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Biology of *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) in the laboratory¹

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Abstract:

The flea beetle *Aphthona nigriscutis* Foudras was introduced to Canada in 1983 and the United States in 1989 for the biological control of leafy spurge, *Euphorbia esula* L. Life history data for *A. nigriscutis* are described based on laboratory studies using insect populations from Barnes County, North Dakota, and Fallon County, Montana. Initial emergence of males and females from spring soil samples was synchronized, but complete emergence for males preceded that of females. For the Barnes County population, ≈80% of the adults were female. When adults were held as mated pairs at 25° C and a photoperiod of 14:10 (L:D) h, male longevity was longer than that of the female with a median longevity of 221 and 124 days, respectively. Oviposition started ≈10 days after female emergence and proceeded at a rate of 4 eggs per day over an average oviposition period of 109 days. Oviposition averaged 537 eggs per female with a range of 103-1,157. Eggs from the Fallon County population started to hatch within 10 days after oviposition when eggs were held at 25° C. Egg hatch averaged near 60% and occurred over a period of ≈8 days. Life history data for *A. nigriscutis* were similar to other univoltine *Aphthona* spp., except *A. nigriscutis* had a female-biased sex ratio, greater male longevity, and higher female oviposition.

Keywords:

Aphthona nigriscutis, adult biology, leafy spurge.

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Introduction

The flea beetle *Aphthona nigriscutis* Foudras was introduced to Canada in 1983 and the United States in 1989 as a biological control agent for leafy spurge, *Euphorbia esula* L. (Rees *et al.* 1995). This noxious perennial weed infests > 1 million hectares with significant infestations on the Great Plains of the United States and Prairie Provinces of Canada (Noble *et al.* 1979, Messersmith and Lym 1983). In pasture and rangeland, leafy spurge displaces desired forages, especially for grazing cattle. Cultural and chemical control methods can be effective but long-term suppression over large areas is often impractical and expensive.

Forty species of *Aphthona* have been reported to associate with spurges (*Euphorbia* sp.) in Europe and Asia (Harris *et al.* 1985, Pemberton and Wang 1989). Six species [*A. abdominalis* Duftschmid, *A. cyparissiae* Koch, *A. czwalinae* (Weise), *A. flava* Guillebeau, *A. lacertosa* Rosenhauer, and *A. nigriscutis*] have been established in the United States (Rees *et al.* 1995). For the univoltine species (i.e., all except *A. abdominalis*), adults usually emerge in June and July, feed on leaf tissues, and oviposit in the soil near the plant base. The 3 larval instars tunnel the root system (Maw 1981) causing damage to primary and lateral roots. These species overwinter as larvae, typically in the 3rd instar. Pupation occurs in the spring. Details of the life history have been described for *A. abdominalis* (Fornasari 1993), *A. cyparissiae* (Sommer and Maw 1982), and *A. flava* (Sommer and Maw 1982).

Maw (1981) compared the occurrence of *A. cyparissiae*, *A. czwallinae*, *A. flava*, *A. lacertosa*, and *A. nigriscutis* in several sites in Europe with different spurge species, vegetational characteristics, and soil texture. *A. nigriscutis* was similar to *A. flava* and *A. cyparissiae* in its frequent association with *Euphorbia cyparissias* (Maw 1981, Sommer and Maw 1982, Pemberton and Rees 1990). *A. nigriscutis* differed in that it favored dry habitats (Maw 1981). All 3 species have been widely distributed and established throughout the northern Great Plains for the biological control of leafy spurge (Rees *et al.* 1995). Detailed knowledge of the life history of *A. nigriscutis* would assist laboratory research and the effective use of this biological control agent in the field. This study reports on adult emergence, longevity, oviposition pattern, oviposition rate, and egg hatch under laboratory conditions.

