Activity 1. Biology of spittlebug species

Rationale

A major barrier for advancing the management of spittlebugs in grasses is the lack of biological information on specific pest species. This has led to a weak understanding of the patterns of variation in this insect group and has contributed to an overgeneralization of this complex pest problem. Describing the patterns of variation in biology across this diverse group will depend on more detailed studies of currently unknown species. Tailoring IPM strategies to the particular species and habitats of concern will also depend on this information. To establish this foundation, results from the studies of Colombia's grass-feeding spittlebugs are summarized and some trends highlighted. In addition, life table analyses were carried out for the first time in spittlebugs. *Zulia carbonaria* was chosen as the model species to establish methodologies to further quantify relevant demographic parameters, overcome technical difficulties in the comparative study of reproductive biology, and compare results to previous life cycle studies. Although not presented here, we have gone on to obtain life table information for *M. andigena* and *P. simulans* as well.

Materials and Methods

Comparative biology. The biology of nine grass-feeding spittlebug species from Colombia were studied from 1996 to 2002 using comparative methodologies previously established at CIAT. To differentiate among the life stages, morphological measures were made of eggs, nymphs and adults. To quantify the life cycle, duration of life stages were followed under controlled conditions. And to begin to describe the reproductive behavior, oviposition site preferences were determined.

To have access to all life stages, small colonies were established in the greenhouse with eggs collected from field-caught adults. To differentiate among the developmental stages, these were characterized morphologically. With the aid of a stereoscope and ocular micrometer, certain aspects of the external morphology were measured for four developmental stages of the eggs, five nymphal instars, both sexes of late instar V (Vb) and both adult sexes.

To quantify the duration of life stages, eggs, nymphs and adults were observed under controlled conditions. For the adults, tenerals (<12 hours old) from the colony were confined in cohorts of four individuals under acetate sleeve cages over pots of the host plant; mortality was assessed daily. For the nymphs, recently emerged first instars (<12 hours old) were placed in individual pots of host plant established with abundant surface roots that nymphs require as feeding sites. Transformation from one instar to the next was determined by direct observation of the nymph itself or the molted exuvia. The mean longevity of each life stage was calculated from observations of 40 individuals. For the eggs, duration of the developmental stages was determined under controlled conditions (27°C, 100% RH, total darkness). Recently laid eggs (<24 hours old) were maintained on moist filter paper in petri dishes and observed daily. The mean duration of each of the four generalized developmental stages was calculated from observations on 100 eggs.

To study oviposition sites as part of the description of reproductive biology, field conditions were replicated in the screenhouse. The soil surface was specially prepared with soil oviposition substrate and 2 g leaf litter was dispersed on top. Each pot was infested with two females and two males from the colony and 10 days later eggs were recovered from three oviposition substrates: soil, leaf litter and the plant surface.

Life table analysis. Life tables were established for spittlebugs for the first time, using *Zulia carbonaria* as the model species. This research was carried out in 2002 under laboratory conditions and in the screenhouse (mean min/min temperature 19.5/29.5°C, and minimum RH of 56.3%). Adult female *Z. carbonaria* were collected in the field and confined in groups of six individuals in each of 10 large petri dishes lined on the bottom with humid filter paper that served as oviposition substrate. Stems of *Brachiaria ruziziensis* held in a microvial filled with water served as a food source. After 24 hours all eggs were removed and put in groups of seven in sectioned dishes. Eggs were kept under controlled conditions (27^{o}C, 100% RH, complete darkness) and observed daily to record survivorship and mortality in each of the four developmental stages. A life table was calculated from these data based on an initial cohort of 100 eggs. Upon emergence, each nymph (<12 hours old) was transferred to the screenhouse and placed in pots of *B. ruziziensis* specially prepared with abundant surface roots for feeding sites and spittle mass establishment. These pots were covered with a lid that had a hole through which the plant stems emerged. Nymphs were observed daily to determine advancement to the next instar through direct observation or detection of the exuvia.

Once adults emerged, data were collected for the female fertility table. Females were maintained under acetate sleeve cages over pots of *B. ruziziensis*. Oviposition substrate (2 cm thick) was provided in an inverted lid fitted in the top of the pot with an opening through which the plant stems emerged. Each pot was infested with one female and two males, the latter replaced when dead to provide continual presence of the opposite sex. Every three days females were transferred to new pots. Eggs were recovered from the soil substrate and the plant stem. Calculations were based on an initial cohort of 30 females.

The fecundity of *Z. carbonaria* females was determined based on the number of eggs laid by the initial cohort of 30 individuals. The fertility table was calculated from the longevity of the females, rate of oviposition and sex ratio (based on previous biological studies). Once the fertility table was constructed the following demographic parameters were calculated. Generation time, T, was the mean period between birth of the parents and birth of the progeny, based not only on duration of the immature and adult stages but on the preoviposition period and duration of the oviposition period. Net replacement rate, Ro, was represented as the mean number of female progeny a female leaves in a generation, a function of survival and fecundity. The innate capacity for increase, r_m, is the potential increase in a population under optimal conditions given an initial cohort.

Results

Comparative biology. Eggs of all species passed through four developmental stages (S1, S2, S3, S4) distinguished by external characteristics and usually accompanied by an increase in size from one stage to the next **(Table 1)**. Eggs were spindle-shaped and varied from white to

creamy yellow to brownish-yellow in color. Generally, in S1 eggs were recently laid with no particular externally visible signs of development. In S2 a spot of red pigment was usually visible and the operculum had darkened to reveal a gray streak below the chorion. In S3 the chorion opened to expose the black operculum and the red spot was usually no longer visible. In S4 two pairs of red spots were visible, the posterior representing the Batelli glands of the abdomen and the anterior representing the eyes of the developing nymph. Each progressive stage was usually accompanied by a statistically significant increase in both length and width. Total egg development time varied from 14.1-18.0 among species, and up to a maximum of 310 days (*A. reducta*) for quiescent or diapausing eggs.

Five instars were confirmed for the nymphs of all species, accompanied by an increase in head capsule width, body, stylet and wing pad length; head capsule width was the most diagnostic because of little overlap among instars **(Table 2, Table 3)**. Stylet length decreased from the fifth instar to the adult in most species. There was sexual dimorphism expressed as the smaller size of male adults and late fifth instars in most species, and a trend toward brighter coloration in male adults in some species **(Table 4)**.

Life cycle varied by 30 days (45.3-75.5) **(Table 5)**. For eggs, nymphs and adults, the range of variation in duration was 14.1-18.0, 26.1-48.4 and 6.2-21.4 days, respectively. Seven of the nine species oviposited primarily in the soil, while *Prosapia simulans* preferred the stem surface and *Zulia pubescens* both stem and soil. Litter was the least preferred substrate receiving 0.0-8.2% of eggs with the exception of 22.7% in the case of *Mahanarva trifissa*.

Life table analysis. Under the conditions of the egg to adult study, mortality was 46.6%, that is 53 of the original cohort of 118 individuals died in the immature stages. The life table parameters for *Z. carbonaria* are summarized in Table 6. During the egg stage, dx (proportion of the original cohort that dies during each life stage) was highest (9.3%) in S1. Nymphs had the highest dx (25.4%) in instar I. The total mortality exhibited during the immature stages was 46.6%, divided in 10.1% and 36.3% for the egg and nymphal stages, respectively. The k-values for immature *Z. carbonaria* under the conditions of this study were 0.27 represented by 0.05 for the eggs and 0.23 for the nymphs **(Table 6, Figure 1)**.

Mean (± S.E.) longevity of the females under the conditions of this study was 34.2±11.6 days **(Figure 2)**. Mean lifetime fecundity was 125.8±82.9 eggs per female, with a fertility of 97.9% **(Table 7)**. During the study oviposition rate peaked near the half life of the females where 16.6% of eggs were recovered **(Figure 3)**. The demographic parameters generation time (T), net replacement rate (Ro), and intrinsic rate of growth (r_m) summarize the behavior of *Z. carbonaria* on *B. ruziziensis* **(Table 8)**. The generation time was calculated as 78.2 days, net replacement rate (Ro) as 125.8 times; the intrinsic rate of natural increase showed that *Z. carbonaria* produces 0.06 females/day throughout the generation time. Finally, finite rate of multiplication was 1.06 females/day.

Discussion

The biological variation exhibited by Colombia's grass-feeding spittlebug complex is relevant to effective pest management. This new information both strengthens certain trends and broadens

the known variation in this diverse and damaging pest complex of Neotropical forage grass and sugar cane.

The survival of *Z. carbonaria* from egg to adult was 21.3% less than that determined for *Prosapia simulans* under the same conditions (CIAT 2001). The highest mortality rate was suffered by the nymphal stage, 26.2% higher than the egg stage. The mortality measured for the immature stages of *Z. carbonaria* (46.4%) was higher than that measured in *P. simulans* where mortality in eggs and nymphs was 2.1 and 19.1%, respectively. The overall k-value for *Z. carbonaria* (0.27) was 0.14 times higher than *P. simulans.* The highest k-value for *Z. carbonaria* was for the nymphal stage while in *P. simulans* the greatest value was for the egg stage.

The longevity of females under these conditions exceeded by 13.8 days the longevity reported in previous biological studies (CIAT 2000). This difference is partially due to the provision of fresh material every 3 days in the case of the present study. Daily fecundity of females (3.7 eggs/day) is lower than that observed in *A. varia* (10.6 eggs/day, unpublished data). Fertility rates were similar to *M. andigena* (94.3%) and *P. simulans* (95.1%). The generation time (78.2 days) is considerably higher that that estimated from field population studies (63.2 days) partially because the latter did not include an estimation of the age of first reproduction and sequence of oviposition as included in the fertility table.

Table 1. Differentiation of egg developmental stages.

Species	Length				Width			
	S1	S2	S3	S4	S1	S2	S3	S4
Aeneolamia lepidior	a	b	c	c	a	b	c	d
Aeneolamia reducta	a	a	b	c	a	b	c	d
Aeneolamia varia	a	a	b	c	a	b	c	d
Mahanarva andigena	a	b	c	d	a	b	c	d
Mahanarva trifissa	a	ab	b	c	a	a	b	c
Prosapia simulans	a	b	c	d	a	b	c	d
Zulia carbonaria	a	a	b	c	a	b	c	d
Zulia pubescens	a	a	b	c	a	b	c	d
Zulia sp. nov.	a	ab	b	c	a	b	c	d

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Table 2. Differentiation of nymphal instars: head capsule width and stylet length.

Species	Head Capsule Width					Stylet Length				
	I	II	III	IV	V	I	II	III	IV	V
Aeneolamia lepidior	-	-	-	-	-	-	-	-	-	-
Aeneolamia reducta	a	b	c	d	e	a	b	c	d	e
Aeneolamia varia	-	-	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	c	d	e	a	b	c	d	e
Mahanarva trifissa	a	b	c	d	e	a	b	c	d	e
Prosapia simulans	a	b	c	d	e	a	b	c	d	e
Zulia carbonaria	a	b	c	d	e	a	b	c	d	e
Zulia pubescens	a	b	c	d	e	a	b	c	d	e
Zulia sp. nov.	a	b	c	d	e	a	b	c	d	e

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Table 3. Differentiation of nymphal instars: anterior wing pad and body length.

Species	Anterior Wing Pad Length			Body Length				
	III	IV	V	I	II	III	IV	V
Aeneolamia lepidior	-	-	-	-	-	-	-	-
Aeneolamia reducta	a	b	c	a	b	c	d	e
Aeneolamia varia	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	c	a	b	c	d	e
Mahanarva trifissa	a	b	c	a	b	c	d	e
Prosapia simulans	a	b	c	a	b	c	d	e
Zulia carbonaria	a	b	c	a	b	c	d	e
Zulia pubescens	a	b	c	a	b	c	d	e
Zulia sp. nov.	a	b	c	a	b	c	d	e

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Table 4. Differentiation of adult sexes.

Species	Head capsule width		Stylet length		Forewing length		Body length				Body width	
							Without wings		With wings			
	M	F	M	F	M	F	M	F	M	F	M	F
Aeneolamia lepidior	a	b	a	a	a	b	a	b	a	b	a	b
Aeneolamia reducta	a	b	a	b	a	b	a	b	a	b	a	b
Aeneolamia varia	-	-	-	-	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	a	b	a	b	a	b	a	b	a	b
Mahanarva trifissa	a	b	a	b	-	-	-	-	-	-	a	b
Prosapia simulans	a	b	a	b	a	a	a	b	a	b	a	b
Zulia carbonaria	a	b	a	a	a	b	a	b	a	b	a	b
Zulia pubescens	a	b	a	b	b	b	a	b	a	a	a	a
Zulia sp. nov.	a	b	a	a	a	b	a	b	a	b	a	b

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Table 5. Duration of life stages and oviposition sites of nine spittlebug species in Colombia

Species	Duration (days)				Oviposition sites [1]		
	Egg	Nymph	Adult	Total [2]	Soil	Litter	Surface of Plant Stem
Aeneolamia lepidior	14.1	35.4	6.2	52.6	79.7	5.7	14.6
Aeneolamia reducta	15.8	261	6.8	45.3	90.4	8.2	1.4
Aeneolamia varia	17.2	30.8	7.2	51.6	97.6	1.7	0.7
Mahanarva andigena	16.4	48.4	21.4	75.5	67.6	0.0	32.4
Mahanarva trifissa	17.0	44.2	6.8	64.6	74.0	22.7	3.3
Prosapia simulans	18.0	45.6	17.8	72.5	17.4	0.0	82.6
Zulia carbonaria	17.4	42.4	19.6	69.6	99.4	0.6	0.0
Zulia pubescens	14.3	38.0	18.4	61.5	40.4	0.4	59.2
Zulia sp. nov.	14.6	42.7	14.2	64.4	10.0	0.0	0.0

[1] Percent of eggs recovered in each substrate.

