Novel techniques for increasing the survival of aestivating biological control insects

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Summary

Red apion, Apion miniatum, is a potential biological control agent for Emex australis, doublegee, a major annual weed in the Mediterranean climatic zones of southern Australia. Offspring from the univoltine weevil emerge from the plants in late spring/early summer. Over summer, the host plant is not available and the adults aestivate. It is desirable to release the red apion in autumn. For insects being held in the laboratory over the summer, we assessed the effects of food availability, temperature and lighting on the individual’s subsequent reproductive output. Survival rates of red apion over the storage period were inversely related to temperature for 5 to 20°C. The most successful storage method was to store the normally leaf-eating insects at 8°C, whilst giving them access to simple carbohydrates. This temperature was just above their lower developmental threshold temperature. Infertility resulted at lower temperatures and fecundity and survival declined at higher temperatures. The techniques outlined have possible applications to other biological control programs.

Keywords: aestivation, Apion miniatum, biological control agent, doublegee, Emex australis, low temperature, reproductive output, summer storage.

Introduction

Typically in classical biological control programs, agents are collected overseas and imported in low numbers into quarantine facilities for host-specificity testing. If suitable, agents are removed from quarantine and a mass-rearing and redistribution program initiated. In this phase, like in any production plant, the aim is to maximize the output of the product without affecting quality, and the net output is inversely proportional to the efficiency of the system. Larger numbers of available agents maximize the chance of establishment with larger release numbers permitted at each site and they allow for releases to be made at more sites.

In Mediterranean climates, the summer is hot and dry with most annual plant species existing in the seed-bank. Many adult insects are able to endure unfavourable environmental conditions by entering a reproductive diapause and even non-quiescent individuals in diapause have been shown to age far slower than non-diapause insects under identical conditions (Tatar & Yin 2001).

Our biological control project targets Emex australis, doublegee, a major weed in the Mediterranean climatic zones of southern Australia, using the red apion, Apion miniatum (Yeoh et al. 2002). This univoltine weevil is from Israel where its native host is Emex spinosa, the lesser jack. Red apion adults aestivate over the summer, becoming reproductive at the break of the season in autumn when their host plant, a winter annual, becomes available. Its host range is restricted to Emex spp. and some Rumex spp. During winter, it is only observed feeding on the leaves and petioles of the host plants and there are no published records of it feeding on nectar or flowers of any species at any time (Scott & Yeoh 1996). In both its native and intended new habitat, there is no suitable green foliage for it to feed upon during the summer.

Other summer aestivating, univoltine weevils, Mogulones geographicus and M. larvatus, are widely established in Australia and are causing significant damage to Paterson’s curse, Echium plantagineum (Swirepik & Smyth 2001). Collecting and releasing teneral adults immediately onto field sites lessens husbandry requirements, but fully exposes them to the
hostile environmental conditions of summer. Despite entering diapause, many still die before reproducing. For the Paterson’s curse project, autumn releases proved to be more likely to establish populations at sites and the insects are usually stored over summer in cages located under shade, with soil, leaf litter/mulch and dead host plants. Similarly, we opted for autumn releases for the red apion and this required us to develop a method of holding our aestivating insects over the summer.

The storage method developed for our red apion (our “host plant” method) gives good survival, but is resource intensive. It requires environmental chambers with light and temperature control, host plants grown out of their normal season and constant husbandry so as to control pests and prolong the life of the plants. We therefore sought an alternative, pest-free and more labour-efficient method. We also wanted a system that would expedite the process of collecting and releasing the apion in autumn as well as a system that could be used by others.

The aims of this paper are to 1) determine the value of supplementary feeding with host plants to the survival of red apion so as to develop a method of holding the mass-reared red apion over the summer and 2) investigate alternative low maintenance, low resource methods of storing the red apion over summer that do not require the use of host plants.

**Methods**

**The value of host plants over summer**

Laboratory observations confirmed that red apion enters a non-quiescent reproductive diapause over summer and will drink and feed if given the opportunity. During this period, *E. australis* is difficult to grow in pots due to the soil temperatures, so in quarantine, breeding colonies were maintained over summer by caging them at very low densities (10 per plant) on *Rumex crispus* plants. Once the mass-rearing phase of the biocontrol program began, it was necessary to increase the densities at which adult red apion were housed over summer.

