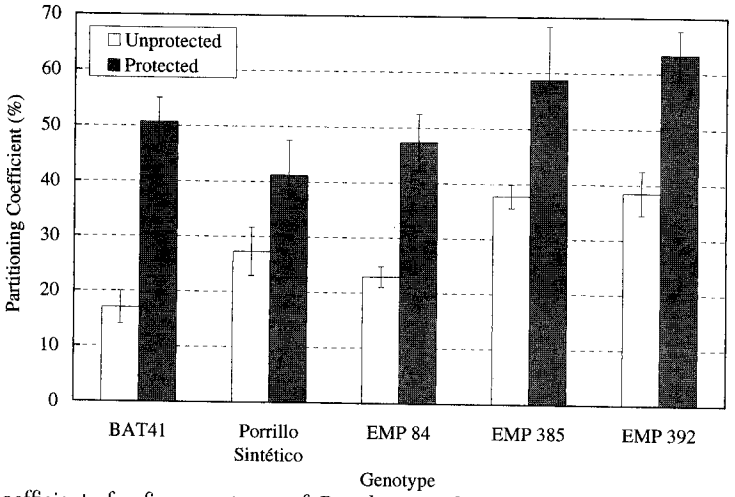

Fig. 4. Partition coefficients for five genotypes of *P. vulgaris* under leafhopper attack and insecticide protection. Partitioning is the ratio of grain yield to crop growth rates. Columns are means ± SEM ($n = 4$).

seed size helped to counter its heavy loss (32%) in pods per plant.

Univariate Comparison of Stylet Penetration. Of the ≈90 h of recordings made on each bean genotype, insects probed between 87 and 93% of the access time (Table 4). Insects made between 1,723 and 2,180 probes on each genotype, equivalent to a rate of 24–29 probes per hour. The nonprobing time was divided between walking (restricted by the length of the wire) and egg-laying behaviors.

Univariate analysis of the many variables generated at the per-event, per-probe, or per-insect levels, which has been traditional in electronic monitoring studies (e.g., Calderón and Backus 1992, Al-Dawood et al. 1996), revealed no distinctive trends that reflected the susceptibility or tolerance of the genotypes studied. However, some variables measured at the per-insect level showed differences among genotypes. Insects on BAT 41 had relatively long probing duration per probe per insect (Table 5) and intermediate probing duration by insect, suggesting that, on average, long moderately-frequent probing prevails on this highly susceptible genotype. In contrast, EMP 385 had short probing duration per probe per insect, yet again, intermediate duration by insect, short, moderately frequent probes prevail. For the other three genotypes, probing duration per probe per insect was intermediate in duration, 5.4–5.9 min on average. Yet EMP 84 had significantly shorter duration by insect ($P > 0.05$). Probing duration per probe per insect combined with number of probes on the three tolerant genotypes suggests that all stimulated medium-length probes, though a moderate number on Porrillo Sintético, a small number on EMP 84, and a large number on EMP 392. However, ANOVA did not detect differences among genotypes for number of probes (Table 5). No difference or pattern of differences provided a straightforward separation of the genotypes into susceptible and tolerant. A more detailed explanation of these univariate results can be found in Serrano (1997).

Comparison of Genotypes by Stylet Penetration Tactic. The pulsing laceration tactic was performed in similar frequencies (no significant differences for the number of probes made during this tactic) on all genotypes tested. The average duration by insect of this tactic was also not significantly different among genotypes ($P = 0.53$). In contrast, the cell-rupturing tactic was performed very differently on BAT 41 and Porrillo Sintético, where significantly fewer probes occurred than on other genotypes (Table 6). In fact, number of probes containing cell rupturing offered the clearest statistical distinction between the EMP and non-EMP genotypes. Lancing sap ingestion was

Table 2. Mean ± SEM ($n = 4$) seed yield (kg · ha^{-1}) of susceptible and tolerant genotypes *P. vulgaris* under attack by *E. kraemeri* (unprotected) and insecticide protected, under field conditions

Genotype	Unprotected	Protected	% Reduction	Yield Index[a]
BAT 41	298.5 ± 34.5a	1,068.5 ± 95.1a	72.2	1.30
Porrillo Sintético	506.4 ± 62.1ab	1,276.7 ± 96.5a	59.8	1.01
EMP 84	592.2 ± 29.5bc	1,474.2 ± 174.5a	59.7	1.00
EMP 385	782.1 ± 71.5cd	1,653.8 ± 220.3a	52.6	0.90
EMP 392	827.7 ± 105.3d	1,570.3 ± 172.5a	47.6	0.87

Within a column, means followed by the same letter do not differ significantly at $\alpha = 0.05$ Ryan–Einot–Gabriel–Welsch multiple F test, (ANOVA $P < 0.05$ [SAS Institute 1988]).