[2] Equivalent of egg + nymph + ½ adult

Table 6. Life table parameters for immature stages of *Z. carbonaria* on *B. ruziziensis.*

Stage	*ax*	*lx*	*dx*	*qx*	*kx*
Egg S1	118	1.000	0.093	0.093	0.042
Egg S2	107	0.907	0.000	0.000	0.000
Egg S3	107	0.907	0.008	0.009	0.004
Egg S4	106	0.898	0.000	0.000	0.000
Instar I	106	0.898	0.254	0.283	0.144
Instar II	76	0.644	0.025	0.039	0.017
Instar III	73	0.619	0.042	0.068	0.031
Instar IV	68	0.576	0.000	0.000	0.000
Instar V	68	0.576	0.042	0.074	0.033
Adult	63	0.534			
Ó			0.466		0.271

ax =number of individuals at start of each life stage
lx =age-specific survivorship
dx =proportion of individuals dying in each life stage
qx =rate of mortality
kx = age-specific mortality (“killing power”)

Table 7. Fecundity of *Z. carbonaria* females in under screenhouse conditions on *B. ruziziensis.*

Day	Total Eggs Laid	Mean ± S.E.	*n* (Females)
3	1	0.03±0.18 a	30
6	88	3.03±8.22 b	29
9	264	9.43±16.00 d	28
12	392	15.08±13.60 e	26
15	626	25.04±13.74 f	25
18	401	16.04±13.34 e	25
21	405	16.88±11.05 e	24
24	431	17.96±17.43 e	24
27	434	17.67±11.31 e	24
30	330	13.75±9.37 e	24
33	231	10.04±7.25 d	23
36	134	6.38±5.53 d	21
39	27	3.86±4.81 c	7
42	16	4.00±2.16 c	4
45	3	3.00±0.00 b	1

Means followed by different letters are significantly different (P<0.05).

Table 8. Demographic parameters for females of *Z. carbonaria* on *B. ruziziensis.*

Parameter	Value
Time to development (days)	59.83
Rate of survival (%)	53.40
Sex ratio (M:F)	1:1
r_m, Innate cpacity for increase (individuals/day)	0.063
Ro, Net replacement rate (progeny/individual)	125.77
T, Generation time (days)	78.20
ë, Finite rate of multiplication (females/day)	1.06

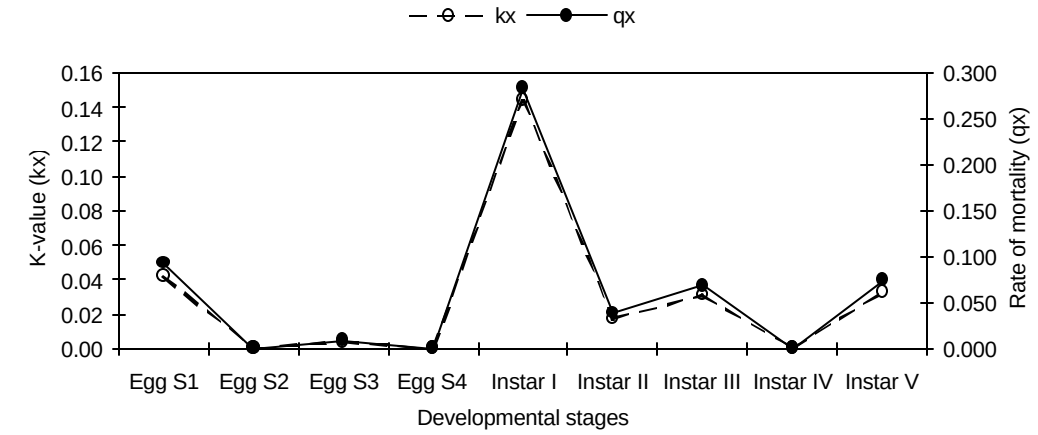

Figure 1. Rate of mortality and killing power for the immature stages of *Z. carbonaria* in laboratory conditions.

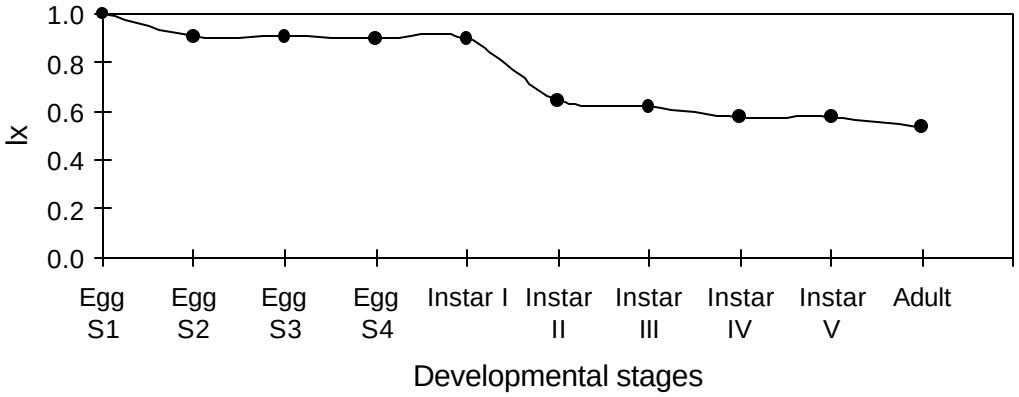

Figure 2. Survivorship curve of *Z. carbonaria* on *B. ruziziensis* under laboratory conditions

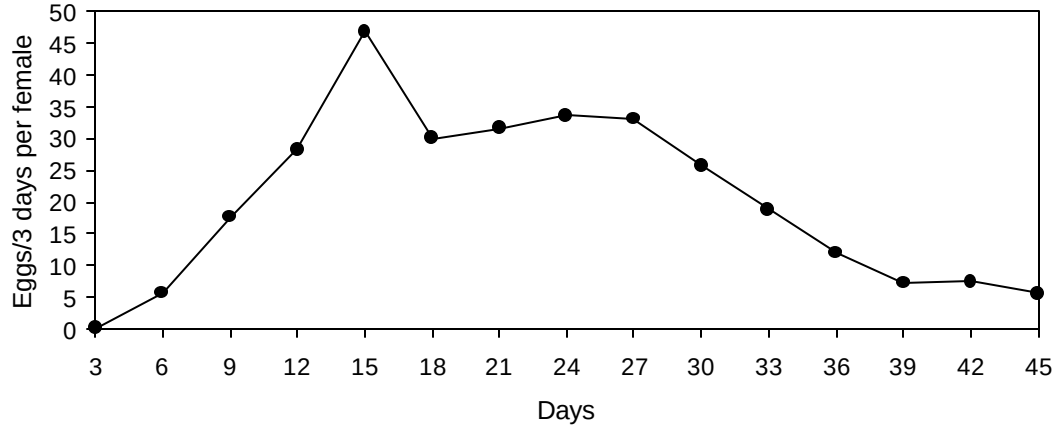

Figure 3. Oviposition rate of *Z. carbonaria* on *B. ruziziensis* under laboratory conditions

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Activity 2. Phenology of spittlebug populations in the field: prediction of the first generation in three ecoregions

Rationale

In ecoregions with a defined rainy season, management of spittlebug populations may depend on suppression of the first population outbreak before the adults lay eggs and contribute to future generations or before they colonize previously uninfested areas of the farm (CIAT 2001). Predicting when and where on the farm the first focal outbreaks occur will help target control tactics and establish control strategies. Given the correspondence between the rainy season and the occurrence of spittlebug nymphs and adults, it should be possible to predict the arrival of the first generation based on the nature and timing of the first rains together with information on the determinants of diapause termination.

Predicting the first generation of insects in regions with a marked seasonality will depend on a detailed understanding of the behavior of the eggs in combination with population dynamics surveys and meteorological data. It is known that the abrupt outbreak of nymphs that occurs at the beginning of the rainy season is not related to the eclosion pattern of eggs under continually humid conditions in the laboratory. It is postulated that eggs terminating diapause are able to enter a state of post-diapause quiescence in which they are still tolerant to drought conditions (Output 2.1.3). These eggs are poised to continue developing and eclose once adequate conditions of moisture return. The nature of the return of the wet season rains, therefore, could determine the synchrony and magnitude of the first generation.

In Colombia, the spittlebug complex varies across different ecoregions, therefore any predictive models need to be tailored according to the diapause syndrome of the major species and the agroecological characteristics each region. In this output we summarize the status of a verbal and quantitative model to predict the time of arrival of the first spittlebug generation.

Materials and Methods

This research was carried out in three contrasting ecoregions of Colombia: the Cauca River Valley (Cauca), Caribbean Coast (Sucre) and Orinoquian Piedmont (Meta). The Cauca Valley features a bimodal precipitation pattern where the rainiest months are March-May and September-November with a mean of 1800 mm/yr; the predominant spittlebug species is *Zulia carbonaria*. The Orinoquian Piedmont is unimodal for precipitation where the rainiest months are March-November and mean annual precipitation is 2730 mm/yr; the predominant spittlebug species is *Aeneolamia varia*. The Caribbean Coast is unimodal for precipitation where the rainiest months are April-October, and mean annual precipitation is 1039 mm/yr; the predominant spittlebug species is *Aeneolamia reducta.* Specific site characteristics are described elsewhere (CIAT 2001).

Three plots of 0.5 ha were selected in each survey site in pastures of *Brachiaria* spp. (Cauc, Meta) and *Bothriochloa pertusa* (Sucre). Each plot was divided into four subplots to facilitate subsampling of the different spittlebug life stages. Surveys were initiated two months before the

rainy season and were carried out twice weekly. Once the rainy season was underway surveys were increased to twice weekly until the end of the first population peak of nymphs and adults.

Nymph surveys comprised counts in two quadrats (0.25 m^2) per subplot, or 8 per plot, in which the occupants (nymphs, teneral adults) of all spittle masses were collected and identified to instar in the laboratory. This provided a measure of the absolute abundance of the different life stages and documentation of the progression of the spittlebug generation through the nymphal instars to teneral adults. Adult surveys comprised 50 sweeps with an insect per subplot, or 4 series of 50 per plot, between 09h00 and 11h00; spittlebug adults were collected and identified in the laboratory to sex and species. This provided a measure of the relative abundance of adults.

In this analysis repetitions were the years in which these population surveys were carried out in the three sites: 3 years for Cauca and Meta and 4 years for Sucre. For each region and year the population fluctuation data for nymphs and adults were analyzed in terms of cumulative insect-days (or cumulative area under the population curve) to control for variation in number and frequency of surveys. From these data we calculated the date of 50% cumulative insect-days as a measure of when that life stage reached peak abundance. This analysis allowed a comparison of the life cycle of each species and the precipitation pattern that originated the first generation. It was considered that the precipitation event that prompted the eclosion of eggs was that which occurred during the diapause egg stage S2; to identify this event we back-calculated from the nymph and adult population peak according to known information on duration of the life stages **(Figure 1)**.

To predict the timing of the first generation, repetitions were the different survey dates and the respective percent cumulative insect-days. This relationship was best described as linear and therefore a simple linear regression model was applied to estimate time of arrival of the first generation in each region. Models were adjusted so that time 0 was considered as the date of precipitation that gave origin to the population.

Results

Using the dates of 50% cumulative insect-days to back-predict the precipitation events that promoted the population appeared adequate for all years in the two unimodal sites (Meta, Sucre). These precipitation events were predicted as being sufficient for promoting the synchronous eclosion of post-diapause quiescent eggs and thereby the first generation of nymphs. For example, in Sucre 1997, the 12.5 and 24.0 mm of rain that fell during a period of 4 days was probably responsible for promoting the first generation of nymphs and adults 28.7±6.7 and 39.3±11.2 later **(Table 1, Figure 1)**. In Meta 1997, 34.0 and 108.9 mm of rain falling in a period of 4 days promoted nymph and adult peaks 32.3±7.3 and 39.3±11.2 days later.

In Cauca the situation was more complicated because it was difficult to identify the precipitation events responsible for initial outbreaks. For that reason analysis of this site is considered more preliminary and it was necessary to analyze population data at the plot rather than farm level. It was estimated that in this region 35.8 and 75.0 mm of rain falling in a period of 4 days was sufficient to promote nymph and adult peaks 44.9±5.9 and 61.1±9.7 days later **(Table 1**).

Table 1. Date of 50% cumulative insect-days and precipitation events that originated first generation spittlebug populations in two regions.

Region	Year	Stage	50% Cumulative Insect-Days (Julian Date)	Precipitation		Difference
				Julian Date[1]	Amount (mm/day)	
Sucre	1997	Nymph	138.3	100	24.0	38.3
		Adult	148.8			48.8
	1998	Nymph	134.6	106	15.3	28.6
		Adult	154.0			48.0
	2000	Nymph	145.5	121	12.5	24.5
		Adult	146.2			25.2
	2001	Nymph	148.4	125	13.7	23.4
		Adult	160.1			35.1
	Mean	Nymph				28.7±6.7
	± (S.E.)	Adult				39.3±11.2
Meta	1998	Nymph	56.5	32	75.1	24.5
		Adult	72.7			40.7
	2000	Nymph	117.1	84	108.9	33.1
		Adult	121.5			37.5
	2001	Nymph	107.2	68	34.0	39.2
		Adult	115.0			47.0
	Mean	Nymph				32.3±7.3
	± (S.E.)	Adult				41.7±4.83
Cauca	1999 P1	Nymph	47	10	75.0	37
		Adult	70			60
	1999 P2	Nymph	60			50
		Adult	71			60
	1999 P3	Nymph	51			41
		Adult	57			47
	2000 P1	Nymph	382*	335	35.8	47
		Adult	413*			78
	2000 P2	Nymph	389*			54
		Adult	403*			68
	2000 P3	Nymph	375*			40
		Adult	390			55
	2001 P3	Nymph	391*	346	68.0	45
		Adult	406*			60
	Mean	Nymph				44.9±5.9
	± (S.E.)	Adult				61.1±9.7

[1]Back-calculated according to the known life cycle.
*Includes 365 days of previous year.

