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Source: Florida Entomologist, 89(1) : 95-97

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2006)89[95:LRPFTL]2.0.CO;2

# LABORATORY REARING PROCEDURES FOR TWO LEPIDOPTERAN WEED BIOCONTROL AGENTS

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Most noxious weeds infesting rangeland are exotic species (DiTomaso 2000). The genus *Centaurea* which contains several species of knapweed is the most abundant group in the western United States (Skinner et al. 2000). In their native habitat of Eurasia, natural enemies have prevented knapweed from becoming an economic problem (Keane & Crawley 2002). However, in the United States where they were accidentally introduced more than 100 years ago, these weeds, in the absence of their natural enemies, have reproduced unchecked and replaced many of the more desirable rangeland vegetation. Biological control of weeds with imported insects and pathogens is safe, environmentally sound, and cost effective (McFadyen 1998), and importation and use of highly host-specific biological control organisms offers considerable promise for weed control. Thirteen insect species have been imported into the US from Eurasia for control of knapweeds (Lang et al. 2000). Releases of some of these species on a Colorado grassland reduced diffuse knapweed by 77% of absolute cover (Seastedt et al. 2003). However, a major constraint to the widespread use of these biocontrol agents is the lack of sufficient numbers of insects for release. Story et al. (1994) produced 20,000 adults of *Agapeta zoegana* on spotted knapweed planted in field cages at a cost of $1.32/insect. Since artificial rearing has been used to mass-produce insects that are comparable to wild populations, at a reasonable cost (Leyva et al. 1995), it would be desirable to develop artificial diets to rear some of the weed-feeding insects.

*Pterolonche inspersa* (Lepidoptera: Pterolonchidae) that feeds both internally and externally on diffuse knapweed roots and *A. zoegana* (Lepidoptera: Tortricidae) that feeds on the roots of spotted knapweed, were selected for this study based on their host specificity and efficacy. Schroeder (1977) considered *P. inspersa* to be one of the most promising candidates for the biological control of diffuse knapweed in North America. In both species, rosettes were preferred for oviposition as well as feeding by newly hatched larvae. In northern Greece, *P. inspersa* was reported to be univoltine, diapausing during the winter months as 3rd instars (Campobasso et al. 1994; Dunn at al. 1989). *A. zoegana*, which has six instars, can complete 2-3 generations per year in Europe (Müller et al. 1988), whereas in British Columbia, it is restricted to only one generation (Muir & Harris 1987). However, there is little information on the nature of the diapause and no descriptions of rearing methodology for either of these species. We report here that both species can be reared from the egg to the adult stage on artificial diet and that diapause can be averted or shortened under our rearing conditions.

Adults of *P. inspersa* and *A. zoegana* were collected from diffuse and spotted knapweed in western Montana during June of 1997. The moths were placed in 500-ml cylindrical paper oviposition containers lined with wax paper, provided with 10% honey for food, and shipped overnight to the USDA-ARS Insect Biocontrol Laboratory, Beltsville, MD. The containers were held at 28° ± 2°C, 55 ± 10% RH and a photoperiodic regimen of LD 15:9. Eggs were removed every third day and transferred to Petri dishes lined with moist filter paper. Newly hatched larvae were placed directly on diet. Because there was no artificial diet available for any insect in the family Pterolonchidae, we obtained a diet developed for the pink bollworm, *Pectinophora gossypiella*; another member of the superfamily Gelechioidea. Similarly, for *A. zoegana*, we obtained diet developed for another member of the Tortricid family, the Eastern spruce budworm, *Choristoneura fumiferana*. Both these diets were purchased from Southland Products, Lake Village, AR. Recipes for the *P. gossypiella* and *C. fumiferana* diets can be found in Bartlett and Wolf (1985) and in Robertson (1985), respectively. Roots of knapweed, obtained from Montana, were washed, freeze-dried, and powdered in a grinder. Diets were prepared with or without 2% root powder. Following testing of the first batch of diets, we reduced the water content by 10% (from 930 ml recommended by Southland Products to 835 ml per liter of diet). Three types of containers; wax coated paper straws (7 mm in

