ENVIRONMENTAL FACTORS AFFECTING THE SURVIVAL OF EGGS AND EARLY INSTAR NYMPHS OF SPITTLEBUGS Zulia entreriana (BERG) AND Deois flavopicta STAL DURING THE RAINY SEASON IN CENTRAL BRAZIL

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RESUMO

Fatores ambientais que afetam a sobrevivência de ovos e ninfas recém eclodidas das cigarrinhas-das-pastagens Zulia entreriana (Berg) e Deois flavopicta Stal, durante a estação chuvosa, no Brasil Central

Observações e testes de campo foram empregados para docu mentar a predação de ovos e ninfas das cigarrinhas-das-pastagens, por cinco espécies de formigas: Solenopsis sp. (1 e 2), Pheidole sp., Canomyrma sp. e, Cyphamurmex rinosus. Essas cin co espécies de formigas carregaram 47 ovos e 15 ninfas durante um período de nove horas de observação, em dois locais. A mortalidade de ninfas em função de predadores, distúrbios do solo, doenças e fatores abióticos foi de 61% em um dos locais estudados. A incidência direta da chuva ocasionou o deslocamento de ovos, tendo sido recuperados menos de 35% dos mesmos, após ficarem expostos à chuva. Neste estudo, a predação efetuada pelas formigas sobre ovos e ninfas recém eclodidas foi o que teve maior efeito sobre o número de cigarrinhas.

INTRODUCTION

In Central Brazil two spittlebug species Zulia entreriana (Berg) and Deois flavopicta Stal are abundant and cause ex tensive damage to introduced grasses, mainly Brachiaria decumbes Stapf. The monocultures of these grasses cover exten-

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sive areas and are vital to the beef cattle industry because they grow well on the relatively infertile soil of the area. These insects feed on the leaves and stems causing leaf yellow ing and a reduction in forage quality and growth.

Diapause spittlebug eggs after having passed through the dry season (June-September) hatch at the beginning of the rainty season (usually near the end of September). Upon hatching, first instar nymphs search out and find stems or grass runners on which to feed. Spittle is produced within a few minutes after the initiation of feeding. In this area of Brazil D. flavopieta usually has three generations a year and Z. entreminant entropy of the first adults usually appear in November and the generations overlap. Eggs are laid from November to May and hatching occurs after 14-18 days except the last generation of adults lays diapausing eggs during March, April, and May (KOLLER & VALÉRIO, 1985).

Only in recent years has research been initiated in order to determine what factors have the greatest influence on survival of spittlebugs in Brazil. It is believed that there are at least two times in the life cycle of spittlebugs when mortality factors greatly influence population density; during the egg stage and at the time of hatching before nymphs become established on plants and form spittle. In fact WIEGERT (1964) studying the meadow spittlebug *Philaenus spumarius* L. stated that estimates of adult mortality were small compared to mortality during all other stages. The present research approach is based on this assumption.

In general grazing is year-long in this area of Brazil and the low value forage resource does not allow for chemical control of spittlebugs to be cost effective. Thus, other means of supressing these insects must be found. One long-term goal is to manipulate the grazing environment and cause micro-habi tat changes which will be unfavorable for the growth and development of spittlebugs. Before this can be accomplished, additional information is needed on the role of predators and environmental factors in regulating spittlebug populations.

For example, ants destroyed nearly all 1st. instar nymphs in one study where nymphs were used to screen grasses for spittlebug resistance. This paper reports on studies involving the survival of eggs and nymphs during the rainy season in Central Brazil with emphasis on the role of ants as predators. According to WIEGERT (1964) both weather and predation are probably the most important regulating factors during the life cycle of spittlebugs.

MATERIALS AND METHODS

Studies on ant predation of eggs and nymphs

Experiment 1: Observations were recorded on ant predation in two separate studies:

