

ECOLOGY, BEHAVIOR AND BIONOMICS

Alternative Methods for Rearing Grass-Feeding Spittlebugs (Hemiptera: Cercopidae)

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Métodos Alternativos Para a Criação das Cigarrinhas-das-Pastagens (Hemiptera: Cercopidae)

RESUMO - Duas metodologias para criação de cigarrinhas-das-pastagens são relatadas e avaliadas, utilizando-se *Aeneolamia varia* (Fabricius) sobre *Brachiaria ruziziensis* Germ. & Evrard. Desenvolveu-se uma nova unidade de criação em pequena escala para manter a cigarrinha nos seus diferentes estágios de desenvolvimento, e assim promover estudos biológicos dos insetos. A unidade possui uma bandeja de plantas com raízes, que são os locais de alimentação e desenvolvimento das ninfas, junto com uma gaiola para emergência dos adultos contendo plantas e substrato para oviposição e obtenção de ovos. Para produção em larga escala, aperfeiçoou-se um método de criação massal, reduzindo-se os insumos e agilizando-se a produção. A principal característica é uma caixa coberta com raízes e condições de microclima adequados para o desenvolvimento de ninfas. A eficiência de produção de adultos a partir de ovos foi de 45,1%, produzindo 1002 adultos/m² por geração, com um período de desenvolvimento ninfal de 36,1 dias. A emergência de adultos foi de 70,5% em uma semana. Esse método para produção massal é uma ferramenta efetiva para produção de cigarrinhas em larga escala, podendo ser utilizado para estudos como de controle com fungos entopatógenos, resistência da planta hospedeira e outros. São discutidas as possibilidades e necessidades futuras para se melhorar a técnica e ajustá-la a outras espécies de cigarrinhas.

PALAVRAS-CHAVE: *Aeneolamia varia*, *Brachiaria*, praga, gramínea forrageira, cigarrinha, técnica de criação

ABSTRACT - Two methodologies for rearing grass-feeding spittlebugs are described and were evaluated with *Aeneolamia varia* (Fabricius) on *Brachiaria ruziziensis* Germ. & Evrard. To promote biological studies, a new small-scale rearing unit was developed to maintain spittlebug life stages in the greenhouse year round. This unit features a plant tray established with roots as feeding sites for nymphal development alongside an adult emergence cage with plants and oviposition substrate for egg collection. For large-scale production, an improved mass-rearing colony was designed to reduce inputs and streamline production. The major feature was a covered box with root and microclimate conditions adequate for nymphal development. Efficiency of adult production from eggs was 45.1%, yield 1002 adults/m² per generation, nymphal development time 36.1 d, and adult emergence 70.5% in a 1-wk period. This mass-rearing method is a more effective tool for reliable and high-level production of spittlebugs for massive screening required for the evaluation of control tactics such as fungal entomopathogens and host plant resistance. The possibilities for further improving these designs and tailoring them to other spittlebug species are discussed.

KEY WORDS: *Aeneolamia varia*, *Brachiaria*, forage grass pest, frog hopper, rearing technique

Grass-feeding spittlebugs (Hemiptera: Cercopidae) are a major biotic limit to forage grass, milk and beef production in rangelands and pastures of the Neotropics (Valério *et al.* 1996). These native pests damage most improved forage grasses such as the widely sown and highly susceptible *Brachiaria decumbens* Stapf, an African grass well-adapted and highly productive under the acid and low fertility

conditions of the humid and subhumid tropics (Keller-Grein *et al.* 1996). Other species from forage grass genera such as *Cenchrus* (Agostini *et al.* 1981), *Cynodon* (Taliaferro *et al.* 1967, Fagan & Picado 1971), *Panicum* (Thomas & Lapointe 1989), and *Pennisetum* (Peck 1998) are highly injured by this pest complex as is sugar cane (*Saccharum officinarum* L.) (Fewkes 1969) and under certain circumstances rice (*Oryza*

sativa L.) (Nilakhe 1985, Souza & Nilakhe 1985). Quantitative impact studies on some spittlebug/forage combinations have documented a very significant negative effect on the yield and quality of forage, and consequently on the establishment and persistence of improved pastures (Valério & Nakano 1987, 1988, 1989). In susceptible forage grasses, severe outbreaks cause die-back of all the above-ground biomass thereby promoting weed invasion and contributing to environmental degradation. On a regional scale, spittlebug attack is estimated to cause a minimum of US\$8.5-34.0 million/year economic damage in Colombia (Holmann & Peck 2002).

