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Full Length Research Paper

Raising *Trissolcus basalis* for the biological control of *Nezara viridula* in greenhouses of Almería (Spain)

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The reduction in use of crop protection products in recent years has led to the appearance of new phytophage pests such as *Nezara viridula* (L.) in the greenhouses of Almería (Spain). Its biological control is, however, possible, and involves the deliberate release of the wasp *Trissolcus basalis* (Woll.), which parasitizes the pest's eggs. The aim of the present study was to help meet peak demand for this wasp by providing a viable reserve of material - the frozen eggs of *N. viridula* - for parasitization, and using stored *T. basalis* adults hatched from these eggs as a source for beginning wasp production in anticipation of such peak moments. The capacity of *T. basalis* to parasitize the eggs of *N. viridula* subjected to freezing and storage at -25°C was therefore assessed, and the possibility of storing (at +15°C) adult parasitoids hatched from these frozen host eggs examined. No significant differences were observed in the use of the frozen or fresh host eggs in terms of percentage parasitism, parasite hatching or parasite mortality. These results confirm that *T. basalis* can be raised using frozen *N. viridula* eggs in order to supply the varying needs of greenhouse crop growers in Almería.

Key words: Biological control, egg storage, parasitoid.

INTRODUCTION

Biological control was originally used for the management of insects, mites and weeds (Debach, 1964), playing its greatest role in the fight against insect crop pests (Laing and Hamai, 1976). Later, biological control was used to manage a wider range of animal pests (Cook and Baker, 1983). Biological control programmes have been used against crop pest insects belonging to the orders Homoptera, Diptera, Himenoptera, Coleoptera and Lepidoptera, among others, with most success enjoyed against the first of these (Greathead, 1986). Greathead and Greathead (1992) recorded that over 500 insect species had been subject of some 1200 biological control programmes. Almost a decade has now passed since biological control was introduced into southeast Spain (Province of Almería), which has the largest greenhouse area in Europe - some 26958 ha (Sanjuán, 2004). It was not until the 2006/2007 growing season, however, that its large scale implementation began (Fundación, 2008).

Almería's greenhouses and the crops they contain provide a unique microclimate for the development of pests and diseases (Castilla and Hernández, 2005), and recent reductions in the use of traditional crop protection products (Ministerio de Medio Ambiente, Medio Rural y Marino, 2008) has led to the appearance of new phytophage pests (Núñez et al., 1991) including *Nezara viridula* (L.). The present work focuses on the relationship between this stinkbug and its parasitic wasp *Trissolcus basalis* (Woll.), and proposes a new method for raising the latter in order to control the former.

N. viridula belongs to the family Pentatomidae of the order Hemiptera (Figure 1); it is the most economically important and most widely distributed of all hemipterans. It is present on all continents bar Antarctica, although it originates from tropical and subtropical Africa, perhaps the Ethiopia area (Ehler, 2002). This polyphytophage insect is commonly found on herbaceous and horticultural crops, but even appears on tree crops (Ceroni et al., 2004). The females of *N. viridula* lays barrel-shaped eggs cemented into ooplaques containing 80-120 eggs each.

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Figure 1. *Nezara viridula*: (a) adult. (b) nymph.

Figure 2. Adult female *Trissolcus basalis*. Picture credited to the University of Hawaii at Manoa (2009).

These are more often found on the underside of leaves. When close to hatching (4 - 9 days after laying) the eggs take on a yellow or clear orange colour. First instar nymphs show gregarious behaviour, remaining in groups often close to the ooplaque. After the fifth instar (taking 24 - 60 days) they eventually become adults that live for about three weeks (Todd, 1989). *N. viridula* can complete 2 - 5 generations a year, depending on the weather. Some adults survive the winter in a dormant state under the bark of trees or in piles of wood (Knight and Gurr, 2007). Dormancy is induced by the photoperiod. Dormant adults usually change from green to a dark rust or brown colour (Musolin et al., 2007).

T. basalis belongs to the family Scelionidae of the order Hymenoptera (Figure 2). A native of Mediterranean Europe it is commonly found in Spain, but it is a cosmopolitan species. It parasitizes the eggs of *N. viridula* but it is not monophagous, having been shown to also parasitize other pentatomid species (Jones, 1988; Colazza and Bin, 1995). The wasp lays an egg inside the egg of the host species. After hatching, the young parasite passes through three larval stages, a pre-pupal and a pupal stage (together taking some 12 - 15 days) before emerging as adult (El-Husseini et al., 2006). Each female lays around 150 offspring over its lifetime (Colazza and Wajnberg, 1998). The species shows a haplodiploid nature that steers it towards a sex ratio in favour of females when the eggs of the host are abundant. Todd (1989), described in detail the ecology and behaviour of *N. viridula*, and recorded *T. basalis* as an important parasitoid of the species. It was first used to control *N. viridula* in Egypt and Australia (1933), and then later in the Antilles (1952 and 1953), South Africa (1980), Brazil (1980) and the USA (1979 and 1981) (Clarke, 1990).

