

Protocol & Techniques

Breeding technique of *Selenothrips rubrocinctus* (Giard, 1901) (Thysanoptera: Thripidae) on roses leaflets

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Abstract. The development of adequate methods for maintaining populations of arthropod organisms in the laboratory has been a challenge due to the characteristics of each species. This work has aimed to define a method for breeding *Selenothrips rubrocinctus* (Giard, 1901) in rose leaflets in order to study this species in the laboratory. A condition which could maintain the leaflets turgor for a longer time was sought, in order to guarantee both the survival and multiplication of the insects, and less influence of abiotic factors. Four types of substrates were tested: a) a filter paper disk moistened with distilled water covering the bottom of a Petri dish and; b) a vegetable sponge moistened with distilled water surrounding the base of the leaflet; c) a potato, dextrose and agar (BDA) in a microcentrifuge tube surrounding the base of the leaflet; and d) hydrogel in a microcentrifuge tube surrounding the base of the leaflet. The filter paper moistened with distilled water allowed 65% of the leaflets to remain turgid over a 10-day period and was the most suitable substrate for thrips breeding. With the results at hand, we described *S. rubrocinctus* breeding in the laboratory. The adopted methodology provided the population density stability of the bred insects, as well as the obtainment of specimens of *S. rubrocinctus* in quantity and quality throughout the entire period of development of studies on the biology of the species.

Keywords: Red-banded thrips, insect multiplication, cut flowers, mass rearing.

Thrips *Selenothrips rubrocinctus* (Giard, 1901) (Thysanoptera: Thripidae) has its origin in African origin but is found worldwide. It is a polyphagous species known as cocoa thrips or red band thrips and it is responsible for high defoliation in crops (Denmark & Wolfenbarger 2016). In Brazil, its occurrence has already been recorded for more than twenty botanical species (Mascarenhas et al. 2016) and, in the state of Bahia, this thrips was found in *Rosa chinensis* (Rosaceae) (Mascarenhas et al. 2016). More recently, in 2019, an infestation by this thrips was identified in rose bushes (*Rosa* spp.) of the cultivars Avalanche and Príncipe Negro which were grown in a greenhouse, in Lavras, Minas Gerais. The rapid population increase of the species in both cultivars allowed us to infer about its adaptive capacity in these hosts and to suspect their potential to reach the crop pest status (Dos Santos et al. 2021). There is still little information in the scientific literature regarding the association of thrips with rose bushes (Mascarenhas et al. 2016). Since it is a species likely to become a pest in this crop, studies on its development and reproductive cycle are important and will serve as a basis for research involving control alternatives for use in management programs.

One of the main challenges in researches which require a large number of insects refers to their multiplication in the laboratory and, in most cases, the breeding methodology is the main bottleneck. Thus, the establishment of a breeding protocol is essential when seeking speed, practicality, and continuous production of insects for experimental purposes (Parra 2015). According to Oliveira et al. (2010) and Cohen (2015), the great diversity of species and the intrinsic particularities of each one of them make this stage of scientific research even more exciting.

For the development of an appropriate methodology for the breeding of a species in the laboratory, the adequacy and quality of the

food to be offered, environmental conditions such as temperature and relative humidity of the air, photoperiod and suitability of the recipients for the breeding of pre-imaginal and adult phases must be considered (Parra 2015). When it comes to the rearing of phytophagous insects, as the thrips, that feed on leaf sap, the degree of difficulty in defining the protocol for in vitro rearing is even greater (Da Silva et al. 2019), and it is especially linked to the aggravations arising from the need for development of artificial diets. In addition, an appropriate methodology must predict the possibility of obtaining a sufficient number of quality specimens for the purpose of the researches, with standardized age, size and sex ratio, as well as the health of the individuals produced. Methodology of breeding for the thrips *S. rubrocinctus* have been relatively little explored so far.

The scarcity of information about *S. rubrocinctus*, mainly associated with rosaceas, has strongly motivated us to establish a method for the breeding of this thrips in roses (*Rosa* spp.), under laboratory conditions. The objective has been to define a way to enhance the longevity of leaflets in an artificial medium by maintaining their turbidity and healthiness for a longer period. The conservation of these characteristics in vitro is relevant since the leaflets are used as oviposition and feeding substrate for thrips and, therefore, cannot compromise researches on the insect's life cycle. The successful maintenance of these conditions implies obtaining data closer to reality regarding the survival and multiplication of the species, with less influence of abiotic factors.

