FORAGE ENTOMOLOGY

Activity 1. Screening Brachiaria hybrids for spittlebug resistance.

Continuous mass rearing of spittlebug species in Palmira and Macagual

This is a continuous activity. A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. Progress made in the logistics of mass rearing of nymphs and in obtaining eggs from adults collected in the field has allowed us to screen *Brachiaria* genotypes for simultaneous resistance to six major spittlebug species: *Aeneolamia varia*, *A. reducta*, *Zulia carbonaria*, *Z. pubescens*, *Mahanarva trifiss*a, and *Prosapia simulans*. Insect material produced in our mass rearing facilities is used for greenhouse evaluations in Palmira and field evaluations in Caquetá.

Contributors: C. Cardona, G. Sotelo.

Identify *Brachiaria* genotypes resistant to spittlebug

Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia*, *A. reducta*, *Zulia carbonaria, Z. pubescens*)

Introduction

The correct identification of resistant hybrids is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. This is why assessment of resistance to spittlebug received special attention in 2003. Based on results obtained in 2001 and 2002, simultaneous but independent screening for resistance to three key spittlebug species was fully implemented.

Materials and Methods: A set of 64 "pre-selected" SX x AP (sexual-by-apomictic) hybrids received from the Breeding Program were evaluated for resistance to *Aeneolamia varia, A. reducta*, and *Zulia carbonaria*. Test materials were compared with six checks fully characterized for resistance to one or more spittlebug species. Plants were infested with six eggs per plant of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs reached the fifth instar stage or adult emergence occurred. Plants (5 per genotype) were scored for symptoms using the damage scale (1, no damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated. Materials were selected on the basis of low damage scores (<2.0 in the 1-5 scale) and reduced percentage survival (< 30%). Those genotypes showing resistance to two or more spittlebug species were reconfirmed in replicated nurseries (10 replications per genotype per spittlebug species).

Results and Discussion: The preliminary screening revealed that 10 of the 64 hybrids showed acceptable levels of resistance to at least two spittlebug species **(Table 1)**. As in previous occasions, fewer genotypes showed antibiosis resistance to *Z. carbonaria.*

Further testing with 10 replications per genotype per insect species allowed us to identify four hybrids combining antibiosis resistance to *A. varia*, *A. reducta*, and *Z. carbonaria* **(Table 2)**. Levels of resistance in this case were comparable to those exhibited by the resistant checks CIAT 36062 and 'Marandú' (CIAT 6294).

Table 1. Sexual-by-apomicitic hybrids selected in 2003 for reconfirmation of resistance to three spittlebug species. Means of five replicates per genotype per spittlebug species.

Genotype	Damage Scores			Percentage Nymph Survival		
	Aeneolamia varia	***Aeneolamia reducta***	***Zulia carbonaria***	***Aeneolamia varia***	***Aeneolamia reducta***	***Zulia carbonaria***
			Hybrids			
BR02NO/0419	1.2	1.6	2.1	3.3	10.0	23.3
BR02NO/0465	1.8	1.1	1.4	30.0	0.0	20.0
BR02NO/0638	1.2	1.6	2.6	23.3	36.7	-
BR02NO/0643	1.1	1.9	3.2	3.3	40.0	-
BR02NO/0644	1.4	1.3	2.2	33.3	20.0	80.0
BR02NO/0649	1.4	1.2	1.6	16.7	3.3	53.3
BR02NO/0756	3.0	1.2	2.0	-	0.0	26.7
BR02NO/0812	2.3	1.6	2.4	-	16.7	56.7
BR02NO/1372	2.4	1.6	1.6	-	20.0	56.7
BR02NO/1485	1.3	1.7	2.5	13.3	20.0	66.7
			Checks[a]			
FM9503/4624 (T)	3.5	2.2	1.5	85.0	25.0	11.7
CIAT 6294 (R)	2.0	1.4	2.5	26.7	25.0	56.7
SX0NO/0102 (R)	1.0	1.1	1.1	0.0	0.0	0.0
CIAT 36062 (R)	1.1	1.2	1.4	1.7	0.0	16.7
CIAT 0606 (S)	5.0	4.3	4.1	95.0	80.0	68.3
BR4X/44-02 (S)	4.9	4.8	3.5	90.0	73.3	70.0
LSD 5%	1.21	1.23	0.94	28.8	22.2	23.7

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Table 2. Sexual-by-apomictic hybrids selected in 2003 for high antibiosis resistance (<30% nymphal survival) to three spittlebug species. Means of 10 replicates per genotype per spittlebug species.

Genotype	Damage Scores			Percentage Nymph Survival		
	Aeneolamia varia	***Aeneolamia reducta***	***Zulia carbonaria***	***Aeneolamia varia***	***Aeneolamia reducta***	***Zulia carbonaria***
			Selected hybrids			
BR02NO/0419	1.0	1.1	1.8	0.0	0.0	5.0
BR02NO/0465	1.0	1.0	1.1	10.0	1.7	18.3
BR02NO/0756	1.4	1.2	1.8	18.3	3.3	21.7
BR02NO/0812	1.0	1.5	2.4	6.7	8.3	28.3
			Checks[a]			
FM9503/4624 (T)	1.6	1.8	1.9	51.7	21.7	30.0
CIAT 6294 (R)	1.2	1.9	2.7	25.0	21.7	63.3
SX01NO/0102 (R)	1.0	1.0	1.1	1.7	0.0	1.7
CIAT 36062 (R)	1.0	1.0	1.5	0.0	0.0	6.7
CIAT 0606 (S)	4.9	4.4	3.8	96.7	68.5	46.7
BRU4X/44-02 (S)	4.8	4.1	3.8	96.7	55.0	75.0
LSD 5%	0.36	0.65	0.68	16.8	17.8	26.3

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia*, *Zulia carbonaria*, *Z. pubescens*, *Mahanarva trifissa*)

Introduction

Assessment of spittlebug resistance under natural levels of infestation in the field is very difficult due to the focal, unpredictable occurrence of the insect. This problem has been overcome since 1998 when we developed a technique that allows us to properly identify resistance under field conditions. Evaluating for resistance under field conditions is important because it allows us to reconfirm levels of resistance identified under greenhouse conditions.