The simple linear regression model to estimate the arrival of nymphs and adults in each region is summarized in Table 2 and Figure 2. There was a statistically significant positive correlation between survey day and cumulative insect-days for all three regions ($P<0.01$). For *A. reducta* in Sucre, the correlation (Pearson correlation coefficient, r) was 0.86 for nymphs (n=40) and 0.77 for adults (n=50). For *A. varia* in Meta the correlation was 0.86 for nymphs (n=32) and 0.84 for adults (n=30). For *Z. carbonaria* in Cauca, the correlation was 0.90 for nymphs (n=57) and 0.80 for adults (n=72).

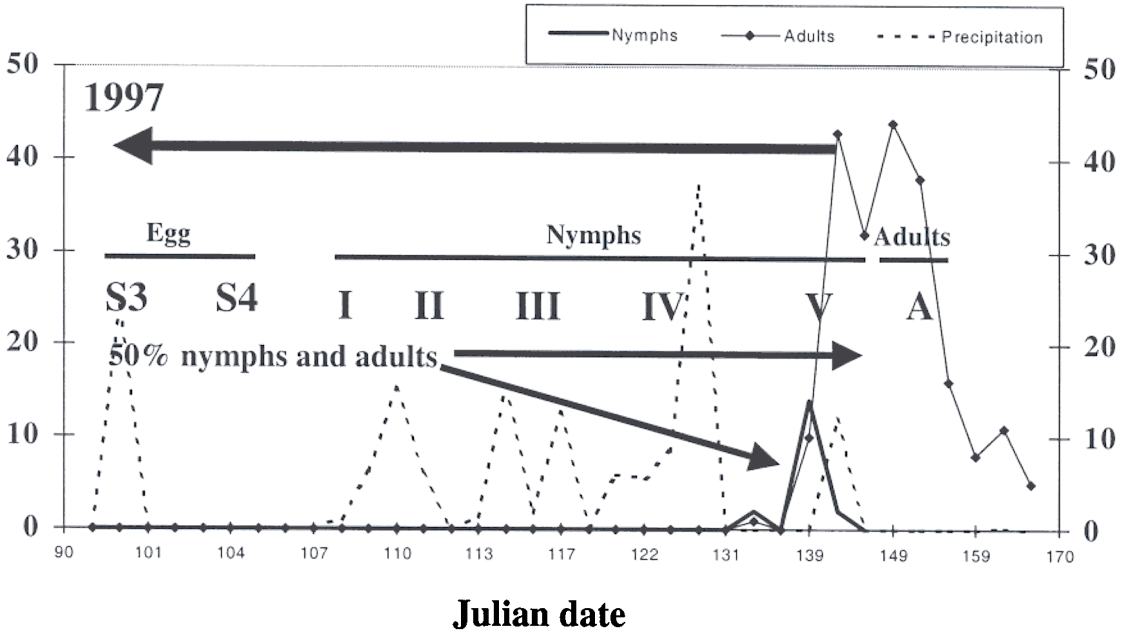

Figure 1. **Precipitation and life cycle of *A. reducta*, Sucre, 1997.**

Table 2. **Model to predict date of arrival of first generation nymphs and adults given known causative precipitation date. T = days to appearance, X = percent of the population expected.**

Region	Life Stage	Formula	Correlation (r)	50% Cumulative Insect -Days (Julian Date)
Sucre	Nymph	T=10.2+35.2(X)	0.86	27.8
	Adult	T=18.9+39.7(X)	0.77	38.9
Meta	Nymph	T=9.7+44.8(X)	0.86	32.1
	Adult	T=19.9+41.4(X)	0.84	40.6
Cauca	Nymph	T=16.7+56.6(X)	0.90	45.0
	Adult	T=25.2+58.2(X)	0.80	54.3

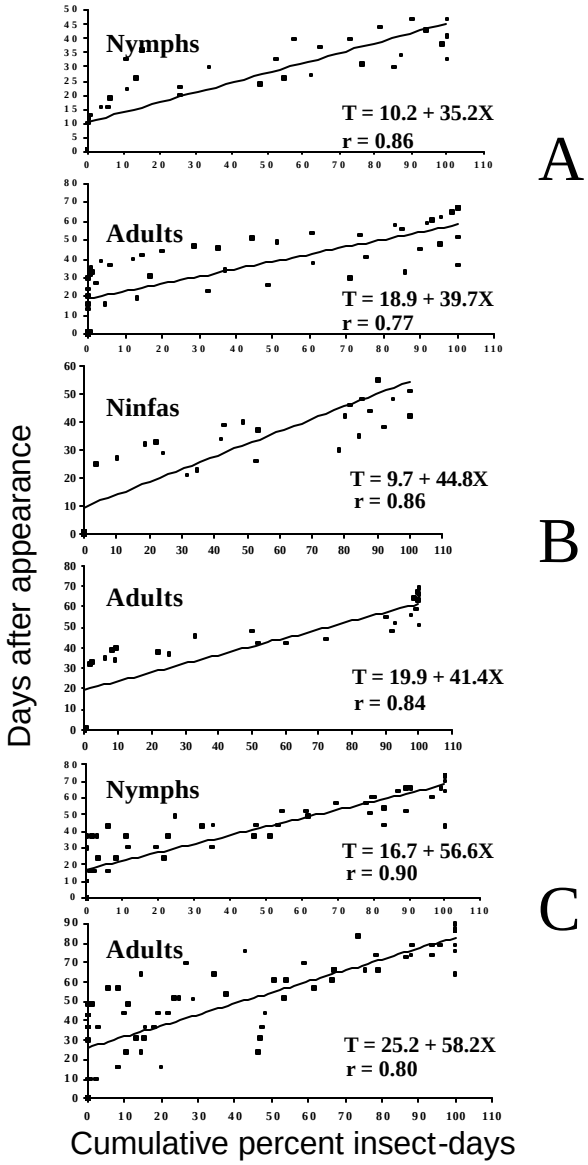

Figure 2. Prediction of the first generation of spittlebugs in Sucre, *A. reducta* (A); Meta, *A. varia* (B) and Cauca, *Z. carbonaria* (C).

Discussion

Information obtained previously (CIAT 2000, Output 2.1.3) indicates that the marked seasonality in Sucre and Meta causes population synchrony in early season spittlebug populations given the number of post-diapause quiescent eggs ready to continue their development upon arrival of the first rain. The difference between the date of 50% cumulative insect-days of appearance of the nymphs and adults and the date of the first major rainfall event can explain the arrival of the first generation population peak based on the duration of the egg life stages S3 and S4 and the five instars and at least a third of the adult longevity for all three species in all three regions. The information obtained suggests that *A. reducta* responds to 12.5-24.0 mm and *A. varia* to 36.4-

112.0 mm of rain. It has been previously determined for *A. varia* that 25 mm was sufficient to promote the first population outbreak (King 1975). Given the bimodal seasonality in Cauca, it will be necessary to broaden the studies to have more certainty in defining the day in which the amount of precipitation was sufficient to promote population outbreak in the case of *Z. carbonaria*.

The quantitative model for predicting the appearance of the three species in the different regions is important because this tool could reduce the need for population surveys or complicated scouting. If it is possible to reduce the abundance and diffusion of the first generation adults the impact from subsequent generations will also be reduced as will economic losses in pasture production, especially in highly seasonal areas such as the Caribbean Coast and Orinoquian Piedmont of Colombia. This prediction will have its greatest impact in combination with control tactics directed at the nymphs before they lead to the highly mobile and injurious adult stage of the first generation.

With all the information collected and analyzed during the development of this study and parallel studies (Output 2.1.3), the following is a verbal model to describe spittlebug phenology. This model should be considered a general theory to explain the population synchronization of spittlebugs in seasonal agroecosystems.

In the Neotropics, grass-feeding spittlebugs synchronize their life cycle with the season most appropriate for their development and reproduction. After an extended dry season the first rains of the wet season stimulate the regrowth of new plant tissue which offers abundant sites that are adequate for nymphal development. Once the insect has established in the field under favorable humid conditions the first generation of females lay eggs which are immediately developing (not diapausing) to take advantage of the favorable food sources and habitat by producing a second generation. With the progression of the wet season the incidence of diapausing eggs increases to up to100% in the last generation in anticipation of the imminent dry season.

It is the immature stage (nymph) of the later generations that is responsible for perceiving the environmental signals or "token stimuli" that indicate that the dry season. The response to this challenge is egg diapause. Once the nymphs have transformed to the adult stage, they are conditioned to lay a higher proportion of diapausing eggs so that the next generation may survive the dry season and return during the next rainy season.

In the temperate zones, photoperiod and temperature are the major regulators of diapause induction. In tropical regions there is less variation in photoperiod and temperature and therefore other factors may be more important in diapause regulation. Both phenology of the plant (greater age, poorer nutritional quality) and soil humidity (drought stress) could be perceived by nymphs and lead to adults that lay eggs with a higher incidence of diapause. Other factors such as temperature should also be explored for their role as token stimuli.

The insect survives the long dry period as eggs temporarily delayed in developmental stage S2, tolerant to drought. Diapause and quiescence are natural phenomena where development is suspended, thereby offering a defense against the adverse environment. The difference is that

eggs in diapause cannot respond directly to the return of wet conditions, while quiescent eggs are able to continue development in direct response to favorable conditions.

In the case of *A. varia*, the majority of eggs in the dry season are in diapause. As the dry season progresses, more and more eggs terminate diapause. The same conditions of drought accelerates this process as shown after 15 and 30 days of complete dryness (Output 2.1.3). When diapause termination occurs under unfavorable dry conditions, the eggs of *A. varia* enter post-diapause quiescence. In this phase, the eggs remain tolerant to drought stress but they are poised to continue their development and eclose in direct response to higher levels of soil humidity. Therefore with the progression of the dry season the proportion of diapause eggs decreases while the proportion of post-diapause quiescent eggs increases.

Few days after the rains the nymph population appears in the field, often in a highly abrupt and synchronous fashion. The life stage responsible for this synchrony is the quiescent eggs. The model to predict the arrival of the appearance of the first generation based on rainfall patterns is summarized in **Table 2**. A large and highly synchronous outbreak would therefore be the result of a long dry season (diapause terminated in the majority of the eggs) ended by an abrupt arrival of rains that soak the pasture completely (stimulating the continued development of quiescent eggs en masse). In contrast, it is predicted that a small and asynchronous initial outbreak would be the results of a shorter dry season with interrupted rains going into the wet season.

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Activity 3. Determinants of egg diapause: postoviposition determinants

Rationale:

The influence of moisture on the induction or termination of diapause has been little studied . The function of moisture as a regulator in the termination of diapause has been documented in insects of the Tropics where temperature and moisture appear to replace photoperiod as the primary regulator of diapause. Field studies on certain species of cercopids have shown a relationship between the arrival of the rainy season and the appearance of the insect's first population peak in the field. It has been suggested that this population synchrony is achieved because in a stage of post-diapause quiescence eggs are capable of responding to the new humid conditions to terminate their development and eclose, thereby contributing to the first generation of nymphs. At least in the Brazilian species *Deois flavopicta,* diapausing eggs enter a physiological period of post-diapause quiescence if conditions are not favorable upon diapause termination. Once such quiescent eggs are subjected to moist conditions, however, development continues and eggs eclose.

To understand and predict the synchrony and timing of the arrival of the first generation outbreak, it is necessary to elucidate the environmental factors that could serve as post-oviposition signals in the termination of egg diapause. For the widespread Colombian species *Aeneolamia varia*, it is predicted that a period of drought, like that experienced by the eggs in the dry season, could serve as a signal to accelerate the termination of diapause and that eggs then enter a state of post-diapause quiescence. This physiological stage would permit the synchronized eclosion of field populations in response to the return of the wet season rains. In this study we sought to determine the influence of drought as a post-oviposition signal in the regulation and termination of diapause in eggs of *A. varia*.

Methodology

The diapause eggs used in this experiment were obtained from a colony of *A. varia* maintained at CIAT that originated 1-2 generations previously from field-caught adults in Villavicencio, Meta. Earlier studies showed that *A. varia* from this region are capable of diapause, increasing the proportion of diapausing eggs as the dry season approaches.

The experiment was performed in two trials. Eggs in the first trial came from females reared in June 2001 while eggs in the second trial came from females reared between December 2001 and January 2002. For each trial, a completely random design was used with three repetitions of the following three treatments: control (eggs continually moist), 15 d drought (eggs exposed to 15 days of dryness and then remoistened), and 30 d drought (eggs exposed to 30 days of dryness and then remoistened). The experimental units were large petri dishes (15 cm diameter, 2 cm height) lined with filter paper, sealed with parafilm and kept under controlled conditions (27ºC, 100% RH, total darkness). Eggs were evaluated twice weekly with the aid of a stereoscope to record the number of eclosed and damaged/dead eggs. Differences in treatments for mortality and days to eclosion were tested with an ANOVA. Trials were analyzed separately due to differences in egg origin and eclosion patterns.

