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RESEARCH

Developmental Biology and Effects of Adult Diet on Consumption, Longevity, and Fecundity of *Colaspis crinicornis* (Coleoptera: Chrysomelidae)

Kentaro Miwa and Lance J. Meinke[1]

Department of Entomology, University of Nebraska - Lincoln, Lincoln, NE 68583
[1]Corresponding author, e-mail: lmeinke1@unl.edu

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ABSTRACT. The chrysomelid beetle *Colaspis crinicornis* Schaeffer (Coleoptera: Chrysomelidae) occurs primarily in the Great Plains region of the United States. Little is known about the biology and ecology of this species, but over the last decade, it has become increasingly common in the corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merrill, agroecosystem of southeastern Nebraska. As part of a larger comprehensive project to understand the natural history and pest potential of this species, laboratory experiments were conducted to study the developmental biology, morphological characters of immature stages, and the effect of adult diet on consumption, longevity, and fecundity. Females readily deposited egg clusters in the soil, and percentage egg hatch was high under laboratory conditions. Larvae and pupae were confirmed to be soil-dwelling stages. *C. crinicornis* has relatively short egg, pupal, and adult stages with the majority of its life cycle spent in the larval stage. Results of choice and no-choice adult feeding experiments indicate that diets of corn or soybean leaves did not significantly affect consumption, longevity, or fecundity of adult *C. crinicornis*, suggesting that corn and soybean leaves are similarly suitable food sources for adults. The ability to effectively utilize tissues from very different plant families as adult food sources suggests that *C. crinicornis* is polyphagous in the field.

Key Words: Chrysomelidae, Eumolpinae, *Colaspis crinicornis*, developmental biology

The leaf beetle genus *Colaspis* (Coleoptera: Chrysomelidae) contains over 200 species and is the largest New World genus in the subfamily Eumolpinae (Riley et al. 2002). Approximately 28 species are currently recognized in the United States and Canada (Riley et al. 2002). Most of what is known about *Colaspis* biology and ecology has been obtained from studies of the few species that can be agricultural pests (Lindsay 1943, Echols 1963, Rolston and Rouse 1965, Ostmark 1975, Balsbaugh 1982, Flynn and Reagan 1984, Oliver 1987, Lopez et al. 2002). Adults generally feed on plant shoot systems, whereas soil-inhabiting larvae feed on below-ground portions of plants (Lindsay 1943, Echols 1963, Ostmark 1975, Lopez et al. 2002). Some species are highly polyphagous, whereas others have more specific host ranges (Riley et al. 2002, Clark et al. 2004).

Colaspis crinicornis Schaeffer is one of the larger species among the yellow-brown, costate *Colaspis* species found in the United States (Fig. 1) and primarily occurs in the Great Plains (Blake 1974, Riley et al. 2003). Museum specimens indicate that *C. crinicornis* records from Nebraska date back to the 1910s, but its presence in crop fields was not recorded in Nebraska or Iowa until the mid-1990s or early 2000s (Bradshaw et al. 2011, L.J.M., personal observation). Population densities of *C. crinicornis* have been increasing during the last decade (L.J.M., personal observation) to the point that the species can be abundant in some locations (Miwa 2014). However, in contrast to some of the *Colaspis* species that have been associated with agricultural crops for many years, little information exists on the natural history and pest potential of *C. crinicornis.* Adults are most commonly found in corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merrill, fields from June through August in southeastern Nebraska (Miwa 2014) although low densities occur outside of crop habitats (K.M., personal observation). Moreover, adult *C. crinicornis* emerge from both corn and soybean fields and readily feed on leaves of both crops (Miwa 2014).

Because of the increasing awareness of *C. crinicornis* by growers in production agriculture, a proactive approach has been taken to understand the natural history and pest potential of this species. As part of a larger, comprehensive study, this article reports the results of laboratory experiments conducted to quantify basic aspects of developmental biology, describe immature stages, and characterize the effect of adult diet on consumption, longevity, and fecundity.

Materials and Methods

Developmental Biology Experiments

Adult Collection. Live adult *C. crinicornis* were collected in cornfields in Nemaha County, NE, for all laboratory experiments using screen emergence cages based on a modified design of Fisher (1980). The inside of each cage was kept free of corn leaves and weeds to minimize feeding by newly emerged beetles. Beetles were recovered from the cages every 3–4 d from inverted glass jars placed at the highest point of the cage. This type of emergence cage was originally developed to collect *Diabrotica* species in corn, but because of the similar behavior exhibited by both *Diabrotica* and *Colaspis* species, involving upward movement from the soil level after emergence, the cage worked well to capture *Colaspis* beetles. Beetles were transported to the laboratory in a cooler, and sex was determined for each beetle under a dissecting microscope based on descriptions given by Chapin (1979) before placing them in oviposition containers on the days of collection. Only active individuals were used in the laboratory experiments, and no evidence of adult infection by natural enemies such as nematodes or fungi was observed during the experiments. Identification of specimens collected during this study was confirmed by E. G. Riley at Texas A&M University, and vouchers are deposited in the University of Nebraska State Museum.

