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SHORT COMMUNICATION

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# Parapause breakage as a key step for the continuous indoor rearing of *Philaenus spumarius*

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## Abstract

The development of an efficient indoor rearing protocol of *Philaenus spumarius* is a key step for research on the meadow spittlebug, as it could reduce researchers' dependency on the availability and collection of univoltine individuals in the field. Here, we implemented the rearing protocol previously described by inducing females' parapause breakage before mating. This new step enables the completion of the life cycle of *P. spumarius* (adult to adult) in 149 days, potentially providing three generations per year under laboratory conditions.

KEYWORDS

diapause, life cycle shortening, meadow spittlebug, ovariole, photoperiod

## 1 | INTRODUCTION

Diapause is defined as a physiological state of suppressed development induced by environmental stimuli, mainly temperature and photoperiod (Beck, 1962), allowing the individuals to deal with environmental fluctuations, synchronizing the life cycle with food and optimal climatic conditions availability (Mansingh, 1971; Müller, 1965, 1970).

The univoltine life cycle of *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), the main European vector of *Xylella fastidiosa* (Cornara et al., 2016, 2017), is characterized by two obligate separate ovarian and overwintering diapauses (Avosani et al., 2021; Müller, 1979). The availability of a single generation per year is one of the main constrains for the research dealing with the tripartite interaction vector-bacterium-host plant. Witsack (1973) related the long preoviposition period in *P. spumarius* with a thermally or photoperiodically induced ovarian dormancy. Namely, he observed that short days accelerate ovarian development, while long days slow it down to complete stagnation, also termed parapause. Morente et al. (2018) succeeded in shortening the *P. spumarius* life cycle in 3 months compared to field conditions in Central Spain. The authors of the mentioned study used eggs laid in June and September, obtaining an F1 generation only from the latter. They proposed that the eggs laid in June were likely not fertilized, as female ovaries were still undergoing an ovarian parapause.

Here, we modified and improved the indoor rearing protocol proposed by Morente et al. (2018) by inducing the breakage of females' parapause before they started to mate. Thus, we were able to speed up the life cycle of *P. spumarius*, which was completed in approximately 5 months (from adult to adult).

## 2 | MATERIAL AND METHODS

We initially obtained F0 adult individuals reared following the methodology described by Morente et al. (2018). Newly emerged adults were promptly sorted by sex in order to avoid mating and caged on 3-week-old *Sonchus oleraceus* L. plants (10 adults per plant). The 180 males used in the assay were kept under controlled conditions: 24:18°C (L/D), a photoperiod of 16:8 (L/D) and 60% RH (hereinafter