diam × 10 cm long), borosilicate glass culture tubes (10 × 75 mm) and 30-ml clear plastic cups with paper lids (BioServ, Frenchtown, NJ) were tested for suitability in rearing both species. All test containers were filled with diet and infested with either 1 or 4 larvae. The straws were placed in desiccators containing water, and together with tubes and cups kept in an environmental chamber maintained at LD 15:9 and temperatures of 30° ± 2°C and 25° ± 2°C during the light and dark cycles, respectively. Subsequently, 131 cups containing pink bollworm diet and 509 cups containing Eastern spruce budworm diet, both with and without 2% root powder were infested with *P. inspersa* and *A. zoegana* larvae, respectively. The containers were examined every 10 days for 50 days. Some of the larvae/pupae were shipped to Sidney, MT. Whereas adults were allowed to emerge from the pupae, the cups with larvae were placed in a refrigerator (3°C) for 86 days to provide for diapause, after which time the cups were returned to 28°C to promote further development.

Hatch was high (> 80%) if eggs were placed on moist filter paper in Petri dishes. Since both species were root feeders, we first chose to test diet-filled straws, thus providing conditions for rearing that simulated the natural environment. However, the straws became moldy even though the diet was prepared and the straws filled in a laminar flow hood. Culture tubes were also inappropriate as rearing vessels because the tubes retained too much moisture and the larvae invariably drowned. Only approximately 10% of *P. inspersa* and 2% of *A. zoegana* reached the 2nd instar in tubes, even in the presence of root powder. Growing larvae in plastic cups with paper lids was the most effective method tested. A comparison of results from experiments in which 1 *vs* 4 larvae were placed in each cup of the appropriate diet showed that no more than one larva survived and grew to maturity. While both species of larvae remained alive for several weeks on diets in which root powder was omitted, insect growth was considerably slower than when root powder was present in the diet. With the incorporation of root powder, the larvae initiated feeding and tunneled into the diet. Whereas 6.1% of *P. inspersa* larvae grew to 3rd or higher instar on diet without the root powder, 33.3% did so when root powder was incorporated into the diet. Larvae molted 4 times and reached the 5th instar in approximately 50 days. These 5th instars contained large amounts of fat and their mean length was 8.6 mm (Fig. 1A). Percent survival of *A. zoegana* was lower than that of *P. inspersa*, i.e., 9.1% of the larvae placed in cups containing the Eastern spruce budworm diet with root powder survived beyond the 3rd instar. The mean length of *A. zoegana* 4th and 5th instars was 6.0 and 9.1 mm, respectively, and it took approximately 45 days for larvae to reach the 5th instar (Fig. 1B). Larvae of both species spun silk sheaths during feeding, and removal from their sheaths often resulted in larval death. Less than optimal percentages of survival were due in large part to high mortality of first instars. In our study, two factors appeared to con-