- (A) This study was originally designed to screen grases for spittlebug resistance using newly-hatched spittlebug nymphs. The following three grasses were grown in separate pastures; B. decumbens, B. brizantha cv. Marandu, and Panicum maximum cv. Tobiatã and were infested with newly hatched (< 1 day old) nymphs of Z. entreriana. In each pasture 5, one m^2 areas, were infested with 50 nymphs/m². The nymphs were placed on the soil in close proximity to grass stems and/or directly on the stems. The infested areas were enclosed in 1 m² saran covered cages. Nymphal counts were made the day following infestion. The cages were infested twice during one week and after the second infestation observations were made on ant predation.
- (B) Observations on ant predation were also recorded from four pastures, Santa Ana and Sebastião near Dourados-MS and California and CNPGC near Campo Grande-MS. At each of the Campo Grande sites two potted plants (B. decumbens) each of which was infested with three first instar nymphs, were placed in the ground so the base of the plant was level with the ground surface. Twenty spittlebug eggs were placed on the soil surface on each side of the plants. On ly eggs were observed in the fields near Dourados. Both eggs and nymphs were observed for a total of three hours at each of the sites near Campo Grande and eggs were observed for a total of 1.5 hours at each of the sites near Dourados. Observations were carried out in 1.5 hour time periods at the Campo Grande sites and plants were reinfes ted with nymphs after the first 1.5 hour period. All ants which were observed carrying away nymphs or eggs were collected and later identified.

Experiment 2: Mirex bait (.1% a.i.) was applied to rangeland near Dourados where both spittlebugs and ants were numerous. Three of six pastures each 1/10 ha in size were treated with Mirex bait at the rate of 10 g/m² once in November and once in January. The untreated pastures served as checks. Twenty egg samples were collected from each of the six pastures on March 20 which was near the end of the oviposition period. A sample consisted of a circular soil core and little 7 mm in diameter and one cm deep. All samples were collected near *B. decumbens* plants. Eggs were separated from the soil and litter particles using the method described by NILAKHE et al. (1984). Studies on spittlebug eggs

Eggs used in these experiments were laid either in circular pans (30 cm in diameter 3.5 cm deep) in the laboratory or directly into pasture soils. In the laboratory eggs were obtained by placing 20 females in cages set over soil-filled pans. Food was placed in the cages allowing the females to live and oviposit during a three-day period. In some tests sterilized litter was compacted on the soil surface. Pans containing eggs were placed in pastures (*B. decumbens*) at the soil level. In others botton-less cages (7 cm in diameter) were in fested with 20 females per cage and eggs could then be deposited directly into the soil. Eggs were counted before and after exposure in all tests. In these tests it was assumed that the average number of eggs counted at the conclusion of oviposition which acted as control was the same as the number exposed in the treatments.

Experiment 3: Four pans of Z. entreriana eggs were placed in the pasture under a coverting which was 15 cm above the ground. In addition eggs from 4 pans were counted and used as control eggs at the end of the 3-day ovipositional period. The eggs placed in the pasture were counted after the 10 day expo sure period.

Experiment 4: Eggs were laid by Z. entreriana into 12 pans. Eight pans were placed in a pasture, four under a covering 15 cm above the ground and four on a level surface completely exposed. Eggs in four pans were counted at the end of the ovipositional period. Pans from the pasture were brought to the laboratory after 14 days and the eggs counted.

Experiment 5: Twelve pans contained eggs of *D. flavopic* ta. Four were placed in a pasture under a covering 15 cm above the ground, four on a level surface completely exposed, and eggs in four pans were counted at the end of the ovipositional period. The eight pans from the pasture were brought to the laboratory after 14 days and the eggs counted.

Experiment 6: Twelve bottomless cages were infested with 2. entreriana females. Eight cages were placed over bare soil and four cages were placed over litter. After oviposition was completed all the cages were removed from the pasture. At that time the soil 1 cm deep was removed from beneath four of the cages over soil and the eggs were counted. The location of the other eight cages was marked and after 10 days the eggs were counted.

Experiment 7: Eggs were laid by Z. entreriana into 12 pans. Six pans were placed in a pasture completely exposed and six were kept in a screen house protected from predators. After four days the eggs were counted in all 12 pans.

Experiment 8: Twelve bottomless cages were infested with Z. entreriana females (A). All cages were placed over soil near grass plants. After oviposition was completed all the cages were removed from the pasture. At that time the soil 1 cm deep was removed from beneath four of the cages and the eggs were counted. The location of the other eight cages were marked and a covering 15 cm above ground was placed over four locations and four locations were left completely exposed. After 12 days the eggs were counted in the 12 locations.

This experiment was repeated using 15 cages (B) but was also slightly modified. After oviposition, the soil one cm deep was removed from beneath five of the cages, five cages were covered with a glass cover, and the location of five cages was marked after the cages were removed. After 12 days the eggs were counted in the remaining 10 locations.