After detecting *N. viridula* on greenhouse pepper crops in Spain, Catalán and Verdú (2005) analysed the crops' fauna to detect possible biological control agents. *T. basalis* and *O. telenomicida* (V.) were found. The former species was recommended for control purposes given its greater longevity and number of progeny under laboratory conditions, as well as its habit of concentrating egg-laying in the first few days of adult life.

The growth of *Trissolcus oenone* (D.), a close relative of *T. basalis*, is linear with respect to temperature (growth range 15 - 35°C) (James and Warren, 1991). The temperature and duration of storage of the host's eggs influence the degree of parasitization achieved, the proportion of parasitoids that hatch, the duration of the development stage, the longevity of the adults, and the daily oviposition rate of *Trissolcus* sp. (Awadalla, 1996). The interaction between *T. basalis* and *N. viridula* depends on the temperature and photoperiod (Jones and Westcot, 2002), and the raising of *N. viridula* is affected by the relative humidity (Hirose et al., 2006). In the laboratory, the larvae of *T. basalis* feed upon the eggs of the host, but adults need to be fed with honey, pollen or nectar, although some only take water (Debach and Rossen, 1991). Although high survival rates can be achieved, the survival of both *T. basalis* and *N. viridula* is better under natural conditions (Rahat et al., 2005; Fortes et al., 2006).

When searching for host eggs *T. basalis* follows allelochemical cues among others; this information could be of use in the improvement of biological control programmes (Lewis and Martin, 1990). The adult wasp begins its search by detecting the synomones released by plants that have come under attack by pests (Colazza et al., 2004). Kairomones secreted by the adults of *N. viridula* help the wasp to localise the egg masses of the host (Colazza et al., 1999; Salerno et al., 2006; Colazza et al., 2007; Dauphin et al., 2009). In addition, *T. basalis* is able to locate *N. viridula* adults via their secretion of volatile substances; indeed, it can even locate the females via the detection of chemical cues in their exreta. This helps to direct the wasp in areas where eggs are most likely to be found (Colazza et al., 2007). Using liquid nitrogen (-196°C), Correa-Ferreira and De Oliveria (1998) managed to store the eggs of *N. viridula* for 12 months and still achieve a post-thawing 95% parasitization rate by *T. basalis*. However, not all biofactories may have the necessary facilities or expertise for using liquid nitrogen.

The use of large quantities of *T. basalis* as part of pest control systems in Almería is seasonal, which means biofactories face production peaks followed by lull periods. This, plus the inability of the wasp to tolerate long storage periods, cause some of the major economic problems with which such biofactories must deal. The aim of the present study was to help meet these peak demands by providing a viable reserve of material - the frozen eggs of *N. viridula* - for parasitization, and using stored *T. basalis* adults hatched from these eggs as a source for beginning wasp production in anticipation of such peak periods.

MATERIALS AND METHODS

The present work was performed in the quality-accredited laboratories of Bioplanet S.C.A. in Cesena (Italy) between October 2008 and June 2009, in collaboration with the technical department of the Hortofrutícola Costa de Almería S.L. company. The biological material used included 78 ooplaques of *N. viridula* collected from a greenhouse aubergine crop, and 800 *T. basalis* adults (50% males and 50% females) produced by Bioplanet S.C.A. The sex of the insects was determined by examining their antennae, which are filiform and upward-pointing in males, and nail-shaped and downward-pointing in females (Figure 2). 39 ooplaques of *N. viridula* obtained from producive units were taken within 24 h of oviposition and each one was then placed in separate test tubes stored in a polystyrene container at -25ºC; therefore, changing partly the technique used by Correa-Ferreira and Moscardi (1993).

After 40 days of storage, the eggs have been set to defrost for 24 h at a temperature of 8°C in a refrigerator. Later other 39 ooplaques were similarly withdrawn within 24 h of oviposition, but not frozen so that a comparison could be made between fresh and frozen material. Once the period of defrosting had finished and taking both, frosted and fresh eggs, different groups were formed, consisting of 5 fresh ooplaques and 5 frozen ooplaques, except for one group that consisted of 4 ooplaques. The groups of 5 and 4 have formed couples and together, have undergone the action of *T. basalis* (choice test) in productive units with a number of 100 adults parasitoids in their inside, sharing the same amount of time from hatching, subdivided into 50 males and 50 females.

After the period of parasitization (approximately 7 days) eggs of *N. viridula* have been collected and the two original groups, fresh and frozen have been reconstructed separately.