Results

In both trials the eclosion curves showed an initial peak for the treatments under drought stress **(Figure 1)**. This initial peak was followed by a period of no eclosion corresponding to the period of drought stress (15 and 30 days). Eggs did not resume development and eclose until after the return of moist conditions. In Trial 2, three defined eclosion peaks were observed in experimental eggs, corresponding in chronological order to the control, 15 d and 30 d treatments. In Trial 1, eggs submitted to drought eclosed in a similar pattern of one major peak in contrast to the control where eclosion occurred sporadically without evidence of synchronized peaks.

No differences in the mortality of diapausing eggs were detected between the two drought treatments **(Table 1)**. These treatments had a higher mortality than the control, varying from 22.9 to 71.8%. The mortality in the control was 5.1 and 3.9% for a trials 1 and 2, respectively.

For the variable of time to eclosion, in Trial 2 the mean for diapausing eggs held under moist conditions was 16.1 days **(Table 1)**. After terminating diapause the eggs continued with their post-diapause development without a period of quiescence given the conditions of adequate humidity. In the drought treatments, the period of eclosion was delayed by approximately the duration of the drought period in comparison to the control.

Compared to Trial 2, Trial 1 eggs were under longer diapause. The eclosion patterns showed a long and relatively constant pattern of eclosion with a mean of 75.3 days for the control **(Figure 1, Table 1)**. In addition, the drought-stressed eggs eclosed earlier than the control, the 30 d earlier than 15 d. Compared to the control, the eclosion curve showed a peak of eclosion a few days after return of moisture, which did not appear in the control eggs.

Table 1. Mortality of diapausing *A. varia* eggs under drought stress.

	Mortality (%)		Mean Days to Eclosion	
Treatment	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
0 days drought (control)	5.1 b	3.7 b	75.3 a	16.4 c
15 days drought	22.9 a	54.5 a	54.0 b	32.0 b
30 days drought	69.7 a	71.8 a	51.0 c	50.0 a

For each trial, means followed by the same letter were not statistically different ($P<0.05$).

Discussion

The initial peak observed in the eclosion curves is probably attributed to eggs already in post-diapause development that were able to continue development and eclose despite the dry conditions. After this brief period, no other eclosion was observed during the period of drought stress. The higher mortality in drought-stressed versus control eggs is attributed to the severity of the drought conditions and to some eggs being in early post-diapause development; in this phase the eggs are sensitive to the lack of moisture because water is required for continued development.

In terms of time to eclosion, Trial 2 showed an early and synchronized pattern of egg eclosion due to the period of drought. According to the control, during the period of drought the majority of eggs would have terminated diapause, completed post-diapause development and eclosed.

Under drought conditions, however, a proportion of eggs survived the drought-stress and delayed development until the return of moist conditions. Eggs that terminated diapause while under drought stress entered a drought-resistant post diapause quiescence, delaying continued development until the return of moist conditions.

The same evidence was obtained from Trial 1. In eggs subjected to drought stress, development was delayed until return of humid conditions at which point development proceeded once again. In addition, this Trial offered evidence for accelerated diapause termination. Besides confirming the idea of post-diapause quiescence with drought-tolerance, these results indicate that a period of drought accelerates the termination of diapause. The eclosion peak caused by the return of moist conditions is the result of a large proportion of eggs that had terminated diapause and entered post-diapause quiescence during the drought period. This proportion was higher in the 30 d drought eggs indicating that a longer period of drought accelerated diapause termination even more. In the Trial 1 eggs that had a longer diapause period, eggs actually eclosed earlier when subjected to drought than when kept under continually moist conditions.

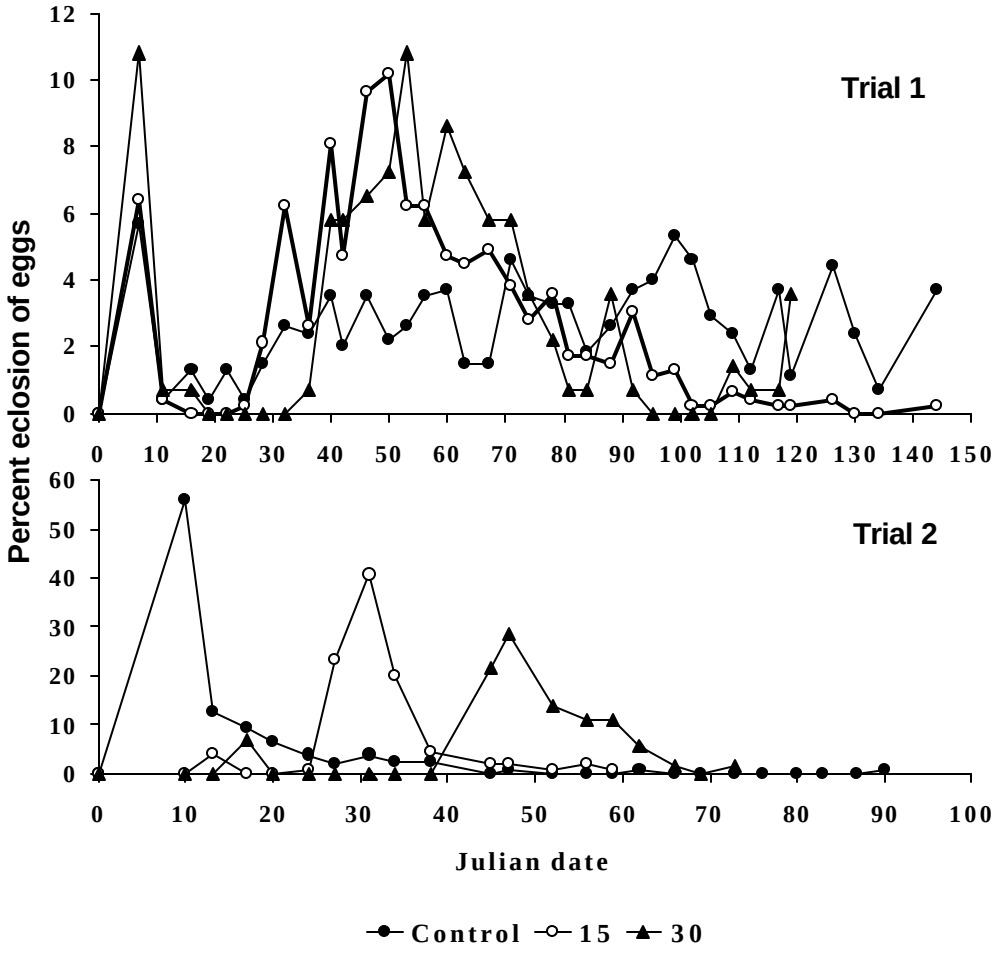

Figure 1. Eclosion pattern of diapausing *A. varia* eggs under drought stress.

The differences in time to eclosion between the eggs in Trial 1 and 2 could be attributed to the origin of the adults that contributed these eggs. Trial 2 eggs came from generations posterior to the adults that provided the eggs for Trial 1. Field studies have shown that the duration of diapause varies among generations and increases in the generations prior to the arrival of the dry season. Although early generations have an decreased incidence of diapause compared to later generations, the duration of this diapause is longer.

Contributors: Ulises Castro, Oscar Yela, Daniel Peck

Activity 4. Taxonomy and distribution of spittlebug species

Rationale

For several reasons our taxonomic foundation for the neotropical grass-feeding spittlebugs is weak. First, a high degree of intra- and inter-specific variation in color patterns complicates species identification. Second, the male genitalia of relatively few species have been described so this key character is not available for species determinations. Third, there are few taxonomists working in the Cercopidae and there are very few regional taxonomic keys. As a result, getting correct species determinations is difficult for researchers working in this pest system. In this output we summarize new information on the taxonomic identity of certain economically important spittlebug species.

Results and Discussion

Identity of *Mahanarva trifissa*. A summary of the identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador (CIAT 2000) left unclear the status of two species, *Mahanarva* sp. from the Amazonian piedmont of Colombia and Ecuador, and *Zulia* sp. from the south Pacific Coast of southern Colombia and northern Ecuador. After a visit to the British Museum of Natural History (London, UK) and discussions with the Homoptera specialist M. Webb, additional information was obtained on the identity of these two species. The undescribed species of *Mahanarva* reported from Caquetá, and previously thought to be a new species, is identified as *Mahanarva trifissa* (Jacobi). This species was originally described as *Tomaspis trifissa* by Jacobi in 1908 based on a single specimen collected in Peru. After studying the type specimen loaned to the British Museum, we determined that the form of the male genitalia, profile of the rostrum and color pattern fell within the range of variation exhibited by specimens of *Mahanarva* sp. from Caquetá and different regions of Ecuador. However, the color pattern expressed by the Peru type is uncommon, exhibited by only one individual of the several dozen observed from Colombia and Ecuador. The locality of the type specimen conformed to the lowlands tropics of the interior (Amazonian Piedmont). This species will be transferred to the genus *Mahanarva* as part of a taxonomic review pending publication by the British Museum. In a pending CIAT publication on the biology and habits of this species (CIAT 1999) we will describe the color pattern variation.

Identity of *Zulia* sp. The status of *Zulia* sp. nov. is still unclear. Whether it is a new species distinct from *Zulia vilior* (Costa Rica, Panama) will depend on more studies of the degree of variation in populations from those two countries and those in Colombia and Ecuador.

Identity of new sugar cane pest. In collaboration with Cenicaña, we investigated a recent report of spittlebugs reaching injurious levels on panela sugar cane in Risaralda (September 2002). This species has been initially identified as *Mahanarva bipars* (Walker). As far as we know this species has not yet been reported from Colombia, has not yet been cited as feeding on sugar cane or other graminoids, and has not yet been studied biologically. Similar to *Mahanarva andigena* in Nariño, spittle masses are aerial (at the base formed by leaf axils). This insect is being managed locally by removing old leaves from the stem and using insecticides (chorpyrifos). An initial summary of this report is being prepared for publication including a

review of known host plants and distribution, taxonomic status, identification, color pattern variation and pest status.

Identity of Venezuelan spittlebug complex. We received specimens from Venezuela for identification. Specimens from Barinas, Venezuela were identified as *Aeneolamia varia* and *Aeneolamia reducta.* The pest complex seems to be similar to the Colombian Llanos (mostly *A. varia*, some *A. reducta* and apparently some *Zulia pubescens*). Spittlebugs are increasing problems in this region of Venezuela and current outbreaks are more severe than anything we have seen over the last five years in our Colombian sites.

Identity of Guatemalan spittlebug complex. A long series of specimens from 28 sites in Santa Lucía Cotzumalguapa, Guatemala was received from collaborators of Cengicaña. The two species present in forage grasses and sugar cane were confirmed as *Prosapia simulans* and *Aeneolamia postica*. *Prosapia simulans* comprised 3.4% of the examined specimens, detected in 10/28 sites. In sites where this species was collected it comprised 3.3-16.7% of the adults present with the exception of one site where they comprised 100%. Various color patterns were observed; males tended to have 3 lateral yellow bands dorsally (one on the posterior edge of the pronotum, two across the wings) while females tended to be darker due to the reduction in the bands. With little exception this species is longer and wider that *A. postica*. *Aeneolamia postica* comprised 96.6% of the examined specimens, detected in 27/28 sites where it comprised 83.3-100% of the adults present. Like *P. simulans* it has two dorsal lateral bands but it differs in having a third anterior band in the form of a "V" along the border of the scutellum; in only a few cases were these bands reduced. *Aeneolamia postica* presented three general color morphs, red comprising 2.5% of specimens, orange 1.6% and yellow 95.8%.

Contributors: Daniel Peck, Jairo Rodríguez

Activity 5. Short-term mass rearing of different spittlebug species

Six spittlebug species (*Aeneolamia reducta*, *A. varia*, *Mahanarva andigena*, *Prosapia simulans*, *Zulia carbonaria*, *Z. pubescens*) were reared in the screenhouse at CIAT to support research **(Table 1)**. Mass-rearing colonies were established according to an improved box design, previously tested and described (CIAT 1999) while small-scale colonies were maintained with the traditional pot methods. Colonies were used to support biological studies of *M. andigena*, *Z. carbonaria* and *Z. pubescens*; diapause studies on *A. varia*; and evaluations of fungal entomopathogens for *A. reducta, P. simulans*, *Z. carbonaria* and *Z. pubescens*.

Table 1. Summary of spittlebug colony maintenance activities.

Species	Months maintained at CIAT (2001-2002)												Rearing Scale		Purpose of Studies	
	N	D	J	F	M	A	M	J	J	A	S	O	Large[1]	Small[2]	Biology	Entomopathogens
A. reducta	X	X	X										X			X
A. varia	X	X	X	X	X	X	X	X	X	X	X	X		X	X	
M. andigena	X	X	X	X	X	X	X	X	X	X	X	X		X	X	
P. simulans	X	X	X	X	X								X	X	X	X
Z. carbonaria		X	X	X			X	X	X	X	X		X	X	X	X
Z. pubescens			X	X	X								X			X

[1]Improved mass-rearing design.
[2]Traditional small scale pot design.

Contributors: Anuar Morales, Oscar Yela, Ulises Castro, Jairo Rodríguez, Daniel Peck.

Activity 6. Maintenance of the entomopathogen collection

The fungal entomopathogen collection was established in 2000 as a resource for studies on biological control of major pest complexes. Maintaining and strengthening this collection is vital for advancing non-toxic alternatives to insecticides and other effective tactics as components of integrated pest management. The three main activities related to this ceparium are (1) isolation, entry and storage of new isoaltes, (2) viability tests, propagation and reactivation, and (3) multiplication for laboratory and field studies on virulence and pathogenicity.