Materials and Methods: Using the experimental unit described in our 1998 Annual Report, the genotypes (usually 10 replicates) are initially infested in the greenhouse with an average of 10 eggs per stem. Once the infestation is well established, with all nymphs feeding on the roots, the units are transferred to the field and transplanted 10-15 days after infestation. The infestation is then allowed to proceed without interference until all nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the 1-5 visual scale utilized in greenhouse screenings. The number of stems per clump is counted before and after infestation and a tiller ratio (tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process) is then calculated. Using this methodology, eight major screening trials (six with *Zulia pubescens*, two with *Mahanarva trifissa*) were conducted in Caquetá in 2003.

Results and Discussion: In **Table 3** we highlight the results of evaluating 32 hybrids for resistance to *Z. pubescens* in comparison with six checks well known for their reaction to *Aeneolamia varia*. As in previous occasions, there was a significant negative correlation ($r = -0.452$; $P < 0.001$; $n = 2273$) between damage scores and tiller ratios. This means that damage scores are useful in predicting tiller losses resulting from intense insect damage. Selected hybrids in **Table 3** showed significantly lower damage scores and significantly higher tiller ratios than the susceptible checks CIAT 0606 and BRUZ4X/44-02.

Table 3. Field resistance to *Zulia pubescens* in selected *Brachiaria* hybrids and checks. Means of six trials, 10 replicates per genotype per trial.

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/1494	2.0	1.04
BR00NO/0755	2.0	0.92
BR00NO/1392	2.0	0.91
BR00NO/1032	2.1	0.89
BR00NO/0604	2.1	0.88
BR00NO/1076	2.1	0.88
BR00NO/1295	2.0	0.88
BR00NO/0036	2.0	0.87
BR00NO/0042	2.1	0.96
BR00NO/0029	2.1	0.86
	Checks[b]	
FM9503/46/024 (T)	1.1	1.04
CIAT 6294 (R)	1.1	1.04
CIAT 36062 (R)	1.1	1.01

Genotype	Damage scores	Tiller ratio[a]
CIAT 6133 (T)	1.8	0.92
CIAT 0606 (S)	3.6	0.46
BRUZ4X/44-02 (S)	3.9	0.47
LSD 5%	0.13	0.12

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

The same set of 32 hybrids was evaluated for field resistance to *Mahanarva trifissa.* Results are shown in **Table 4**. Resistant hybrids exposed to *M. trifissa* performed significantly better than the checks both in terms of damage scores and tiller ratios.

Table 4. Field resistance to *Mahanarva trifissa* in selected *Brachiaria* hybrids and checks. Means of two trials, 10 replicates per genotype per trial.

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/0587	2.1	1.16
BR00NO/1494	2.1	1.07
BR00NO/1392	2.0	1.06
BR00NO/0106	2.0	1.01
BR00NO/0078	2.1	1.00
BR00NO/0049	2.1	0.97
BR00NO/1733	2.1	0.96
BR00NO/0235	2.1	0.96
	Checks[b]	
FM9503/46/024 (T)	1.1	0.98
CIAT 6294 (R)	1.1	1.22
CIAT 36062 (R)	1.0	1.03
CIAT 6133 (T)	1.8	0.99
CIAT 0606 (S)	3.8	0.41
BRUZ4X/44-02 (S)	4.3	0.28
LSD 5%	0.19	0.16

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Contributors: C. Cardona, G. Sotelo, A. Pabón, and J. W. Miles.

Activity 2. Identify host mechanisms for spittlebug resistance in *Brachiaria.*

Mechanisms of resistance to five spittlebug species

Introduction

We have shown in previous reports that resistance to one spittlebug species does not necessarily apply to other species. We have also shown that the mechanisms of resistance vary. In 2003 we finalized the characterization of antibiosis and tolerance to *Aeneolamia reducta*, the most important species in the Caribbean zone. What follows is a summary of what we know about host plant resistance mechanisms to five major spittlebug species present in Colombia.

Materials and Methods: Several experiments were conducted and are reported herein. As test materials we used four germplasm accessions well known for their reaction to *Aeneolamia varia*: the susceptible checks CIAT 0606 and CIAT 0654 and the resistant checks CIAT 6294 ('Marandú') and CIAT 36062 (a hybrid-derived clone). These four host genotypes were also used to compare their resistance to other spittlebug species. CIAT 0654 and CIAT 36062, highly susceptible and resistant, respectively, were used in antibiosis studies. Tolerance studies were conducted with CIAT 0654, CIAT 6294, and CIAT 36062. *A. varia*, *A. reducta*, and *Z. carbonaria* were mass-reared on plants of CIAT 0654 in a screen-house. Mature eggs were used to infest test plants in the different experiments. In the case of *Z. pubescens* and *M. trifissa*, large numbers of adults were collected in the field with a sweep net and transferred to muslin cages in a screen-house to feed on potted plants of CIAT 0654. Adults were allowed to oviposit and eggs were separated from the soil. As with other spittlebug species, test plants were infested with mature eggs. All tests were conducted in a glasshouse at a mean temperature of 24^{o}C (range, 19-27^{o}C) and mean relative humidity of 75% (range, 70-90%).