The effects of supplementary watering and the provision of host plants over summer on red apion’s survival were assessed using adults that had just entered aestivation. The experiment was set up as a three-level, three replicates. Each of 6 replicates consisted of 10 aestivating insects that do not require the use of host plants. All cages contained a 70 cm long × 10 cm diameter trunk section of stringy bark bottlebrush (*Callistemon* sp.), dead *E. australis* foliage/stems, *Acacia cyclops* stems/ seeds/leaves, hessian material, crumpled paper towelling and mulch/leaf litter made from local trees (predominantly *Eucalyptus* spp.). All cages were located under a stand of *Eucalyptus citriodora* at CSIRO, Perth, Western Australia. Ants were recognized as potential predators and were prevented from entering the cages by trays of water, layers of oil and applications of tanglefoot. The over-summer survival was assessed at the commencement of the autumn rains (May) when individuals were recovered from the cages by attracting them to *E. australis* plants.

**Alternatives free of host plants**

The need to provide host plants to aestivating red apion over summer has associated high labour and resource investments. Storing the red apion at lower than ambient temperatures without host plants was therefore investigated. Two separate experiments were conducted: the first using a range of temperatures with no supplementary feeding and the second with a range of temperatures, but with supplementary feeding with simple carbohydrates.

**Experiment 1 – no supplementary feeding**

This commenced in summer 1999/2000 and was designed as a 4 × 3 factorial ANOVA with insects held at 5, 10, 15 or 20°C and given a 0:24, 14:10 or 24:0 (hours light: dark) photophase. The effect of light on survival and reproductive output was also incorporated into this experiment as fridges could substitute for environmental chambers if storage within the dark was acceptable. Storage with constant light has been shown to maintain aestivation in grasshoppers (*Pener & Broza* 1971). Each of 6 replicates consisted of 10 aestivating individuals. They had been previously housed using the host plant method as above for 1 month. Insects were housed within “vial” cages made from Cospak 40 Dram styrene vials (142 mL volume). A cotton wick was inserted into a water source through a 8 mm hole in the base: crumpled paper towel within the top half of the vial provided roosts for the insects and terylene voile in place of the lid gave ventilation. Heavy gauge nylon mesh (10 mm aperture) separated the water wick from the paper. The vial cages and their water source were then placed inside a 30 × 20 × 20 cm lightproof cardboard box. Relative humidity within the boxes was 75 ± 5%. A 20 cm × 20 cm metal conduction plate ensured the internal temperature matched the set temperature (within 0.5°C). A non-heat producing 12 volt, 6 cp white LED light source was attached to the metal plate and the lighting regime (0, 14 or 24 hours at 0.1 to 0.3 µE/s/m² within the box) controlled by timers on the power supply.

The number of live and dead insects within each vial cage was assessed monthly from the outside of the vial. Most insects came out of the paper to die and their
bodies could be easily seen on the base of vial. The experiment was terminated after 129 days as the winter rains had started and field releases begun. Surviving insects were weighed and measured. From then onwards they were maintained at ambient temperatures and with natural, but diffused daylight whilst their fecundity and fertility were assessed.

For assessing fecundity and fertility, we pooled individuals from different replicates within the same treatment. Each female was treated as a replicate and her egg production/hatch recorded until her death. Females were paired with males from the same treatment at 5°C but at 10°C there were no surviving males so males housed using the host plant over-summer storage method were substituted. The experimental design became unbalanced due to the absence of female survivors and the failure of some females to lay eggs. The fecundity and fertility of the individuals housed in the vial cages was therefore compared to those that were housed using the host plant over-summer storage method by treating the data as a one-way ANOVA (after pooling all homogenous factors identified within the initial two-way ANOVA).

Pairs of red apion were held on leaves of *E. australis* using 20 mm diameter clip-on cages. Each week, the pairs were transferred to a new leaf and the number of eggs laid was counted. At 2 and 4 months post-removal from the vial cages, the eggs were checked for viability. Eggs laid over a 3-day period were individually identified and then monitored daily (*in situ*) for up to 2 weeks. All surviving red apion were killed 6 months post removal. At this stage in the field, the breeding season had finished and all host plants were dead.