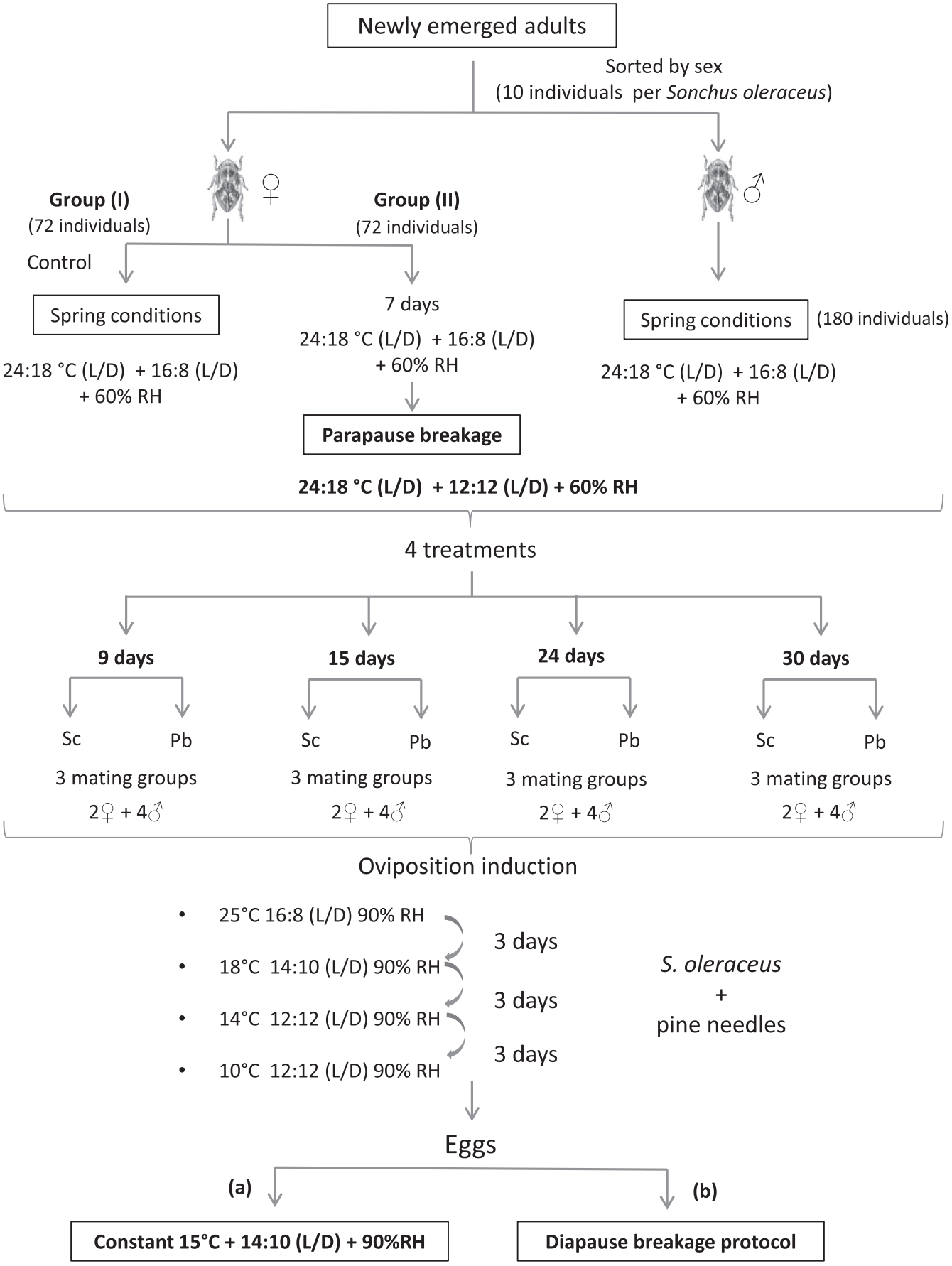


**FIGURE 1** Methodology applied in the study (from newly emerged adults to the temperature treatments applied to the eggs). Sc: spring conditions, Pb: parapause breakage

referred as 'spring conditions') (Figure 1). Immediately after emergence, females were separated in two groups: the group (I) (72 females used as control) was reared under spring conditions, while the group (II) (72 females) was split in three cages (24 females caged on four *S. oleraceous* plants per cage) and subjected to a treatment aimed at inducing the ovarian parapause breakage (hereinafter referred as 'parapause breakage treatment') (Figure 1). Briefly, females of group (II) were kept for a week in a growth chamber under a temperature of 24:18°C (L/D), a photoperiod 16:8 (L/D) and 60% RH. Thereafter, photoperiod was shortened to 12:12 (L/D) while maintaining temperature and humidity constant (Figure 1). Females belonging to each group (I and II) were furtherly split in four treatments (six females per group), each one exposed to the short daylight for a different time period, namely 9, 15, 24 and 30 days. Similarly, males and females under spring conditions were subjected to the treatment short daylight for different time periods (group I: photoperiod 12:12 (L/D) and group II: photoperiod 16:8 (L/D) (Figure 2).