To evaluate antibiotic effects, cohorts of no fewer than 900 individuals of each of the five species under study were established on each of two host genotypes well characterized for their reaction to *A. varia*: CIAT 0654 (highly susceptible) and CIAT 36062 (highly resistant). Cohorts were established by infesting 150 single-plant units with 6 eggs of the respective species per unit as described above. Following eclosion, a sample of two or three tubes per host genotype was examined daily to determine the fate of 12 or 18 individual insects. Nymphal instars and their duration were determined from measurement of the width of the head capsule of every nymph recovered (dead or alive). Survival rates were calculated. The dry weight of each nymph was recorded. Daily sampling continued until all surviving nymphs reached adulthood.

To study tolerance we initially compared the response of the susceptible CIAT 0654 and the *A. varia*-resistant CIAT 6294 ('Marandú') to increasing levels of infestation with nymphs of *A. varia*, *M. trifissa*, *Z. carbonaria*, and *Z. pubescens*. *A. reducta* was not included in these studies. Thirty-day-old plants of CIAT 0654 and CIAT 6294 were exposed to 0, 2, 3, 5, 7, or 10 nymphs per plant of each of the spittlebug species. The 48 host genotype- insect species-infestation level treatment combinations were randomly assigned to single-plant experimental units within 10 complete blocks. Plants were infested with mature eggs and the infestation was allowed to proceed until all nymphs were mature or adult emergence occurred. Plants were then scored for damage using the 5-point scale described above and the percentage nymphal survival recorded.

Aboveground dry weight of plants was recorded following drying in an oven at 40°C. Percent weight loss was calculated (relative to the uninfested controls). We calculated functional plant loss indexes for each infestation level based on plant weight loss and damage response. Using the same general methodology, we conducted one more tolerance test in which plants of CIAT 0654 (susceptible) and CIAT 36062 (resistant to *A. varia*) were submitted to increasing levels of infestation (0, 2, 3, 5, 7, or 10 nymphs per plant) with each of the following species: *A. varia*, *A. reducta*, *M. trifissa*, *Z. carbonaria*, and *Z. pubescens*. We used a randomized complete block design with 10 repetitions per species-infestation level-host genotype combination. Damage scores, nymph survival, and above ground plant dry weights were recorded. Functional Plant Loss Indices were calculated.

All data were analyzed using the general linear model procedure. Means were separated by least significant difference (LSD: $\alpha = 0.05$) only when the overall F test was significant ($\alpha = 0.05$). Percentage nymph survival was transformed to arcsine square root of proportion; percentages of dry weight loss were transformed to square root. Means and standard errors of untransformed data are presented. Antibiotic effects for the different spittlebug species were assessed by comparing nymphal instar duration and nymph weight between the susceptible and resistant host genotypes by paired *t*-test. To compare survivorship of nymphs on susceptible and resistant host genotypes, median survival times were calculated using the Kaplan-Meier test. The Cox-Mantel survival test was used to compare survival distributions on susceptible and resistant host genotypes. Tolerance to the different spittlebug species was assessed by comparing mean percentage survival, mean damage scores, and mean percentage plant dry weight loss of five infestation levels between the susceptible and resistant host genotypes by paired *t*-test within spittlebug species.

Results and Discussion: ***Antibiosis tests.*** Relative to the susceptible control, CIAT 0654, there was a significant delay in development time of nymphs of *A. varia*, *A. reducta*, and *Z. pubescens* reared on CIAT 36062 **(Figure 1)**. No such effect was found in the case of *Z. carbonaria*. Mortality of second instars of *M. trifissa* was so high, that we were unable to calculate developmental times for this species.

Nymphal survival on the resistant CIAT 36062 was least for *M. trifissa* and greatest for *Z. carbonaria*. Survival of all species was less on CIAT 36062 than on the susceptible CIAT 0654. The Kaplan-Meier survival test revealed significant effects (no overlapping confidence intervals) of the resistant genotype on the median survival times of *A. varia*, *A. reducta*, and *M. trifissa* and, to a lesser extent, *Z. pubescens* populations. Survival time of *Z. carbonaria* was not affected by the resistant genotype. Calculation of the Cox-Mantel survival statistic showed differences at the 1% level of significance between CIAT 0654 and CIAT 36062 **(Table 1)** in terms of survival rates for *A. varia*, *A. reducta*, and *M. trifissa*, and at the 5% level of confidence for *Z. pubescens* (a lower level of antibiosis). No difference was found in the case of *Z. carbonaria* meaning that there is no antibiosis to this species in CIAT 36062. This was confirmed when survival rates of *Z. carbonaria* and *Z. pubescens* on CIAT 36062 were compared. The Cox-Mantel survival test statistic (2.8) was positive and significant at the 1% level, indicating that CIAT 36062 is more favorable to *Z. carbonaria* than to *Z. pubescens*.

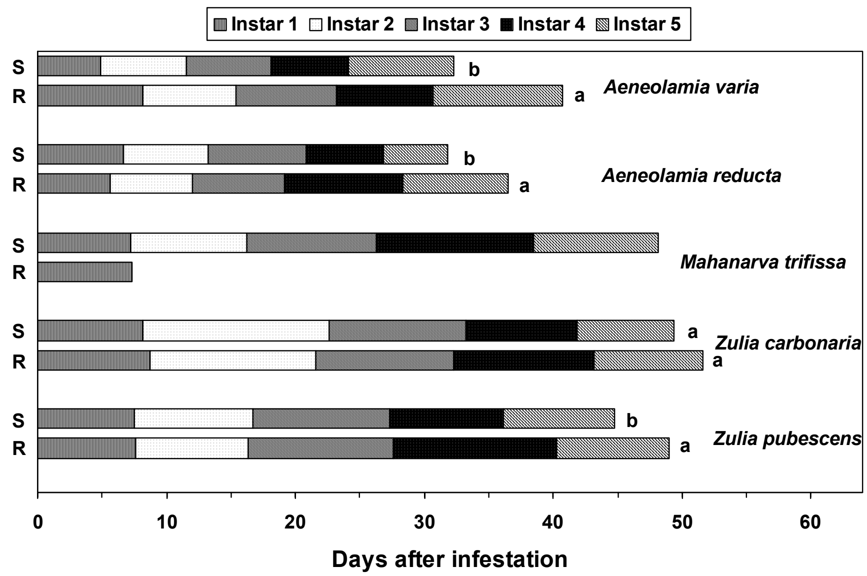

Figure 1. Duration of nymphal instars of five spittlebug species reared on susceptible (S, CIAT 0654) or resistant (R, CIAT 36062) *Brachiaria* genotypes. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within species. *Mahanarva trifissa* was not analyzed due to very high mortality of second instars.