A particular challenge to management of this pest complex is the diversity of taxa involved and habitats affected. Dozens of species from at least 11 genera attack graminoids from the southern United States to southern Brazil, across humid to subhumid regions, and extensive to intensive grazing systems. Spittlebugs are pests of increasing concern in many regions including the hillsides and forest margins of Colombia, pastures of northern Argentina, and sugar cane of Central America and Ecuador (DCP, unpublished). Unfortunately, the biology and ecology of relatively few economically important species has been studied. Advances in management thereby suffer from ineffective tailoring of control tactics to local species composition and habitat conditions.

One limitation is the lack of appropriate rearing methodologies. Mass-rearing is a necessary tool for evaluation of control techniques. As methodologies improve for the evaluation of fungal entomopathogens (CIAT 1999) and the screening of germplasm for host plant resistance (Cardona *et al.* 1999), throughput is increasingly dependent on a reliable, continuous and high-level production of eggs, nymphs and adults. Streamlining the rearing methodology will help achieve these requirements as well as increase efficiency and reduce inputs of materials, labor and space. Small-scale colonies are another tool that would be useful for conducting biological studies in the laboratory and greenhouse, especially for resource-limited research groups such as regional universities.

Earlier techniques for rearing spittlebugs have confronted the challenges of providing grass roots for nymphal feeding sites, maintaining high humidity for nymphal development, and efficiently recovering eggs from oviposition substrate. Fewkes & Demidecki-Demidowicz (1971) reared *Aeneolamia varia saccharina* (Distant) and *Aeneolamia postica jugata* (Fowler) on roots of maize plants that perforated fibrous pots bedded onto plant nutrient solution; moist filter paper served as oviposition substrate. McWilliams & Cook (1975) reared nymphs of *Prosapia bicincta* (Say) on pearl millet grown in vermiculite with commercial plant food, and obtained eggs from cotton oviposition substrates. Pacheco & Silva (1982) obtained 27.9% survivability of *Deois flavopicta* (Stal) nymphs reared on uprooted clumps of *Brachiaria plantaginea* (Link) Hitchc. kept in glass beakers with water. Neto & Pavan (1984) achieved 48-100% survivability of *Deois* sp. nymphs grown on potted *Digitaria decumbens* Stent held in a water tray under protective screen cages to maintain high humidity. Magalhães *et al.* (1987) raised nymphs of *Deois incompleta* (Walker) on potted *Brachiaria humidicola* (Rendle) Schweik. and used filter paper as an oviposition substrate.

Lapointe *et al.* (1989) is the most recent report of an improved mass-rearing technique. A key aspect was producing favorable conditions for nymphal development featuring abundant surface roots and high humidity in pots covered by aluminum lids. The second major advance was preparing a soil oviposition substrate that permitted efficient recovery of eggs through sieving and decanting. Current work with this traditional methodology at CIAT (International Center for Tropical Agriculture, Cali, Colombia) yields an efficiency of approximately 50% (proportion of eggs recovered as adults) and overall production of 800 adults/m² per generation in the case of *Aeneolamia varia* (Fabricius) (R. Pareja, CIAT, personal communication). Finally, CIAT (1997) reported on a new technique for nymphal rearing that essentially reproduced the high humidity conditions and surface root availability of the pot in a larger box environment thereby reducing soil, space and pot requirements. Although only evaluated on a small scale, this technique showed promise for integration into an improved mass-rearing colony.