Materials and methods

Aphthona nigriscutis for the emergence, longevity, and oviposition studies were collected in May (1994 and 1995) as late stage larvae and pupae in soil samples. The samples were collected from a 1989 release site established by Bob Carlson and Don Mundal (North Dakota State University, Fargo, ND) in Barnes County, North Dakota. Five undisturbed samples (15 cm deep and 10.5 cm diameter) were placed into each of 2 plastic buckets. A bouquet of leafy spurge was changed 2-3 times per week to supplement shoots that grew from the soil samples. The soil was watered periodically. Buckets were covered with a fine screen and held at 25° C with a photoperiod of 14:10 (L:D) hours. Beetle emergence was checked daily.

Adults were sexed and paired within 24 hours of collection from the soil samples. Twenty and 19 male-female pairs were established in 1994 and 1995, respectively. Pairs were held in cages of clear acrylic tubing (7 cm long and 5 cm diameter) with a 44-mesh nylon screen cemented to one end. The open end of the tube was forced into sieved (particle diameter <180 µm), moist soil held in the bottom of a petri dish (60 mm diameter by 15 mm deep). Narrow furrows were made in the soil to serve as oviposition sites. Cages were held at 25° C, a photoperiod of 14:10 (L:D) hours, and 80% RH. On Monday, Wednesday, and Friday, each pair was transferred to a new cage along with a fresh-cut stem tip (5-8 leaves) of leafy spurge. The stem tip rested on the moist soil in the bottom of the cage. Eggs were isolated from soil, leaf material, and cage surfaces by rinsing the cage and petri dish with water over a sieve (250-µm openings). Eggs and debris were recovered on the screen and transferred to moist filter paper. Eggs were counted using a dissection microscope. Beetle mortality was recorded on the dates of transfer. Cages were maintained using the same methods until both individuals of the pair were dead. Data were checked for normality and summarized using SAS/Assist and Insight (SAS Institute 1991).

Aphthona nigricutis adults used for the egg hatch study were collected in June 1994 from a 1990 release site established by Neal Spencer (Agricultural Research Service, Sidney, MT) in Fallon County, Montana. Approximately 1,000 beetles were placed in a screened, wooden framed cage (30 cm wide by 30 cm deep by 60 cm long) with bouquets of fresh-cut leafy spurge. The cage was held at 25° C, a photoperiod of 14:10 (L:D) hours, and 60% RH. Leafy spurge bouquets were changed 3 times weekly. To facilitate egg collection, 2 petri dishes (150 mm diameter by 20 mm deep) were filled with sieved (particle diameter < 180 µm), moist soil and placed under the leafy spurge bouquets. The soil surface was scored with a knife to create 7-8 narrow furrows per dish. These dishes were changed weekly. After the colony was maintained for 1 month, a subsample of the weekly egg collection was acquired by offering the adults new dishes for a 24-hour period. The eggs from these new dishes were isolated from the soil as described previously, selected without bias (i.e., for size, color, or shape), and transferred with a fine wet brush to moist filter paper. Two or 3 groups of 100 eggs were transferred weekly for 5 consecutive weeks to moist filter paper and held in a covered petri dish at 25° C in darkness. Egg groups were checked daily for hatch until all eggs had hatched or perished. Regression analysis was conducted using SAS/Assist (SAS Institute 1991).

Leafy spurge used for adult food was collected from untreated natural infestations near the Northern Grain Insects Research Laboratory, Brookings, SD, or raised in the greenhouse (Lym 1992) with initial stem tip cuttings from a NGIRL infestation. Stem tips and bouquets used as food were from young shoots (i.e., <30 cm long, before flowering).

Results

Adult emergence from the 1994 and 1995 soil samples held at 25° C was similar (Fig. 1). The 10 samples collected each year yielded 229 and 283 adults, respectively. Adult populations were 84% female in 1994 and 78% female in 1995. Both sexes emerged on the 1st day of adult collection. During both years, female emergence extended over a

longer time interval than male emergence. Male emergence was 80% complete after 13 days, whereas female emergence required \approx 21 days to reach 80%.