Table 1. Survivorship parameters for nymphs of five spittlebug species reared on susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes.

Spittlebug species	Number tested: On CIAT 0654	Number tested: On CIAT 36062	C[a]
Aeneolamia varia	480	480	4.8**
Aeneolamia reducta	420	480	6.4**
Mahanarva trifissa	708	246	9.7**
Zulia carbonaria	744	720	1.4ns
Zulia pubescens	648	648	2.2*

**, Significant at the 1% level; *, significant at the 5% level; ns, not significant.
a C is the test statistic for the Cox-Mantel two-sample survival test (CIAT 0654 versus CIAT 36062).

Antibiosis to *A. varia*, *A. reducta*, and *Z. pubescens* in CIAT 36062 was also manifested by the reduced weight of surviving 4th and 5th instar nymphs, and adults. No effect on nymphal or adult weight of *Z. carbonaria* was detected. Other manifestations of antibiosis were the occurrence of minute 2nd, 3rd, and 4th instar nymphs, staggering of developmental times, and reduced spittle production by surviving nymphs. Also, we found that nymphs reared on CIAT 36062 usually leave the spittle and wander over the soil surface, eventually dying of dehydration. We found no deformation of nymphs or adults nor did we detect obvious disruptions in the molting process. The level of antibiosis resistance in CIAT 36062 clearly differs by spittlebug species and can be classified as follows: very high for *M. trifissa*, high for *A. varia* and *A. reducta*, moderate for *Z. pubescens*, and absent for *Z. carbonaria*.

Tolerance tests. CIAT 6294 expressed clear antibiosis to *A. varia* and *M. trifissa* as the mean nymphal survival of five infestation levels was significantly lower than the mean for the susceptible control CIAT 0654 **(Figure 2)**. However, survival of *Z. carbonaria* or *Z. pubescens* nymphs was high on both genotypes at all levels of infestation, indicating lack of antibiosis in CIAT 6294 to these two species. These results were consistent with those obtained in resistance reconfirmation tests. CIAT 6294 plants suffered less damage and less plant dry weight loss than the susceptible control at all levels of infestation **(Figure 3)**. As in previous studies, visual damage scores predicted biomass loss. Since survival of the *Zulia* spp. nymphs did not differ between the genotypes, we interpret the lower damage scores and lower plant dry weight losses caused by *Z. carbonaria* and *Z. pubescens* on CIAT 6294 as tolerance.

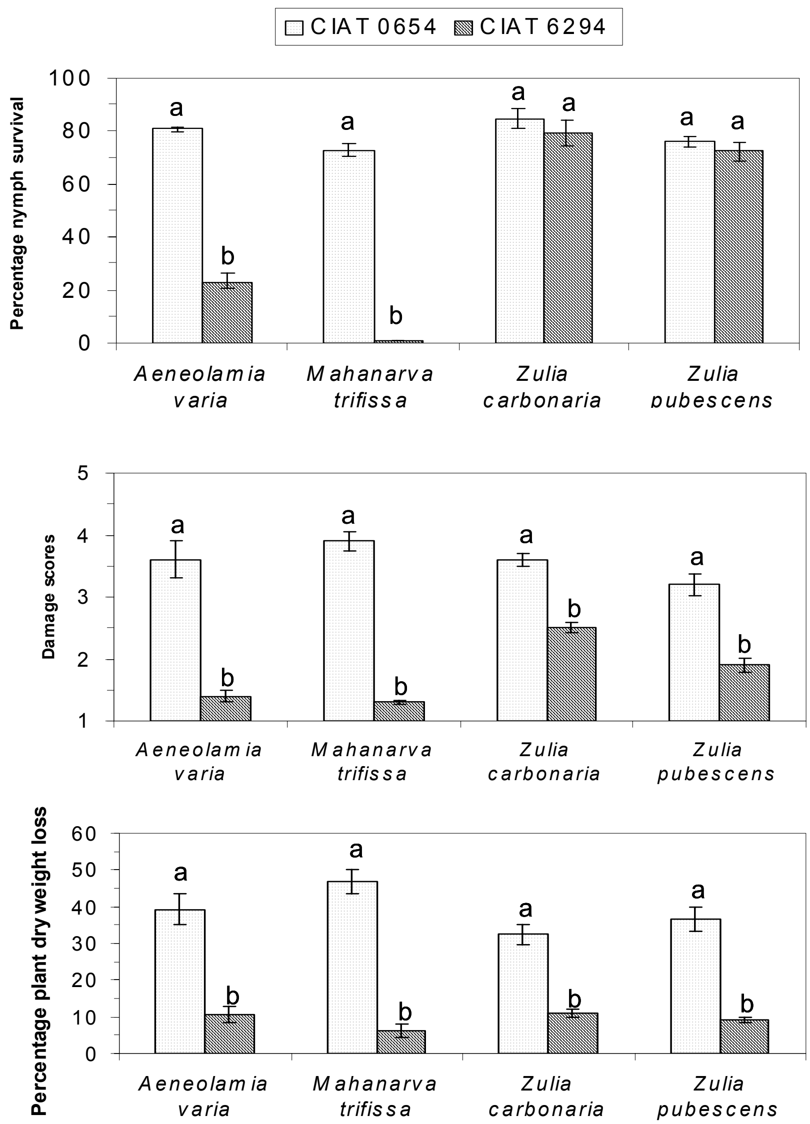

Figure 2. **Response of susceptible (CIAT 0654) or resistant (CIAT 6294) *Brachiaria* genotypes to attack by nymphs of four spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ (*P* < 0.05). Pair-wise comparison by *t*-test within spittlebug species.**