Here we report on two advances in spittlebug rearing technologies. The first is a new small-scale rearing unit. We sought to develop a unit that met several general characteristics: adaptability to a variety of spittlebug species and genera; low labor, space and time requirements for operation by small research groups; and effective maintenance of all life stages to support year-round studies despite the absence of the insect in the field during the dry season. An important component of our design strategy was to combine nymphal rearing and egg laying chambers thereby eliminating a major bottleneck in the traditional mass-rearing colony used by CIAT.

The second is an improved mass-rearing protocol to support studies where a large, dependable, year-round source of eggs, nymphs and adults are required for evaluations. We implemented and evaluated a new mass-rearing technique that integrated recent advances built on more than 15 years of experience at CIAT where the Tropical Grasses and Legumes Project has been raising *A. varia* for studies on host plant resistance. The objective was to establish a reliable and more economical methodology for mass production of spittlebug eggs and adults.

Materials and Methods

Insect and Plant Material. Both rearing techniques were developed and evaluated with the spittlebug *A. varia* on *Brachiaria ruziziensis* Germ. & Evrard (CIAT accession 0654). This cercopid is the most damaging species in the extensive Eastern Llanos and the Amazonian Piedmont of Colombia, while *B. ruziziensis* is an acceptable host that is responsive to planting conditions and the establishment of secondary roots for nymphal feeding. All insects used in this study were obtained from CIAT's long-established colony that is periodically reinvigorated with insects from the field and managed with the traditional methodology of Lapointe *et al.* (1989); all grass was obtained from field plots at CIAT's campus, Palmira, Valle del Cauca, Colombia.

Small-Scale Rearing Unit. The oviposition component of

the unit was built around a frame of aluminum posts (height 48 cm) (Fig. 1) whose legs were placed in water dishes to exclude ants. Plant trays (61.0 x 30.6 x 3.4 cm l/w/h) were fitted at the top and bottom (16 and 46 cm) of the chamber (Fig. 1C). The frame was wrapped on the outside with shade fabric (black polypropylene, 63% shade) to form walls, reduce light and elevate humidity. As the adult food source, stems and foliage of potted host plants entered the unit through lateral slits on two sides (Fig. 1F). A tray with specially prepared soil oviposition substrate was fitted in the bottom level while the top was covered by either a plant tray that served as a lid or one that was prepared with roots that housed late instars (Fig. 1A). The oviposition substrate and egg extraction techniques were identical to those described in Lapointe *et al.* (1989) where the soil substrate with eggs was washed in water and passed through a series of sieves to remove particulates, followed by egg extraction using flotation in a saline solution.

Plant trays were specially prepared to provide feeding and development sites for nymphs. The bottom of each tray was latticed with holes that allowed roots to descend. Trays were planted with 21 plants (each with 5–7 stems) in a 2-cm layer of soil. The best root growth was obtained by using young material with partially pruned roots and foliage and planted in contact with the bottom of the tray in soil fertilized with N:P:K (15:15:15) at 2 g/l water. After five days this tray was stacked on top of another with a dusting of fertilized soil to stimulate root growth. About three weeks after transplant the space between the trays filled with enough roots for infestation with eggs, each tray receiving 500 eggs 1–2 d from hatch. The eggs, in groups of 50 on pieces of cut filter paper, were placed on the lower tray with the upper tray of roots replaced on top.

Three weeks after infestation the lower tray was removed and the upper tray was mounted on a wooden frame (58 x 29 x 10 cm l/w/h) (Fig. 1B). This frame allowed the roots to descend and thereby give the adults more space to emerge. The dark and high humidity conditions also helped to maintain root quality. Just before adult emergence this frame was positioned

alongside the aluminum oviposition chamber. Adults moved from the frame to the relative light of the oviposition chamber through a long opening (1.5 x 56.0 cm) on one side of the frame that abutted a slit in the wall of the oviposition chamber (Fig. 1D).