Oviposition Containers. A male and a female *C. crinicornis* were placed in each polystyrene oviposition container with silty clay loam soil and an adult food source. Containers were similar in design to that described by Campbell and Meinke (2010) and were 5.9 cm by 5.9 cm by 7.8 cm (length by width by height) with a 0.6-cm deep lid (ShowMan Box, Althor Products, Windsor Locks, CT). Soil was sifted through a 60-mesh sieve and then autoclaved. Approximately 50 ml of soil was placed in each container as an oviposition medium, and the soil was moistened with distilled water (about 27% moisture by volume).

Fig. 1. An adult *C. crinicornis* collected in southeastern Nebraska in 2012.

The soil was mixed in the container creating variously sized soil clumps as preliminary experiments indicated that *C. crinicornis* seldom oviposited in compacted soil with a smooth surface. Because adult *C. crinicornis* were found more commonly in cornfields than in any other type of habitat at the initiation of this study, a piece of corn leaf was placed in each container as the adult food source which was replaced every 3–4 d.

Egg Incubation Dishes. Eggs from each female were transferred with a camel hair brush and were placed on the soil surface in a covered polystyrene Petri dish (standard Petri dish: refers to 100 mm in diameter and 15 mm in height throughout the article, Thermo Fisher Scientific, Waltham, MD). The same type of autoclaved soil used in oviposition containers was used for egg incubation. Distilled water was added to achieve approximately 30% moisture by volume, and soil was smoothed out before eggs were placed on the soil surface to provide contact moisture, as eggs are sensitive to desiccation (K.M., unpublished data). Petri dishes were wrapped with Parafilm M (Bemis Company, Neenah, WI) and were kept at 23.0 ± 1.7°C. Neonate larvae were subsequently collected as they eclosed for use in laboratory experiments.

Female Fecundity on Corn. Eggs were collected from individual females over their life span to quantify the number of eggs laid per female, percentage of ovipositing females, percentage egg hatch, and duration of the egg stage. Female beetles each paired with a male were placed singly in oviposition containers as described under Oviposition Containers, above, and provided with corn leaves. Eggs were recovered by careful search of the soil every 3–4 days until the females died. In 2010, males were not replaced if they died before females, while one replacement male was added per female if the original male died before the female in 2011. Fresh corn leaves were provided after each search.

Log-transformed eggs laid per female and arcsine transformed percentage egg hatch were statistically compared between years using PROC TTEST in SAS software 9.2 (SAS Institute 2008). A two-way analysis of variance was used to analyze year and size of log transformed egg cluster main effects plus potential year by cluster size interaction (PROC MIXED, SAS Institute 2008). A chi-square goodness of fit test was used to compare the proportion of females that oviposited in 2010 versus 2011 (PROC FREQ, SAS Institute 2008). Nontransformed means are presented.

Larval Rearing and Development. In 2010, numerous attempts were made to rear *C. crinicornis* larvae using various types of substrate (e.g., soil, vermiculite, potting mix, and peat moss) with different moisture levels and larval food sources (e.g., seedlings of corn, soybean, and wheat, *Triticum aestivum* L., and larger plants of red clover, *Trifolium pretense* L., and alfalfa, *Medicago sativa* L.). However, most of these attempts resulted in little to no success. Based on results obtained in 2010, improved rearing methods were established in 2011and are reported in this article. Thirty neonate larvae were placed in each larval rearing container, which was a 59-cm^2 plastic cup with a lid (Solo Plastic Souffle Portion Cups, Solo Cup Company, Lake Forest, IL). Peat moss was used as the substrate and was sifted through a 4-mesh sieve before being autoclaved. Distilled water (∼20% moisture by volume) was added to peat moss in a larger container to prepare substrate for multiple larval rearing containers. Because larvae appeared to have difficulty moving if the substrate was loose, moist peat moss was compressed and filled to two-thirds the height of each container. Two germinated corn seeds (Pioneer Brand hybrid 34A15) were placed in each container. Five pin holes were made on each lid to allow slight ventilation. Containers were incubated at 23.0 ± 1.7°C for approximately 7 d to allow corn roots to grow inside containers before larvae were introduced. Two germinated corn seeds were added approximately every 10 d to adequately provide fresh roots for larvae. After 30 d, larvae were placed in new containers with fresh peat moss and germinated corn seeds. In 2011, larval survival was recorded at 30-day intervals for up to 270 d after larval eclosion. Head capsule width at the widest point and body length were measured 30, 60, and 90 d after larval eclosion.