[a] Values above 1 are considered susceptible; and below 1, tolerant to leafhoppers.

Table 3. Mean ± genotypes exposed to field conditions

Genotype	Plan
BAT 41	210.
Porrillo Sintético	195.
EMP 84	178.
EMP 385	201.
EMP 392	212.
BAT 41	221.
Porrillo Sintético	185.
EMP 84	194.
EMP 385	203.
EMP 392	190.

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Genotype
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Porrillo Sintético
EMP 84
EMP 385
EMP 392

Table 3. Mean ± SEM ($n = 4$) yield components of *P. vulgaris* genotypes exposed to protected from attack by *E. kraemeri*, under field conditions

Genotype	Yield component			
	Plants/plot	Pods/plant	Seeds/pod	Seed size[a]
Unprotected plots				
BAT 41	210.3 ± 23.6a	7.6 ± 0.7c	2.3 ± 0.2b	11.8 ± 0.3d
Porrillo Sintético	195.0 ± 8.5a	8.6 ± 0.8bc	4.1 ± 0.3a	14.8 ± 0.3c
EMP 84	178.3 ± 3.3a	12.1 ± 0.4a	4.1 ± 0.1a	16.5 ± 0.3b
EMP 385	201.3 ± 1.0a	12.3 ± 0.5a	3.8 ± 0.2a	18.3 ± 0.3a
EMP 392	212.3 ± 10.4a	9.4 ± 0.7b	4.2 ± 0.4a	17.5 ± 0.3a
Protected plots				
BAT 41	221.0 ± 6.6a	12.9 ± 0.7a	3.2 ± 0.1b	15.3 ± 0.1d
Porrillo Sintético	185.5 ± 2.1a	13.3 ± 0.4a	5.5 ± 0.2a	16.3 ± 0.8cd
EMP 84	194.5 ± 7.6a	13.4 ± 0.9a	5.3 ± 0.1a	18.8 ± 0.3b
EMP 385	203.5 ± 5.9a	14.2 ± 0.5a	4.8 ± 0.1a	21.0 ± 0.7a
EMP 392	190.0 ± 27.3a	13.8 ± 1.3a	5.2 ± 0.1a	18.0 ± 0.4bc

Within columns, means followed by the same letter did not differ significantly according to Ryan–Einot–Gabriel–Welsch multiple F test ($\alpha = 0.05$) (ANOVA $P < 0.05$ [SAS Institute 1988]).

[a] Weight of 100 seeds.

also significantly different ($P < 0.05$) among genotypes (Table 6).

Principal Components Analysis. Three principal components were retained from the analysis (Table 7). The first principal component loaded a combination of frequency and duration of the pulsing laceration tactic. The duration of cell rupturing also loaded into this principal component, although negatively, which suggests that performance of these two tactics may be mutually exclusive. That is, when an insect spent most of its probing time performing pulsing lacerating, it was likely to have little time left to perform rupturing because the total access time to plants was constant. The interpretation of this principal component is behaviorally sound, therefore it was retained for further analysis and called the "pulsing laceration score" (p). The second principal component was composed of the frequency of cell rupturing, and was interpreted as the "cell rupturing score" (r). Had the duration of this penetration tactic loaded into this principal component, its interpretation would have been straightforward. However, because of its negative loading in the previous one, it still makes behavioral sense to interpret this principal component as a score of the cell rupturing ingestion tactic. The third principal component is easily interpretable as the "lancing ingestion score" (l), because both the frequency and duration of the lancing sap ingestion tactic

Table 4. Total probing duration and total number of probes made by *E. kraemeri* females while electronically monitored on susceptible and tolerant genotypes of common beans

Genotype	Total probing duration, min	% of access time	Total no. probes
BAT 41	4,560.0	91.2	2,180
Porrillo Sintético	4,454.2	89.1	1,926
EMP 84	4,384.6	87.7	1,723
EMP 385	4,532.3	90.7	2,180
EMP 392	4,655.2	93.1	2,136

Table 5. Mean ± SEM ($n = 30$) probing duration per probe (*PDPI*), number of probes (*NPI*), and probing duration per insect (*PDI*) performed by *E. kraemeri* females while electronically monitored on susceptible and tolerant genotypes of *P. vulgaris*