Experiment 9: Eggs were laid by Z. entreriana in 24 soil filled clay pots that measured 17 cm across the top and 12 cm deep. After oviposition was completed the pots were pla ced into four treatments as follows: A - pots were left out-doors, exposed to direct sunlight and received natural precipitation. B - Same as A except these pots received 100 ml more of water once a day, on Monday, Wednesday, and Friday. C -- Pots placed under a roof with no direct sunlight or natural precipitation but 100 ml of water was applied once a day on Monday, Wednesday, and Friday. D - Same as C except 100 ml of water was applied twice a day and everyday except Saturday and Sunday. All pots were protected from surface predators such as ants by a water barrier. Temperatures at the soil surface in each treatment were taken at 0800 hrs. and 1300 hrs. Monday through Friday. The number of hours of direct sunlight was es timated each day for treatments A and B. After eight days the eggs in each pot were counted and the percent hatch determi ned.

Studies on spittlebug nymphs

Circular pans, as used in the studies with eggs, were filled with moist soil and seeds of *B. decumbens* were planted in a circle around the outside edge of the pans. When the seedling plants were about 2 cm tall, cages in the center of the pans were infested with 20 *Z. entreriana* females. When hatching occured the seedlings were 6-8 cm tall and first ins tar nymphs could establish on the seedlings after moving only a short distance on the soil surface. Experiment 10: Twelve pans containing spittlebug eggs and seedling plants were held in the laboratory until two days before hatching was expected and then were placed in a pasture. Four pans were placed under a covering which was 15 cm above the ground, four were covered completely with nylon cloth for protection against predators and scavengers, and four were placed in the open, completely exposed. When hatching began the number of nymphs that established on the plants in each pan was recorded daily for three days at which time the test was destroyed by dogs.

Experiment 11: This experiment was similar to no. 10 except that most of the eggs were allowed to hatch in the laboratory before they were placed in the pasture. The nymphs in all 12 pans were counted and then the pans were divided into two groups, each containing six pans and about the same number of nymphs. Six pans were completely covered with nylon and six partially covered. The area around the base of the partially covered pans was left open to allow excess for predators and scavengers. A daily record was kept of the number of nymphs alive in each pan for 17 days. This experiment was also carried out near Dourados-MS but only eight pans were used. Four were completely covered with nylon and four partially covered. The pans were in the pasture at Dourados for eight days.

RESULTS AND DISCUSSION

Predation by ants

Experiment 1: A - Nymphal counts following the first day of infestation revealed only one nymph present out of 750 ori ginal nymphs placed in the cages. After the cages were infested the 2nd time direct observations showed that ants were rea dily carrying the nymphs away. We were aware that nymphal sur vival may be poor on some-grasses such a *B. brizantha* (NILAKHE & PASCHOAL 1985) however, the failure of nymphs to establish on *B. decumbens* was a surprise.

B - Observations of ants preying on eggs and newly hatched spittlebug nymphs was limited to a few hours at two sites. However, these studies have documented that ants indeed do carry off both eggs and nymphs. The number of eggs and nymphs carried off by different species of ants is shown in table 1.

Evidently several species of ants feed on insect eggs as active predators or scavengers. In Texas the imported fire ant *Solenopsis invicta* Baren has been reported to consume 0.4

Heliothis eggs per ant (McDANIEL & STERLING 1979). These authors report that a conservative estimate is that 5 ants removed two eggs every 24 hours, thus ants would rank as . major predators of Heliothis eggs. Direct observations and radioactive eggs were used in Florida soybean fields to determine pre dators of velvetbean caterpillar eggs (BUSCHMAN et al., 1977). These authors reported that ants and earwigs were observed most frequently as eggs predators. Ants belonging to three ge nera (Pheidole, Conomyrma and Solenopsis) which were reported as egg predators in Florida of velvetbean caterpillar eggs are also recorded in Table 1 as predators of spittlebut eggs in Brazil. Since ants are one of the most numerous insects in Brazil, their effect on spittlebug populations could be signi ficant. KEMPF (1972) records over 400 species of ants in the state of Mato Grosso.

TABLE 1 -	Observations	on ant preda	tion of	f spittlebug	eggs and	1st instar
	nymphs (Deoi	s flavopicta)	in B.	decumbens pa	astures ¹ .	