To iterate our way to the most appropriate design, we evaluated three versions of the rearing unit that allowed us to compare contrasting conditions of root exposure and light gradient (Fig. 2). The first version was the complete unit as described above where adults were recovered from the oviposition chamber after emerging from the wooden frame where nymphs completed development. We tested a second version thought to increase emergence from the dark conditions under the wooden frame by allowing adults to emerge into a higher light environment. This consisted of just the nymphal rearing unit where adults exited the wooden frame into a large emergence cage (61.5 x 31.0 x 30.0 cm l/w/h) covered in white nylon mosquito netting. The third version was as the unit was originally conceived, consisting of a single unit without use of the wooden frame; the root tray with nymphs was placed directly on top of the oviposition chamber three weeks after infestation. This version gauged the capacity of roots that were more exposed to support nymphal development and adult emergence.

Six paired repetitions of versions 2 and 3 were evaluated on separate dates from October 1997 to February 1998 whereas five repetitions of version 1 were evaluated later from August 1998 to June 1999. Differences among these three versions in efficiency (measured as the proportion of infested eggs that emerged as adults) and sex ratio of emerging adults were tested using the Tukey-Kramer HSD test while deviations from a 1:1 sex ratio were tested using the Wilcoxon signed-rank nonparametric test (SAS Institute 1989).

Mass-Rearing Colony. Host plants were brought from the field, washed, partially pruned of roots and foliage, as above, and planted into 750 cm³ pots. After two weeks, 16 plants were transplanted to the nymphal rearing box made of a wooden frame (120 x 60 x 10 cm l/w/h) with 4–5 cm soil on the bottom.

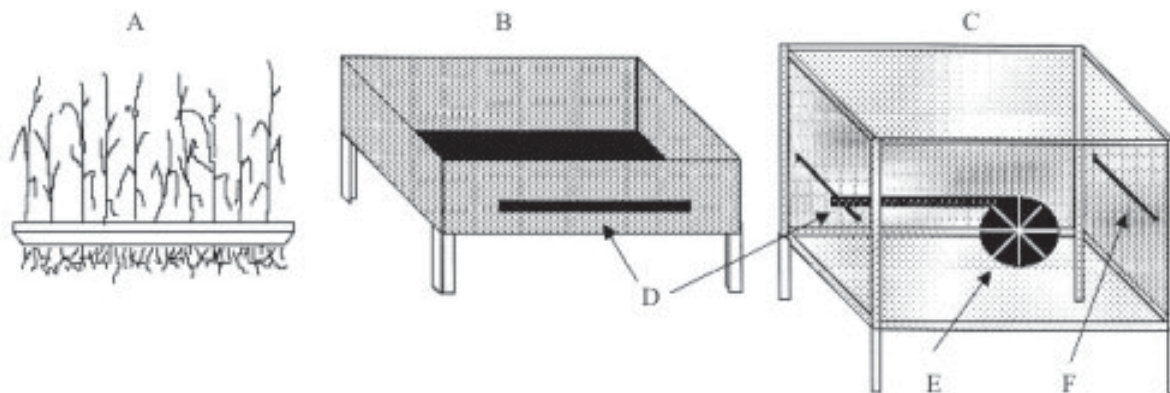


Figure 1. Components of the spittlebug rearing unit. The tray with roots and nymphs (A) is put on top of the wooden frame (B). New adults exit the frame and enter the oviposition chamber (C) through a lateral opening where the two components abut (D). The oviposition chamber is equipped with a tray of oviposition substrate on the bottom, lateral slits to enter stems of potted host plant (F) and a circular entrance to allow manipulation inside (E).