At all levels of infestation, survival of nymphs on CIAT 36062 was much less than on the susceptible control for *A. varia*, *A. reducta*, and *M. trifissa*, but only moderately less for *Z. pubescens*. *Z. carbonaria* nymphs survived equally well on the two genotypes **(Figure 3)**. Thus, expression of antibiosis in CIAT 36062 was dependent on spittlebug species. CIAT 36062 suffered significantly less damage (expressed as damage scores or plant weight loss) than the susceptible control at all levels of infestation with *Z. carbonaria* **(Figure 3)**. Since *Z. carbonaria* nymphs survived equally well on both genotypes, we interpret the mechanism of resistance to *Z. carbonaria* in CIAT 36062 as tolerance.

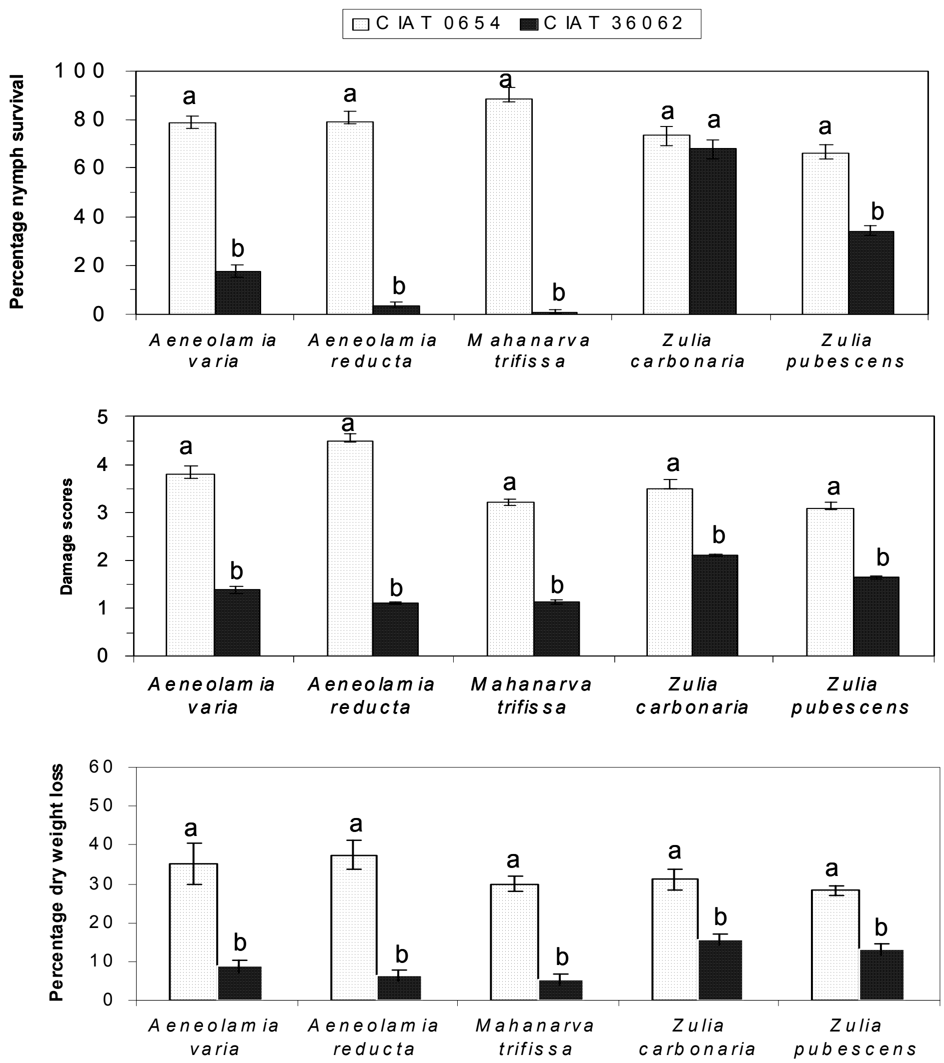

Figure 3. Response of susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes to attack by nymphs of five spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within spittlebug species.

We also calculated a functional plant loss index to measure tolerance to both *Z. carbonaria* and *Z. pubescens*. Losses were highest for the susceptible control, CIAT 0654, at all levels of infestation **(Figure 4)**. Losses caused by both species on CIAT 6294 and on CIAT 36062 were lower at all infestation levels. These results suggest the presence of true tolerance to *Z. carbonaria* in CIAT 6294 and CIAT 36062, true tolerance to *Z. pubescens* in CIAT 6294 and a combination of tolerance coupled with antibiosis as mechanisms of resistance to *Z. pubescens* in CIAT 36062.