In 2002, 26 new accessions were added to the collection for a total of 184. Of this total, 82 were originally isolated from spittlebugs (Homoptera: Cercopidae), 29 from white grubs (Coleoptera: Scarabaeidae), 63 from whitefly (Homoptera: Aleyrodidae) and burrower bug *Cyrtomenus bergi* (Hemiptera: Cydnidae) and 10 from other hosts.

In 2002 this germplasm bank was a vital resource for studies on (1) the characterization of select isolates for field trials to manage grass-feeding spittlebugs (Outputs 2.2.3, 2.2.4) and (2) evaluation and biological characterization of isolates against life stages of the whitefly *Aleurotrachellus socialis.*

Contributors: Anuar Morales, Rosalba Tobón, Daniel Peck.

Activity 7. Screening fungal entomopathogens for virulence to spittlebugs: characterization of isolates selected for field evaluations

Rationale

Four isolates were selected from CIAT's fungal entomopathogen collection for experimental field trials designed to test application techniques. These isolates are the three *Metarhizium* and one *Paecilomyces* strains screened from 49 isolates as the most virulent to adults of *Aeneolamia varia* (CIAT 2000). CIAT 054 and CIAT 055 are *Metarhizium* sp., CIAT 007C *Metarhizium anisopliae* and CIAT 009 *Paecilomyces farinosis.* Before deploying in the field, these isolates must be characterized for their biological and virulence activity on different species and life stages of spittlebugs. Variation in virulence among adults of four species (*A. reducta, A. varia, Z. carbonaria, Z. pubescens*) was described elsewhere (CIAT 2001) as were the results of studies to determine the LC_{50} and LC_{90} on nymphs of *A. varia*. Here we summarize the results obtained for adults and nymphs of *P. simulans*.

Methods and Materials

Evaluation methods for adults and nymphs were based on previously established protocols (CIAT 2000). For adults, evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (15 cm diameter) covered by acetate cylinders (40 cm tall, 15 cm diameter). These plants were infested with 10 adult tenerals (<24 hours old) obtained from colonies maintained at CIAT. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 conidia/ml) with an airbrush and compressor (10 PSI). All four isolates were evaluated. Ten repetitions (pots) were evaluated for each isolate and a control (water with tween at 0.05%). After spraying, plants and insects were maintained in a growth chamber (27±2°C, RH 80±10%). Virulence was evaluated 5 days later when all insects were scored as alive, dead, and dead with evidence of mycosis. Dead insects with no visible signs of fungus attack were stored in petri dishes with moist filter paper for 3-4 days to ascertain whether they were infected with fungus. Differences were evaluated with an ANOVA and Tukey multiple range test.

For nymphs, evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 weeks after planting with *B. ruziziensis*, surface roots were sufficiently established for nymph development and egg infestation. Eggs of *P. simulans* about to hatch were prepared for treatments and infestation by placing 10 on each of 10 small pieces of filter paper in a petri dish that corresponded to one treatment. Nine different concentrations of conidial suspensions ($1x10^4$, $5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $5x10^6$, $1x10^7$, $5x10^8$, $1x10^9$ conidia/ml) were prepared for the three *Metarhizium* isolates (CIAT 007C, CIAT 054, CIAT 055) with a control (water and tween at 0.05%). Applications were made on the substrate before infestation and on the eggs in petri dishes before infestation. An airbrush and compressor (10 PSI) were used at a volume of 1 ml for substrate and <1 ml for direct egg application. Plants were maintained in the greenhouse until evaluation of mortality 30-32 days after infestation. During this period, plants were fertilized twice (just before and 15 days after infestation) with urea at 2g/l. There were ten repetitions per treatment. Mortality data were analyzed with Probit (SAS).

Results

Results are shown for *P. simulans* alongside results previously obtained from identical studies in other species (CIAT 2001). The selected isolates of *Metarhizium* did not give good control of *Prosapia* or *Zulia*, compared to *Aeneolamia* **(Figure 1)**. The most virulent isolate against *P. simulans* was CIAT 054 with 35.6% mortality.

The mortality recorded at the different concentrations demonstrated virulent activity of each isolate on nymphs of *P. simulans*. As expected, mortality increased with concentration **(Figure 2)**. Probit analysis showed low X^2 values but X^2 probabilities a little high **(Table 1)**. The lowest LC_{90} was obtained for CIAT 054 ($2.7x10^8$ conidias/ml) followed by CIAT 007C (3.4×10^8 con/ml) and CIAT 055 (6.4×10^8 con/ml).

Table 1. Probit analysis of mortality caused by three isolates of fungal entomopathogens on nymphs of *P. simulans*.

Isolate	n	LC_{50} (95% CI)	LC_{90} (95% CI)	X^2	Prob X^2	b±S.E.
CIAT 054	765	$1.01x10^7$ ($5.7x10^5$-$2.7x10^7$)	$2.7x10^8$ ($7.4x10^7$-$1.3x10^{10}$)	10.9	0.092	0.39±0.05
CIAT 055	637	$1.58x10^5$ ($5.9x10^5$-$3.5x10^7$)	$6.4x10^8$ ($1.4x10^8$-$1.6x10^{14}$)	7.06	0.219	0.34±0.06
CIAT 007-C	593	$1.04x10^5$ ($4.4x10^3$-$1.1x10^6$)	$3.4x10^8$ ($6.1x10^7$-$3.4x10^9$)	3.8	0.429	0±0.17

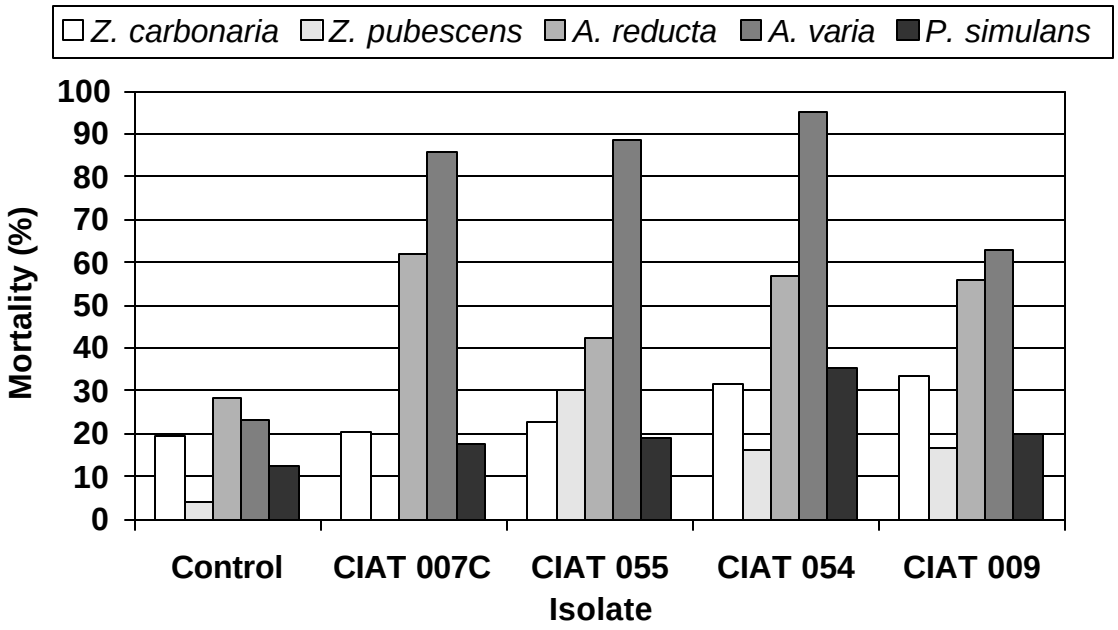

Figure 1. Mortality (absolute percent) of four isolates of fungal entomopathogens on five spittlebug species. Means followed by different letters are significantly different at P<0.05. * CIAT 007C was not evaluated on *Z. pubescens*.

Discussion

It is important to determine the LC_{90} to establish an appropriate concentration for field applications. This reduces the risk of applying material that is too low in concentration to have any effect in the field or too high that material and production time is wasted.

These results confirm that virulence of fungal entomopathogen strains varies among spittlebug species. Deploying these pathogens as agents of biological control therefore depends on an understanding of the species complex in the area where control is desired, selecting isolates specific to spittlebug species, and reassessing the broad effectiveness of commercial products. On the other hand, results indicate that the diverse collection of isolates in CIAT's ceparium probably has strains highly virulent to species other than *A. varia*, which up to this point has been used as the model species for developing evaluation methodologies. The most efficient screening process might therefore be evaluating a diversity of isolates to the particular spittlebug species of interest, rather than using preselection (with a model species such *A. varia*) with subsequent confirmation of high control on other species.

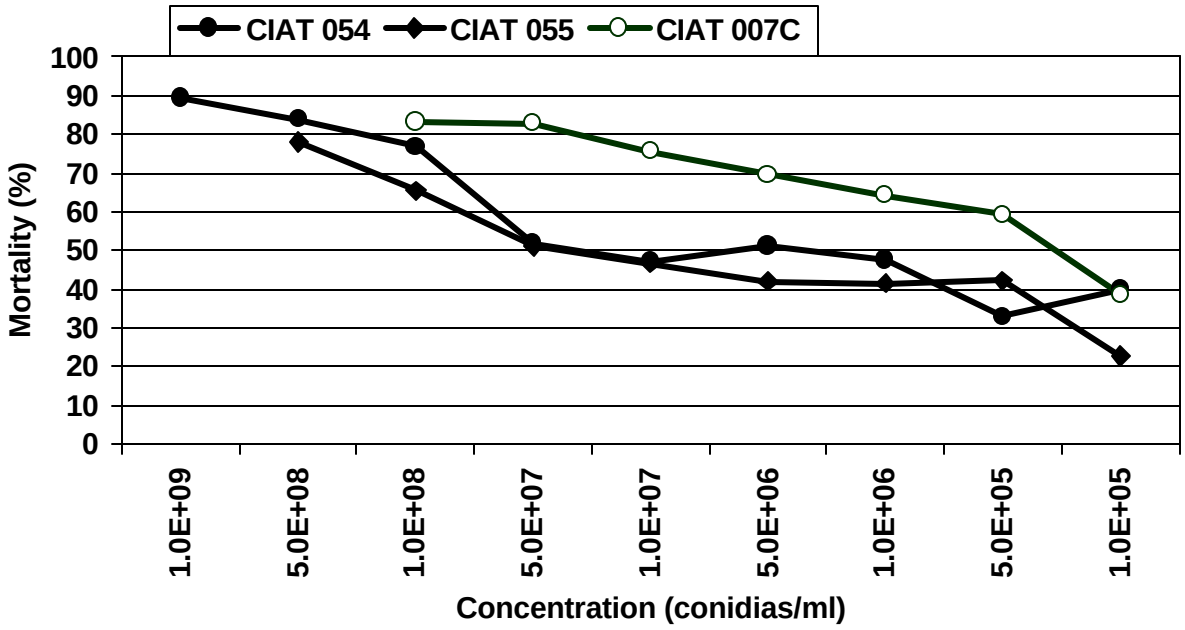

Figure 2. Mortality in *P. simulans* caused by of three fungal entomopathogen isolates at different concentrations.

Contributors: Anuar Morales, Rosalba Tobón, Oscar Yela, Daniel Peck.

Activity 8. Field evaluation of spittlebug entomopathogens in two contrasting regions

Rationale

In general, previous attempts to evaluate the efficiency of fungal entomopathogens as biological control agents of spittlebugs in pastures have focused on laboratory assays. The few that have gone to the field have demonstrated highly variable and low levels of control due to a variety of factors including poor evaluation and application techniques. Aspects such as the number of applications and the timing of applications in relation to phenology of the life stages have received no special attention.

To seriously evaluate the potential of fungal entomopathogens as an alternative for managing pasture spittlebugs, we are combining a detailed knowledge of the biology and phenology of spittlebugs with a series of studies to collect, screen, characterize, and formulate select isolates for deployment in field trials. In previous work (CIAT 1999, 2000, 2001) we have established and strengthened a collection of fungal entomopathogens at CIAT that currently houses 84 isolates of 10 fungal genera originating from almost every region in the country where the pest has been detected. In addition, an improved design for a mass rearing colony of *A. varia* was developed to provide adults of this species and others for evaluations. Finally, a reliable and rapid method to evaluate fungal entomopathogens against spittlebug nymphs and adults has been established. With these protocols we have information on the lethal concentration of the two most promising isolates and variation in their efficiency on other spittlebug species.

The objective of this phase of the project is to establish an application methodology for fungal bioinsecticides. In this report, we summarize the field trials established in two contrasting ecoregions of Colombia, the Amazonian Piedmont and the Cauca River Valley to evaluate the effect of timing and frequency of applications.

Materials and Methods

The Amazonian Piedmont ecoregion (Caquetá) is continuously humid, corresponding to presence of spittlebug nymphs and adults throughout the year with little population synchrony. In this site the number of applications required to achieve an effect was evaluated. The Cauca River Valley ecoregion (Valle del Cauca) is a highly seasonal site with bimodal precipitation and here spittlebug nymphs and adults are present only during the rainy months but with high population synchrony. In this site the timing of the applications in relation to the insect's life cycle was evaluated. The premise is that the diverging environmental conditions of these two ecoregions will require different strategies and control tactics for management of spittlebugs in pastures.