Genotype	Probing duration per probe, min.	No. Probes per Insect[a]	Probing duration per insect, min
BAT 41	7.5 ± 2.0a	72.7 ± 11.7a	152.0 ± 2.9ab
Porrillo Sintético	5.4 ± 1.1b	64.2 ± 10.0a	148.5 ± 2.1ab
EMP 84	5.9 ± 1.2b	57.4 ± 9.5a	146.2 ± 3.2b
EMP 385	3.9 ± 0.6c	72.7 ± 9.5a	151.1 ± 2.7ab
EMP 392	5.8 ± 1.6b	71.2 ± 9.5a	155.2 ± 1.9a

Means within columns followed by the same letter are not significantly different according to Ryan–Einot–Gabriel–Welsch multiple F test ($\alpha = 0.05$) ANOVA $P < 0.05$ (SAS Institute 1988).

loaded significantly to it. The principal components analysis of the penetration tactics almost completely explained the variance in the data; the combination of the first three components explained 99.93%. Thus, although the other four variables loaded to the rest of the principal components, the proportion of variance left unexplained was so negligible that they were not retained.

In principal component terms, a "0" level for a score means that the performance of that penetration tactic matched the overall average of its performance on the five genotypes. Therefore, the more positive the principal component score for any of the three tactics, the more the plant stimulated its performance; the more negative the score, the less the plant stimulated that tactic's performance. When we compared the principal component scores as new variables via ANOVA, there were significant differences ($P < 0.01$) among genotypes for the cell rupturing score (Table 8) that best matched the classification as tolerant or susceptible genotypes based on field data. The susceptible control BAT 41 and Porrillo Sintético had negative values, whereas the tolerant genotypes had the highest

Table 6. Mean ± SEM ($n = 30$) number of probes (frequency) and mean duration (±SEM, $n = 30$) of stylet penetration tactics performed by *E. kraemeri* females while electronically monitored on susceptible and tolerant common bean genotypes

Genotype	Pulsing Laceration	Cell Rupturing	Lancing Ingestion[a]
No. of probes during stylet penetration tactic[b]			
BAT 41	67.9 ± 11.6a	9.0 ± 1.3a	4.1 ± 0.4a
Porrillo Sintético	64.6 ± 9.9a	8.7 ± 1.3a	3.7 ± 0.5a
EMP 84	51.5 ± 9.1a	14.3 ± 2.2b	4.2 ± 0.5a
EMP 385	65.9 ± 9.2a	15.6 ± 1.5b	2.9 ± 0.3b
EMP 392	64.6 ± 9.2a	15.5 ± 1.6b	4.6 ± 0.4a
Duration of Stylet Penetration Tactic, min.[b]			
BAT 41	66.8 ± 8.7a	55.7 ± 7.6a	29.2 ± 5.0a
Porrillo Sintético	59.5 ± 7.7a	68.7 ± 7.2b	24.2 ± 4.6b
EMP 84	49.9 ± 7.1a	67.5 ± 6.2b	28.9 ± 5.6a
EMP 385	56.9 ± 7.3a	75.8 ± 6.8c	18.9 ± 4.6c
EMP 392	65.5 ± 7.7a	68.2 ± 8.1b	21.5 ± 3.2c

[a] Means followed by the same letter do not differ significantly at $\alpha = 0.06$ Ryan–Einot–Gabriel–Welsch multiple F test (ANOVA $P < 0.06$ [SAS Institute 1988]).

[b] Within columns, means followed by the same letter do not differ significantly at $\alpha = 0.05$ Ryan–Einot–Gabriel–Welsch multiple F test (ANOVA $P < 0.05$ [SAS Institute 1988]).

Table 7. Conformation of principal components (PC) for stylet penetration data obtained from *E. kraemeri* on common bean genotypes

Variable	Loadings		
	PC 1	PC 2	PC 3
Frequency pulsing laceration	0.97[a]	0.09	−0.15
Duration pulsing laceration	0.76[a]	0.16	0.07
Frequency cell rupturing	−0.10	0.99[a]	0.10
Duration cell rupturing	−0.71[a]	0.23	0.09
Frequency lancing sap ingestion	−0.09	0.11	0.96[a]
Duration lancing sap ingestion	−0.19	−0.12	0.93[a]
% variance explained	96.88	99.75	99.93

Only those variables with a significant loading on a principal component are shown. Ten variables and 150 observations were included in this analysis.