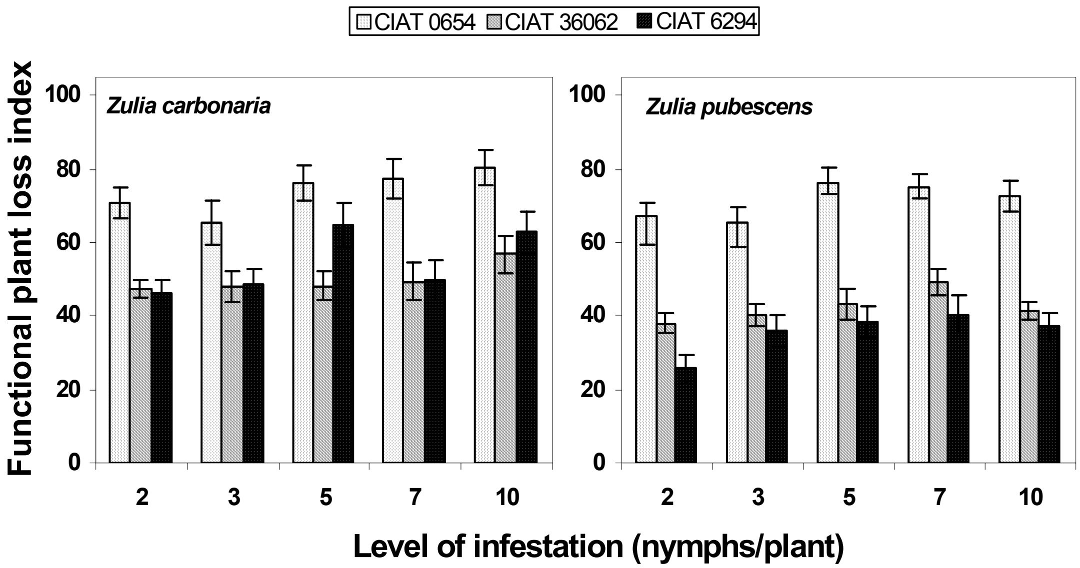

Figure 4. Functional plant loss indices (percentage) for susceptible (CIAT 0654) or resistant (CIAT 36062, CIAT 6294) *Brachiaria* genotypes exposed to five levels of infestation with each of two spittlebug species.

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Activity 3. Effect of mixed infestations on resistance expression in selected *Brachiaria* genotypes.

Introduction

As explained in the 2002 Annual Report, we can identify spittlebug nymphs with absolute precision by means of RAPD-PCR DNA analysis or by comparison of esterase banding patterns. Using these techniques we have been able to detect mixed infestations in commercial fields and to measure percentage survival of different species when mixed infestations by two or more species occur. This in turn has allowed us to study how different species combinations affect resistant expressions in selected resistant or susceptible genotypes.

Materials and Methods: In 2003 we measured the effect of single species infestation as opposed to mixed infestations by infesting plants with eggs of two or more spittlebug species in different proportions. The infestation was allowed to proceed until adult emergence occurred. Plants were then scored for damage and the surviving nymphs were collected and identified to species level by comparison of esterase banding patterns or, in some cases, by RAPDs-PCR analysis. Percentage survival was calculated for each spittlebug species.

Results and Discussion: We will highlight results of studies on the effect of mixed *Aeneolamia reducta* - *Zulia carbonaria* infestations. These are two of the most important spittlebug species present in Colombia. As shown in **Figure 1**, when the resistant genotype CIAT 36062 is exposed to *Zulia carbonaria* alone or when *Z. carbonaria* predominates in the mixture, damage scores increase so that the genotype is classified as intermediate resistant rather than resistant. This was not the case with the hybrid SX01NO/0102, the most resistant hybrid tested to date for resistance to five spittlebug species.

Most important, we detected significant and differential antibiosis effects **(Figure 2)** when mixed populations of *A. reducta* and *Z. carbonaria* in different proportions were used to infest plants of the resistant genotypes CIAT 36062 and SX01NO/0102 **(Figure 2)**. At all levels of infestation, survival of *A. reducta* on both resistant genotypes was significantly reduced to levels below the cut-off point for resistance rating (< 30%). On the contrary, the survival of *Z. carbonaria* nymphs on CIAT 36062 was significantly higher, in some cases well above the 50% level used to classify genotypes as susceptible. The hybrid SX01NO/0102 showed intermediate resistance to *Z. carbonaria* at two of the levels of infestation tested. Again, these findings emphasize the need to characterize resistance to as many species as possible and illustrate the need to breed for multiple antibiosis resistance.

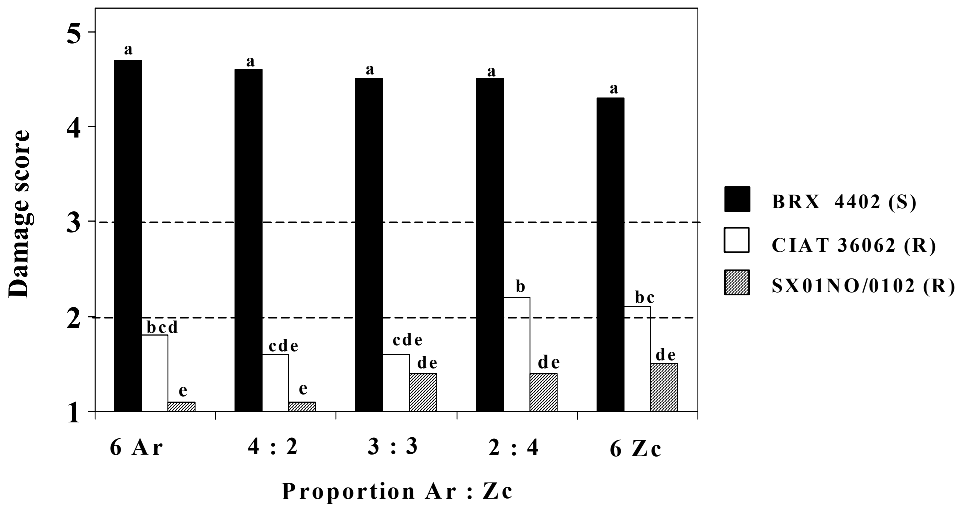

Figure 1. **Damage scores recorded on susceptible (S) and resistant (R) *Brachiaria* genotypes exposed to individual or simultaneous attack by nymphs of *Aeneolamia reducta* (Ar) or *Zulia carbonaria* (Zc). Dotted lines represent cut-off points for resistance (< 2) and intermediate ratings (2 -3) in a 1 - 5 damage score scale. Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.**