		N	umber	of Eggs	and	Nymphs 1	Remove	ed ²		
Ant	CAMPO GRANDE					DOURADOS				
species	CNPGC		FAZ. CALIF.		SANTA ANA		SÃO SEBASTIÃO			
	eggs	nymphs	eggs	nymphs	eggs	nymphs	eggs	nymphs	total	
Solenopsis sp1		3			1				4	
Solenopsis sp2							6		6	
Pheidole sp	2	6			12		11		31	
Conomyrma sp			4	6					10	
Cyphomyrmex rinosus					=		11		11	

¹ Observations were made in Sept. and Oct. 1984. Observation time totaled 6 hrs at the Campo Grande sites and 3 hours at the Dourados sites.

 2 All nymphs except one were taken before they produced spittle.

Other authors have reported that several species of ants forage for a 3-4 hour period in the morning and also in the afternoon (CARROLL & JANSEN, 1973; CLARK & COMANOR 1973, and ROGERS, 1974). Since it has been observed that spittlebug usu ally hatch during the night or early morning they would be especially susceptible to predation by foraging ants (FAGAN & KUITERT, 1969). Field observations also indicate that most spittlebug nymphs fall prey to ants before the spittle is for med. Therefore, the time span between hatching and the formation of spittle by first instars is critical for survival.

Results of the study involving the application of mirex bait to control ants are inconclusive. More spittlebug eggs were expected in plots treated with mirex but in all the samples collected (120) only 17 eggs were recovered, 10 from trea ted plots and 7 from untreated plots. Shortly after the bait was applied ants were observed carrying the bait and later it was found stored in ant tunnels. However, rain showers occurred shortly after the bait was applied in November and January. It is possible that rain reduced the toxic effect of the bait.

Spittlebug egg survival

A total of six experiments were carried out with non-dia pause eggs to determine egg survival under field condition and attempt to identify the factors causing mortality. The results of these experiments which show the average number of eggs recovered per 20 females before and after exposure to field conditions is shown in Table 2. In most experiments the average number of eggs recovered that were exposed but protec ted from effects of rain showers was equal or higher than the average number recovered following oviposition (control) (Table 2). Evidently the differences in the number of eggs laid in the pans was great. However, in all but one experiment (Experiment 8) the average number of eggs recovered that were fully exposed to environmental influences differed significan tly from the average number of eggs recovered following ovipo sition (Table 2). When results of experiments 4, 5, 6, 7, and 8 are combined the actual reduction in recovered eggs between those in control groups and those fully exposed was 66%. Also when the results of experiments 4, 5, and 8 (experiments with the treatment labeled partial exposure) are combined the reduction in recovered eggs between those protected from rain showers by a cover and those that were fully exposed was 66%. This egg mortality is considerably higher than that reported for Neophilaenus lineatus (L.) by WHITTAKER (1971). He reported egg mortality to range from 25-31%. The large difference between eggs recovered after exposure and those protected from the effects of rain showers is believed to be due to the displacement effects of numerous showers that occur during the time eggs are being laid. For example, in experiments 4 and 5 only 2 eggs were recovered after being completely exposed for 14 days but 482 eggs were recovered from the pans that were protected from rain showers. Heavy rain showers occurred during both experiments with a total of 98.00 mm recorded during

the time span of experiment 4 and 70. 1 mm during experiment 5. In experiment 7 where only 23.9 mm of precipitation was recorded the ratio between exposed eggs and protected eggs was closer. A total of 223 eggs were recovered following oviposition compared to 31 after exposure. The intensity of the rain showers probably has a greater effect on egg displacement than the amount of precipitation or the duration of the showers. Eggs displaced by rain showers could be washed down soil cracks, moved to low areas in the field, buried under soil and litter, or be left fully exposed (PICKLES, 1933).

Experiment	Oviposition	Precip. (mm)	Exposure (days)	Mean number of eggs recovered/20 °'s ¹			
N?	media			Treatment ²	x S.E.		
3.	litter + soil	-	10	Control Partial Exposure	30.50a ± 3.80 19.25b ± 2.02		
4	soil	98.0	14	Partial Exposure Control Full Exposure	64.50a ± 0.65 30.75b ± 4.64 0.25c ± 0.25		
5	litter + soil	70.1	14	Partial Exposure Control Full Exposure	56.00a ± 10.68 31.50b ± 6.76 0.25c ± 0.25		
6	litter + soil	52.1	10	Control Full Exposure-litter Full Exposure-soil	84.00a ± 30.63 9.50b ± 1.55 2.25b ± 1.11		
7	litter + soil	23.9	4	Control Full Exposure-litter	37.17a ± 2.18 5.17b ± 1.70		
A	soil	48.5	12	Partial Exposure Full Exposure Control	76.25a ± 10.26 68.50a ± 9.02 52.75a ± 4.17		
8 B	soil	-	12	Control Partial Exposure Full Exposure	7.2 a ± 4.84 7.2 a ± 2.78 0.6 a ± 0.40		

TABLE 2 - Survival of spittlebug eggs following exposure to environmental influences, Campo Grande-MS, 1985.