Each group of eggs was evaluated by the following parameters:

Percentage of parasitization.
Percentage of hatching.
Percentage of failed hatching.

Storage of *T. basalis* adults

With the newly-hatched *T. basalis*, produced with fresh and frozen eggs, 15 products similar to those used for commercial purpose (bottles of 100 cc, suitable for direct use in the field), different by group, frozen and fresh, with 105 individuals within each. With the aim of providing food to adults during the period of storage, inside the cap of each bottle, it has been placed a feeding ring soaked in a sugary liquid substance. The products thus obtained were stored in an air-conditioned environment (15°C temperature, also used by Foerster and Doetzer, 2006), but with 55% relative humidity and absence of light. After 10, 20, 30 days in each of the bottles, the controls of mortality of the individuals have been executed. After 10, 20, 30 days in every one of the bottles the controls of mortality of the individuals have been executed (Figure 3). All data were validated by tests for normality and homocedasticity, and analysed using the Mann-Whitney U test employing STATISTICA 8.0.

RESULTS AND DISCUSSION

No significant differences were seen in the percentage of

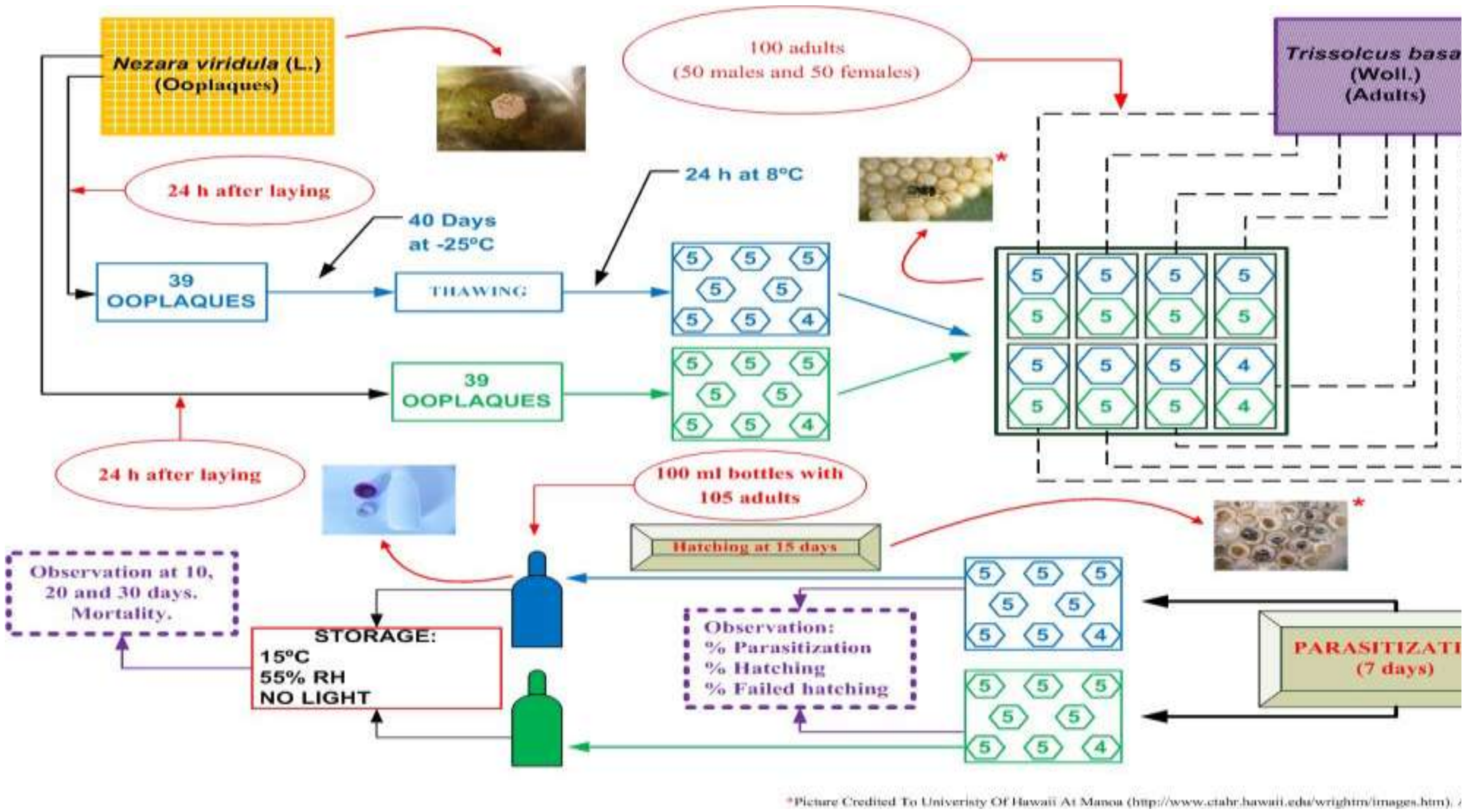

Figure 3. Parasitization of frozen Nezara viridula eggs and storage of adult *Trissolcus basalis*.