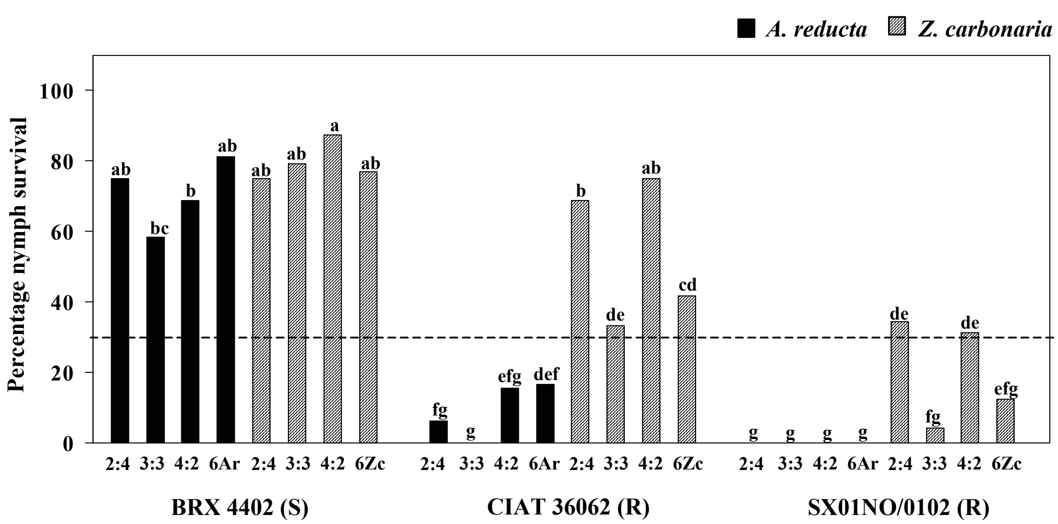

Figure 2. **Levels of antibiosis (reduced percentage nymph survival) detected when plants of susceptible (S) or resistant (R) *Brachiaria* genotypes were infested with *Aeneolamia reducta* or *Zulia carbonaria* or combinations thereof (*A. varia*: *Z. carbonaria*). The dotted line represents the cut-off point for resistance rating (< 30% percentage survival). Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.**

Contributors: A. Pabón, G. Sotelo, and C. Cardona.

Activity 4. Interactions between strains of five spittlebug species and resistance expression in selected *Brachiaria* genotypes

Introduction

The protective properties of insect-resistant cultivars may be overcome by the development of resistance-breaking strains of a given insect species. These are insect populations that possess an inherent genetic capability to overcome plant resistance. Typically, biotypes develop as a result of selection from the parent population in response to exposure to the resistant cultivar. It may occur that the genetic capability of an insect to overcome resistance is so great that resistance is nullified before the resistance cultivar is grown in a large geographical area. This is why it is important to obtain information on the reaction of resistant genotypes to as many geographical strains of a given insect pest as it is possible. We initiated a series of experiments aimed at measuring the response of resistant cultivars to populations of *A. varia*, *A. reducta*, *Z. pubescens*, *Z. carbonaria*, and *M. trifissa* collected in several different areas of Colombia. For the first time, we also generated information on resistance to *Prosapia simulans* (Walker).

Materials and Methods: All trials were conducted using test materials well known for their reaction to *Aeneolamia varia*. We evaluated the susceptible checks CIAT 0606 and CIAT 0654, the resistant checks CIAT 6294 and CIAT 36062, and two new sexual hybrids SX01NO/0102 and SX01NO/0233 classified as highly resistant to *A. varia* in previous studies. The *A. varia*-CIAT colony combination was used as the standard check in all trials. Screening for resistance was conducted using standard methodologies. Plants were infested with six eggs per plant of the respective spittlebug species-geographical combination and the infestation was allowed to proceed without interference until all nymphs reached the fifth instar stage or adult emergence occurred. Plants (20 per genotype) were scored for symptoms using the damage scale (1, no damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated.

Results and Discussion: We have conducted four trials. At this point, we will highlight results obtained with geographical strains of *A. varia* and *Z. pubescens*. We will also report on our first-ever screening for resistance to *P. simulans*.

The reaction of six genotypes to attack by nymphs of three strains of *A. varia* is shown in **Table 1**. No significant genotype x strain interaction was detected for damage scores or percentage nymph survival, meaning that resistance ratings did not change when the genotypes were exposed to different strains of *A. varia*. Similarly, no significant genotype x strain interaction was detected when susceptible and resistant genotypes were exposed to attack by nymphs of *Z. pubescens* **(Table 2)**.

Table 1. Reaction of selected *Brachiaria* genotypes to strains of *Aeneolamia varia* from two geographical areas of Colombia.

Origin of strain	Genotypes[a] BRUZ4X-44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO/0102	SX01NO/0233
	Damage scores					
Florencia, Caquetá	3.9b	3.8b	1.2a	1.3a	1.1a	2.0a
V/vicencio, Meta	4.2a	3.7b	1.3a	1.1a	1.0a	1.3b
CIAT colony	4.9a	4.6a	1.3a	1.1a	1.0a	2.2a
Mean	4.3A	4.0A	1.3C	1.2C	1.0C	1.8B
	Percentage nymph survival					
Florencia, Caquetá	75.8b	86.6a	17.5b	0.0c	2.5b	26.7b
V/vicencio, Meta	90.8a	71.1b	35.8a	9.2a	6.7a	14.2c
CIAT colony	86.1a	87.1a	19.8b	4.7b	6.3a	40.7a
Mean	84.2A	81.6A	24.4B	4.6C	5.2C	27.2B

Means of 20 replicates by genotype by insect strain. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

Table 2. Reaction of selected *Brachiaria* genotypes to strains of *Zulia pubescens* from three geographical areas of Colombia.