Total Adults

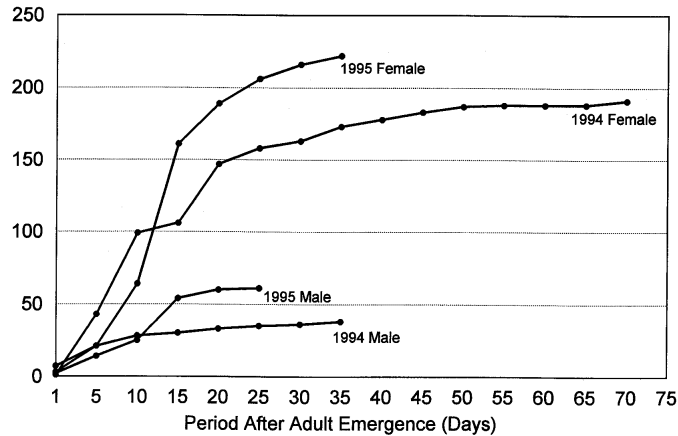


Fig. 1. Cumulative emergence of *A. nigriscutis* males and females from soil samples held at 25° C in 1994 and 1995.

Adult longevity distributions were not normal, except for 1994 males and 1995 females. Male longevity was more variable and the median longevity was longer as compared with the female (Table 1). When the 1994 and 1995 data were combined, median longevity for males was 221 days (range, 25-387) and for females was 124 days (range, 47-258). Sixty percent of the adults lived >120 days.

Table 1. Longevity (d) of *A. nigriscutis* adults.

Year	Sex	Median	25 th percentile	75 th percentile	Percentile difference
1994	Male	233	99	279	180
1995	Male	174	83	245	162
1994 and 1995	Male	221	96	259	163
1994	Female	93	90	164	74
1995	Female	162	111	191	80
1994 and 1995	Female	124	90	191	101

All pairs produced eggs. Females started ovipositing within 4-16 days after eclosion with a mean starting time of 10 ± 3 days. Initial oviposition was not significantly different for the 2 years ($F = 0.1502$; $df = 1, 37$; $P = 0.7006$). Mean oviposition per female was

537 ± 262 eggs (mean ± SD) and was not significantly different for the 2 years ($F = 0.0167$; $df = 1, 37$; $P = 0.8979$). The range of female oviposition for both years was 103-1,157. The period of oviposition from the 1st to the last egg laid per female averaged 109 ± 49 days and was not significantly different for the 2 years ($F = 0.6337$; $df = 1, 37$; $P = 0.4311$). The pattern of oviposition was similar for both years (Fig. 2) with 90% of the oviposition complete after ≈100 days and the average number of eggs per day of female oviposition at 4 ± 2.

Eggs Per Female

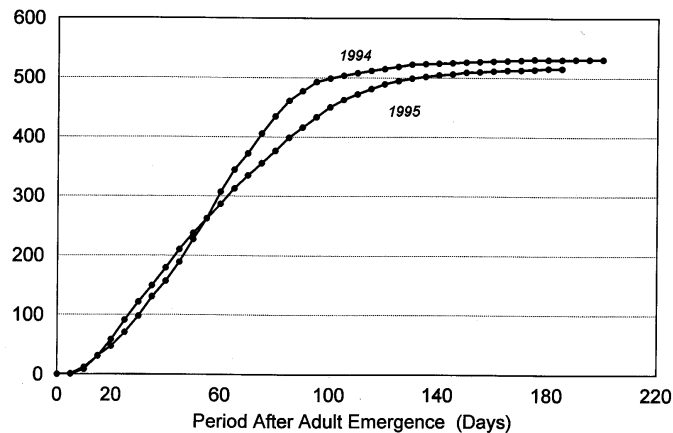


Fig. 2. Cumulative oviposition of *A. nigriscutis* females for 1994 ($n = 20$) and 1995 ($n = 19$).

