Biological Control 60 (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

The effect of larval predators *Thanasimus dubius* (Coleoptera: Cleridae), produced by an improved system of rearing, against the southern pine beetle *Dendroctonus frontalis* (Coleoptera: Curculionidae)

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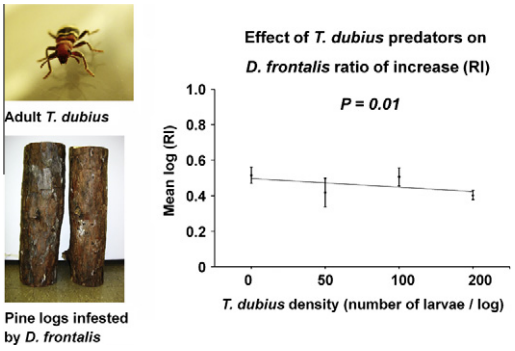
HIGHLIGHTS

- Evaluations of *Thanasimus dubius* predators against southern pine beetle previously limited by rearing methods.
- Refreshing the larval diet for predators every 5 days improves the rearing system efficiency.
- Sorbic acid added in the larval diet can reduce female fecundity.
- Sorbic acid may not be useful to improve predator rearing efficiency.
- Release of larval *T. dubius* resulted in reduction of prey ratio of increase.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 May 2010
Accepted 17 August 2011
Available online 28 August 2011

Keywords:
Predator–prey interaction
Bark beetle
Biological control
Preservative
Larval diet
Performance

ABSTRACT

The southern pine beetle, *Dendroctonus frontalis* (Coleoptera: Curculionidae) (SPB), is known to be a major bark beetle pest of pines throughout the southeastern United States. A common predator of bark beetles, *Thanasimus dubius* (Coleoptera: Cleridae), has been suggested to play a prevalent role on SPB dynamics. Evaluations of *T. dubius* have been limited by rearing methods; an artificial diet for larval *T. dubius* exists, and preservatives such as sorbic acid could help to maximize diet shelf-life and enhance the efficiency of the rearing system. The effects of sorbic acid at different concentrations (0%, 0.1% and 0.2%) in the larval diet for *T. dubius* were measured, and the effects of increased feeding time intervals (2–3 *vs.* 5 days) on predator performance evaluated. In addition, an experimental bioassay was conducted where newly hatched *T. dubius* larvae were released at four densities (0, 50, 100, and 200 per log) on pine logs infested by SPB. Sorbic acid in the diet reduced female fecundity (by 20–40%), but did not affect adult *T. dubius* size or longevity. However, using this preservative may not be necessary because it had no effect on the overall efficiency of the rearing system, while refreshing the larval diet every 5 days (compared with 2–3 days) did improve its efficiency, even without sorbic acid. The release of larval *T. dubius* resulted in a highly significant effect on the SPB ratio of increase (*RI*). This experiment was facilitated by the improvements in our rearing methods.

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1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2011.08.006

1. Introduction

Bark beetles kill more mature pines in North America than any other insect pest (Price et al., 1992). Using semiochemicals, bark beetles aggregate through a synchronized mass attack that permits them to overwhelm the defenses of the host tree (Wood, 1982). Adults drill galleries while feeding inside the phloem layer of the attacked tree, and inoculate pathogenic fungi that also contribute to its death (Raffa and Berryman, 1983; Paine et al., 1997). Although many species colonize dying or weakened trees, some aggressive or "primary" species can kill healthy trees (Payne, 1980; Raffa and Berryman, 1983). Among these aggressive species, the southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae), has long been an important pest of pine forests in the southeastern United States (Hopkins, 1899; Price et al., 1992). SPB can infest several species of pines, but of the major species, loblolly (*Pinus taeda* L.) and shortleaf (*Pinus echinata* L.) are preferred (Payne, 1980; Price et al., 1992).

It has been hypothesized that fluctuations in SPB populations are driven by exogenous factors (such as drought) that reduce the ability of the tree to resist bark beetle attack (King, 1972; Lorio and Hodges, 1977). However, recent studies suggest that these fluctuations have a strong endogenous component, with cycles in SPB abundance possibly driven by interactions with natural enemies (Reeve, 1997; Turchin et al., 1999; Reeve and Turchin, 2002; Friedenberg et al., 2008). Clerid beetles (Coleoptera: Cleridae) are predators that likely affect the dynamics of SPB (Reeve, 2000) and other bark beetles (Moeck and Safranyik, 1984; Schroeder and Weslien, 1994; Weslien, 1994; Lawson et al., 1997). A very common clerid predator in northeastern America, *Thanasimus dubius* Fabricius, has long been thought to generate high levels of SPB mortality (Thatcher and Pickard, 1966; Mignot and Anderson, 1969). Experimental manipulations of predator densities have demonstrated an effect of adult *T. dubius* on adult SPB survival (Reeve, 1997) and on the survival of the pine engraver *Ips pini* Say (Aukema and Raffa, 2002; 2004). Experiments using logs, artificially infested with *Ips grandicollis* Eichhoff and varying numbers of *T. dubius* eggs, have shown that larval *T. dubius* can reduce *I. grandicollis* larval survival (Reeve and Turchin, 2002), but further tests using eggs or larval *T. dubius* are required to ascertain its effect on larval SPB.