The potential reproductive output over the red apion’s lifetime under each storage regime was estimated by multiplying the average over-summer survival rate by the average fecundity rate by the average egg fertility observed for that regime.

**Experiment 2 – supplementary feeding with sugars**

This commenced in the 2000–01 summer period and was designed as a 5 × 2 factorial experiment. The temperature range was similar (5, 8, 10, 15 and 25°C), but with the addition of an 8°C treatment because the lower developmental threshold temperature of red apion, during the egg to adult phase, had been determined to be 7.0°C (unpublished data). The second factor was the effect of supplementary feeding with simple carbohydrates (sugar and honey). Although adult red apion are only reported to feed upon foliage, this was included because escaped aestivating individuals were noticed feeding upon honey. Insects were either given both white sugar cubes and Australian honey (Wescobee) – or given neither. Three replicates each consisting of 20 random individuals were set up for all cells except at 25°C with carbohydrates, where only 10 males per replicate were set-up and at 25°C without carbohydrates, where no replicates were set. A fully balanced design was not utilized so as to conserve our breeding stocks. All individuals had been stored for 1 month using the host plant over-summer storage method prior to being set up in this experiment.

Experiment 2 utilized over-summering storage “capsules” specifically designed to hold moderately large numbers of insects and to facilitate the transport and release of the red apion at the start of the new breeding season. They were constructed from 1.25 L polyethylene terephthalate (PET) plastic drink containers (bases removed). An inverted plastic lid containing potting mixture was taped to the bottom of the container to act as a cage floor. This was to reduce the saprophytic fungal build-up observed in the smaller vial cages used in experiment 1. An open-celled foam plug blocked a 6 cm diameter access hole bored through the side of the bottle. This plug provided the only ventilation to the cage. Sugar cubes were placed on the potting mix at the base of the cage and the honey was smeared on the inner surface of the plug. Bundles of paper towelling were secured within the top half of the cage by wire. Water was provided *ad libidum* via a cotton wick protruding through the lid of a water filled 16-dram vial placed within the cage. The light regime, via florescent tubes, was 16 hours light: 8 hours dark. Cages were placed within brown paper bags to subdue the lighting (intensity inside bags 5–13 µE/s/m²), elevate humidity (to 80 ± 10%) and prevent visual disturbances.

The red apion individuals were removed from their over-summer storage capsules when the *E. australis* plants became available in the field (180 days after set up). The egg production and hatch rates were then assessed, as for experiment 1, until the plants were no longer available in the field (December).

**Statistical analysis**

Statistica 99 was used for all statistical analysis. All averages are expressed as means ± SE. In tables, the sample size is shown in parentheses.

**Results**

**The value of host plants over summer**

There was almost complete mortality with an average survival of only 0.1 ± 0.1% when insects were housed for a 4-month period at summer ambient temperatures (average shade temperature = 21°C, range 6–40°C) in the tubs with assorted potential over-summering sites, but with neither host plants nor water. Supplying water gave slightly, but not significantly better results (5.1 ± 4.4%). Supplying host plants with green foliage did, however, significantly (*F*<sub>2,6</sub> = 21.1, *P* < 0.01, Tukey HSD) improve survival to 67.1 ± 6.1%.

Based upon these results, our method of storing red apion over summer was further refined. To accommodate the numbers mass reared, more insects needed to
be housed within smaller cages using potted Rumex plants, but at 500 to 1000 red apion/cage, plants retained green foliage for only a fortnight. Floor space became limiting within the cages, but removing dead plants from the cages required us to disturb insects that were hiding in the dead leaves. The survival rate dropped to 48.3 ± 8.36% (n = 5).

When smaller cages (30 × 40 × 50 cm) containing 500 to 1000 apion were held within an environmental chamber at 15°C with 14 hours light/day, insect activity was slowed and plant life prolonged. Under these conditions, only one potted R. crispus plant per month needed to be introduced and old pots did not need to be removed. Paper towelling, when folded and hung inside the cages, was as good as or better than any other refuge substrate tried. Plastic covers loosely placed over the cages reduced desiccation by minimizing air movement. This has become our regular or proven method of holding red apion over summer, and gives 74.4 ± 2.48% (n = 26) survival. For future reference, this will be referred to as the “host plant” method.

