NOTE

Winter Survival of *Cassida rubiginosa* (Coleoptera: Chrysomelidae), a Biological Control Agent of Canada Thistle[1]

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Cassida rubiginosa Müller, a thistle-feeding shield beetle native to the Palearctic Region of Europe and Asia (Zwölfer and Eichorn 1966. Z. Angew. Entomol. 58: 394-397), was accidentally introduced into eastern North America around 1901 (Barber 1916. Proc. Entomol. Soc. Wash. 18: 113-127). It is currently distributed south to Virginia and west to southern Michigan and Ohio. Its host range in North America includes several economically important thistles—*Carduus thoermeri* Weinmann, musk thistle; *C. acanthoides* L., plumeless thistle; *Cirsium arvense* (L.) Scop., Canada thistle; *C. discolor* (Muhl.) Spreng, field thistle; and *C. vulgare* (Savi) Tenore, bull thistle (Ward and Pienkowski. 1978. Ann. Entomol. Soc. Am. 71: 585-591).

Adult beetles of *C. rubiginosa* enter diapause in the fall and emerge the following spring for reproduction. Overwintered *C. rubiginosa* adults appear on the host plant in late winter upon the first occurrence of warm weather. The overwintering location of the beetles and proportion surviving each winter in Virginia is unknown. The prehibernal and hibernal activity of this insect was studied in the Ojcow National Park in southern Poland (Kosior and Klein. 1970. Acta. Zool. Cracov. 15: 315-340). In Poland, adult beetles were observed migrating from thistle to forest floor litter on a south-facing slope from late August to early October. Kosior (1975. Acta. Zool. Cracov. 20: 251-393) studied the overwintering habits of Cassidinae in Poland. He concluded that location of a favorable hibernation site was probably determined by greater insolation and higher air and soil temperatures. Palij and Klepikova (1957. Entomol. Oboz. 36: 75-95) observed that the majority of Cassidinae hibernate in forest litter. Overwintering mortality of *C. rubiginosa* has not been reported.

A crucial factor in determining the efficacy of a biological control agent is the ability of the agent to maintain its numbers. Data on reproduction and mortality, and the factors that influence these dynamic processes, provide important clues about a given

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population. Mortality due to incomplete development and parasitism (Ang and Kok. 1995. J. Entomol. Sci. 30: 9-17), and the reproduction of *C. rubiginosa* (Spring and Kok. 1997. Environ. Entomol. 26: 876-881) were studied. This report is on the winter survival and probable overwintering site of *C. rubiginosa.*

This research was conducted at the Kentland Farm in Montgomery Co., VA over a 2-yr period. In September 1992 (year 1), a randomly selected Canada thistle plant was caged in aluminum screening (18 mesh, 0.76 by 0.76 by 1-m) and replicated four times. Cage bases were buried about 15 cm beneath the soil surface. Fifty field-collected adult beetles were placed in each of the four cages. Soil and ambient temperatures were measured daily using a Campbell Scientific CR-21 micrologger (Campbell Scientific, Inc., Logan, UT) located at a weather station approximately 500 m from field cages. During months with heavy precipitation, snow-covered roads and fields often prevented access by truck to the field cages. To maintain consistency on snowfall data source, data on snowfall were reported from the National Oceanic and Atmospheric Administration's (NOAA) Blacksburg SE station. The NOAA Blacksburg SE station is located approximately 9.3 km from the field cages. In late-February, before new Canada thistle shoots emerged, potted musk thistle plants were placed inside each cage to provide food for beetles emerging from diapause. On 6 May 1993 vegetation in each cage was removed and examined for presence of *C. rubiginosa.* The cage interiors (including the soil) were also thoroughly examined for beetles.