Origin of strain	Genotypes BRX 44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO/0102	SX01NO/0233
	Damage scores					
Darién, Valle	3.9a	4.2a	2.0a	1.0b	1.1a	1.5b
Popayán, Cauca	3.8a	3.3b	2.1a	1.2ab	1.1a	1.3b
S. José de Fragua, Caquetá	4.3a	4.1a	2.1a	1.4a	1.3a	2.6a
Mean	4.0A	3.9A	2.1B	1.2C	1.2C	1.8B
	Percentage nymph survival					
Darién, Valle	71.6a	55.9ab	34.1b	2.8b	0.9c	4.6b
Popayán, Cauca	44.3b	45.5b	47.5a	11.6a	6.7b	5.0b
S. José de Fragua, Caquetá	74.1a	69.0a	34.2b	8.8a	21.6a	47.2a
Mean	63.3A	56.8A	38.6B	7.7D	9.7D	18.9C

Means of 20 replicates by genotype by insect strain. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

Table 3 summarizes results of our first screening for resistance to *Prosapia simulans*. Susceptible (CIAT 0606, BRX-44-02) and resistant genotypes (CIAT 6294, CIAT 36062, SX01NO/0102, and SX01NO/0233) differed for damage scores for all spittlebug species tested. *P. simulans* caused more damage than *A. varia* and *M. trifissa* on the resistant genotype CIAT 6294 ('Marandú'). Using our resistance classification, CIAT 6294 would be classified as resistant to *A. varia* and *M. trifissa* (damage scores: 1-2) but intermediate to *P. simulans* (damage scores: 2.1-3.0). SX01NO/0233 was intermediate to all three species tested. Survival of nymphs of *A. varia* and *M. trifissa* was significantly lower on the *A. varia*-resistant genotypes than on the susceptible controls CIAT 0606 and BRX-44-02 **(Table 3)**. Survival of *P. simulans* nymphs was significantly higher on CIAT 6294 than on the other resistant genotypes suggesting that antibiosis resistance to this species is absent in 'Marandú'. Using our resistance classification, CIAT 6294 would be classified as susceptible (> 50% survival) to *P. simulans*. The relatively low levels of damage caused by *P. simulans* on CIAT 6294 could be the result of tolerance to this species.

Table 3. Response of selected *Brachiaria* genotypes to attack by nymphs of three spittlebug species.

Spittlebug species	Spittlebug species		
	Mahanarva trifissa	***Aeneolamia varia***	***Prosapia simulans***
	Damage scores		
BRX-44-02	4.2aA	4.9aA	4.3aA
CIAT 0606	3.5bB	4.6aA	4.3aA
CIAT 6294	1.2cB	1.3cB	2.4bA
CIAT 36062	1.1cA	1.1cA	1.3cA
SX01NO/0102	1.0cA	1.0cA	1.5cA
SX01NO/0233	2.1bcA	2.2bA	2.3bA
	Percentage nymph survival		
BRX-44-02	55.0aB	86.1aA	90.8aA
CIAT 0606	36.0aC	87.1aA	79.2bA
CIAT 6294	0.0bC	19.8cB	65.0bA
CIAT 36062	0.0bB	4.7dA	14.2cA
SX01NO/0102	0.0bA	6.3dA	6.7dA
SX01NO/0233	34.5aA	40.7bA	5.0dB

Means of 20 replicates by genotype by insect species. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

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Activity 5. Mechanisms of resistance to adults of five spittlebug species and sub-lethal effects of antibiosis on adults of spittlebug

Introduction

Varying levels of antibiosis resistance to nymphs of several spittlebug species have been well characterized in a number of resistant *Brachiaria* genotypes. The effects of antibiosis on the biology of nymphs have also been studied. Not much is known about possible direct effects of antibiotic genotypes on the biology of adults. Even less is known about sub-lethal effects (i. e. reduced oviposition rates, reduced longevity, prolonged generation times, reduced rates of growth, etc.) on adults resulting from nymphs feeding on antibiotic genotypes. We initiated a series of studies aimed at measuring how antibiotic genotypes may directly or indirectly (through sub-lethal effects) affect the biology of adults of *A. varia*.

Materials and Methods: Initially, we conducted two experiments aimed at measuring how feeding on an antibiotic genotype affects the biology of adults of *A. varia*. Later on, we initiated a comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the biology of resulting adults. For this, a number of life tables will be constructed. Treatment combinations are shown in **Table 1**.

Table 1. Treatment combinations to study possible sub-lethal effects of intermediate and high levels of nymphal antibiosis on adults of *Aeneolamia varia*.

Nymphs reared on:	Adults feeding on:	Null hypothesis
BRX 44-02[a]	BRX 44-02	Absolute check
BRX 44-02	CIAT 06294	A genotype that is moderately antibiotic to nymphs does not affect adults
BRX 44-02	CIAT 36062	A genotype that is highly antibiotic to nymphs does not affect adults
CIAT 06294	BRX 44-02	Intermediate antibiosis to nymphs does not affect resulting adults
CIAT 06294	CIAT 06294	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 06294	CIAT 36062	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype
CIAT 36062	BRX 44-02	High antibiosis to nymphs does not affect resulting adults
CIAT 36062	CIAT 06294	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 36062	CIAT 36062	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype

[a] BRX44-02 is susceptible to *A. varia*. CIAT 6294 and CIAT 36062 show intermediate and high levels of antibiosis resistance to nymphs of *A. varia*, respectively.

Results and Discussion: This work is in progress. Results will be presented in full in 2004.

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Workshops and Conferences

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