[a] Variables that constitute each principal component (significant loading).

positive values. Significant differences ($P < 0.05$) were also found for the lancing sap ingestion score, but clear segregation into susceptible and tolerant genotypes did not occur. No differences ($P = 0.58$) were found for the pulsing laceration score. However, we note that two out of three tolerant genotypes had negative scores for laceration, whereas both of the susceptible ones had positive scores.

The Stylet Penetration Index (*SPI*). Using the scores produced by principal components, we devised a simplified stylet penetration index as follows: $SPI = 1 - [(r + l) - p]$.

The index was subtracted from unity to make the values comparable among genotypes and with the CIAT yield index used with data from the field trial. Stylet penetration index values can be individually calculated for each insect monitored. Therefore, averages and associated measures of variability were obtained for each cohort of insects on a particular genotype. Similar to the yield index, 1.0 is considered the decision level. A value >1.0 indicates that a genotype is "behaviorally susceptible," i.e., it stimulates performance of such damaging feeding that, in the absence of a healing or compensatory response, the plant would show symptoms of hopperburn. Below 1.0, the plant can be considered "behaviorally tolerant," in that it stimulates feeding tactics that are much less damaging, or an amount below a physiological threshold for the initiation of macroscopic hopperburn symptoms.

The values for the stylet penetration index were calculated for each insect monitored (Table 8). BAT 41 and Porrillo Sintético had values above the decision threshold of 1.0. All EMP-coded lines had values below this decision line. Porrillo Sintético had a significantly higher index value ($P > 0.05$) than any other genotype, including the susceptible control. EMP 84 had the lowest index value of all the genotypes tested. All three tolerant genotypes had values significantly lower than the susceptible control.

The values for the stylet penetration index are plotted with the CIAT yield index in Fig. 5 for comparison. Because the yield index is calculated once per field trial, whereas the stylet penetration index can be calculated for each individual insect and therefore be averaged, we cannot statistically compare the findings of the two indices for each genotype. Causal relationships between the two indices can only be inferred, not conclusively established, unless a larger number of genotypes is compared. Both indices classified BAT 41 and Porrillo Sintético as susceptible genotypes, and the EMP-coded lines as tolerant. BAT 41, EMP 385, and EMP 392 all had relatively similar classifications with both indices. However, the stylet penetration index classified EMP 84 as highly tolerant, while the yield index placed it in the susceptible category (although very close to the decision line, Table 1).

Discussion

Field Experiment. Our data provide a consistent view of genotypic responses to heavy leafhopper attack under field conditions, similar to previous findings for the same genotypes (Kornegay and Cardona 1991, Cardona and Kornegay 1999). The density of the adult leafhopper population was extremely high during this field experiment and not significantly different among the genotypes. Differences in nymphal infestation probably reflect levels of ovipositional antixenosis also selected for in at least EMP 385 and EMP 392 (Kornegay et al. 1986, Cardona and Kornegay 1999). The high nymphal infestation observed in EMP 84 especially at 40 and 50 d after planting reflects its tolerance sensu Painter (1951). Under an insect pressure similar or greater than that on the susceptible BAT 41, this genotype has significantly higher seed yield than the susceptible control. Differences in unprotected yield observed in this experiment can,

Table 8. Mean ± SEM ($n = 30$) scores generated by principal components analysis of stylet penetration tactics and stylet penetration index by *E. kraemeri* on susceptible and tolerant genotypes of common beans (see text for description of the stylet penetration index)

Genotype	Pulsing laceration score (p)	Cell-rupturing score (r)	Lancing sap ingestion score (l)	Stylet penetration index values
BAT 41	0.193 ± 0.2a	−0.361 ± 0.1a	0.283 ± 0.1a	1.27 ± 0.3a
Porrillo Sintético	0.019 ± 0.2a	−0.478 ± 0.1a	−0.298 ± 0.3b	1.80 ± 0.2b
EMP 84	−0.203 ± 0.2a	0.195 ± 0.3b	0.147 ± 0.1ab	0.46 ± 0.3c
EMP 385	−0.075 ± 0.2a	0.350 ± 0.2b	−0.194 ± 0.2ab	0.77 ± 0.3c
EMP 392	0.065 ± 0.2a	0.294 ± 0.2b	0.062 ± 0.1ab	0.71 ± 0.2c

Within columns, means followed with the same letter do not differ significantly at $\alpha = 0.05$ Ryan–Einot–Gabriel–Welsch multiple F test (ANOVA $P < 0.05$ [SAS Institute 1988]).