Caquetá. Studies in Caquetá were carried out in collaboration with four undergraduate thesis students from the Universidad de la Amazonía. Five plots of 1200 m^2 were selected and marked at the C.I. Macagual of Corpoica. Each plot was in a separate pasture of predominately *Brachiaria decumbens* forage grass with a history of spittlebug attack. Each plot was subdivided into subplots (100 m^2) for application of treatments. Adults were evaluated weekly with 20 sweeps of an insect net per subplot while nymphs were counted in two 0.0625-m^2

quadrats. In the laboratory nymphs were scored to instar and adults to sex and species. Population surveys began 23 March 2001 and ended 10 May 2002. Applications began 22 September 2001 and were made every 2 weeks until 8 February 2002. Treatments were in a completely randomized block design with five repetitions and are summarized in Table 1.

Table 1. Experimental treatments applied in C.I. Macagual, Caquetá to test the effect of application frequency on spittlebug abundance.

Treatment code	Treatment	Frequency[1]
1a	Cepa CIAT 054	2
1b	Cepa CIAT 054	1
1c	Cepa CIAT 054	0.5
2a	Cepa CIAT 007-C	2
2b	Cepa CIAT 007-C	1
2c	Cepa CIAT 007-C	0.5
3a	Chemical control	2
3b	Chemical control	1
3c	Chemical control	0.5
4	Absolute control	0

[1]Frequency is the number of applications per month

Applications were made with a traditional backpack sprayer fitted with a curtain nozzle. The volume of application was set at 2 liters per subplot. The two isolates were CIAT 054 (*Metarhizium* sp.) and CIAT 007C (*Metarhizium anisopliae*), selected from among 49 isolates as the most virulent to adult *A. varia*. Concentration of the suspension was $8.95x10^7$ and $3.61x10^8$ conidias/ml for the isolates CIAT 054 y CIAT 007C, respectively, equivalent to the LC_{90} for *A. varia* determined in greenhouse studies (CIAT 2001). The chemical control was Malathion applied at 1.5 liters/ha.

Efficiency of the isolates was calculated as the cumulative area under the population curve for adults and nymphs for each treatment over the months of the study. The slope of the line was calculated before and after the start of the treatments. Differences among treatments in the cumulative area under the curve and the slope of the line were analyzed with ANOVA and a multiple range test.

Valle del Cauca. Studies in the Cauca Valley were carried out at Hacienda Piedechinche, El Cerrito and established as in Caquetá except plots were 1600 m^2 to accommodate 16 subplots of 100 m^2. Nymph and adult surveys were identical to Caquetá and were carried out weekly from 25 January 2001 to 26 July 2002.

Due to security problems and a change in management practices that led to a sharp drop in spittlebug populations, it was decided to carry out this study in Valle del Cauca instead of Cauca where originally anticipated. Unfortunately, we had no previous understanding of the phenology of the pest in the new site. While *Z. carbonaria* predominated in Cauca, *Prosapia simulans* was most abundant in the new site, with some presence of *Z. carbonaria* and *Z. pubescens*. *Prosapia simulans* had been reported as a newly detected pest species in South America, Colombia and the Cauca Valley (CIAT 2000).

The original application regime was scheduled as three treatments (CIAT 054, Malathion, control) applied 1, 2, 3, 4, 5 or 6 weeks after the start of the first generation outbreak to compare when in the life cycle of the insect a bioinsecticide would be most effective. Because the insect population was low and early life stages were more difficult to detect than anticipated, applications were never made. Nevertheless, population data are reported in this summary since the phenology of *P. simulans* has not yet been described in Colombia or in a seasonally bimodal ecoregion.

Results

Caquetá. A total of 2,603 nymphs and 22,786 adults were examined in population surveys. According to adult specimens *A. varia* was most abundant with 98.5% of the population, followed by *Z. pubescens* with 1.7% and *M. trifissa* with 0.27%.

The months of lowest nymph and adult abundance were April and March, respectively, corresponding to the months of highest precipitation **(Figure 1)**. The greatest incidence of nymphs and adults was in September. From March 2001 to May 2002 a mean of 52.3 nymphs/1.5 m^2 and 441.8 adults/240 sweeps was documented.

Table 2. Slope of the cumulative area under the population curve for adults of *A. varia* under different treatments applied in C. I. Macagual, Caquetá.

Treatment	Slope[1]		Treatment	Slope[1]	
2c-Before	1.64	a	1c-After	0.78	abcde
1b-Before	1.62	ab	4 -After	0.69	abcde
1a-Before	1.56	ab	3b-After	0.76	abcde
4 -Before	1.54	abc	1b-After	0.63	bcde
3b-Before	1.34	abcde	2a-After	0.60	cde
1c-Before	1.12	abcde	2b-After	0.59	cde
3c-Before	0.92	abcde	2c-After	0.58	cde
2a-Before	0.90	abcde	3a-After	0.55	de
2b-Before	0.88	abcde	1a-After	0.52	e
3a-Before	0.78	abcde	3c-After	0.50	e

[1]Values followed by the same letter are not significantly different (P<0.01)

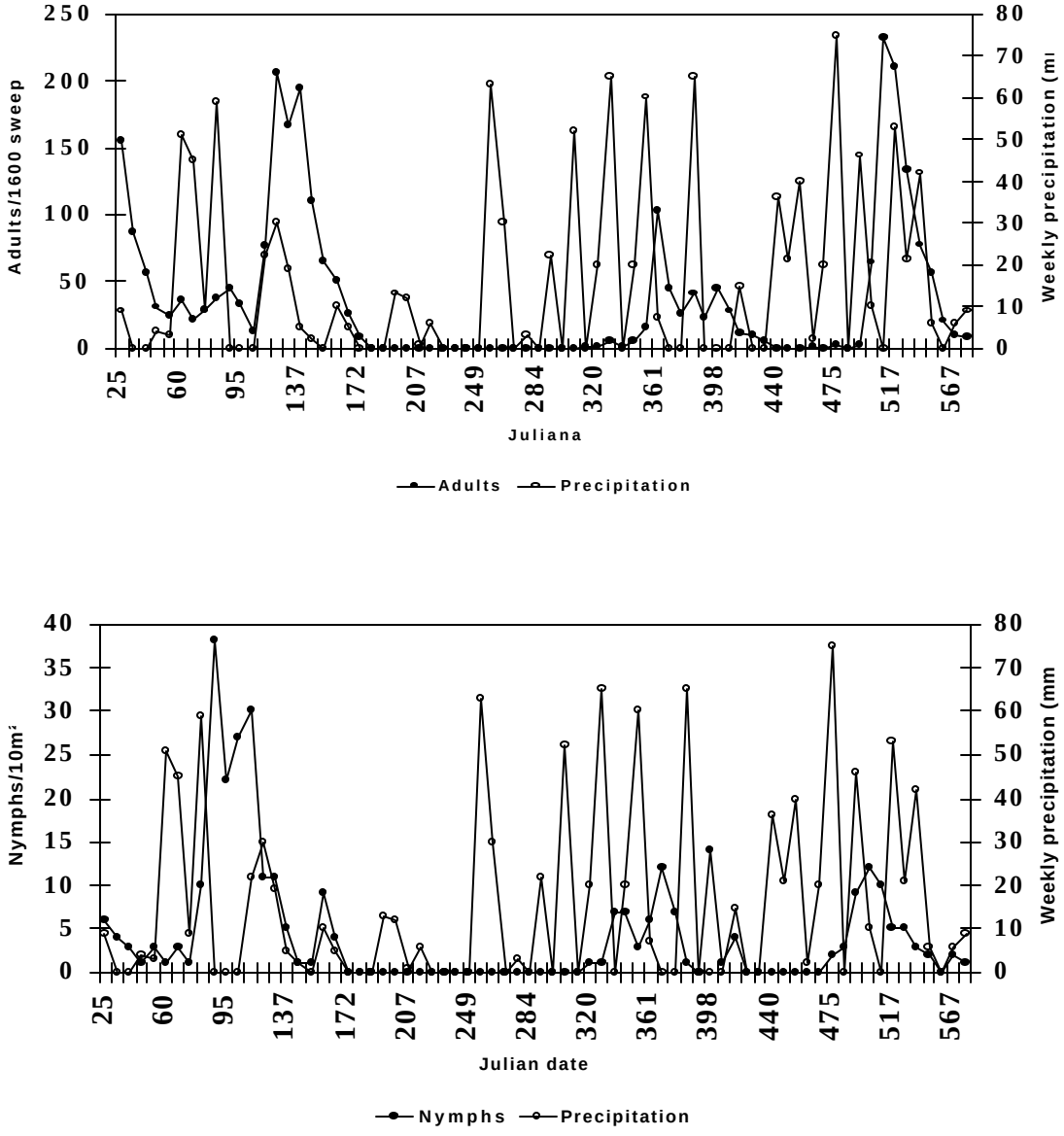

Figure 1. Precipitation and population fluctuation of nymph and adult spittlebugs in C.I. Macagual, Caquetá, 2001-2002.

There was no effect of treatment detected for the final cumulative area under the population curve for nymphs or adults **(Figures 2, 3)**. Separating the analyses into isolate and spittlebug species, the greatest effect was on *A. varia*, although not significantly higher. The insecticide control and CIAT 054 had the lowest cumulative area. For CIAT 054 there were some differences detected; 1 or 2 applications a month showed a greater effect than 0.5 applications per month **(Figure 4)**.

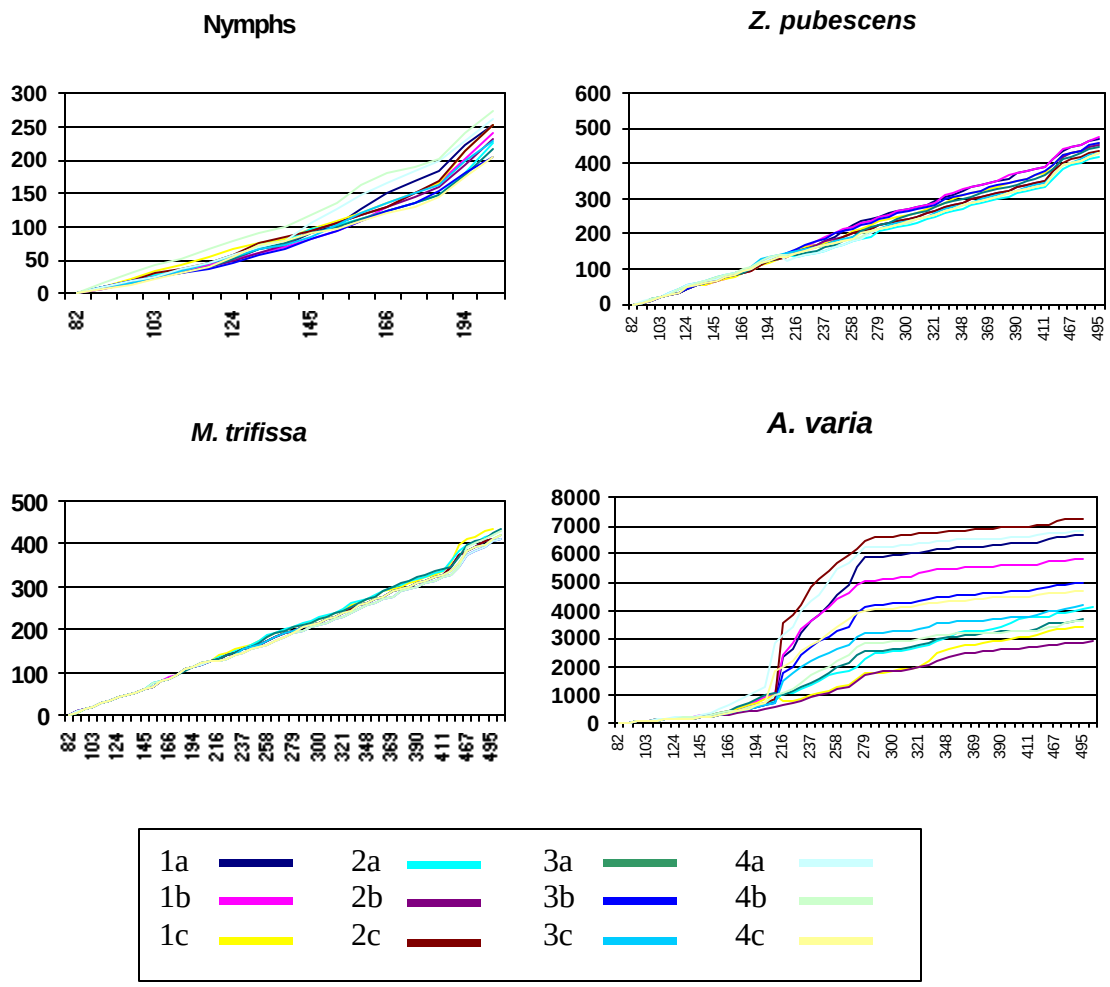

Figure 2. Cumulative area under the population curve (X axis) versus Julian date (Y axis) for adult spittlebug species under different treatment application in C.I. Macagual, Caquetá.