The study has been conducted at the Laboratório de Controle Biológico com Entomófagos (LCBE) of the Department of Entomology (DEN)/Escola de Ciências Agrárias de Lavras (ESAL)/Universidade Federal de Lavras (UFLA), Lavras (MG), at 25 ± 1 °C, 70 ± 10% RH and 12h photophase, with lighting from 9W LED lamps with 6.5000K color

temperature.

In a first step, a bioassay was carried out to define the most suitable substrate for maintaining both the turgor and durability of the rose leaflets after being removed from the plant. Cultivar Avalanche leaflets, taken from the middle or upper third of the rose plant were used.

Four substrates were tested: a) a filter paper disk moistened with distilled water covering the bottom of a Petri dish (10 cm x 1 cm); b) a piece of vegetable sponge moistened with distilled water surrounding the leaflet base; c) a potato, dextrose and agar (BDA) stored in a microcentrifuge tube and surrounding the leaflet base; and d) hydrogel placed in a microcentrifuge tube and surrounding the leaflet base (Fig. 1). A completely randomized experimental design (DIC) with 20 replications was used. The leaflets conservation status was monitored over ten consecutive days, as this period corresponds to the average duration of the embryonic stage of *S. rubrocinctus* (Soesanthy et al. 2012).

Quality states were attributed to the leaflets. We took into consideration: I) turgid leaflet: the one that maintained its turbidity and green color, that is, in the state close to that collected from the plant; II) discolored leaflet: the one that showed partial or total loss of turbidity and green color (yellowish color); III) dry leaflet: the one whose state varied from dry to brittle; and IV) fungal leaflet: the one with the beginning of saprophytic fungal developmental structures (Fig. 2).

The data obtained for the time elapsed from the setting up the experiment until the beginning of leaf turgor loss were analyzed by survival analysis (Survival Model in Statistic). We used Log-logistic model estimators. Turgor curves for each tested substrate were compared by contrast ($p < 0.05$). The free software "R Statistics" (R Core Team 2020) was used in the analyses. In this evaluation, only the turgid and discolored states were considered since the others were not suitable for use in the *S. rubrocinctus* breeding.

From the results obtained in the test to define the substrate for the breeding of thrips, it was found that the filter paper moistened with distilled water was the most suitable for providing better leaflets conservation and turbidity maintenance of 65% of them for up to ten days of evaluation ($\chi^2 = 29.33$, $df = 3$, $p < 0.001$). The others (35%) were discolored at the end of the period evaluated.

The vegetal sponge caused the beginning of the leaflets yellowing from the 5th day (75%) and, at the end of ten days, the discolored ones reached a percentage of 85%, 5% moldy and only 20% turgid. The medium composed of potato, dextrose and agar (BDA) and the hydrogel were inadequate for the leaflets maintenance for the studies of the life cycle of *S. rubrocinctus*. The BDA caused discoloration on the first day and, at the end of the evaluation period, only 25% of them were turgid, 15% were discolored and 60% were dry. In the hydrogel, on the 9th evaluation day, 100% of discolored leaflets and 70% of dry ones were added on the tenth day (Fig. 3).