**Alternatives free of host plants – 1. no supplementary feeding**

With only water supplied, the rate of decline in population size was inversely proportional to the temperature at which the red apion populations were stored with high mortality (41%) already being noticed at the highest temperature (20°C) after only 1 month (Fig. 1a). Comparison of survival curves using Cox proportional hazard models also found significant, but smaller influences due to the lighting regime (Wald statistic (WS) = 18.1, n = 720, p < 0.001 for light and WS = 460.6, n = 720, p < 0.001 for temperature). The survivorship rates between red apion housed under constant light or under a 14:10 (light:dark) diurnal light regime made no difference to the pre-oviposition period (F5.29 = 1.15, p = 0.36). Placing insects in complete darkness for the entire summer affected lifetime fecundity (F2.43 = 3.29, p < 0.05). Insects exposed to constant dark laid only 42 ± 8.0 eggs whereas those in constant light laid on average 106 ± 20.9 eggs (Tukey HSD, p < 0.05). A diurnal rhythm in the lighting was not essential (Table 1). Although red apion stored with a light source at 5°C laid approximately the same number of eggs as the females from the host plant method (all approx. 75 eggs/female), the average egg hatch rate was lower at only 43.7% compared with 93.8% for females from the host plant method. Females stored at 10°C had reasonable egg hatch (85.3%).

By combining the estimates of survival, fecundity and fertility (Table 1), it becomes obvious that red apion populations do not fare well when housed under laboratory conditions with only water. Although increasing survival to acceptable levels occurred with reduced temperatures, decreases occurred in fecundity and fertility so that even under the best conditions offered (5°C and constant light) offspring production was only 38.5% of that obtained under the host plant method.

**Alternatives free of host plants – 2. supplementary feeding with sugars**

The survival of red apion, when housed with only water over summer mirrored that of the previous experiment (Table 2). At 5°C, it was approximately the same as that observed using the host plant method (73%). Egg lay from colonies stored at 5°C with water was only half of that of females from the host plant method, but this was not significant due to high individual variation. Egg hatch was significantly lower at only 43.7% compared with 93.8% as that observed using the host plant method (73%).

Egg lay from colonies stored at 5°C with water was only half of that of females from the host plant method, but this was not significant due to high individual variation. Egg hatch was significantly lower at only 43.7% compared with 93.8% as that observed using the host plant method (73%).

The provision of simple carbohydrates virtually eliminated mortality regardless of the temperature, with over 93% survival occurring at even the highest tested temperature (25°C). Survival by itself is not, however, a good predictor of the success of the storage method for at 5°C with honey, eggs laid were not viable, with less than 2% hatching. As a result, less than 0.1 offspring could be produced from every initial female. An increase of only 3°C saw significant increases in both egg lay and hatch. Females stored at 8°C with honey each laid 73 more eggs than the 42 eggs/female
observed from those stored using the host plant method. The hatch rate from females stored at 8°C with honey was equal to that of those stored using the host plant method (both approx. 81%), but with a significantly better survival rate (98% versus 73%), the net result being an increase in lifetime reproductive output of 360%. For colonies stored at 8°C with honey over summer, 90 larvae can be expected from each initial female. Females housed during the summer using our host plant method had a reproductive output of only 25 larvae/female. At temperatures higher than 8°C, fecundity dropped, so that at 15°C with honey, the lifetime reproductive output was 71% of that observed from females stored using our host plant, high maintenance method. As no females were set up at 25°C, the results are unknown at this temperature.