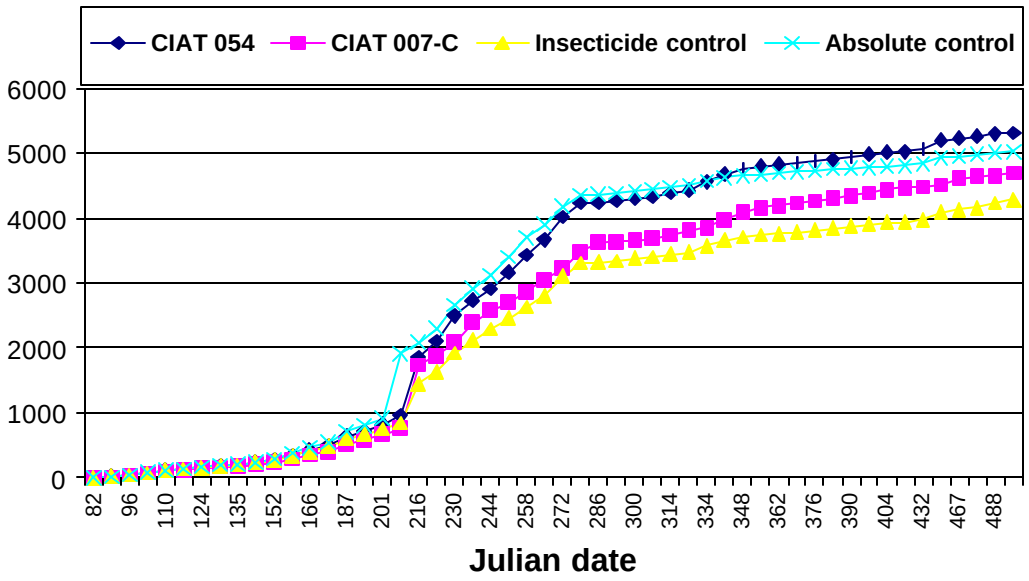

Figure 3. Cumulative area under the population curve (X axis) for adult spittlebugs in each of the treatments applied in C.I. Macagual, Caquetá.

Analysis of variance detected differences in the slope of the cumulative population curve measured before and after the treatments. For example, CIAT 007C applied once per month had a slope of 1.64 before application of the treatment and a slope of 0.58 afterwards, suggesting that the treatment reduced the velocity of population increase.

Under this same analysis no effect of treatments was observed on the nymph population. Analysis of the cumulative area under the curve showed a similar growth for all instars in all treatments including the insecticidal and absolute controls. This result could be explained by ineffective deployment of the applications because the products may not have penetrated the spittle mass itself, or penetrated the sward to their feeding sites near the soil surface and litter layer

Valle del Cauca. A total of 309 nymphs and 2,687 adults were examined in population surveys and all belonged to *P. simulans*, even though *Z. carbonaria* and *Z. pubescens* were detected in these sites previous to the start of surveys.

Population analysis of the nymphal life stages at the farm level (all plots summed) and plot level showed two distinct population peaks in 2001 and one for the first semester of 2002 **(Figures 5, 6, 7)**. Analysis at the plot level showed that population peaks occurred in the same period across the three plots, March and April of 2001 and May 2002 **(Figure 6)**. Variation in abundance at the farm level was established by comparing the number of nymphs collected in the surveys. Plot 2 had 46.0% of the total and Plot 5 4.2%, representing the plots of highest and lowest incidence, respectively. At the farm level, over the entire survey period the mean abundance was 3.3 nymphs/2 m².

Population analysis of *P. simulans* adults at the farm level showed distinct population peaks in February and May 2001 and January and May 2002 **(Figure 5)**. The same peaks appeared in each of the separate plots and corresponded to nymph peaks indicating that they originated from local nymph populations rather than through immigration **(Figures 6, 7)**. Like nymphs, Plots 2 and 5 had the highest and lowest incidence of adults with 35.3 and 4.0% of total adults captures, respectively. At the farm level, over the entire survey period the mean relative abundance was 27.8 adults/320 sweeps.

In the first year of evaluation, nymphs and adults were not detected in the field from July to October, which corresponded to the conditions of drought that preceded and occurred during that period **(Figure 8)**. In the same fashion population peaks of nymphs and adults corresponded to periods of rain that preceded and occurring during that period

Discussion

Although the information obtained from field applications does not permit solid conclusions, certain aspects should be highlighted because they will help to orient future studies on fungal entomopathogens. In ecosystems characterized by continual humidity and continual presence of spittlebugs throughout the year, applications should be directed towards diminishing the velocity of population growth with frequent and regular applications over a brief period of time. This inundative use of biocontrol should initially maintain the population and then slowly cause population decline as the fungus establishes and propagates in the environment. Biological control will not cause a drastic reduction of populations but a slow and longer lasting decline; and it will not contaminate the environment. We believe that integrating elements such as controlled grazing with an improved formulation and delivery system of the product directed towards the nymphs should produce better results.

These results confirm the idea that to establish a spittlebug control program it is indispensable to have a detailed understanding of the habits and behavior of the particular species at that particular site. For example, in Valle del Cauca, we did not know that detection of the early instars of *P. simulans* would be so difficult (compared to other species) because these stages were well-hidden in the soil surface, often a few centimeters below in cracks and fissures. That behavior is relevant to making decisions about when and how to control the first generation of spittlebugs.

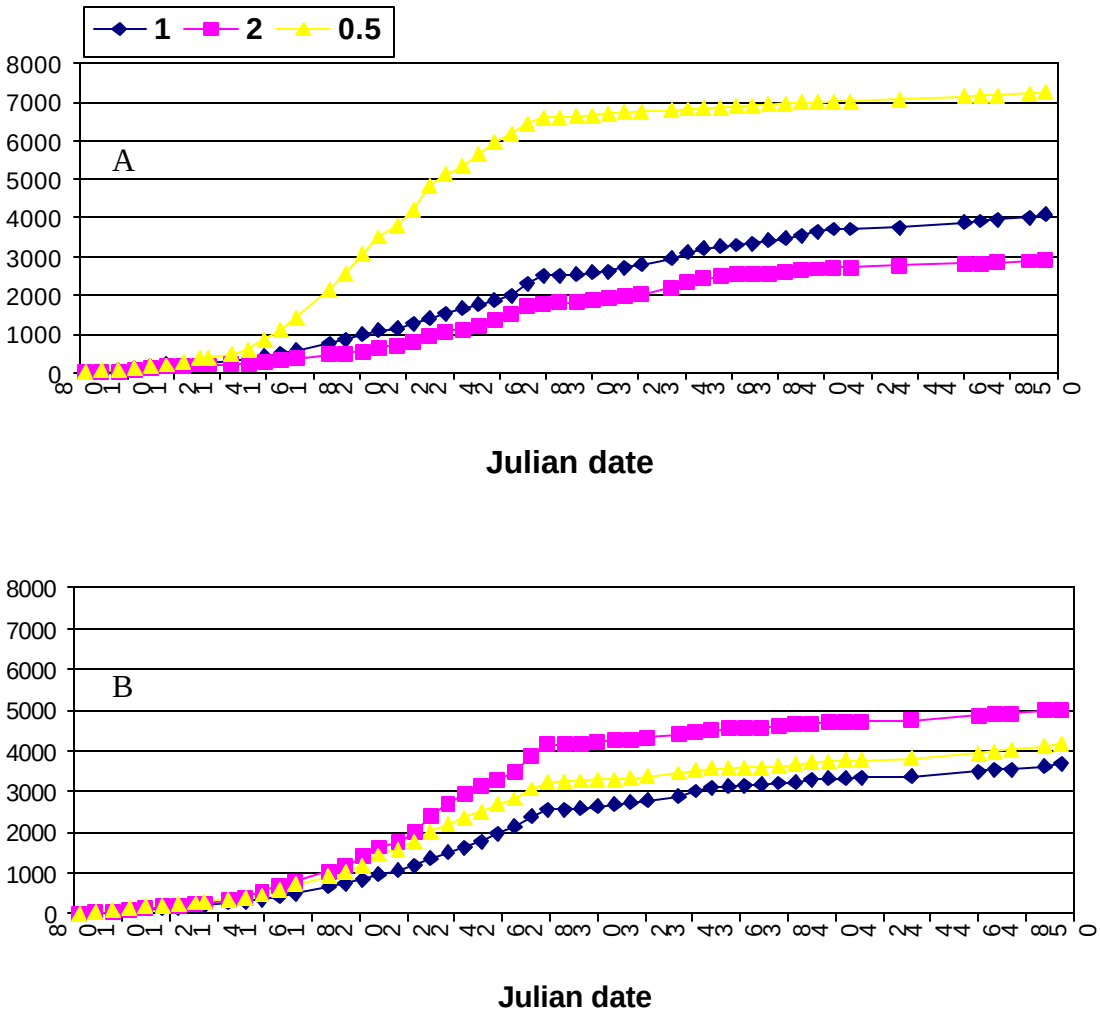

Figure 4. Cumulative area under the population curve (X axis) for adults of *A. varia* according to application frequency. A) CIAT 054. B) Insecticide control.

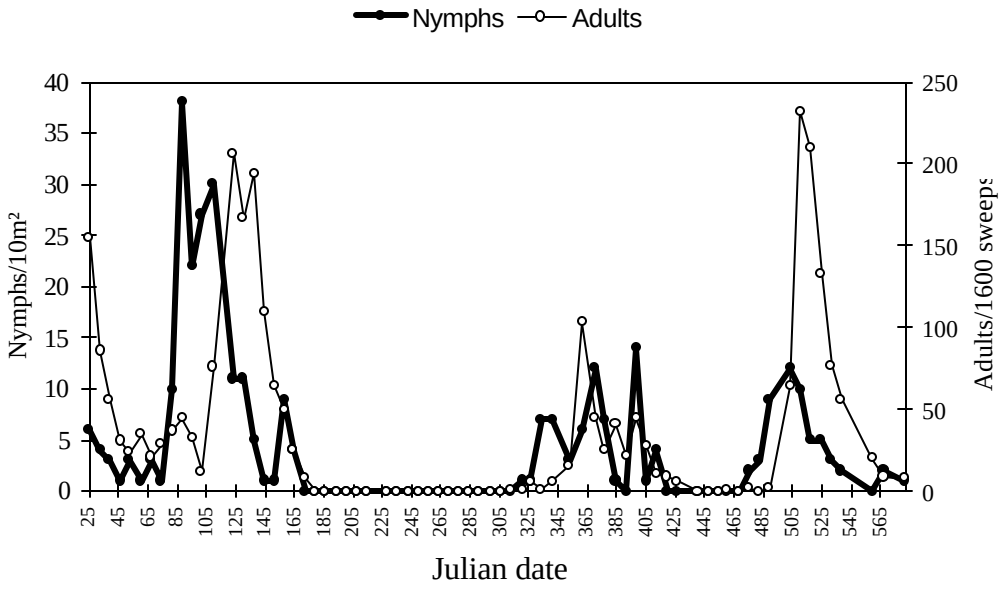

Figure 5. Population fluctuation curve for nymphs and adults of *P. simulans* in Piedechinche, Valle del Cauca, 2001- 2002.

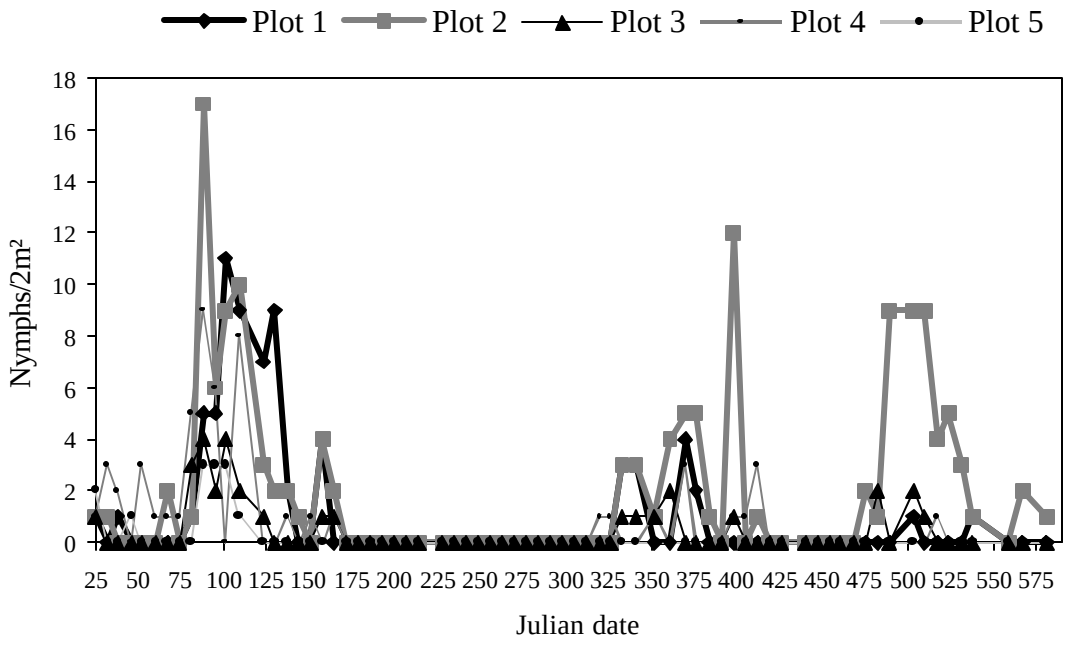

Figure 6. Population fluctuation curve of *P. simulans* nymphs in five plot of *B. decumbens*, Piedechinche, Valle del Cauca, 2001- 2002.

