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SHORT COMMUNICATION

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Continuous indoor rearing of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*

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Abstract

The phytosanitary emergence triggered by *Xylella fastidiosa* introduction in Europe urgently calls for research on its main vector, the meadow spittlebug, *Philaenus spumarius*. The difficulties faced in altering *P. spumarius* life cycle setting up a continuous indoor rearing under artificial conditions, obtaining a continuous availability of insects for laboratory trials, represent a great limit for research. Here, we propose a methodology to rear *P. spumarius* in the laboratory allowing the supply of nymphs and adults before they become available in the field. This crucial step forward will permit to overcome the seasonality barrier, reducing time and efforts currently required for experimentation on the meadow spittlebug. The proposed methodology would allow producing the data urgently required to fill the knowledge gap and finally set up an effective and environmentally friendly control strategy of *P. spumarius*.

1 | INTRODUCTION

European entomological research is spending great efforts to gather essential data for managing an insect that rose from its condition of almost a naturalistic curiosity to become one of the major agricultural threats throughout the Old World: the meadow spittlebug *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae). Indeed, the spittlebug is to date considered the main vector of the bacterium *Xylella fastidiosa* (Wells, 1987) to olive and other bacterium host plants in the South Italian outbreak (Cornara et al., 2016, 2017) and in other Mediterranean countries where *X. fastidiosa* is present, such as Spain and France (Cruaud et al., 2018; Morente & Fereres, 2017). Spittlebugs are widely spread in Europe as opposed to sharpshooters, which are the major vectors of *X. fastidiosa* in the Americas (EFSA, 2015). *Xylella fastidiosa* epidemics in Europe are causing devastating economic, environmental and socio-anthropological effects (Almeida, 2016; Colella, 2016; Martelli, Boscia, Porcelli, & Saponari, 2016).

Effective containment of bacterial spread strongly relies on vector management, and, therefore, on a detailed knowledge of the vector itself (Almeida, Blua, Lopes, & Purcell, 2005). Nevertheless, biological, ecological and behavioural studies about *P. spumarius* are currently scattered and continuous research efforts are urgently needed (Cornara, Bosco, & Fereres, 2018). However, a major limitation for conducting research on *P. spumarius* is the difficulty in continuously rearing the spittlebug under controlled conditions. Field and laboratory trials, especially those focusing on spittlebug management, imply the use of a high number of individuals. Currently, efforts for continuous rearing of the meadow spittlebug in indoor conditions have failed, and research activities are limited to the period when the insects become available in the field or in enclosed environments under natural conditions (meso or macrocosmos) (Plazio et al., 2016). Furthermore, extensive field-collection of nymphs is costly, time-consuming and risky (high mortality rate during transport from the field and acclimation to laboratory conditions). *Philaenus spumarius* is a univoltine species that undergoes to obligate separate ovarian and overwintering diapause (Müller, 1979). Several authors have tried to qualitatively and quantitatively understand the effect of different climatic variables on oviposition, eggs hatching and nymphal development (Chmiel & Wilson, 1979; Masters, Brown, Clarke, Whittaker, & Hollier, 1998; Medler, 1955; Weaver & King, 1954; West & Lees, 1988; Zajac, Hall, & Wilson, 1989). To the best of our knowledge, only Stewart and Lees (1988) succeeded in breaking the diapause and shortening the life cycle of the meadow spittlebug, by reducing day length and temperature. However, all the data and methods previously reported are heterogeneous and inconsistent

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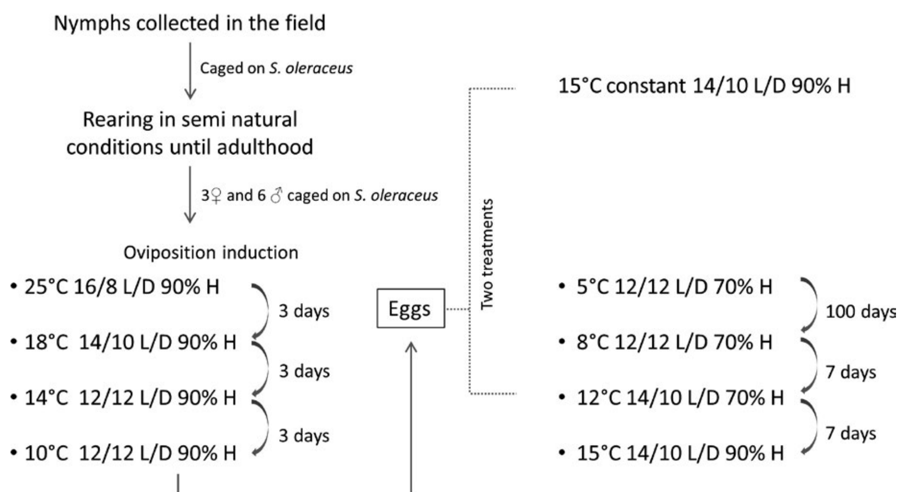

FIGURE 1 Methodology of rearing of *Philaenus spumarius* under climatic controlled conditions

and need to be further investigated (Cornara et al., 2018). Therefore, our main goal was to develop a continuous indoor-rearing protocol that could facilitate studies on the biology, ecology and management of *P. spumarius* and the transmission of *X. fastidiosa*. Here, we report our results on ovarial and overwintering diapause breakage for a first *P. spumarius* rearing method under controlled laboratory conditions.