Discussion

The duration of our storage experiments matched that of the local summer dry period. Individuals must survive the full period in order to breed, as the required host plants do not become available until after the rains begin. Our results indicate that without some supple-

Table 1. Lifetime reproductive output from red apion stored with only water and under various lighting regimes/temperatures over summer.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Light (hours)</th>
<th>% survival</th>
<th>No. of eggs laid1</th>
<th>% egg hatch2</th>
<th>No. of offspring per initial female</th>
<th>As % of host plant method</th>
</tr>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>63.3 ± 8.82 (6)</td>
<td>42.3 ± 8.69 (12)</td>
<td>36.9 ± 15.34 (8)</td>
<td>9.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>80.0 ± 5.16 (6)</td>
<td>92.9 ± 21.23 (14)</td>
<td>40.5 ± 12.08 (11)</td>
<td>30.1</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>71.7 ± 6.01 (6)</td>
<td>92.7 ± 21.34 (12)</td>
<td>54.9 ± 12.40 (8)</td>
<td>36.4</td>
<td>38.5</td>
</tr>
<tr>
<td>All 5 pooled</td>
<td></td>
<td>71.7 ± 4.06 (18)</td>
<td>76.8 ± 11.09 (38)</td>
<td>43.7 ± 7.48 (27)</td>
<td>24.1</td>
<td>25.5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3.3 ± 2.11 (6)</td>
<td>32.0 (1) 3</td>
<td>[88.3 (0)] 2</td>
<td>0.9</td>
<td>1.0</td>
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<tr>
<td></td>
<td>14</td>
<td>8.3 ± 3.07 (6)</td>
<td>99.8 ± 20.63 (8)</td>
<td>88.3 ± 4.87 (7)</td>
<td>7.3</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>21.7 ± 6.54 (6)</td>
<td>192.5 ± 35.06 (2)</td>
<td>74.9 ± 15.80 (2)</td>
<td>31.2</td>
<td>33.0</td>
</tr>
<tr>
<td>All 10 pooled</td>
<td></td>
<td>11.1 ± 3.01 (18)</td>
<td>110.5 ± 20.63 (11)</td>
<td>85.3 ± 4.97 (9)</td>
<td>10.5</td>
<td>11.1</td>
</tr>
<tr>
<td>All vial treatments</td>
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<td>20.8 ± 3.52 (72)</td>
<td>84.4 ± 9.88 (49)</td>
<td>46.7 ± 6.60 (31)</td>
<td>9.8</td>
<td>10.4</td>
</tr>
<tr>
<td>Host plant method</td>
<td></td>
<td>75.7 ± 3.74 (12)</td>
<td>133.2 ± 20.73 (19)</td>
<td>93.8 ± 1.64 (13)</td>
<td>94.5</td>
<td></td>
</tr>
</tbody>
</table>

1 Pooled by light regime for comparing to the host plant storage method. (x + 0.5)0.5 transformed data.
2 x3 data transformation applied.
3 No data as no eggs laid – given same value as 10°C 14 hours light.
Means with same letters are not significantly different at 5% (one-way ANOVA).
mentary supply of energy, the red apion population would go to extinction if the mean temperature of their selected or available microclimate was 15°C or higher. Assuming red apion’s selected microclimate is at ambient temperature, this means survival would not have been possible with water alone in either the area from which the red apion was originally collected (Israel) nor the main target area for our biocontrol program (the Western Australian wheat-growing region). In both these regions, mean summer temperatures exceed 22°C.

Supplying simple carbohydrates resulted in an incredible increase in survival rates for red apion stored over the summer period at what could be considered “normal summer temperatures”. This would imply that these insects do in fact feed on some sort of nectar or sugar source in the wild. The absence of a suitable substitute may be the reason why, despite considerable effort, this insect appears not to have established in Australia (Yeoh et al. 2002). It should, however, be emphasized that nothing is known about the behaviour of the red apion in its native environment and the need to feed on sugar at warmer temperatures may in fact be an artefact of us providing the insects with totally unnatural microclimates during the summer period.

Host records for red apion have almost exclusively been restricted to plants within Rumex spp. and Emex spp. with the exception of an insect being “found” on blackcurrant. In this case, there was no mention of red apion feeding (Scott & Yeoh 1996). The adults typically produce “shot holes” in the leaves, but when plants are senescing, green foliage becomes rare. At this stage, they eat anything green, including petioles, stems, seed and flowers. Despite working on the red apion for almost a decade, whilst it is in its reproductive phase we have never seen it feeding on any nectar sources from any non-host plants. We have not even noticed it selectively feeding on the flowers or nectar of Emex although this may be because the flowers are small and insignificant.