In September 1993 (year 2), the overwintering study was repeated with one Canada thistle plant in the center of each cage. A hinged door was added to the cage and weather stripping (6 mm) was placed at the cage opening/door interface to prevent gaps through which beetles could escape. Aluminum flashing (15 cm high) was used to partition the cage floor into four equal sections, and straw, brick, decaying wood, or leaf litter was placed in each of the sections. Orientation of harborages within each cage was randomized. The layer of leaf litter and straw in each cage was approximately 10 to 15 cm deep. Hardwood logs from a fallen tree in decay for 3 to 4 yrs were used as decaying wood harborages. Bricks of even size were stacked together (allowing small spaces between them) for the brick harborages in each cage. The harborage component of the experiment was added to determine if any preference existed among the four materials as an overwintering site. One hundred and fifty adult field-collected beetles were placed inside each cage. Beetle behavior was observed every other day until activity inside the cages appeared to cease. Soil and ambient temperatures were measured daily as in the previous season. In early March 1994, each of the harborages was examined for presence of beetles. The number of beetles (dead and live) found in each harborage was noted. Potted musk thistle plants were placed inside each overwintering cage (in the center, where all four harborage sections were joined) to provide food for beetles that may have been missed during counting. The cages were reexamined weekly until no additional beetles were recovered. Comparisons between mean overwintering survival for years 1 and 2 were made using the Mann-Whitney *U*-test. Data on harborage occupation were analyzed with one-way analysis of variance (ANOVA) (Zar 1984. Prentice-Hall, Englewood Cliffs, NJ. 718 pp.), and the Tukey-Kramer multiple comparisons test (Zolman 1993. Oxford Univ. Press, Oxford, NY. 343 pp.) was used for mean separations.

Survival of overwintered beetles for the first year (1992/93) was 20.5 ± 17.2% (range, 10 to 46%). Precipitation and melting snow combined with field topography caused 3 of the 4 overwintering cages to remain flooded from mid-December until mid-April. The only cage that did not experience flooding at any time during the winter had the highest (46%) survival rate. Survival for the 3 flooded cages was 16, 10, and 10%. The mean monthly soil temperatures ranged from 2.1 to 14.3°C between October of 1992 and April of 1993 (Fig. 1a). Total monthly snowfall (Fig. 1c) ranged from 0 to 45.7 cm but was somewhat filtered by the cage screening. As a result, only about 50% of the reported snowfall reached the cage floor.

Survival for the second year (1993/94) was 21.3 ± 3.6% (range, 17.3 to 26.0%) and was not significantly different ($P > 0.05$) from survival in the first year (20.5 ± 17.2%). Of a total of 600 beetles released into the cages, 210 (35%) were recovered intact. Of those that were recovered, the most beetles (both dead and live) were found in leaf litter (96) and straw (73) harborages (Table 1). The decaying wood and brick harborages contained 17 and 7 beetles, respectively. Seventeen beetles were recovered from nonharborage areas within the cage. Number of beetles found in the straw and leaf litter harborages were significantly ($P < 0.05$) greater than the number of beetles found in the brick and wood harborages. There were no significant differences ($P >$ 0.05) for beetles found in leaf litter and straw harborages, or in brick and wood harborages. Some beetles not detected before emergence from diapause were collected from potted musk thistle placed inside the cage (or elsewhere inside the cage). The mean monthly soil temperatures ranged from −1.1 to 19.6 °C between October 1993 and April 1994 (Fig. 1b). Total monthly snowfall ranged from 0 to 25.4 cm but was filtered by the cage screening. No cages were flooded at any time during the winter in the second year.

The 46% survival observed for the non-flooded field cage in year 1 indicates that flooding was most likely responsible for the lower survival (12 ± 3.5%) observed in the three other field cages in the same year. Kok (1976. Environ. Entomol. 5: 1105-1108) examined the overwintering mortality of caged *Rhinocyllus conicus* Froel. (Coleoptera: Curculioridae) in Virginia over a 2-yr period and reported significantly higher mortality (33.3% and 41.7%, respectively) in cages without sufficient drainage when compared with control. Kung et al. (1992. J. Econ. Entomol. 85: 1695-1700) studied diapause survival of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and noted that higher mortality was generally observed in wet treatments than in dry ones and suggested that heat may be transferred away from beetles more rapidly in wet soil. Cool, low temperatures and high soil moisture would have also made the beetles more susceptible to attack by fungal pathogens.

