A method for continuous rearing of grapevine leafhoppers, *Erythroneura* spp. (Hemiptera: Cicadellidae)

J. Saguez, C. Vincent

Abstract—We report a two-step method to rear three leafhopper species, *Erythroneura elegantula* Osborn, *Erythroneura vitis* (Harris), and *Erythroneura ziczac* Walsh, for experimental purposes on grapevines. The first step involves rearing adults on grapevines for reproduction, oviposition, and egg development. The second step involves rearing nymphs on isolated leaves placed in Petri dishes lined with agar. No mortality was observed and adults emerged in about three weeks. The three *Erythroneura* species were reared for 10 consecutive generations with minimal resources.

Résumé—Nous rapportons une méthode en deux étapes pour élever trois espèces de cicadelles, *Erythroneura elegantula* Osborn, *Erythroneura vitis* (Harris), et *Erythroneura ziczac* Walsh, aux fins d'expérimentations sur vigne. La première étape consiste à élever les adultes sur des plants de vigne, pour permettre la reproduction, la ponte, et le développement des œufs. La seconde étape consiste à élever les nymphes sur feuilles de vigne isolées en boîtes de Pétri contenant de l'agar. Aucune mortalité n'a été observée et les adultes ont émergé en environ trois semaines. Les trois espèces d'*Erythroneura* ont pu être élevées avec succès pendant 10 générations consécutives avec un minimum de ressources.

Species of *Erythroneura* Fitch (Hemiptera: Cicadellidae) are the most commonly captured leafhoppers in Canadian vineyards and in several states in the United States of America (Metcalf 1968; McKenzie and Beirne 1972; Wells and Cone 1989; Paxton 1990; Martinson and Dennehy 1995; Bostanian *et al*. 2003; Costello 2008). Owing to their abundance, they are important pests of grapevines, *Vitis vinifera* L. (Vitaceae), capable of inducing serious losses. *Erythroneura elegantula* Osborn (western grape leafhopper), *E. vitis* (Harris), and *E. ziczac* Walsh are mesophyll feeders that induce chlorosis, dehydration of leaves, reduction of photosynthesis, and early leaf drop. Heavy infestations of leafhoppers can defoliate grapevines, sometimes causing mortality (Cate 1975). Although *Erythroneura* spp. have been reared on whole plants for experimental purposes (Paxton 1990; Olsen *et al.* 1998), no information has been published on extended laboratory rearing. Likewise, other cicadellids that attack grapevines, such as *Scaphoideus titanus* Ball or species of *Homalodisca* Stål, have only been reared on whole grapevines (Lessio *et al*. 2009) or other host plants (Dugravot *et al.* 2008; Lauzière and Sétamou 2010) for short periods.

During experimentation with *Erythroneura* spp., we required a constant supply of physiologically synchronized individuals for more than a year. Initially, we reared leafhoppers on whole grapevines grown in greenhouses, but this was tedious and costly. High densities of leafhoppers quickly damaged grapevines and required much plant material. In this study, we report a two-step method that allows the rearing of several generations of three *Erythroneura* spp. throughout the year under controlled conditions.

To establish colonies, in August 2008, adult leafhoppers (mixed species) were collected with a sweep net in a commercial vineyard in Dunham (45°06′N, 72°51′W), Quebec, Canada, and

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transported to the laboratory in plastic bags containing grapevine leaves. In the laboratory, the leafhoppers were released on one-year-old grapevine plants ('Seyval Blanc') in a Plexiglas cage (50 cm × 50 cm × 50 cm, with one side screened) in a controlled environment chamber (23 ± 0.5 °C, 16 h light at 1400 ± 10 lm/m^2 : 8 h dark, 60% ± 2% humidity). The plants were watered three times a week.

Approximately three weeks later, nymphs emerged from eggs that had been laid on the leaves. We separated specimens based on morphological characteristics (size and color). Specimens of the three most abundant *Erythroneura* species (subsequently identified as *E. elegantula, E. vitis*, and *E. ziczac*) were transferred to grapevine plants in other cages. For each species, samples of nymphs and adults were sent for identification to Agriculture and Agri-Food Canada (Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario). Our specimens of *E. elegantula* are the first recorded in Quebec vineyards.