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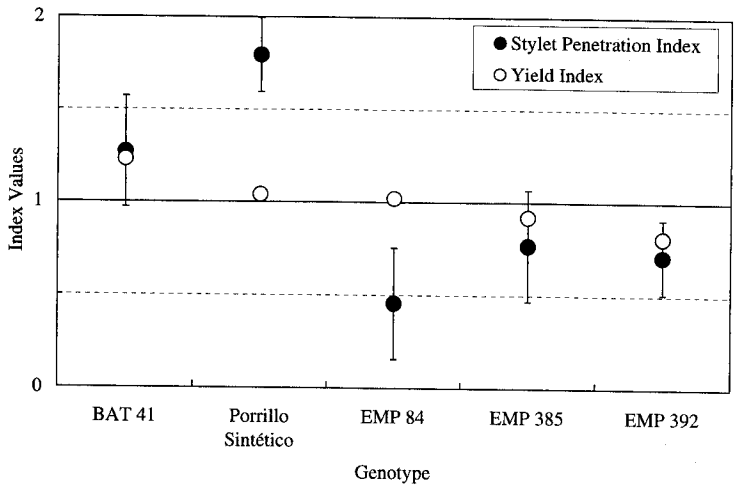

Fig. 5. Comparison of values for the stylet penetration and yield indices for five genotypes of *P. vulgaris*. For the stylet penetration index, each dot represents an average mean ± SEM (n = 30).

therefore, be attributed to genotype-specific responses to leafhopper attack. Hopperburn scores were high in the susceptible genotypes and the significant differences observed for these measurements could be attributed to the expression of genotypic differences to hopperburn.

In general, tolerant genotypes yielded more than susceptible genotypes under leafhopper attack by balancing their yield components in ways that allowed them to sustain higher insect populations with less damage. For example, tolerant plants increased the number of pods per plant and lost comparatively less seed weight. Additionally, tolerant genotypes have probably been selected for their physiological ability to respond to leafhopper attack. These include both larger canopies, reflected in the increased crop growth rates in protected plots, and the tolerant plants' ability to maintain higher crop growth rates under leafhopper attack. *Empoasca* leafhoppers are known to cause severe reductions in crop growth rates. Hutchins and Pedigo (1989) found that alfalfa, *Medicago sativa* L., plots infested with *E. fabae* had a lower crop growth rates during the 7- to 14-d regrowth period compared with uninfested plots. Tolerant beans had larger partitioning coefficients than did the susceptible plants. These large coefficients were maximally expressed in protected plots, but were also retained under leafhopper pressure (especially for EMP 385 and EMP 392). Physiological characteristics associated with increased tolerance may be related to the vascular compensatory response following leafhopper probing shown by Serrano and Backus (1998), which may allow some tolerant genotypes to maintain vascular flow during the critical (van Schoonhoven et al. 1978) seedling stage.

Rationale for Stylet Penetration Tactics. Electronic monitoring allows an extremely fine dissection of the stylet penetration behavior of a piercing-sucking insect, into smaller and smaller "pieces," or waveform types. Yet, behavioral analysis based on individual waveforms can sometimes be too reductionistic to be useful. It ignores the fact that the performance of a given behavior is influenced by the performance of previous behaviors. It also can eliminate any "emergent properties" born of sequential combinations of waveforms that could provide a fuller picture of leafhopper probing behavior. It is well documented in aphid electronic monitoring studies (e.g., McLean and Kinsey 1964, Tjallingii 1988) that combinations of waveforms are performed in stereotypical sequences. In the case of *Empoasca* spp., analysis of patterns of waveforms plus histological correlation from several studies (Kabrick and Backus 1990, Ecale and Backus 1995b, Njihia 1996) and multivariate analysis (Serrano 1997) provide converging lines of evidence for a repertoire of at least three stereotypical, sequential combinations of waveforms. We have termed these "stylet penetration tactics." We propose that *Empoasca* spp. leafhoppers are capable of varying the proportion of their time spent in each of these three tactics; thus "mixing and matching" their choice of feeding behaviors to optimize nutritional gain on each different host plant. Herein we present data supporting application of such information to host plant resistance studies.