Survival (21.3 ± 3.6%) for the second overwintering season (during which flooding did not occur), was half that of the 46% observed for the non-flooded cage in the first year. Less widely fluctuating (range, 1.1 to 3.2°C) monthly soil temperatures the first year (Fig. 1a) is most likely responsible for the difference in survival. The most critical period for insects passing the winter is the fall and early spring (Holmquist 1931. Ecology 12: 387-400). An overwintering study involving the Colorado potato beetle revealed that the greatest mortality occurred in late fall, when the beetles were physiologically unprepared to withstand extreme low temperatures, and in the spring, when they were unable to restore sufficient water balance (Kung et al. 1992, Milner et al. 1992. J. Econ. Entomol. 85: 1701-1708). Lee (1989. Bioscience 39: 308-313) also stated that widely fluctuating temperatures of fall and spring are often responsible for overwintering mortality among insects. Mild temperatures can cause a rapid loss of cold hardiness (Lee 1989) leaving the insect vulnerable to returning low temperatures (Moore and 1991. Amer. Entomol. 41: 111-118). Fig. 1b illustrates the range with which soil temperatures fluctuated in the second year, particularly in fall and spring.

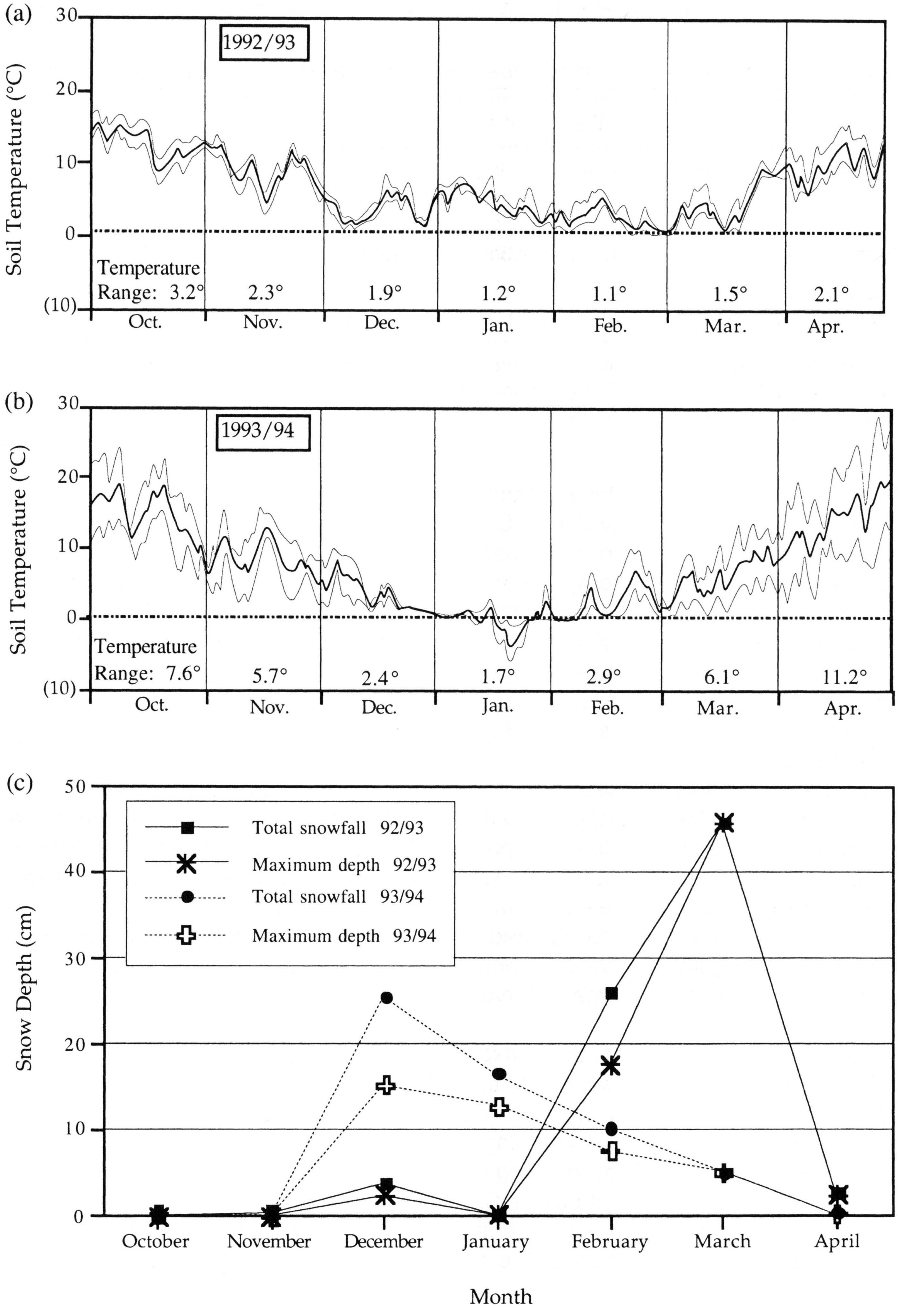

Fig. 1. Average daily soil temperatures and snowfall for winter 1992/93 (a) and 1993/94 (b). Soil temperatures taken at depth of 50 mm; data are averages of 24 one-hour observations. Upper, middle and lower line represent the maximum, mean and minimum soil temperatures, respectively. Data on snowfall (c) are from the National Oceanic and Atmospheric Administration, Blacksburg SE station, VA.

Table 1. Mean number of *C. rubiginosa* recovered from overwintering harborages

Condition upon recovery	Treatment* (mean ± SE)						
	Leaf litter	Straw	Wood	Brick	Other**	*F*	*P*
Live	13.3a ± 3.0	11.8ab ± 2.2	2.0c ± 0.9	0.8c ± 0.5	4.3bc ± 0.8	10.77	0.0003
Dead	10.8a ± 1.6	6.5ab ± 1.2	2.3bc ± 1.3	1.0c ± 0.7	—	12.97	0.0004
Live and dead	24.0a ± 2.6	18.3a ± 2.6	4.3b ± 1.9	1.8b ± 0.5	4.3b ± 0.8	27.44	<0.0001

* Mean ± SE per replicate based on four replicates (n = 600); means with similar letters in same row are not significantly different (P = 0.05, Turkey-Kramer test).