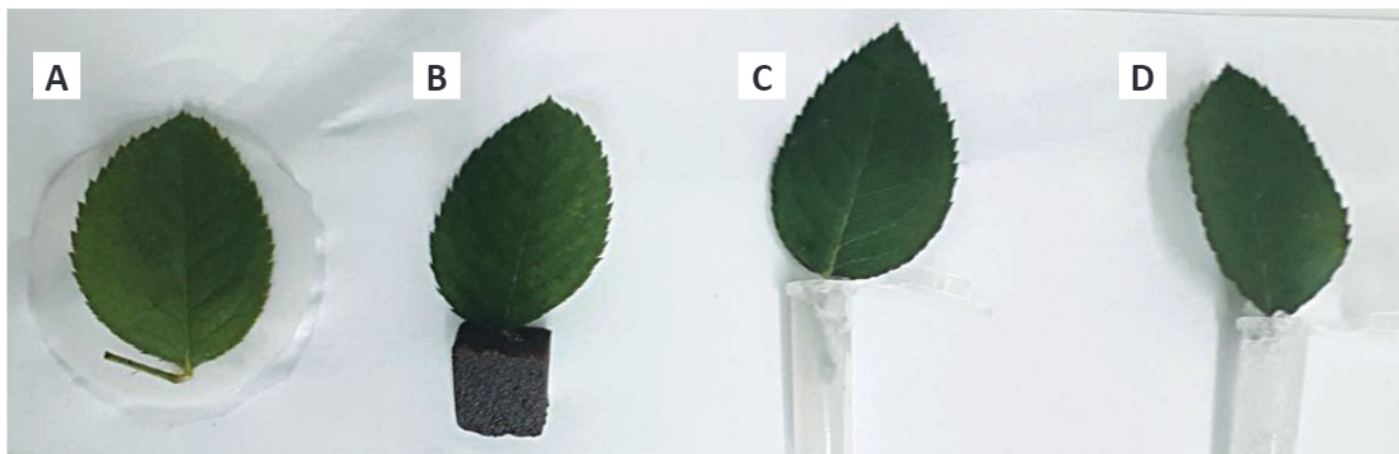


Figure 1. Tested methods for obtaining the most suitable substrate for the maintenance of leaflets of rose cultivar Avalanche in laboratory. **A.** Treatment 1 - Wet filter paper; **B.** Treatment 2 - Vegetable sponge; **C.** Treatment 3 - Potato dextrose agar medium; **D.** Treatment 4 - Hydrogel.