Mature Larvae, Pupae, and New Adults. To study the late larval stage, pupal stage, and early adult stage in the laboratory, overwintering larvae were collected in cornfields in May and June 2012 at the University of Nebraska Agricultural Research and Development Center (ARDC) in Saunders County, NE. Full-sized larvae were recovered from the soil on corn root systems and were placed in the same larval rearing setup as described earlier after being transported to the laboratory. Pupae were normally kept in the larval rearing containers because deformation and mortality sometimes resulted when they were removed from pupal cells. However, some pupae were removed for measurements. Containers were monitored daily, and duration of larval, pupal, and teneral adult stages in the pupal cells and the interval between adult exit from the pupal cell to initiation of feeding were recorded when individuals could be observed through the side or bottom of the containers.

General morphological descriptions were made of eggs and neonate larvae obtained in the laboratory from field-collected beetles and pupae collected as overwintering larvae in the field. A more detailed description of full-sized larvae was made based on five individuals obtained from adults collected in Nemaha County, NE, during the summer of 2011. Morphological measurements were taken using an Olympus SZX16 stereomicroscope, Olympus DP26 camera, and Olympus cellSens v. 1.6 imaging software (Olympus, Tokyo, Japan).

Adult Feeding Experiments

Experimental Insect and Food Plants. Because *C. crinicornis* can be abundant in both cornfields and soybean fields (Miwa 2014), beetle feeding preference between corn and soybean leaves and the effects of food on longevity and fecundity of *C. crinicornis* were evaluated through a series of laboratory experiments in 2012. Newly emerged adults were collected in cornfields at the ARDC using the same type of emergence cages described earlier, and beetles were recovered from cages every 2 d. Pesticide-free corn (Pioneer brand hybrid P1151XR, DuPont Pioneer, Johnston, IA) and soybean (Pioneer brand variety 93M11) leaves were collected at the ARDC on the day each replication of each experiment was initiated. The top two or three leaves on vegetative-staged corn and top two or three trifoliates of soybean plants in the vegetative or early reproductive stages were used as adult food. Beetles placed in plastic containers and leaves stored in sealed plastic bags were transported in a cooler. The sex of each individual was determined under a dissecting microscope before the start of each replicate of each experiment, and only active beetles were used. Beetles were held at 23.0 ± 1.7°C during experiments.

Adult Feeding Preference: Corn Versus Soybean. To determine adult feeding preference between corn and soybean leaves, 72-h choice and no-choice tests were conducted. Each food leaf was cut to 50 mm

by 50 mm for both tests. In choice tests, an isolated beetle received 2,500 mm^2 each of corn leaf tissue and soybean leaf tissue placed approximately 5 mm apart in a large Petri dish (refers to 150 mm in diameter and 15 mm in height, VWR, Radnor, PA). In no-choice tests, each beetle was provided 2,500 mm^2 of either corn or soybean leaf tissue centered in each standard Petri dish. Leaves were placed on moist filter paper to keep them turgid for the duration of the experiments. Distilled water was sprayed on plant material to provide humidity, and dishes and lids were sealed with Parafilm at the start of each replicate. At least four individuals of each sex were tested on each of four dates for both choice and no-choice experiments (choice: 22 June, 27 June, 29 June, and 4 July 2012; no-choice: 27 June, 29 June, 1 July, and 4 July 2012). After each 72-h period, a digital scanner (HP Scanjet 3500c, Hewlett-Packard Company, Palo Alto, CA) was used to scan every piece of leaf onto a computer (Dell OptiPlex 580, Dell, Round Rock, TX). ImageJ 1.46p software (Rasband 1997–2014) was used to measure feeding areas on each leaf.

Data were statistically analyzed using PROC GLIMMIX in SAS software 9.2 (SAS Institute 2008). Analysis of variance was conducted to compare leaf areas consumed per beetle. The choice tests were treated as a split-plot design arranged in randomized complete block with date as the blocking factor. Sex was the whole-plot factor, whereas food type was the split-plot factor. In no-choice tests, the experimental design was a 2 by 2 factorial in randomized complete block. The two factors were sex and food type, and date was the blocking factor. Feeding area data were log-transformed before analyses, and nontransformed data are presented. The 0.05 level of significance was used in all analyses.