** Other = Places within cage not including leaf litter, straw, wood or brick.

The monthly range of soil temperature variations was <3.5°C in the first year, but varied widely between 1.7 and 11.2°C the second year.

Leaf litter was the preferred hibernaculum followed by straw (Table 1). Beetles in the straw and leaf litter harborages were typically found in a layer of loose topsoil and partially decayed vegetation approximately 10 cm under the surface of the straw or leaves. Significantly fewer (P < 0.05) beetles were found in brick and wood harborages. This concurs with the literature on the overwintering of Cassidinae in Poland. In Poland, *C rubiginosa* was observed migrating from the meadows in which it feeds and reproduces to nearby forests where it spends the winter under leaf litter (Kosior and Klein 1970).

Several of the dead beetles recovered in spring from the overwintering field cages were not intact (elytra only) or partially decayed. Arthropod predators were present in the cages in fall and were likely responsible for some of the mortality of this type; however, decay suggests that some beetles died before the onset of cold temperatures. Insectivorous mammals also were present in the field cages for both years. Shrews and field mice had constructed burrows providing them access to cage interiors and may be partly responsible for the low recovery of beetles in the spring. This would indicate that some of the observed mortality was caused by factors other than a lack of cold hardiness.

The results obtained from these experiments may not reflect the exact overwintering survival of *C. rubiginosa* due to the artificial conditions inherent in field caging. Confinement of the beetles does not allow them the freedom to choose the hibernacula most appropriate for maximizing winter survival. Additionally, cage screening prevents some snowfall from reaching the ground thereby limiting some of the insulating properties afforded by snowfall. Caging also provides protection from wind and possible harborage disruption. Data from these experiments, however, do represent the minimum survival likely under the experimental cage conditions present in this study.

These results indicate that less than one in four beetles survived winter conditions in both years. Leaf litter was a preferred hibernaculum and inability to access preferred hibernating locations combined with fluctuating winter temperatures may result in heavy mortality of *Cassida rubiginosa*.