Derivation of the Stylet Penetration Index. Our previous research on the cause of hopperburn by *Empoasca* leafhoppers has correlated each stylet penetration tactic with specific plant injury responses. Most importantly, there is a direct relationship between performance of pulsing laceration and the cellular abnormalities in vascular tissues that lead to the most severe, systemic symptoms of stunting and chlorosis (Kabrick and Backus 1990, Ecale and Backus 1995b, Serrano and Backus 1998). Anatomical signs of damage are apparent within hours of laceration in alfalfa (Ecale and Backus 1995b) and common beans (Serrano and Backus 1998). That pulsing laceration is of fundamental importance to *E. kraemeri* feeding is supported by findings reported herein, because it oc-

curs in all genotypes regardless of their degree of tolerance.

Cell rupturing appears to be less severely damaging to the plant (Njihia 1996), because it is primarily associated with a decrease in photosynthetic capacity of nonvascular tissues, probably exacerbating systemic vascular damage. Although less well understood, lancing sap ingestion (which is similar to the behavioral sequences of more typical, phloem-ingesting, sheath-making leafhoppers) probably causes loss of phloem sap. We estimate (and the findings from the susceptible BAT 41 herein support this view) that performing the lancing sap ingestion tactic is less damaging than the pulsing laceration tactic, but is slightly more damaging than the cell rupturing tactic because it could have systemic effects.

Because *E. kraemeri* spent >90% of the access time in our experiments probing, any decrease in the duration of pulsing laceration performed was behaviorally compensated for by increasing the duration of either or both of the other tactics. Thus, for the purposes of electronic monitoring, performance of cell rupturing or lancing sap ingestion could mitigate that of the more damaging behavior, pulsing laceration. We therefore chose to use the scores for cell rupturing and lancing sap ingestion as additive values, and the score for pulsing laceration as a subtractive value. Thus, our stylet penetration index weights the scores equally, but adds or subtracts them from one another, based upon present knowledge of the degree of cellular damage caused. A measure of "behavioral tolerance" should include the combined effect of all three stylet penetration tactics. For example, a genotype such as Porrillo Sintético that does not greatly decrease the insect's laceration behavior can still be considered tolerant, because it proportionally increases the amount of both cell rupturing and lancing sap ingestion. This is in accordance with its classification as intermediate by yield trials, and may be related to the physiological "rusticity" that has been attributed to this particular cultivar (White and Izquierdo 1991) (see below).

Other authors have attempted to use data from electronic monitoring to develop indices of resistance. Haniotakis and Lange (1974) created a "Relative Resistance Index" derived from data from AC electronic monitors to assess the resistance of sugar beets to the green peach aphid, *Myzus persicae* (Sulzer). Similarly, Hollbrook (1980) developed an "index of acceptability" while evaluating cultivars of potato for probing behaviors by the green peach aphid with an AC electronic monitor. However, no successful attempts were made to use multivariate analysis of electronic monitoring data for genotype separation until the work of Caillaud et al. (1995). They used discriminant function analysis and separated wheat genotypes by their degree of resistance to the cereal leaf aphid, *Sitobium avenae* (F.), with data from DC-electropenetration graphs. Their results, and those presented here, strongly support the view that data from electronic monitors of insect feeding (both AC and DC) can have important application in plant resistance to insects (also discussed in Van Helden and Tjallingii 1999). Our study is the first to successfully use principal component analysis to "boil down" all probing behavior into one single value that is statistically comparable among genotypes, and to derive from that an index of resistance.

Synthesis of Field and Electronic Monitoring Results for Comparison of Genotypes. For behavior results, this synthesis relies entirely on the principal component scores in Table 8, because they represent the most complete numerical summary of these behaviors. Also, although our principal component scores for the laceration tactic were not detected as significant by ANOVA, it has been shown that lacerating behaviors elicit most of the symptoms associated with hopperburn, as previously discussed. Small differences in the laceration tactic undetectable by our analyses might have a significant effect on the plant responses associated to stylet wounding and, ultimately, to tolerance to leafhopper attack.

Under leafhopper attack, the highly susceptible BAT 41 received the greatest performance of pulsing laceration of all five genotypes. Also, although there was high mitigation by lancing sap ingestion, there was virtually no performance of the most important mitigation behavior, cell rupturing. In the field, BAT41 showed the highest hopperburn scores, and its already-slow crop growth rate was strongly reduced. Its yield reduction was the largest of any genotype, and its partitioning coefficient was markedly reduced, indicating the low efficiency of the hopperburned plant to convert photosynthate to economic yield. It did not seem to balance damage by any yield component measured. There was no evidence of either physiological tolerance or extreme sensitivity to the initiation of hopperburn symptoms by probing. Thus, performance of uncompensated laceration to vascular tissues initiates the greatest degree of hopperburn damage. This complex combination of factors explains how BAT 41 could lose ≈70% of its potential yield because of leafhoppers.