¹ Means are based on 4, 5, or 6 replications with 20 %'s/replication.

S.E. = standard error.

Means within a column followed by the same letter are not significantly different (P<0.05, Duncans multiple range test).

Control = Eggs were counted following oviposition.

Partial Exposure = Eggs were covered or partial covered in the field and thus not exposed to rain showers.

Full exposure = Eggs were fully exposed to environmental conditions.

Abiotic factors, such as those tested in experiment 9, also affect non-diapause egg survival and hatching. This experiment was designed to test the effect of a range of possible environmental conditions on egg hatch. The treatment variables involved differences in the amount of direct sun the eggs received, differences in soil surface temperature, and differences in the amount of water the eggs received. The per cent egg hatch recorded under these different treatments is shown in Table 3.

	Av. Temperature (°C)		Av. hours	W	%	
Treatment	0800	1300	Sunshine day	Precip. (mm)	Addit. water (ml)	hatch
A	24.8	29.5	4 hrs 33 min	78.8	0	10.7
В	24.9	29.5	4 hrs 33 min	78.8	400	75.6
с	22.4	24.3	0	0	400	69.1
D	22.5	24.7	0	0	1400	74.7

TABLE 3 - Effects of certain environmental factors on the hatching of non diapause eggs of *Zulia entreriana*, Campo Grande-MS, 1985¹

Treatments A and B were placed outdoors in direct sunlight and treatments C and D were placed in a protected area with no sun or natural precipitation.

Treatments B and D received the most water and also had the highest percent hatch. Treatment C received regular amounts of water but not as much as treatments B and D and the percent hatch was 69%, only slightly less than B and D. The hatch was lowest (11%) in treatment A which received only natural precipitation and none was recorded the last four days of the test. There was little differences in percent hatch between treatment B, the exposed site, and treatments C and D,

shady sites. Therefore it appears that hatching will be successful as long as the soil remains moist, even under exposed, sunny, conditions. The temperature averaged slightly higher (2.4°C at 0800 and 5.0°C at 1200) at the sunny site but again egg hatch appeared not to be affected as long as adequate moisture was present. Non-diapause eggs usually hatch in 14--18 days during the rainy season so it is doubtful that soil moisture will be a limiting factor during this period. However, if precipitation does not occur for several weeks egg hatch could be prolonged.

Spittlebug Nymphal Survival

Experiment 10 was initiated in order to determine the mor tality of eggs hatching in the field. Nymphal counts were made following egg hatch in the field in three different treatments. During the 3-day period after eggs had started to hatch no nymphs were observed in four pans of eggs that were fully exposed, two were observed in the pans under cover, and 61 were observed in the pans that had been covered with nylon cloth. These results show that a high nymphal mortality can occur during the short time between hactching and the formation of spittle for protection.

Mortality of newly hatched nymphs was also examined under field conditions (Experiment 11). A daily record of the number of nymphs alive on grass seedlings exposed to field condition compared to the number of nymphs alive that were pro tected with nylon cloth at two locations, Campo Grande and Dou rados is shown in Figure 1. At Campo Grande most of the eggs hatched in the laboratory and the nymphs established on the seedlings before the pans containing the nymphs were taken to the field. However, additional hatching occurred once the pans were placed in the field. At Campo Grande 83 additional eggs hatched in the field and the nymphs established on seedlings in the covered cages. No additional nymphs were observed to survive in the partially covered cages. At Dourados 158 additional eggs hatched and the nymphs established in the covered cages. No additional nymphs were observed in the partially covered cages. This indicates the high mortality that must often occur when hatching begins.