Field or laboratory trials to assess the efficiency of *T. dubius* as a biological agent against bark beetles would require a system of rearing that is able to produce sufficient numbers of *T. dubius* predators in good condition (Van Driesche and Bellows, 1996). Larval *T. dubius* can be reared using an artificial diet that resembles the nutritional and chemical composition of *I. grandicollis* larvae. This diet is presented to the insects inside Parafilm® capsules (Reeve et al., 2003), which provides a more efficient system than known alternatives, which require live prey that are obtained by collecting bark beetle larvae from infested pine logs (Mignot and Anderson, 1969; Nebeker et al., 1980; Lawson and Morgan, 1992). The rearing method using capsules of diet could be improved by increasing the shelf-life of the diet so that the larvae could be fed at longer intervals. A potential solution is to add preservatives such as formalin, methyl paraben, benzoic acid or sorbic acid in the larval diet to protect it from microbial contaminations (Alverson and Cohen, 2002; Pleau et al., 2002).

Although microorganisms can have detrimental effects on insect growth and survival, it also is possible that preservatives could affect insect performance. Antifungals have been identified as reducing the fecundity and survival of reared insects in some cases (Bass and Barnes, 1969; Alverson and Cohen, 2002), and some (e.g. formalin), are notorious human carcinogens (Sun, 1981). Thus, the overall preservative utility needs to be assessed by also considering its potential deleterious effects on reared insects. Among typical preservatives, sorbic acid appears to be one of the most effective and least toxic when used to rear insects such as the tarnished bug, *Lygus hesperus* Knight (Alverson and Cohen, 2002), the rice grasshopper, *Oxya yezoensis* Shiraki (Konno, 2004) and the potato beetle *Leptinotarsa decemlineata* Say (Martin, 2004). Concentrations of preservatives also can affect antifungal control and insect toxicity. High concentrations may increase deleterious effects and affect insect fitness (Bass and Barnes, 1969; Clancy, 1991; Alverson and Cohen, 2002). Thus, there is a need to find the best preservative and its optimal concentration for control of fungal growth and insect performance.

Overall, this study had three objectives. First, to evaluate the effect of sorbic acid on the performance of *T. dubius* larvae reared on artificial diet. Second, to examine whether longer intervals between feedings, facilitated by the addition of a preservative, could affect *T. dubius* quality. To accomplish these two objectives we measured the effects of sorbic acid on predator performance at three concentrations in the larval diet (0%, 0.1% and 0.2%) for two intervals between feedings (2–3 *vs*. 5 days). Predator performance was evaluated by mean larval developmental time, adult emergence rate, adult longevity, female fecundity, and elytral length. The third objective was to assess the effect of augmenting larval predator densities on SPB survival in naturally infested pine logs. Newly hatched *T. dubius* larvae, reared on artificial diet (plus sorbic acid), were released at four densities (0, 50, 100, and 200) on loblolly pine logs collected from trees naturally infested by SPB and brought to the laboratory. Results are discussed considering the use of this predator as a biological agent against SPB and the potential development of augmentative releases.

2. Materials and methods

2.1. Insects and rearing system

A laboratory colony of *T. dubius* was established in 2000 with wild individuals trapped in the Desoto National Forest, Chickasawhay Ranger District, Mississippi, using multiple-funnel traps (Lindgren, 1983) baited with frontalin and turpentine (Strom et al., 1999; Reeve et al., 2009). The colony was later replenished in 2004 with individuals caught from the same location. Adults were held in cylindrical plastic containers (21 cm high × 21 cm diameter) and fed with adult cowpea weevil *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), a factitious prey (Nebeker et al., 1980). Water was provided with a wet piece of sponge. All predators (adults and larvae) were maintained in an incubator at 23.0 ± 0.5 °C and a photoperiod of 16L/8D. Predator eggs were collected on folded paper towels. Larvae were fed a meridic diet that is a mixture of foodstuffs and other ingredients that has been used to rear *T. dubius* (Reeve et al., 2003). This diet was presented in Parafilm® capsules, that were made using two layers of Parafilm and a 96-well tissue culture plate (R 2508, Sigma, St. Louis, MO) as described in Reeve et al. (2003). In the present study, each capsule contained approximately 0.3 ml of diet. To avoid cannibalism, newly hatched larvae were kept individually in 50 mm Gelman Petri dishes covered with a Kimwipe® tissue. Each larva was provided with one or two Parafilm® capsules of diet and a moistened sponge every 2–3 days (Reeve et al., 2003). When larvae turned purple, indicating a readiness to pupate, they were provided with a piece of sterilized pine bark. After the larvae entered the bark, the bark pieces were placed in 17 × 12 × 6 cm plastic containers. This allowed collection of newly emerged adults (checked every 2 days) that were then placed in plastic containers (21 cm high × 21 cm diameter) for holding until needed.