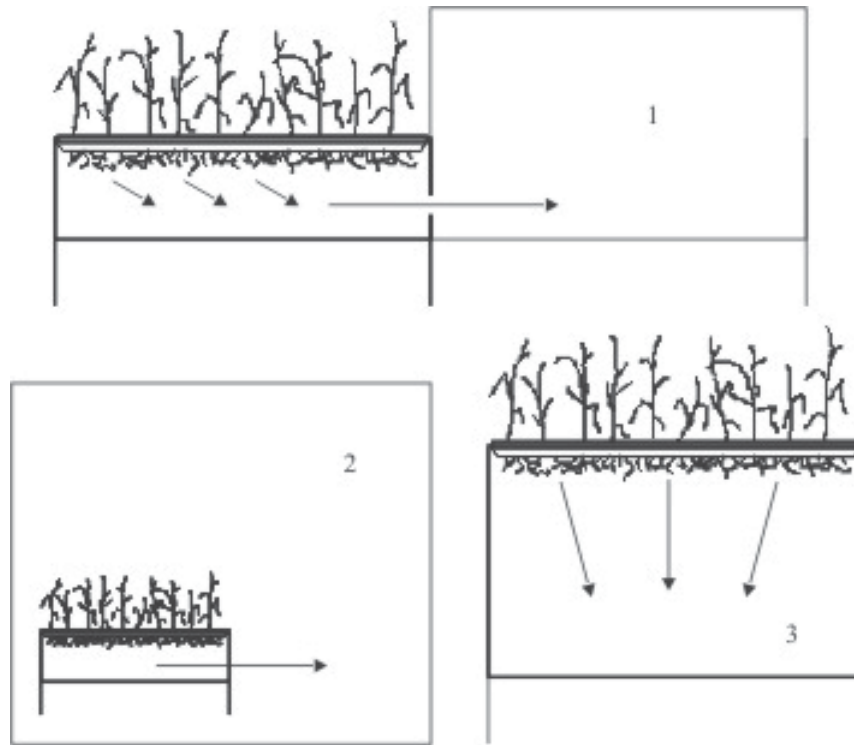


Figure 2. Three versions of the rearing unit assessed for adult emergence. Nymphs completed development on grass roots, and adults were recovered from an abutting oviposition chamber (version 1), an emergence cage (version 2) or directly below (version 3). Arrows point from spittle masses to where the adults were recovered.

Roots of the transplants (each with 5–7 stems) were placed onto small mounds of soil. These were covered with inverted pots, bottoms removed, to promote root growth and improve anchoring of the plant to the substrate. After one week, the pots were removed, and the box was covered with a double layer of cloth formed of black shade cloth below and white cotton material above. Stems and foliage emerged from 16 openings in the cloth. This arrangement provided dark and humid conditions that stimulated the continued development of roots down the mounds of soil. These surface roots are required by early instars for feeding sites and are therefore one of the most critical steps in the rearing process (Fig. 3).

Immediately after covering the box with the cloth, plants were infested with eggs 1–2 d from hatch. Eggs were applied in groups of 50 on pieces of moist filter paper placed near the roots. Applications of urea (2%) were made four and six weeks after transplanting from the field, and plants were watered as required with care taken not to wash away eggs or spittle masses. Four to five weeks after infestation or at first adult emergence, a large emergence cage (120 x 60 x 60 cm l/w/h) was placed over the box to prevent escape. Adults were collected daily with an aspirator from the foliage and from the rooting area below the cloth. Those adults not required for screening tests were transferred to oviposition cages for the



Figure 3. Components of the mass-rearing colony, including box with host plants anchored to mounds of soil and covered with shade cloth (A), root and spittle mass development (B) and emergence cage for adult capture (C).

collection of eggs. Nymphal rearing boxes and adult oviposition chambers were protected from ants by placing the feet of their supporting tables in dishes filled with water.

To measure the efficiency of adult production from eggs, two boxes were infested each week over 31 consecutive weeks from 17 July 1998 to 15 March 1999 with the exception of three weeks when shortage of eggs prevented infestations. Two egg infestation levels were evaluated: 100 eggs per plant, or a total of 1600 eggs per box ($n = 16$), and 150 per plant, or a total of 2400 per box ($n = 13$). We also measured the success of egg hatch under these conditions to gauge how efficiency depended on egg mortality. During five consecutive generations a subsample of eggs (approximately 800) were placed on moist filter paper and divided among four petri dishes situated in the corners of the box. Hatching success under the colony conditions was compared with another group kept under ideal incubation conditions (darkness, 27°C, 100% RH). A two-sample t-test was used to evaluate differences in efficiency and total adult production between the two infestation levels, and to evaluate egg mortality between the two hatching conditions.