Porrillo Sintético stimulated the most damaging combination of feeding behaviors of all the genotypes. Although average performance of pulsing laceration occurred Porrillo Sintético also stimulated very little of both types of mitigating tactics. Performance of the very important cell rupturing tactic was especially low, more than for any other genotype. The yield index classified this genotype very close to the decision threshold line. Thus, we infer that physiological tolerance to injury counterbalanced the damaging behavior, increasing overall yield. This makes sense because Porrillo Sintético is considered by bean physiologists to be capable of adapting to a wide variety of environments, and of maintaining yield under several types of stress, at poor sites (Voysest and García 1984, White and Izquierdo 1991). This generalized stress tolerance, "rusticity," is thought to be the result of physiological characteristics such as recuperative growth (possibly caused by photosynthate and N remobilization), greater partitioning to root growth (required for drought tolerance), high tissue concentra-

tions of phenolic compounds with inhibitory effects on a broad range of pathogens or pests, and increased pod retention and seed filling (White and Izquierdo 1991). Thus, rusticity may explain why this genotype shows less marked hopperburn symptoms and how it can have relatively higher (≈40%) unprotected yield than BAT 41. However, Porrillo Sintético is still comparatively more damaged than the truly tolerant genotypes, and it yielded significantly less (between 15 and 40%) than they did. Compared with the EMP-coded genotypes under leafhopper attack, Porrillo Sintético sustained heavy losses of pods per plant, but it counterbalanced by not losing seeds per pod and by maintaining seed size. In contrast, its reduction in the partitioning coefficient (33% compared with unprotected) was similar to that of EMP 392 and EMP 385.

EMP 84 stimulated the lowest performance of pulsing laceration, combined with moderately mitigating performance of both cell rupturing and lancing sap ingestion. Thus, behaviorally, EMP 84 stimulated the least damaging combination of behaviors of all genotypes in the study. Yet, its yield was intermediate, and the yield index revealed more susceptibility than EMP 385 or EMP 392. In fact, EMP 84 was not significantly different from Porrillo Sintético for hopperburn scores and yield under leafhopper attack. Like Porrillo Sintético, unprotected EMP 84 balanced yield by maintaining a comparatively large crop growth rate, large number of pods per plant, many seeds per pod and a large seed size, compared with protected. However, its partitioning coefficient was reduced by 50% compared with protected (20% more than the reduction suffered by Porrillo Sintético). Thus, its ultimate ability to convert photoassimilate into yield was severely impaired, implying a degree of physiological sensitivity to even the relatively nondamaging behaviors performed.

Overall, the two highest-yielding genotypes, EMP 385 and EMP 392 induced somewhat similar stylet penetration behaviors; EMP 392 stimulated more mitigation by lancing sap ingestion than did EMP 385, despite also stimulating more pulsing laceration. Both stimulated high performance of cell rupturing. The two genotypes also had similar yield-balancing characteristics. Both showed the lowest hopperburn scores and the highest unprotected yield, of all genotypes in this experiment. Under leafhopper attack, both genotypes lost fewer pods per plant and the least seed weight of all other genotypes studied. These two genotypes also had faster crop growth rates that were maintained under leafhopper infestation, and larger partitioning coefficients that were maintained similarly large (≈40%), under attack by leafhoppers. Yet, even these tolerant genotypes showed appreciable symptoms of hopperburn (although significantly lower than the other genotypes), and considerable yield reduction under leafhopper attack (almost 50%). These data also support the hypothesis that uncompensated pulsing laceration is the set of behaviors most related to hopperburn causation, and that it may be responsible, at least in part, for some of the plant responses induced by probing that lead to the development of hopperburn.

Thus, both behavioral tolerance and behavioral susceptibility to *E. kraemeri* feeding can occur because of mixed proportions of the three feeding tactics. Behavioral susceptibility occurs either because the plant stimulates strong performance of pulsing laceration combined with only moderate mitigation, or because it stimulates low to average laceration with very weak mitigation. Behavioral tolerance occurs because weak performance of laceration is combined with some to moderate mitigation, or average laceration is combined with very strong mitigation. Tolerance can also involve physiological or cellular responses to cope with the heavy amount of probing received, as is the case with EMP 385 and EMP 392 (Serrano and Backus 1998).