After the short daylight treatment, adults were sorted in mating groups of four males and two females (from the parapause breakage treatment or the spring conditions) and caged on *S. oleraceus* plants (one group per plant) with the pot substrate covered with dry pine needles as oviposition substrate (Weaver & King, 1954). Then, mating groups were subjected to four changes of temperature and photoperiod every 3 days from 25°C and photoperiod 16:8 (L/D) to 10°C and photoperiod 12:12 (L/D), keeping RH constant (90% RH) (Figure 1). Mating groups were kept at 10°C, a photoperiod of 12:12 and 90% of humidity until all the individuals were dead. We repeated the procedure three times (from mid-June to mid-September) with three replicates (cages) for the control group (spring conditions) and three replicates (cages) for each experimental group (parapause breakage) (thus three cages with four males and two females each) per time (defined as 'temporal replicate' in Table 1 and Figure 2).

The egg masses obtained were split in two groups. The group (a) was maintained at 15°C, photoperiod 14:10 (L:D) and humidity close to 90% (Weaver & King, 1954) until eclosion, while the group (b) was exposed to the diapause breakage protocol described by Morente et al. (2018) (Figure 1). Briefly, egg masses were kept for 100 days at 5°C and photoperiod 12:12. Thereafter, temperature and daylight hours were gradually raised to 15°C and 14:10 (L/D) (90% RH maintained constant). Egg hatching was monitored once per week, and the emerged nymphs were moved to a 3-week-old *S. oleraceus* plant (5–10 nymphs per plant) and reared at 15°C, photoperiod 14:10 L/D and 90% RH. All *S. oleraceus* plants used for nymphs were replaced weekly. The adults emerged were caged on 3-week-old *S. oleraceus* plants at 24:18°C L/D of temperature and a photoperiod of 14:10 L/D.

## 3 | RESULTS

Females subjected to the parapause breakage treatment for either 24 days or 30 days produced viable eggs, while females exposed to short daylight for 9 and 15 days did not. We additionally monitored the development of the nymphs obtained from females exposed to the parapause breakage (reduced daylight) for 24 days until adult emergence. In the case of females subjected to the parapause breakage treatment, first egg masses were observed 2 weeks after the formation of the mating pairs. Eggs were laid continuously for a period of approximately 2 months. The eggs maintained at 15°C constant began hatching roughly 2 months after the oviposition, while eggs under a temperature of 5°C for 100 days, then 15°C, took at least 3 months to hatch (Table 1). Data obtained from the short daylight of 24 days with the different rearing protocols tested, that is number of eggs laid and hatched, nymph's survival, and adult's emergence and survival, are summarized in Table 1. Egg hatching occurred progressively in both cases. First adults of *P. spumarius* emerged ca. 5 months after the beginning of the experiment (newly emerged F0 females exposed before mating to short daylight for 24 days) (Figure 3).

On the contrary, the first oviposition by females kept under spring conditions (group (I)) occurred 2 months after the formation of the mating pairs (Table 1). In this case, eclosion took place from 3 to 5 months after the oviposition regardless of the temperature and first adults emerged roughly 5 weeks after hatching. Therefore, when females were kept under spring conditions, the meadow spittlebug required approximately 7 months and 2 weeks to complete its life cycle. Moreover, females exposed to the parapause breakage treatment produced more than twice the eggs than females under spring conditions, with an eclosion rate of 71%, and an adults' emergence of 54% (Table 1).

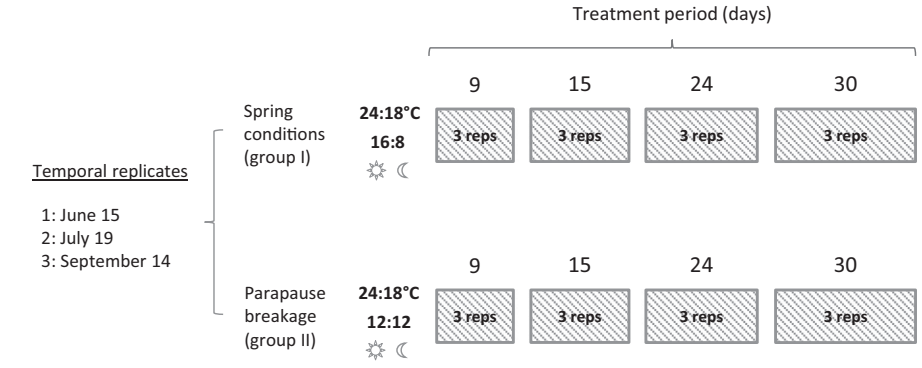


**FIGURE 2** Experimental design. In every temporal replicate (June 15, July 19 and September 14), females of *Philaenus spumarius* were subjected to four short daylight treatments: 9, 15, 24 and 30 days. Three replicates (reps) were used for the group I (spring conditions) and three for the group II (parapause breakage)