During the mass-rearing colony studies, mean daily temperature was $26.3 \pm 6.5^\circ\text{C}$ (min/max 17/36°C) and relative humidity $78.8 \pm 19.1\%$ (min/max 53/97%).

Results

Small-Scale Rearing Unit. The efficiency of adult emergence from eggs was significantly affected by conditions related to the exposure of the roots. About 2.1 times more adults emerged after developing under the more protective conditions of the wooden frame and exiting to the large emergence cage (version 2) than from developing under the more exposed conditions of the top of the oviposition chamber (version 3) (Table 1). This could be explained by direct or indirect effects on nymph survivorship. Under the conditions of the wood frame, higher humidity may have favored spittle mass maintenance. In addition, increased darkness and higher humidity may have enhanced root vigor and quality and/or maintained these characteristics for a greater period of time during the relatively long development period of nymphs. When exposed at the top of the oviposition chamber, roots visibly deteriorated and senesced more rapidly than on the wooden frame. Maintaining healthy roots for nymphal feeding is therefore a critical component of successful rearing.

Adult emergence was also significantly affected by conditions that related to light gradient. Sixty percent of eggs

successfully hatched, completed nymphal development and exited the wooden frame into the large emergence cage (version 2) while only 18.9% exited the wooden frame into the oviposition chamber (version 1) (Table 1). From these data we infer that the reduced light in the oviposition chamber (walled with black shade cloth) did not attract adults as effectively as the emergence cage (walled with white netting). Manipulating light gradients is therefore another aspect relevant to colony maintenance.

Taken together, these data suggest that although the wooden frame might enhance nymph survivorship through root maintenance, the advantage is counterbalanced by adults unsuccessful in exiting into the shaded oviposition chamber. Efficiency did not differ between version 1 and version 3 (Table 1), which suffered from low light gradient and high root exposure conditions, respectively. Efficiency was highest under the conditions of version 2 with high light gradient and low root exposure.

The male:female sex ratio varied from 1.05 to 1.24 among versions but in no case differed from 1:1 (Prob>|t|=0.62, 0.44, 0.16 for versions 1, 2 and 3, respectively).

Mass-Rearing Colony. Adults from each generation emerged over a period of approximately two weeks (Fig. 4). The first adult collection date represented the accumulation of individuals over 2–4 d. However, 70.5% of adults emerged within seven days after this first collection date, an acceptable level of population synchrony especially if eggs are collected weekly to initiate a new generation.

Mean time to median adult emergence was 36.1 ± 0.54 d (31–47). Mean time from egg infestation to first and last adult emergence was 31.6 ± 0.49 d (25–41) and 40.6 ± 0.69 d (35–60), respectively.

Efficiency varied widely throughout the beginning of the study (weeks 1–20) as small adjustments were made to arrive at the final methodology detailed in the methods (Fig. 4). Such variation should be expected when implementing this mass-rearing design for the first time until the rhythm of management is established. Mean efficiency during this initial period was $28.4 \pm 2.81\%$ (13.67–55.81). In the last third of the study (weeks 21–28) efficiency stabilized to a mean of $45.1 \pm 1.07\%$ (35.94–51.56). During this last period, a mean of 1002 ± 23.8 adults/m² (798.6–1145.8) was produced for each generation.

For infestation levels of 100 and 150 eggs per plant, the mean (\pm SE) number of adults produced per box was 601 ± 105 and 678 ± 107 , respectively, but no significant difference

Table 1. Production of adults [mean \pm SE (range)] from 500 eggs of *A. varia* in three versions of the small-scale rearing unit.

Version	n	Adults recovered	Efficiency	Male:female sex ratio
Version 1 (Complete unit)	5	94.4 \pm 14.81a (48–134)	18.9 \pm 2.96a (9.6–26.8)	1.05 \pm 0.097a (0.77–1.09)
Version 2 (Wooden frame)	6	299.8 \pm 26.70b (220–372)	60.0 \pm 5.34b (44.0–74.4)	1.06 \pm 0.049a (0.88–1.22)
Version 3 (Oviposition chamber)	6	142.3 \pm 25.09a (51–232)	28.5 \pm 5.02a (10.2–46.4)	1.24 \pm 0.118a (0.77–1.55)

Within columns, values with different letters are statistically different ($P < 0.05$).