Comparison of the Yield and Stylet Penetration Indices. For three out of five genotypes (BAT 41, EMP 385, and EMP 392), the yield and stylet penetration index values were very close. For those, it is likely that the type and amount of probing damage performed is the best predictor of all subsequent physiological effects leading to reductions in yield. Thus, variations induced in the stylet penetration behaviors are probably directly reflected in yields, as indicated by the yield index. For the other two genotypes (Porrillo Sintético and EMP 84), inherent physiological responses by the plant to stylet penetration probably play a role in dictating yield outcome. Porrillo and EMP 84 both arrived at similar yield levels (borderline between susceptible and tolerant) by two different mechanisms. One stimulated highly damaging behavior but physiologically withstood the onslaught; the other triggered much less damaging behavior but was sensitive to what it received. For all five genotypes, the stylet penetration index provided valuable information that was unavailable from the yield index alone. It was serendipitous that Calderón and Backus (1992) chose exactly these two genotypes for initial tests of the role of probing behavior in tolerance; any other genotypes would probably have failed without the stylet penetration index.

There are some advantages and disadvantages of the stylet penetration index in comparison with the yield index. For disadvantages, the stylet penetration index by itself does not provide any information on the yield performance of a genotype. Considering that genotypes are currently selected exclusively based on unprotected yield and macroscopic hopperburn symptoms, this means that more detailed studies would be needed to correlate the penetration tactics with yield losses observed under field conditions. Additionally, the stylet penetration index is very sensitive to small changes in probing behavior. This is why the largest discrepancies between the values of the two indices occurred with Porrillo Sintético and EMP 84. So, it will probably be better suited for use in later generations within the breeding program. If used during early segregating generations, the risk exists that variability associated with segregating traits may confound ef-

fects on probing behavior or a larger sample size would be required making it more time consuming.

However, the stylet penetration index offers significant advantages over the current procedures. In addition to providing a way to segregate genotypes by their degree of tolerance, the stylet penetration index allows selection on the basis of type of "behavioral tolerance." For instance, one goal for breeders should be to select for germplasm that induces significant reductions in pulsing laceration and ideally, increases in mitigating behaviors. Another advantage offered by the stylet penetration index is its lower sensitivity to the environmental effects inherent in field experiments. This makes the index less susceptible to environmental "noise" and probably to the genotype by environment interaction, but at the same time, more comparable among experiments or genotypes and more repeatable than field experiments. Use of the stylet penetration index could save time in the long run, because there is less risk of having "bad" crop seasons when natural leafhopper infestations are low or erratic (van Schoonhoven et al. 1985). Another advantage of the stylet penetration index is that it is nondestructive. When needed, seedlings can be tested for 3 h, then grown in a greenhouse to obtain their seeds and a generation could be advanced, with the additional gain in time. From the perspective of the recurrent selection-breeding program, the stylet penetration index could also be used for parental selection (Kornegay and Cardona 1991).

Although the measurement, classification, univariate statistical analysis, reclassification, then multivariate statistical analysis of probes was an extensive and time-consuming aspect of this project, such would not be the case for future use of the stylet penetration index. Now that this work is completed, future studies can dispense with painstaking, event-by-event measurement of waveforms, in favor of measurement of whole tactics within and among probes. In combination with a standard computer program for principal component analysis and generation of stylet penetration index values, this rapid waveform analysis could allow electronic monitoring of leafhoppers on bean plants to be used as a simple genotype-screening method. We estimate that a sufficient number of plants per genotype could be monitored, measured and statistically analyzed in 2 wk, a considerable savings in time compared with traditional yield trials in the field.

In conclusion, our work represents the first time that electronic monitoring of leafhopper probing behavior has been used to generate a simplified index that permits segregation of genotypes by tolerance. Its success is probably because of the unusually direct nature of feeding damage to host plants by *Empoasca* leafhoppers. The stylet penetration index generated in this study matched the classification of three out of five genotypes obtained with the CIAT yield index, and explained the mechanism of tolerance in the other two genotypes. It has the advantage of providing additional information on the mechanisms of tolerance, as well as potentially reducing the selection process in a given generation from a few months (under field conditions) to a few weeks. It will be necessary to do additional testing of the stylet penetration index with a larger number of genotypes, to fine-tune its protocols for routine genotype evaluation. Such a stylet penetration index would be a useful supplement to field testing in some stages of a bean-breeding program.

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