The idea of supplementary feeding our phyllophagous insect with simple carbohydrates was initiated only because escapees within our over-summer holding room were found feeding upon honey used in a different experiment. If the red apion does feed upon nectar/pollen prior to aestivating, it is probably not unique, as Mogulones larvatus with its predominantly phyllophagous adults, also has been noted feeding upon flowers prior to aestivation. For this species, the host plant’s flowers are only available in early summer and the survival rate of M. larvatus over summer and within cages declines with the length of time the insect is required to stay within aestivation (Matthew Smyth, Paul Wilson, pers. comm.). Programs such as these are perhaps the most likely to benefit from supplementary feeding with simple carbohydrates.

Conversely, M. geographicus adults, prior to entering aestivation, feed only on the leaves of Paterson’s curse (Echium plantagineum) even when flowers are available (Paul Wilson, pers. comm.). However, it may still be of benefit to supply green plants grown out of season or to drop the storage temperature.

Red apion adults are entering diapause to endure summer drought and storing them at low temperatures may seem counterintuitive. Entering a state of diapause, feed only on the leaves of Paterson’s curse (Echium plantagineum) even when flowers are available (Paul Wilson, pers. comm.). However, it may still be of benefit to supply green plants grown out of season or to drop the storage temperature.
response to a period of unfavourable environmental conditions regardless of whether the stress is heat or cold and in both cases it is believed to be initiated by suppression of juvenile hormone levels (Tatar & Yin 2001). Although our biotype of red apion has evolved with triggers that initiate the onset of reproductive diapause prior to the onset of summer, other biotypes exist in England (Scott & Yeoh 1996) where they presumably enter a winter diapause to survive. The danger of manipulating the insect’s summer temperature is that the selective pressure for entering aestivation may no longer apply so that this beneficial trait gradually disappears in culture. Once released in the field, the selection pressure would be reinstated and this problem could be overcome by periodically adding field-collected individuals to any breeding colonies.

Red apion stored at the lowest temperature (5°C) had good survival but its overall reproductive output was poor due to fecundity and fertility problems. At only a few degrees warmer (i.e. at 8°C), the best reproductive yields occurred. This temperature was 1°C warmer than the calculated lower developmental threshold temperature for immature stages of this insect. Presumably it was warm enough to either permit any necessary development or prevent any permanent damage to the insect’s reproductive organs whilst cool enough to reduce somatic senescence to a minimum. Caution should be taken when exposing insects to cold temperatures as even 10 minutes exposure to 2.0°C has been shown to significantly reduce the lifetime fecundity and delay oviposition in the bruchid Callosobruchus sibirnotatus (Mbata et al. 1998). It was suspected that the short-term exposure to low temperature disrupted normal oocyte maturation.

Our host plant method of storing red apion over the summer, although providing a consistent result both from cage to cage and from year to year, is labour intensive. It requires host plants grown out of season, pests such as aphids, mealy bugs, thrips, spiders, caterpillars and plant pathogens are a problem and there are also issues with either over-watering (insects under pots drowned) or under-watering the plants. As biocontrol practitioners, we also have a duty of care to the collaborating farmers that are providing the field release sites. We cannot transfer weeds, pests and diseases to their property. Extracting 500 red apion from the dead and dying food plants, moths, aphids, spiders and other contaminants takes 2–3 hours/cage and has to be done just prior to going to the field site. Releasing red apion from over-summer capsules can be done in the field and takes 5 minutes/capsule. This allows for releases to be made as soon as germination of E. australis occurs at the sites, giving red apion the largest possible period of time to breed.

The lack of information on red apion’s natural behaviour over summer necessitated the development of novel techniques before the release program could begin in earnest. Enhancements have resulted in methods that have the potential for improving other similar biocontrol programs current or future.

During 2001–02, we conducted a full-scale implementation of the capsule method (18,700 red apion stored and then released), but the adults failed (less offspring produced than adults released). It is possible that local drought conditions were responsible for the poor yield, but experiments are currently under way to investigate if other factors such as slight modifications to the capsule’s design and set up procedures (e.g. being directly placed into the capsules rather than being housed with host plants at 15°C for 1 month) or the presence of symbionts (e.g. Wolbachia) may have been responsible. The findings of these experiments will be reported upon in another publication.

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References