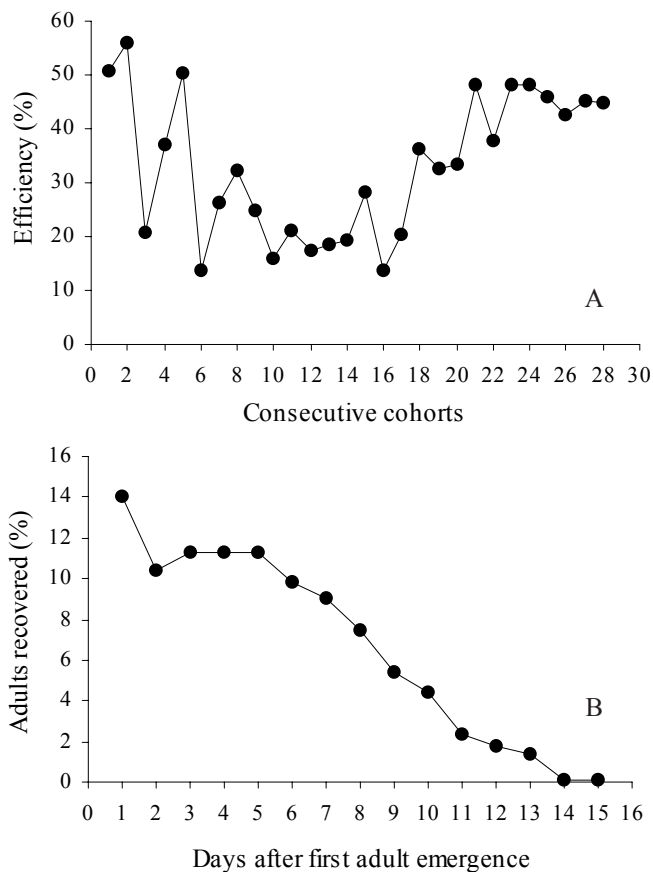


Figure 4. Efficiency (emerged adults from infested eggs) over 28 consecutive weekly cohorts (A) and mean daily emergence rates of adults (B) from the mass-rearing colony of *A. varia*. The first collection date in graph B is the sum of individuals emerged over the first 2–4 d.

was detected (t-test, $df = 56$, $P > 0.1$). Efficiency, however, did differ between infestation levels. Efficiency was $37.6 \pm 2.00\%$ (9.0–51.6) for 100 eggs per plant but only $28.7 \pm 2.70\%$ (4.8–64.8) for 150 eggs per plant (t-test, $df = 56$, $P < 0.01$).

There was no difference in the eclosion success of eggs under conditions of the colony or incubation (t-test, $df = 8$, $P > 0.9$). Mean hatching success was $90.85 \pm 2.35\%$ (81.28–97.37) and $90.39 \pm 2.81\%$ (77.88–96.69) for the colony and incubation, respectively. The conditions of the colony were therefore optimal for the successful emergence of first instars.

Discussion

The small-scale rearing unit offers an alternative for small research groups addressing the biology of different spittlebug species through in situ studies. With minor modifications the unit should be practical and effective for maintaining life stages of different grass-feeding spittlebug species and genera year round.

As originally conceived (version 3), the unit does not have a high efficiency. Only 28.5% of eggs emerged as adults probably as a consequence of inferior conditions for root

and nymph development. But when roots and nymphs were protected under more favorable conditions of light and humidity (version 2), survival was enhanced and efficiency improved to 60%. Yet when adults were lured to an adjacent oviposition chamber with a low light gradient, efficiency fell to 18.9%. From these results it is concluded that the most superior design for combining nymphal development with adult oviposition sites would be the wooden frame in tandem with an oviposition chamber that was walled with white material instead of shade cloth. The wood frame would provide superior conditions for nymph development and root maintenance, while the higher light gradient would lure more adults to the oviposition substrate. Such a unit would be of moderate cost (US\$40–50) and require little space (0.5 m²) and management. Further advances in efficiency could be achieved through more effective manipulation of root quality, number of eggs infested, and light conditions.