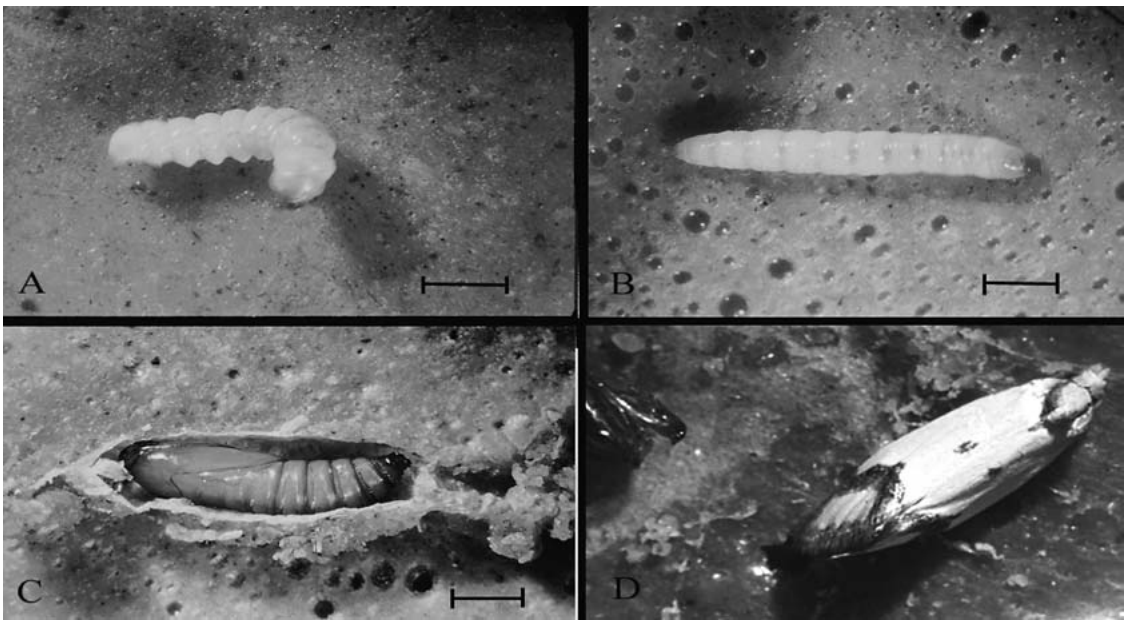


Fig. 1. *Pterolonche inspersa* and *Agapeta zoegana* reared on their respective artificial diets. A. 5th instar of *P. inspersa*. Note the accumulated fat in the body. B. 5th instar of *A. zoegana*. C. pupa of *A. zoegana*. Scale bars = 2 mm. D. Adult *A. zoegana* newly eclosed from the pupa.

tribute to high mortality; drowning in droplets of moisture and lack of immediate feeding. Lowering the moisture content of the diets by 10% and allowing the diets to dry for one hour before infesting with larvae reduced mortality. Although 2% root powder was incorporated into the diets, apparently it did not provide sufficient stimulation for a large percentage of the newly hatched larvae to feed. Because the young larvae of both species mine in the rosette leaves before they tunnel into the roots (Müller et al. 1988), it is possible that some chemical factor present in the leaves, but absent in the roots, may be acting as an initial feeding stimulant. It is suggested that in future studies, two diets, one with root powder as used above and a second one with 2% freeze-dried powdered young leaves of corresponding plants be prepared and poured as two layers into 22 × 52 mm plastic tubes (BioQuip, Gardena, CA) or cups.

We observed three *A. zoegana* pupae (Fig. 1C) which had apparently developed without undergoing diapause. The remaining *A. zoegana* and all of the *P. inspersa* larvae entered diapause. Approximately 30 days after removing both species of larvae from the refrigerator, the first pupa appeared and within a week, the first adults began to emerge (Fig. 1D). Twenty-three and 37 adults of *A. zoegana* and *P. inspersa*, respectively, emerged from late April to early September. These adults were placed in mating cups and eggs that were laid were used to establish an $F_2$ generation. Data from studies performed in Europe indicate that *P. inspersa* is a univoltine species and showed that this insect enters diapause as 3rd instars (Schroeder 1977). In our investigations, some larvae developed to the adult stage without entering diapause, indicating that it is possible to select individuals from a population and use these insects to develop a non-diapause strain. We recognize that this study was not a comprehensive one. However, the basic information that we have obtained can be useful in developing mass rearing techniques for exotic biocontrol agents such as the two lepidopterans used in this study.

We thank Jim Story and Linda White (Montana Agricultural Experiment Station, WARC, Corvallis, MT) for collection and shipment of insects to Sidney, MT. Thanks are also due to Dr. Lincoln Smith, USDA, ARS, Albany, CA, and Dr. David Kazmer, USDA, ARS, Sidney, MT for critically reviewing an earlier version of the manuscript. Mention of a proprietary product is not an endorsement by the US Department of Agriculture.

## Summary

Laboratory rearing methods for *P. inspersa* and *A. zoegana*, introduced into North America for the control of the exotic knapweeds, *Centaurea* spp., were developed. We used known diets for the pink bollworm and Eastern spruce budworm and added 2% knapweed root powder to these. After 45-50 days, we obtained some 4th and 5th instars of both species, which apparently either averted or shortened the diapause for these individuals.

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