**TABLE 1** Number of egg masses, eggs, nymphs hatched and adults emerged for the parapause breakage treatment (photoperiod 12:12 and temperature 24:18 L/D for 24 days) and the spring conditions treatment (photoperiod 14:10 and temperature 24:18 L/D for 24 days)

| | | | No. of egg masses | | No. of eggs | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Parapause breakdown | | Spring conditions | |
| Temporal replicate | No. of females | Date of oviposition | Parapause breakdown | Spring conditions | 15°C | 5°C | 15°C | 5°C |
| 1: June 15 | 6 | July–05 | 2 | 0 | 7 | 5 | 0 | 0 |
| | | July–23 | 4 | 0 | 15 | 17 | 0 | 0 |
| | | July–31 | 4 | 0 | 13 | 14 | 0 | 0 |
| 2: July 19 | 6 | August–07 | 4 | 0 | 30 | 25 | 0 | 0 |
| | | August–28 | 3 | 0 | 4 | 5 | 0 | 0 |
| | | September–14 | 1 | 2 | 7 | 0 | 4 | 3 |
| 3: September 14 | 6 | September–27 | 1 | 3 | 0 | 3 | 9 | 8 |
| | | October–04 | 4 | 2 | 16 | 12 | 9 | 13 |
| | | October–11 | 0 | 3 | 0 | 0 | 6 | 5 |
| Total | | | 23 | 10 | 92 | 81 | 28 | 29 |
| % | | | | | | | | |

*Note:* Egg masses obtained from F0 females were split in two groups, the first stored at 15°C and the second under the diapause breakage conditions (indicated as 5°C) (Morente et al., 2018). Date of hatch includes the first and the last day of egg hatching per date and treatment (15°C and 5° together). Percentages (in bold): eggs hatching rate (no. nymphs hatched/no. eggs laid) and adults emergence rate (no. adults emerged/no. of nymphs) per treatment.