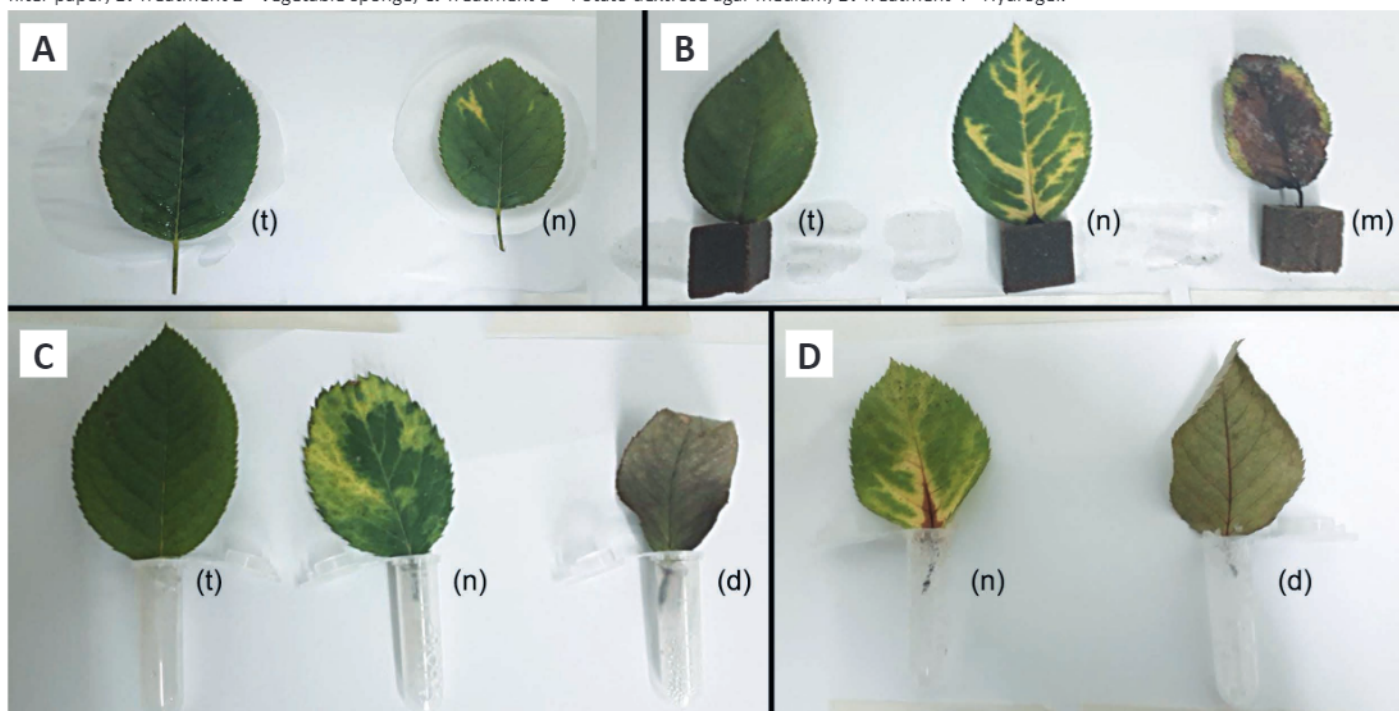


Figure 2. Leaflets on the 10th evaluation day, according to the methodologies tested to define the most suitable substrate for the maintenance of leaflets of rose cultivar Avalanche in laboratory. **A.** Treatment 1 - Wet filter paper; **B.** Treatment 2 - Vegetable sponge; **C.** Treatment 3 - Potato dextrose agar medium; **D.** Treatment 4 - Hydrogel. (t) turgid; (n) nutrient deficiency - (discolored); (m) moldy; (d) dry. Environmental conditions: 25 ± 2 °C, 70 ± 10 % RH and 12h photophase.

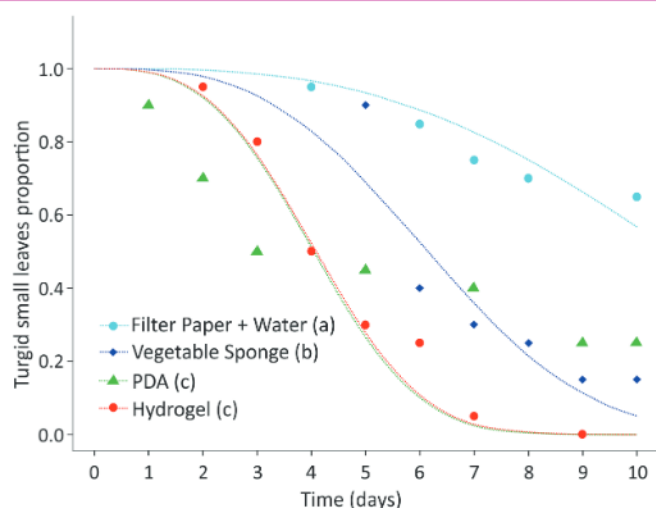


Figure 3. Conservation status of rose leaflets cultivar Avalanche based on the proportion of discoloration caused over ten days of evaluation. Each color represents a treatment. Values followed by the same letter have no significant difference by the contrast test ($p < 0.05$). Environmental conditions: $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 12h photophase.

Upon identifying that the filter paper moistened with distilled water was the most effective substrate (in addition to being economical) for rose leaflets maintenance, the breeding of *S. rubrocinctus* was carried in the laboratory. Petri dishes (15 x 1.5 cm) containing leaflets collected from the middle or upper thirds of roses of the cultivar Avalanche were used. The leaflets were placed on two superimposed discs of filter paper (150 mm), and moistened daily with distilled water, until saturation. To avoid possible contamination by microorganisms, the plant material was disinfected with sodium hypochlorite (0.5%), washed in distilled water and dried with a paper towel before being used in the breeding.

The breeding maintenance was carried out every 72 hours as well as the leaflets replacement by newly collected ones, for the stages of nymphs, pre-pupa, pupa and thrips adults, kept in rearing plates separately. The egg-laying leaflets were maintained until the nymphs hatch, with an average embryonic period of 10 days, making this breeding methodology relevant, as it identifies the better substrate in order to keep the leaves turgid for all the necessary period. The leaflets with eggs come from oviposition carried out by females in the pretri dishes (Fig. 4).