2 | MATERIAL AND METHODS

Last instar of *P. spumarius* nymphs were collected in Constantina (Spain) in May 2017 on the Asteraceae: *Picris echioides*, *Sonchus oleraceus*, *Crepis* sp., *Ditrichia* sp. and *Calendula arvensis*, the Umbelliferae *Daucus carota*, the Fabaceae *Medicago* sp., and the Boraginaceae *Echium plantagienum*. Nymphs were caged in plastic and mesh cages on 3-week old *S. oleraceus* L. plants (20 nymphs/plant) until adulthood. *Sonchus oleraceus* plants came from seedlings reared in a growth chamber under a temperature of 24–20°C (L/D) and a photoperiod of 16–8 L/D. The cages were maintained in a screenhouse without temperature or humidity control (from now on called semi-field conditions). Once emerged, adults were sorted in small mating groups (six males and three females), and transferred to *S. oleraceus* plants (one group per plant) with the pot substrate covered with dry pine needles as straw in order to increase the probability of oviposition (Weaver & King, 1954). Thereafter, once at the beginning of June (three replicates), and once in late August (four replicates), we carried out the oviposition induction tests in the laboratory. Briefly, the cages were transferred to an environmentally controlled growth chamber (Sanyo MLR-351H) with an initial constant temperature of 25°C and photoperiod 16/8 L/D. Temperature and photoperiod were gradually reduced every 3 days until reaching 10°C and photoperiod 12–12 L/D (as shown in Figure 1). The eggs obtained were subjected to two different hatching induction conditions: in the first treatment, the eggs were stored at a constant temperature of 15°C and photoperiod 14/10 L/D; in the second, the eggs were exposed to 5°C and photoperiod 12–12 L/D during 100 days, with a successive gradual temperature and photoperiod raise until reaching 15°C and photoperiod 14–10 L/D. Humidity inside the growth chambers was kept as high as possible during the entire process (close to 100%). The remaining insects caged in semi-field conditions (Table 1) were used as control. The protocol is graphically described in Figure 1.

3 | RESULTS

We consistently succeeded in inducing oviposition in both June and September under growth chamber conditions. Oviposition occurred 2 days after adult exposure to 10°C and photoperiod 12–12 L/D. Eggs laid in September successively exposed to the 5°C treatment hatched at the beginning of January. The resulting nymphs were caged on 2 week-old *S. oleraceus* plants, with the plants replaced weekly, and exposed to 15°C, photoperiod 14–10 L/D, and 100% humidity. The first adults emerged in mid-February. Therefore, we

TABLE 1 Rearing conditions for cages in semi-field (from May 2017 to February 2018)

Month	Mean T (°C)	Max T (°C)	Min T (°C)	HR (%)
May	19.89	26.72	11.5	43.67
Jun	26.37	33.57	17.74	34.13
Jul	26.77	34.07	17.57	34.19
Aug	26.55	33.6	17.97	34.77
Sep	20.89	28.28	12.59	38.9
Oct	17.6	25.95	9.39	49.67
Nov	8.81	16.33	2.52	61.76
Dec	5.74	11.81	0.21	71.6
Jan	5.94	11.91	0.96	79.12
Feb	5.4	11.07	0	64.39

succeeded in shortening the completion of *P. spumarius* life cycle of 3 months compared to natural conditions in Central Spain (Morente, Moreno, & Fereres, 2017). Conversely, eggs laid in September and exposed to a constant temperature of 15°C, photoperiod 14–10 L/D did not hatch. Similarly, none of the eggs oviposited in June without any following treatments hatched, very likely because of female ovarian parapause (Witsack, 1973).

In semi-field conditions (Table 1), oviposition was observed in the cages starting from the end of October, with a peak in late November-beginning of December, and last oviposition at the beginning of January. First nymphs emerged in mid-February, 1 month and a half later than the nymphs from the hatching induction test. Data on temperature (mean, maximum and minimum) and humidity to which semi-field reared individuals were subjected are reported in Table 1.

4 | DISCUSSION

We succeeded to artificially disrupt both ovarial and overwintering diapause, shortening the life cycle of *P. spumarius* and obtaining both nymphs and adults, several months before they become available in the field. This first approach to the indoor rearing of *P. spumarius* is a crucial step in the development of containment measures of *X. fastidiosa*-related diseases based in the effective control of the meadow spittlebug in the infected zones. Thus, further ongoing experiments aim to determine how many cold days are needed to break egg diapause. Thus, our final aim is to obtain a completely artificial continuous mass-rearing method with at least two complete biological cycles per year. Such methodology would reduce the seasonality dependence speeding up the research process. Finally, the methodology described in this study could be further tested with other species belonging to the Cercopoidea superfamily, considered to be the most important vectors of *X. fastidiosa* throughout Europe (EFSA, 2015).

Until recently, the main interests in *P. spumarius* were to determine the ecological features and the genetic control of its polymorphism. Because *P. spumarius* is one of the main vectors for *X. fastidiosa* epidemics in Europe, it is now considered one of Europe's most important pests (Cornara et al., 2018). Accelerating its rearing will speed up biological research on this insect and develop environmentally and economically sustainable pest control strategies. Many aspects of the spittlebug life cycle are currently unknown. Overall, the methodology presented here represents a useful tool, and a basic step towards mass rearing of the main vector of *X. fastidiosa*. This will facilitate and speed up the *X. fastidiosa* sustainable containment strategy.

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AUTHOR CONTRIBUTION

AF, DC, MM and AM conceived research. MM, DC conducted experiments. MM, DC, AM and AF wrote the manuscript. AF, AM secured funding. All authors read and approved the manuscript.

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