At Campo Grande mortality decreased at about the same ra te in both groups of nymphs and near the end of the test the mortality was due to the inability of the seedlings to support 2nd instar nymphs. At Dourados the mortality was much greater in the group of exposed nymphs, mainly because most of the eggs had not hatched when the pans of nymphs and eggs were ta ken to the field. Thus, more newly hatched nymphs were exposed to predation, dessication, etc. At Campo Grande mortality was determined by using the day with the highest total nymphal count (234) in the covered cages and comparing it with

7.1

the number of nymphs 10 days later (206). This shows mortality to be 12%. The mortality in the partially covered cages du ring this time was 73%. The difference (61%) can be considered the effect of predators, scavengers, disease, and abiotic factors on newly hatched nymphs. However, WHITTAKER (1971) re ported nymphal mortality of *Neophilaenus lineatus* in England to be no more than 5%. He stated that nymphal mortality does not seem to be a very important source of mortality because the spittle protects the nymphs from most predators. This also appears to be generally true for spittlebug nymphs in central Brazil except for newly hatched nymphs.

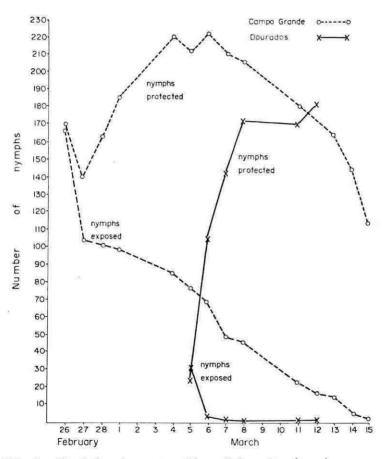


FIG. 1 - Nymphal and egg mortality of Z. entreriana in cages completely protected from predators and scavengers vs mortality in cages partially open to predators and scavengers at Campo Grande-MS and Dourados-MS, 1985.

Observations indicate that great differences occur in adult densities and eggs laid even within pastures composed of the same grass species. These differences occur because (1) females select a favorable egg laying enrivonment (HEWITT, 1985) and areas unsuited for oviposition are ignored (2) once the eggs are in the pasture other environmental influences ei ther favorable or unfavorable operate to determine the number of eggs hatching and survival of nymphs to the adult stage. For example, mortality factors which operate in the life cycle of spittlebugs are shown in Table 4. Certainly these mortality factors will vary with time and place. In the present study the predation of spittlebug eggs and newly hatched nymphs, mainly by ants, appeared to have greatest effect on spit tlebug numbers. Intensive rain showers displaced spittlebug eggs but the actual egg mortality could not be determined.

Life Stage	Causative Agents	Operative Period		
Egg (non-diapause) (a) Eggs consumed or destroyed	invertebrate predators and parasites			
(b) Eggs failed to hatch	inviability, disease, dessication	14 - 18 days		
<u>Nymph</u> (a) Hatching period	invertebrate predation, disease, weather influences	Time of hatch up to 2 hours.		
(b) Spittle period	vertebrate and inverte brate predators, para- sites, disease	25 – 40 days		
Adult (a) Mobile period	vertebrate and inverte brate predators, para- sites, disease	8 - 16 days		

TABLE 4 - Mortality	factors which may operate during the spittlebug	life
cycle and	influence population density.	

It should be understood that, in general, a very favorable habitat exists for spittlebugs in central Brazil. Very few stressful influences are present. The habitat is relatively stable with year-long grazing, there is a minimum of crop rotations and land renovations, and the food is unlimited during the rainy season when the spittlebugs are active. We would like to suggest that this relatively stable pasture environment and the year-long grazing management plan needs to be reevaluated in terms of maximizing beef production and pro viding an unfavorable habitat for spittlebugs. Little is know about the effects of crop rotation, grazing pressure, or rota tional grazing on insects such as spittlebugs and ants. This study points out the need for additional research on the fac tors that determine ant numbers. Also, the advantages or disa vantages of increasing ant numbers needs to be evaluated as a possible means of spittlebug suspression.

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ABSTRACT

Observations and tests were used to document predation of spittlebug eggs and nymphs by five species of ants: *Solenopsis* sp.1, sp.2, *Pheidole* sp., *Canomurma* sp., and *Cyphamyrmex* rino sus. These five species carried off 47 eggs and 15 nymphs during an observation time of nine hours at two sites. Nymphal mortality resulting from predators, scavengers, disease, and abiotic factors at one site was determined to be 61%. Eggs we re displaced by rain showers and less than 35% were recovered following exposure. In this study predation of spittlebug eggs and newly hatched nymphs by ants had the greatest effect on spittlebug numbers.