Percentage of hatch from 1,100 eggs collected over 5 weeks from the Montana population of *A. nigriscutis* averaged 60 ± 5% and did not significantly change over a 5-week period ($F = 1.2127$; $df = 1, 9$; $P = 0.2994$). The average time to 1st hatch for eggs held at 25° C was 10 ± 1 days. The average period of hatch was 8 ± 2 days. Although egg hatch was not routinely determined for *A. nigriscutis* from North Dakota, the hatch from 132 eggs collected over a 5-week period from 1 pair in 1995 averaged 68%.

Discussion

Aphthona nigriscutis, *A. cyparissiae*, and *A. flava* are frequently associated with *Euphorbia cyparissias* in Europe including several sites in Hungary where these species are sympatric (Maw 1981). Life history data for *A. cyparissiae* and *A. flava* (Maw 1981, Sommer and Maw 1982, Pemberton and Rees 1990) were similar in many respects; with *A. nigriscutis* data from this study. This includes the synchrony of male and female eclosion, mating shortly after eclosion, 1st oviposition at ≈1 week after eclosion, a relatively

uniform rate of oviposition over the major portion of the oviposition period, and a similar percentage for egg hatch.

Differences in the life history of *A. nigriscutis*, *A. cyparissiae*, and *A. flava* were observed with respect to the population sex ratio, adult longevity, and oviposition per female. Sommer and Maw (1982) and Fornasari (1996) reported a sex ratio near 1:1 for adult populations of *A. abdominalis*, *A. chinchih* Chen, *A. cyparissiae*, *A. czwalinae*, *A. flava*, *A. seriata* Chen, and *A. venustula* Kutschera. Adult populations of *A. nigriscutis* in Montana and Canada have been observed to be near 90% female (Neal Spencer, personal communication) and a 1995 collection from Campbell County, South Dakota, was 97% female (unpublished data). These observations are consistent with the collection of near 80% females from the 1994 and 1995 North Dakota soil samples. Unequal sex ratios are not unusual for insect populations (Bedford 1980, Hoy 1985, Hamerski and Hall 1988) but if this contributes to a low mating frequency (e.g., < 45% of the females were mated in the 1995 Campbell County, South Dakota, population), population growth may be depressed. Additional samples and comparisons are needed from North American and European populations of *A. nigriscutis* before a female-biased sex ratio is considered typical for this *Aphthona* species.

Median adult longevity for *A. nigriscutis* was near 7 months for males and 4 months for females. In contrast, *A. cyparissiae* and *A. flava* adults maintained under laboratory and field conditions lived 3-4 months (Maw 1981). The longer longevity of *A. nigriscutis*, especially the males, may be a characteristic of the species however, more likely, it was a response to the favorable rearing conditions in this study. Adults were handled with great care and fresh leafy spurge leaves were always available from field or greenhouse grown plants. To support laboratory studies, additional data on adult longevity should be collected for *A. nigriscutis* from several populations. This is especially important because of the large variation in longevity values observed in this study. For field studies, adult longevity would rarely exceed 4.5 months in the northern Great Plains (i.e., adult eclosion in June to a killing frost by mid-October). If *A. nigriscutis* adult populations do not persist until a killing frost, factors that are reducing adult longevity should be evaluated. With oviposition uniformly distributed over 3-4 months, female longevity is critical to sustaining the insect population.

Mean oviposition for an *A. nigriscutis* female held in the laboratory was 537 eggs, with 21% of the females laying >800 eggs. This mean is about double the mean oviposition reported for laboratory held females of *A. cyparissiae* and *A. flava* (Maw 1981) and 9 times that reported for *A. abdominalis* (Fornasari 1993). The *A. cyparissiae* and *A. flava* data were based on females that survived 3 months. This is similar to the mean oviposition period observed for *A. nigriscutis* (i.e., 109 days). Although additional data from other *A. nigriscutis* populations should be collected, the oviposition potential of *A. nigriscutis* appears to be about double that of *A. cyparissiae* and *A. flava*.

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