parasitization achieved or the percentage hatching of wasps with respect to the use of fresh or frozen *N. viridula* eggs (Table 1). Neither were significant differences seen in adult *T. basalis* mortality over a 30 days storage period, or in terms of total mortality, with respect to the use of fresh or frozen *N. viridula* eggs (Table 2). Although Debach (1964), Laing and Hamai (1976), Cook and Baker (1983), Greathead (1986) and Greathead and Greathead (1992) indicated the biological control of insect crop pests to be viable, it was not until the 2006/2007 season that it was widely adopted in Almería (Fundación Cajamar, 2008). Pesticides had been used extensively up to this point, but the EU's banning of numerous chemical agents and the demands made by destination markets for greater respect for the environment required new protection

Table 1. Percentage parasitization, percentage hatching and percentage failed hatching of *Trissolcus basalis* reared in frozen and fresh *Nezara viridula* eggs. Averages (µ), standard deviations (σ), coefficient of variation (cv) and distribution (F).

Eggs of *Nezara viridula*		Parasitization	Hatching	Failed hatching
	µ (%)	91.58a	91.34a	8.66a
Fresh	σ	+6.06	+5.97	+5.97
	cv (%)	6.62	6.53	68.89
	µ (%)	91.88a	92.08a	8.51a
Frozen	σ	+8.94	+7.38	+8.05
	cv (%)	9.73	8.01	94.63
F		6.05	0.28	11.54
Significance		n.s.	n.s.	n.s.

a: not significant at P≤0.05 (Mann–Whitney U test).

Table 2. Mortality of *T. basalis* adults reared in frozen and fresh *N. viridula* eggs at different times. Averages (µ), standard deviations (σ), coefficient of variation (cv) and distribution (F).

Adults of *T. basalis* stored for 30 days at 15ºC		Mortality at 20 days	Mortality at 30 days	% Total mortality
	µ	2.00a	5.07a	6.73a
Hatched from fresh Nezara viridula eggs	σ	+1.77	+2.76	+2.73
	cv (%)	88.64	54.55	40,55
	µ	1.73a	4.80a	6.22a
Hatched from frozen Nezara viridula eggs	σ	+1.53	+2.76	+2.33
	cv (%)	88.49	57.43	37,43
F		0.24	0.21	0.29
Significance		n.s.	n.s.	n.s.

a: not significant at P≤0.05 (Mann–Whitney U test).

measures be found. A reduction in the use of pesticides was achieved (Ministerio de Medio Ambiente, Medio Rural y Marino, 2008), but new phytophage pests, such as *N. viridula* (Catalán and Verdú, 2005), began to appear in greenhouses (Núñez et al., 1991). Fortunately, Todd (1989), Clarke (1990), Catalán and Verdú (2005) and Lauman et al. (2008), showed that *T. basalis* affords a potential biological means for this pest's control. The present results reflect the great parasitic affinity of *T. basalis* for *N. viridula* eggs described by Lewis and Martin (1990), Colazza et al. (2007), Lauman et al. (2009) and Dauphin et al. (2009). They also show that the laboratory rearing of this wasp for release in the commercial greenhouses of Almería is possible.

The protocol followed modifies that of Correa-Ferreira and Moscardi (1993) with respect to the use of frozen *N. viridula* eggs, and Foerster and Doetzer (2006) with respect to the storage of *T. basalis* adults. The modifications made take into account the indications of James and Warren (1991), Awadalla (1996), Jones and Westcot (2002), and Hirose et al. (2006). The proposed method of using host eggs frozen for 40 days and storing the adults that hatch from them is simpler than that described by Correa-Ferreira and Oliveira (1998). The percentage parasitization obtained was slightly lower than that achieved by the latter authors (91.9% compared to 95%), but the freezing technology required is nothing more than a conventional freezer, which is cheaper and for many biofactories more practical than dealing with the liquid nitrogen containers required in Correa-Ferreira and Oliveira's method. The proposed method allows the peaks and troughs of demand in the control of *N. viridula* (which occurs in Almería's greenhouses between spring and mid-autumn) to be easily navigated. The feeding ring containing sugars used in the present work, which was earlier described by Debach and Rossen (1991), Rahat et al. (2005) and Fortes et al. (2006), guarantees the survival and quality of the *T. basalis* adults reared until their release in the greenhouse. In conclusion, this method for the laboratory raising of *T. basalis* using frozen *N. viridula* eggs is capable of meeting the peak demand for this parasitic wasp by the greenhouses of Almería.

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