Because high densities of leafhoppers quickly reduced the vitality of grapevines and, consequently, nymphal mortality increased, we developed a novel two-step method to rear grape leafhoppers. The first step (as described above) was based on whole grapevines grown in pots enclosed in cages. Adults were released on plants for mating and oviposition. In the second step, nymphs were transferred onto leaves placed in Petri dishes for development to adults.

The rearing conditions on plants favoured mating and oviposition. Photoperiod is an important factor for gonad development and mating with photophases <14 h inducing reproductive diapause (Wells and Cone 1989). High humidity and plant irrigation reduce sensitivity of *Erythroneura* specimens to water stress (Costello 2008). When grapevines appeared unhealthy, leaves were removed and branches and stems were pruned. The pruned plants were thoroughly washed to eliminate leafhopper honeydew and eggs laid on the woody parts or in the soil. The plants regenerated in a few weeks under greenhouse conditions.

Most eggs of all three species were laid on the abaxial surface of leaves. Eggs of *E. ziczac* were laid in clusters of three to five, as observed by Wells and Cone (1989). Despite high leafhopper density or plant dehydration, eggs were always surrounded by undamaged green tissue. Eggs of *E. vitis* were laid individually on leaf ribs; each was covered by a brownish capsule. Eggs of *E. elegantula* were also laid singly but were difficult to observe owing to the absence of surrounding green tissue or covering capsules (Wells and Cone 1989).

Upon emergence, nymphs were transferred to a prepared Petri dish (90 mm diameter). First, agar powder (Anachemica Canada Inc., Montréal, Quebec, catalog No. 02116-300) was dissolved in distilled water at a concentration of 0.7% (*w*/*v*) and boiled until complete dissolution. Then, 25 mL of this solution was poured into the bottom of a Petri dish. When the agar started to solidify, a grapevine leaf was deposited on its adaxial surface. Using a brush, approximately 30 neonates (nymphs ≤24 h) were transferred onto the abaxial surface of the leaf. The Petri dish cover was lined with a filter paper (No. 1, 90 mm diameter, Whatman®, Maidstone, England) and placed over the Petri dish. The closed Petri dish was inverted so that the filter paper was beneath the leaf and could collect honeydew and humidity by gravity. Filter papers were changed daily and nymphs were transferred to new leaves in new Petri dishes weekly. Upon emergence, adults were released onto a new plant to allow oviposition.

We determined the nymphal development time (period between egg hatching and adult emergence) for the three species of *Erythroneura*. The development of at least 60 neonates per species was recorded daily. In contrast to Wells and Cone (1989), who reported mortality associated with molting, all nymphs reared using our method survived. *Erythroneura elegantula*, *E. vitis*, and *E. ziczac* had average nymphal developmental times of 21.2 ± 1.4, 20.5 ± 2.0, and 20.0 ± 1.5 days, respectively. Our results are in agreement with Olsen *et al.* (1998) who reared leafhoppers on whole grapevines under different temperatures and recorded nymphal developmental times of 18.0 ± 1.0 days at 25 °C and 26.5 ± 1.9 days at 21 °C for *E. elegantula* and 16.9 ± 1.4 days at 25 °C and 24.3 ± 1.4 days at 21 °C for *E. ziczac*. The best criterion to calculate instar duration is the presence of exuviae. Unfortunately, we

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were unable to count exuviae because they were often lost, especially at the beginning of nymphal development; only the exuviae of the last instars were fixed to the leaves.

Our method allowed continuous rearing of specimens of three *Erythroneura* species from August 2008 to May 2010 (*i.e.*, over 10 generations with minimal resources). Transfer of leafhoppers to Petri dishes containing agar prevented dehydration of the whole plant leaves. We used this method to physiologically synchronize *Erythroneura* individuals for laboratory studies. This method also can be used to isolate individuals when several species of leafhopper are collected from the field or to start new colonies after colony contamination.

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