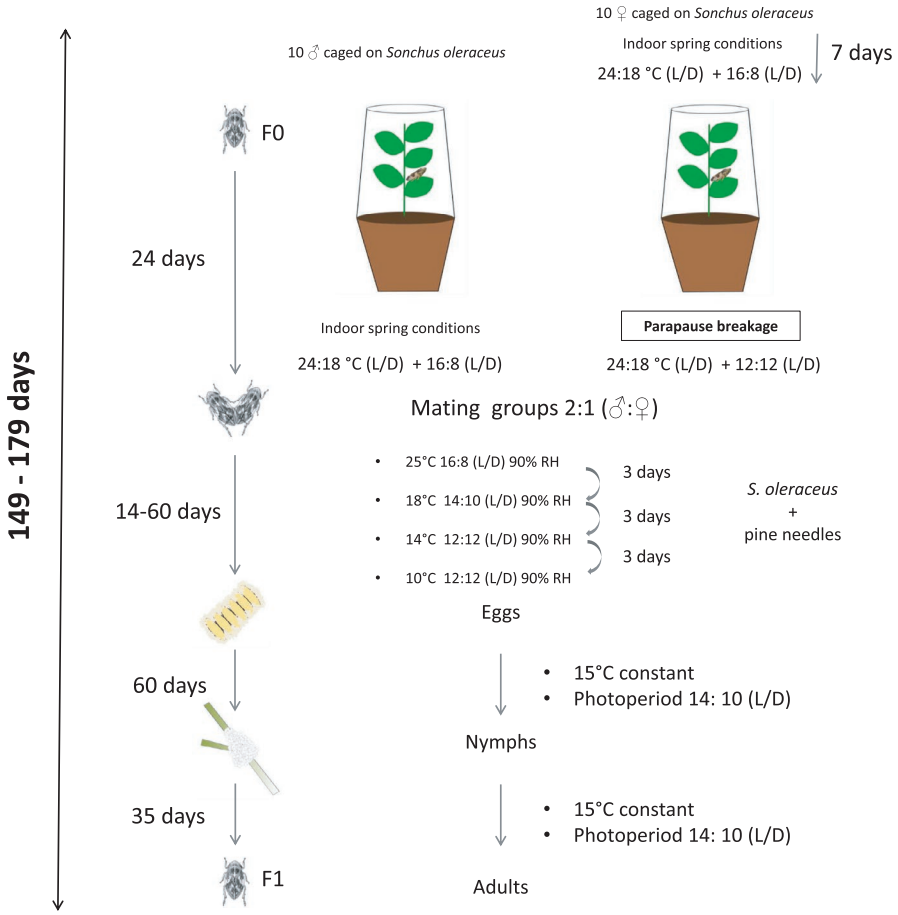


**FIGURE 3** Protocol of indoor rearing of *Philaenus spumarius*. Improvement of the Morente et al., 2018 protocol by adding the parapause breakage and the egg temperature treatment of 15°C constant

| Date of hatch | | No. of nymphs | | | | No. of adults emerged | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Parapause breakdown | | Spring conditions | | Parapause breakdown | | Spring conditions | |
| Parapause breakdown | Spring conditions | 15°C | 5°C | 15°C | 5°C | 15°C | 5°C | 15°C | 5°C |
| October–30/November–20 | — | 7 | 5 | 0 | 0 | 7 | 5 | 0 | 0 |
| October–22/November–16 | — | 15 | 11 | 0 | 0 | 9 | 0 | 0 | 0 |
| October–15/January–01 | — | 13 | 4 | 0 | 0 | 3 | 3 | 0 | 0 |
| October–11/December–13 | — | 25 | 15 | 0 | 0 | 15 | 9 | 0 | 0 |
| October–30/December–05 | — | 1 | 3 | 0 | 0 | 0 | 2 | 0 | 0 |
| October–11/October–22 | Not hatched | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Not hatched | February–07/February–28 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 |
| December–20/January–11 | February–21/February–28 | 2 | 0 | 9 | 11 | 1 | 0 | 0 | 0 |
| — | January–11/February–14 | 0 | 0 | 6 | 2 | 0 | 0 | 2 | 0 |
| | | 66 | 38 | 18 | 14 | 36 | 19 | 2 | 0 |
| | | **71%** | **47%** | **64%** | **48%** | **54%** | **50%** | **11%** | **0%** |

## 4 | DISCUSSION

Here, we demonstrate that the ovarian parapause breakage, that is the exposure of newly emerged females to short daylight conditions for at least 24 days, is a key step in order to shorten the life cycle of *P. spumarius* and obtain more than one generation per year, consistently with previous studies (Witsack, 1973). This protocol permits to remarkably shorten the female's receptiveness to 24 days, while in nature this process lasts several months (Avosani et al., 2021; Weaver & King, 1954). Hence, females are fertile in 31 days and first eggs are laid 40 days after the adults emergence. Additionally, we observed that females exposed to the parapause breakage treatment laid overall more eggs in a shorter time than those not exposed to short daylight conditions, with F1 adults showing a greater survival rate (Table 1).