The improved mass-rearing colony is a more effective tool than the traditional CIAT colony for massive screening required in studies of host plant resistance and fungal entomopathogens. The improved design requires less space, soil and labor, yet maintains a similar efficiency. Production of 1002 adults/m² per generation is about 1.25 times the production obtained by the traditional colony. About 15 person-hours are required per week to maintain two boxes, but economy of scale would allow a doubling of adult production with five additional hours a week, largely dedicated to transplanting material from the field. With practice, the variation in adult production from one cohort to the next is low, thereby ensuring a continuous and dependable supply of insects for evaluation. Adult emergence is sufficiently synchronous that in a single week period 70.5% emerge.

An additional advantage is that the emergence cage makes new adults easier to capture. The improved design may also suffer less from pests, diseases and other contamination. Over a one-year period the traditional colony experienced several isolated outbreaks of fungal entomopathogens, whereas the improved colony experienced none. The particular temperature and humidity conditions of the covered pots housing nymphs in the traditional colony may be better for propagation of pathogens. Other occasional pest problems included toads, spiders and earwigs; ants were largely excluded when legs of the tables were put in water traps.

The time of nymphal development is only 2–4 d longer than the traditional method. Biological studies carried out under the conditions of the traditional pot method (mean daily temperature $26.2 \pm 1.5^\circ\text{C}$ and RH $84.8 \pm 3.0\%$) showed a mean nymph duration of 30.8 d for *A. varia* (CIAT 1999) compared to 36.1 d in the improved design, which included the 1–3 d for eclosion after infestation. It is expected that certain minor modifications would be required to tailor this improved methodology to other species of grass-feeding spittlebugs. *Zulia carbonaria* (Lallemand), for example, is more than twice the size of *A. varia* and should be infested at lower rates to obtain the highest efficiency. Variations of the rearing unit, and the traditional and improved mass-rearing designs have already been used successfully at CIAT for additional Colombian species including *Mahanarva andigena* (Jacobi),

Mahanarva trifissa (Jacobi) *Prosapia simulans* (Walker), *Z. carbonaria*, *Zulia pubescens* (Fabricius) and *Zulia* sp.

In the mass-rearing colony, increasing the efficiency of production depends on fine-tuning levels of egg infestation and adjusting fertilizer additions in consideration of host plant deterioration. Elevating egg infestation from 100 to 150 per plant did not increase overall adult production significantly but did decrease the efficiency of adult production. Under the high infestation level, plant vigor was drastically reduced by the time nymphs developed to fifth instars and many individuals were left to wander apparently without adequate feeding sites. Fifth instars quickly and successfully established on fresh plant material when the most damaged 1–2 plants were replaced with new plants removed from their pots. Late instars of *A. varia* perform well at the base of plant stems rather than roots so the new material appeared to increase survivorship until production of the teneral spittle mass and emergence of the adults. Additional gains in efficiency could be obtained through improved infestation techniques that more evenly distribute eggs on the available roots. A promising technique is temporarily drying eggs and applying them to the base of the host plant with a salt shaker or other applicator with small holes (R. Pareja, CIAT, personal communication)

A major bottleneck that remains in streamlining the mass-rearing is the collection of adults for transfer to oviposition chambers. Although adults can be collected much more efficiently from under a single emergence cage in the improved design, rather than from many individual pots as in the traditional design, the process is still a laborious. Continued improvements in spittlebug mass-rearing will therefore include features such as (1) light gradients to lead adults from nymphal development sites to oviposition sites, (2) a technique to evenly distribute eggs onto the soil and roots, (3) a protocol to terminate egg diapause thereby utilizing the proportion of eggs that are discarded because of delayed development, and (4) any other technique to increase the rate and synchrony of development.

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