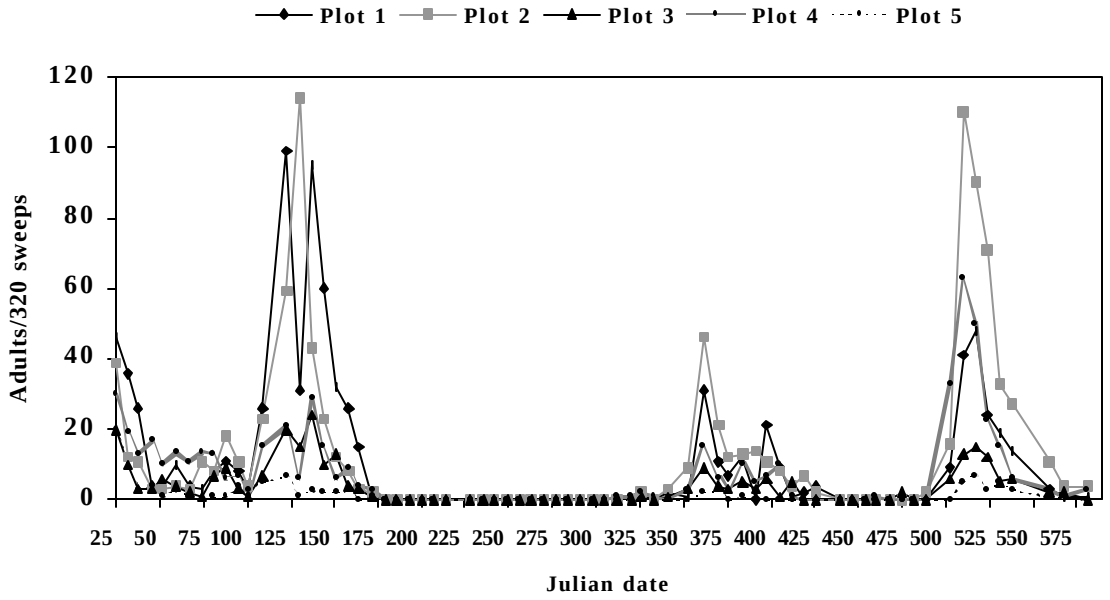

Figure 7. Population fluctuation curve of *P. simulans* adults in five plot of *B. decumbens*, Piedechinche, Valle del Cauca, 2001- 2002.

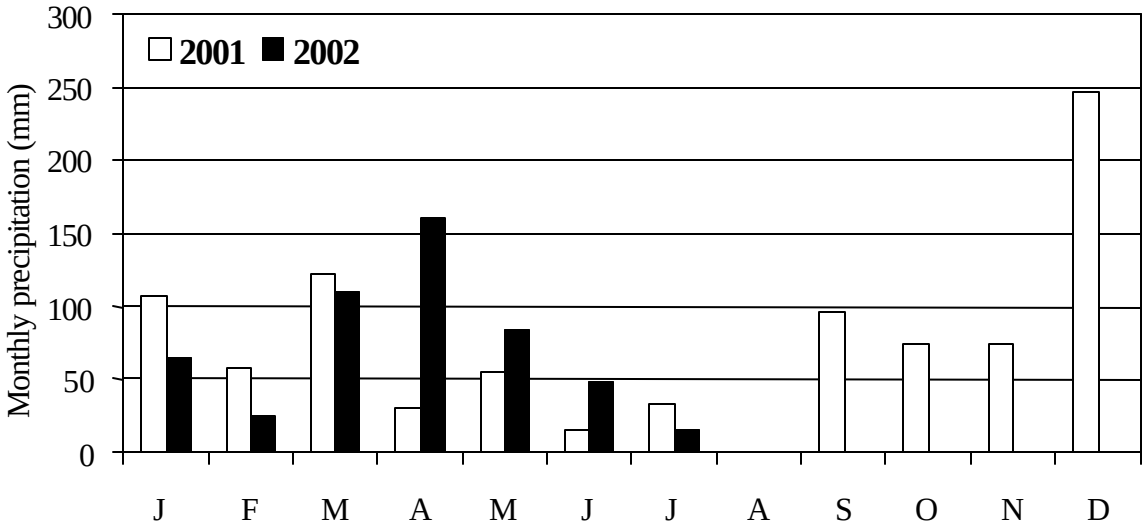

Figure 8. Monthly precipitation (mm) in Piedechinche, Valle del Cauca, 2001- 2002.

Contributors: Anuar Morales, Jairo Rodríguez, Ulises Castro, Oscar Yela, Daniel Peck (CIAT), Daniel Corradine, Germán Chacon, Fabio Obregón, Orlando Narváez (Universidad de la Amazonía).

Activity 9. Facilitate communication through Newsletters, Journal, Workshops and Internet

CIAT field day on spittlebug biology and management

Five field days on spittlebug biology and management were carried out to transmit the most recent research results throughout Colombia. With organizational help from local collaborators, four CIAT support staff traveled to these sites to present a series of talks and field visits. The field day was designed for a diverse group of stakeholders including professionals, technicians, and students of agronomy, biology and veterinary sciences, as well as ranchers, administrators and farm managers who desired to learn more about this group of insects and strategies and tactics for their management. A total of 293 people received training in these events:

- Universidad del Sucre, Sincelejo (Sucre), 31 October 2001, 60 participants
- Universidad San Martín, Barranquilla (Atlántico), 2 November 2001, 35 participants
- Universidad de la Amazonía, Florencia (Caquetá), 6 November 2001, 136 participants
- C. I. La Libertad, Villavicencio (Meta), 9 November 2001, 18 participants
- CIAT, Palmira (Valle del Cauca), 11 March 2002, 44 participants
- Universidad Nacional, Palmira "47 Foro Entomológico" 26 September, 2002, 104 participants

Contributors: Anuar Morales, Ulises Castro, Jairo Rodríguez, Francisco López, Daniel Peck.

Activity 10. Prepare technical bulletin on spittlebug bioecology and management

Contributors:

To further transmit recent research results on the bioecology and management of grass-feeding spittlebugs, a first draft of a technical bulletin was prepared with the following citation:

- Peck, D. C., U. Castro, F. López, A. Morales & J. Rodríguez. El Complejo Salivazo de los Pastos en Colombia. Boletín Técnico, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Manuscript in preparation, 24 pp.

This manuscript is now being advanced for submission to the CIAT graphics arts section for publishing. It features two sections. The first section summarizes the status of information on the bioecology and management of spittlebugs in the following subsections: Introduction, Diversity and Distribution, Biology, Recognition of the Life Stages, Behavior, Ecology, Natural Enemies, Management Tactics and Management Strategies.

The second section summarizes our knowledge of the spittlebug complex in the varying ecoregions of Colombia where they are economically important: Caribbean Coast, Orinoquian Piedmont and Eastern Llanos, Amazonian Piedmont, Cauca River Valley, and Pacific Coast

Activity 11. Bibliographic database on spittlebug bioecology available on webpage

Rationale

A major limitation to advances in the management of spittlebugs in forage grasses and sugar cane is difficult access to information. There are no published reviews of the insect family Cercopidae despite its economic significance in forage grasses and sugar cane. Such material exists for other groups of economically important Homoptera such as the leafhoppers, planthoppers, aphids, scales and whiteflies, but students of the spittlebugs and froghoppers must turn to articles and gray literature to acquire an understanding of this group of insects. Second, reviews of the biology and management of spittlebugs are inadequate. The few that exist are not widely disseminated, are outdated, and contain overgeneralizations and erroneous information, particularly regarding taxonomy. Third, much of the available information is in gray literature sources that are difficult to access. The quality of research from small and isolated universities or research teams is challenged by not being able to acquire the information necessary to support studies on this pest group.

Methods

To start to overcome some of the limitations in information dissemination, we are strengthening our reference collection on the Cercopoidea. References have been gathered over the last 11 years. In 2001 we began working with the CIAT Information Services to make this information source available on-line and here report advances in that endeavor.

Results and Discussion

We currently have physical copies of 688 references related to the superfamily Cercopoidea. Of these, 478 are directly related to spittlebugs in graminoids, 325 related to forage grasses, 146 related to sugar cane and 26 related to other graminoids such as rice and turfgrass. At present, all references are housed alphabetically in filing cabinets of the Spittlebug Bioecology and IPM Research Group and by the end of the year will be housed in the CIAT library.

All citations have been entered into an electronic database (EndNote) and most have now been edited and corrected for consistency and accurateness. Key words have been assigned to each citation to facilitate searching from within the program software (CIAT 2001). Categorical labels were also assigned to facilitate subgrouping of references in the initial on-line database (CIAT 2001). The initial version of the on-line database is a rigid (non-searchable) database divisible into categories with relevant references listed alphabetically. This version will probably be available on the CIAT web page by the end of the year and will include information on how to order copies of references from the CIAT library.

Contributors: Daniel Peck, Mariano Mejía, Carlos Saa, Edith Hesse

Activity 12. Symposium on spittlebug to communicate research results to Pronatta

Two symposia were carried out to transmit the results of two Pronatta projects to a Pronatta review board and the public. The period of execution of each project was 9 February 2000 to 6 August 2002. Project titles were "Establecimiento de hongos entomopatógenos como alternativa para manejar el salivazo de los pastos" and "Avances en el manejo del mión de los pastos (Homoptera: Cercopidae) mediante el pronóstico e interpretación de la primera generación."

The first symposium was 5 August 2002 at CIAT with a review panel of three Pronatta staff. This featured presentations of research results, discussion of impact and a review of the final technical reports.

The second symposium was carried out 26 September 2002 at the Universidad Nacional in Palmira as the 47th Foro Entomológico "Taxonomía, distribución y manejo del salivazo de los pastos (Homoptera: Cercopidae)." A total of 27 professionals and 77 students attended this event that featured presentations and a round table discussion.

Contributors: Daniel Peck, Anuar Morales, Jairo Rodríguez, Ulises Castro.

Activity 13. Publications, conferences, courses, training, workshops and official trips.

Publications

Holmann, F. & D. C. Peck. 2002. Economic damage of grassland spittlebugs in Colombia: a first approximation of impact on animal production in *Brachiaria decumbens*. Neotropical Entomology 31(2): 275-284.

Morales, A. y D. C. Peck. 2002. Avances bioecológicos hacia el conocimiento y manejo del salivazo de los pastos (Homoptera: Cercopidae) en Colombia. Simposio de Plagas de los Pastos en Colombia y su Manejo. In: Memorias, XXIX Congreso de la Sociedad Colombiana de Entomología, Montería, Colombia. p. 180-188.

Peck, D. C. 2002. Desafíos y perspectivas para el manejo integrado de la candelilla (Homoptera: Cercopidae) en pastos tropicales, pp. 37-49. In: R. Tejos, W. García, C. Zambrano, L. Mancilla & N. Valbuena [eds] Memorias, VII Seminario Manejo y Utilización de Pastos y Forrajes en Sistemas de Producción Animal, UNELLEZ, Barinas, Venezuela, 14-16 de marzo de 2002.

Peck, D. C. 2001. Diversidad y distribución geográfica del salivazo (Homoptera: Cercopidae) asociado con gramíneas en Colombia y Ecuador. Revista Colombiana de Entomología 27(3-4):129-136.

Peck, D. C. 2002. La distribución y reconocimiento del salivazo de los pastos (Homoptera: Cercopidae) en la Costa Caribe de Colombia. Pasturas Tropicales 24(1):in press.

Peck, D. C., A. M. Pérez & J. W. Medina. 2002. Biología y hábitos de *Aeneolamia reducta* y *A. lepidior* en la Costa Caribe de Colombia. Pasturas Tropicales 24(1):in press.

Peck, D. C., A. M. Pérez, J. W. Medina, J. Rojas & M. Barrios. 2002. Fluctuación poblacional y enemigos naturales de *Aeneolamia reducta* en la Costa Caribe de Colombia. Pasturas Tropicales 24(1):in press.

Peck, D. C., A. M. Pérez, J. W. Medina, M. Barrios & J. Rojas. 2002. Fenología de *Aeneolamia reducta* en la Costa Caribe de Colombia. Pasturas Tropicales 24(1):in press.

Rodríguez Ch., J., D. C. Peck & N. Canal. 2002. Biología comparada de tres especies de salivazo de los pastos del género *Zulia* (Homoptera: Cercopidae). Revista Colombiana de Entomología 28(1):17-25.

Rodríguez Ch., J., U. Castro, A. Morales & D. C. Peck. 2002. Biología del salivazo *Prosapia simulans* (Walker) (Homoptera: Cercopidae), nueva plaga de gramíneas cultivadas en Colombia. Revista Colombiana de Entomología (in press).

Conferences and courses

VII Seminario Manejo y Utilización de Pastos y Forrajes en Sistemas de Producción Animal, UNELLEZ, Barinas, Venezuela, 14-16 March 2002.

CGIAR System-Wide IPM Programme Taskforce meeting on "Soil Biota, Fertility and Plant Health", 6-8 February 2002, CABI, UK.

XXIX Congreso de la Sociedad Colombiana de Entomología. Montería, Colombia, July 2002. (Ulises Castro, Jairo Rodríguez, Anuar Morales)

Taller Internacional "Ingenieria Genética para la Agricultura Colombiana. Bogotá, Colombia. 12-13 September 2002. (Jairo Rodríguez)

Training courses and workshops

Field day on the Bioecology and Management of Spittlebugs. CIAT, Palmira, Colombia, 11 March 2002 (four other field days carried out only by support staff).

Official trips

United States, 2002
United Kingdom, February 2002
Venezuela, March 2002

Partnerships

Cornell University, United States
Universidad de la Amazonia, Colombia

Staff List

Senior Research Fellow: Daniel Peck, Ph.D., Entomology/Ecology
Research Associates and Assistants: Anuar Morales, Entomology; Ulises Castro, Entomology; Jairo Rodríguez, Entomology.
Technicians: Rosalba Tobón, Entomology
Workers: Oscar Yela, Entomology; Gerson Fabio Vélez, Entomology
Trainees: Nora Valbuena, Professor, Universidad Nacional de los Llanos Ezequiel Zamora, Venezuela
Pregraduate Students: Fabio Andrés Obregón, Germán Chacón, Orlando Narváez, Daniel Corradine