The protocol proposed by Morente et al. (2018) for the indoor rearing of *P. spumarius* already reduced the time required to complete the spittlebug life cycle in 3 months compared to field conditions observed in Central Spain (roughly 1 year). The addition to the previous protocol of the parapause breakage treatment before mating pairs allowed us to furtherly shorten this time, with one generation (from F0 adult emergence to F1 adult emergence) requiring approximately 5 months for completion (Figure 2) and therefore potentially providing almost three generation per year under controlled conditions. Moreover, in contrast to Morente et al. (2018), we obtained nymphs from the eggs exposed to 15°C. According to Weaver and King (1954), we observed an earlier hatching date of eggs exposed to a constant temperature of 15°C which, hatched 1 month earlier than the eggs under the diapause breakage treatment. Thus, the protocol proposed in this study including the female's parapause breakage and a shorter period of egg development are essential to shorten the life cycle of *P. spumarius*. In addition, a greater eclosion rate was observed for egg masses kept at a constant temperature of 15°C than the diapause breakage treatment.

In conclusion, the protocol of Morente et al. (2018) can be improved by exposing newly emerged females to the parapause breakage treatment and by keeping the egg masses obtained at a constant temperature of 15°C, a photoperiod of 14:10 and 90% RH (Figure 3). Eventually, the progressive overlapping of different generations under indoor conditions may result in a constant supply of nymphs and adults throughout the year.

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### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

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

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in https://drive.google.com/file/d/1_3chqwy0LELjH6VTuib9YevxhV_r3tZX/view?usp=sharing.

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## REFERENCES

Avosani, S., Franceschi, P., Ciolli, M., Verrastro, V., & Mazzoni, V. (2021). Vibrational playbacks and microscopy to study the signalling behaviour and female physiology of *Philaenus spumarius*. *Journal of Applied Entomology*, *145*(6), 518–529. https://doi.org/10.1111/jen.12874

Beck, S. D. (1962). Photoperiodic induction of diapause in an insect. *The Biological Bulletin*, *122*(1), 1–12. https://doi.org/10.2307/1539316

Cornara, D., Cavalieri, V., Dongiovanni, C., Altamura, G., Palmisano, F., Bosco, D., Porcelli, F., Almeida, R. P. P., & Saponari, M. (2016). Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants. *Journal of Applied Entomology*, *141*, 80–87. https://doi.org/10.1111/jen.12365

Cornara, D., Saponari, M., Zeilinger, A. R., de Stradis, A., Boscia, D., Loconsole, G., Bosco, D., Martelli, G. P., Almeida, R. P. P., & Porcelli, F. (2017). Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. *Journal of Pest Science*, *90*, 521–530. https://doi.org/10.1007/s10340-016-0793-0

Mansingh, A. (1971). Physiological classification of dormancies in insects. *The Canadian Entomologist*, *103*, 983–1009. https://doi.org/10.4039/Ent103983-7

Morente, M., Cornara, D., Moreno, A., & Fereres, A. (2018). Continuous indoor rearing of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*. *Journal of Applied Entomology*, *142*, 901–904. https://doi.org/10.1111/jen.12553

Müller, H. J. (1965). Probleme der insektendiapause. *Verhandlungen Der Deutschen Zoologischen Gesellschaft*, *13*, 192–222. https://doi.org/10.1111/j.1439-0418.1967.tb03843.x

Müller, H. J. (1970). Formen der Dormanz bei Insekten. *Anzeiger Für Schädlingskunde*, *43*, 128. https://doi.org/10.1007/BF02040933

Müller, H. J. (1979). Effects of photoperiod and temperature on leafhopper vectors. In K. Maramorosch (Ed.), *Leafhopper vectors and plant disease agents* (pp. 29–94). Elsevier. https://doi.org/10.1016/B978-0-12-470280-6.50006-4

Weaver, C. R., & King, D. R. (1954). Meadow spittlebug, *Philaenus leucophthalmus* (L.). *Ohio Agricultural Experiment Station Bulletin*, *741*, 1–99.

Witsack, W. (1973). Experimental and ecological investigations on forms of dormancy in homoptera-cicadina (Auchenorrhyncha). 2. On ovarian parapause and obligatory embryonic diapause in *Philaenus spumarius* (L.) (Aphrophoridae). *Zoologische Jahrbücher. Abteilung Für Anatomie Und Ontogenie Der Tiere Abteilung Für Anatomie Und Ontogenie Der Tiere*, *100*, 517–562.

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