To monitor the efficiency of the applied method, specimens of *S. rubrocinctus* were watched daily through the observations of each development stage, change processes, as well as the sanitary status of the plates. The monitoring of the embryonic period was possible to determine as a result of the female *S. rubrocinctus* releasing a darkened secretion on the posture performed, emphasizing that this is the crucial phase for the success of the adopted methodology. And based upon identification of the phases of *S. rubrocinctus* life cycle, in addition to the presence of exuvia, the following characteristics were observed (Fig. 5): a) from the 1st to the 2nd instar: the sharp red color of the belt present in the first two uromers (the color of the belt in the second uromer is not so prominent in the 1st instar); b) from 2nd instar to pre-pupa: the position of the antennae, which face forward, and the developing wings are noticeable; c) from pre-pupa to pupa, the antennae are turned backwards; d) from pupa to adult, individuals take on a dark coloration. Simultaneously, we evaluated the mean development time, in days, of the egg stages (11 ± 0.10), 1st instar (1.5 ± 0.05), 2nd instar (5 ± 0.11), pre-pupa (1 ± 0.00), pupa (2 ± 0.05) and adult stage (10 ± 0.50).

Through the monitoring of subsequent generations, the adopted methodology has proved to be suitable for the breeding of thrips, because the aforementioned method has provided the means to increase its breeding and keep it in good health conditions as well as promoting the multiplication of *S. rubrocinctus*, and consequently its use in research work.

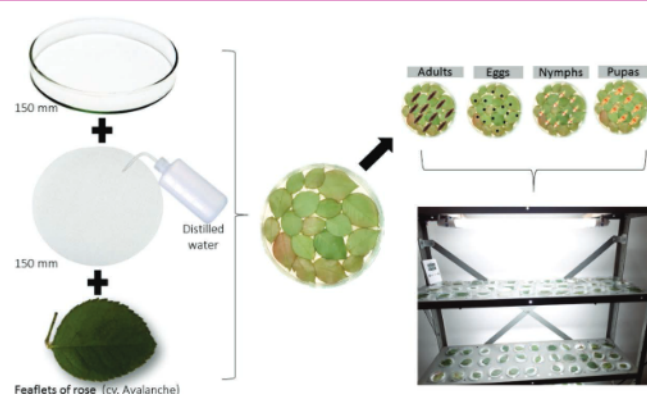


Figure 4. The setting of immature and adult *Selenothrips rubrocinctus* reared on rose cv. Avalanche. Environmental conditions: $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 12h photophase.

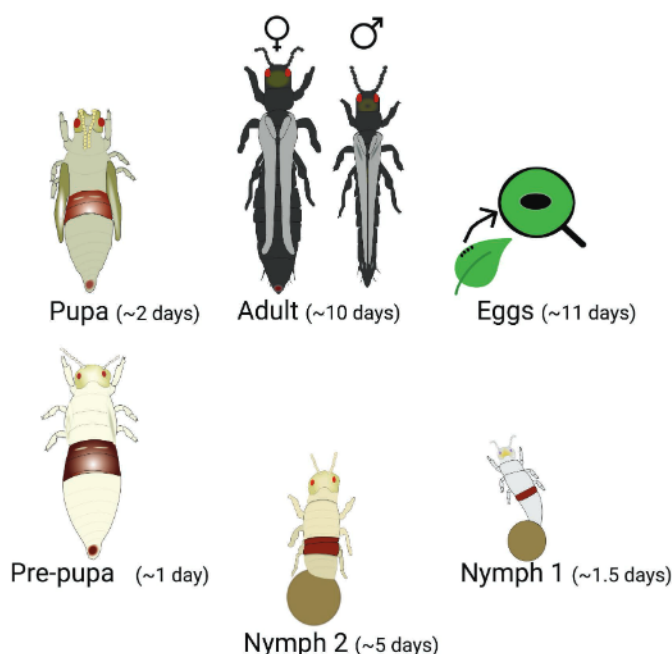


Figure 5. Life cycle of *Selenothrips rubrocinctus* bred on rose cv. Avalanche. Environmental conditions: $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 12h photophase.

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Authors' contributions

All authors contributed to the conception and design of the study. Preparation of material, insect rearing and cultivation of rose bushes, data collection, entry of the first version and final manuscript formatting were carried out by JRS. Guidance on conducting experiments and correcting previous versions of the article by BS. Guidance, tabulation and statistical analysis of the data, interpretation and correction of previous versions of the manuscript were carried out by MMPPH. Aid in the preparation of material, insect rearing and cultivation of rose bushes by LGS. Aid in the identification of the stages of the life cycle of *S. rubrocinctus* by LCPS. All authors read and approved the final manuscript.

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