Effect of Food Plant on Adult Longevity. To test the effect of food plant treatments on longevity of adult *C. crinicornis*, individual beetles were placed in a Petri dish with a corn leaf, a soybean leaf, both corn and soybean leaves, or no food on a moist filter paper sealed with Parafilm. At least three individuals of each sex for each food treatment were tested on each of four dates (20 June, 25 June, 29 June, and 2 July 2012). Food was replaced every 3 d, and each dish was monitored daily until beetles died. Distilled water was mist-sprayed in each dish to provide humidity at the start of each replicate and every time food was replaced. Longevity was measured in *d* from field collection in emergence cages to death of the beetle.

Longevity was statistically compared among food treatments. The experimental design was a 2 by 4 factorial in randomized complete block with date as the blocking factor. The two factors were sex and food treatment (corn, soybean, both, and no food). Longevity data were log-transformed before analysis, and nontransformed data are reported.

Effect of Food Plant on Female Fecundity. To understand the effect of food plant treatments on fecundity, each female was placed with a male and host material (corn, soybean, both corn and soybean, or no food) in the oviposition containers as previously described in the developmental biology experiments. Food was replaced every 3–4 d, and eggs were recovered from soil on the d food was replaced. Eggs from a subset of females were held to measure survival to larval eclosion in egg incubation Petri dishes as previously described.

The percentages of ovipositing females were arcsine transformed and statistically compared among food treatments in a randomized complete block with date as the blocking factor. The number of eggs laid per ovipositing female was also analyzed among food treatments in the same design with square-root transformation. The percentage egg hatch was compared among food treatments in a randomized complete block design after arcsine transformation. Data presented are nontransformed.

Results

Developmental Biology Experiments

Description of Immature Stages and Associated Behaviors.

Eggs. Newly deposited eggs were pale yellow to bright yellow and elongated-ovoid (Fig. 2). The mean size of 30 freshly deposited, randomly selected eggs (five eggs from each of six females) were 0.71 ± 0.01 mm in length and 0.29 ± 0.002 mm in width (error terms are SEM, used throughout the article). The egg chorion was delicate and semi-transparent without any distinctive sculpturing. Eggs adhered to each other. Embryonic development could be observed through the egg chorion. Color was uniform throughout the egg when it was first deposited. As an embryo began to develop, a clear area appeared on one end of the egg and gradually expanded to occupy one-fourth to one-fifth of the egg. The clear area then disappeared, and the entire egg clouded again. Two dark dots, which were probably developing mandibles, appeared toward one end of the egg. Shortly before larval eclosion, movement of the larva inside the egg was visible. Among the egg clusters for which oviposition date could be determined (i.e., eggs visible through oviposition containers), the mean days to egg hatch was 8.00 ± 0.91 (range: 6–10 d, $n = 4$) in 2010 and 8.40 ± 0.51 (range: 7–10 d, $n = 5$) in 2011.

Larvae. Larvae were scarabaeiform with well-developed thoracic legs and an orange head capsule. The body was white or pale yellow throughout most of the larval stage (Fig. 3). However, newly hatched larvae had white head capsules and yellow, transparent bodies (Fig. 4). Mean head capsule width of neonate larvae was 0.21 ± 0.003 mm (range: 0.19–0.23 mm, $n = 18$), and mean body length was 1.47 ± 0.49 mm (range: 0.90–1.07 mm, $n = 18$) when measured on the day of larval eclosion (three offspring from each of six females were measured). The head capsules turned orange after 1 or 2 d, while bodies became increasingly white and less transparent. Neonate larvae normally dispersed shortly after eclosing from eggs, crawling on the

Fig. 2. An egg cluster of *C. crinicornis.*

Fig. 3. A full-sized *C. crinicornis* larva.

surface of soil in the egg incubation dishes or moving into the soil. After transfer to larval rearing containers, neonate larvae did not appear to initiate feeding immediately but wandered around in the substrate. High mortality was observed among larvae, especially during the first 30 d after egg hatch (Fig. 5). After 30 d, mortality rate declined although survival continued to decrease gradually over time (Fig. 5). The surviving larvae fed externally on roots, consuming root hairs and tissues. Larvae were near full-sized 90 d after egg hatch. The mean head capsule width of these larvae was 1.29 ± 0.08 mm (range: 1.11–1.49 mm, $n = 5$), whereas the mean body length was 8.02 ± 1.03 (range: 6.24–10.65, $n = 5$) (Fig. 6). Larger larvae were more mobile and fed more vigorously on root tissues, cutting small corn roots. None of the larvae from eggs collected in the laboratory pupated successfully, even though a small number of individuals overwintered at room temperature in the prepupal stage.

Fig. 4. An egg cluster, empty egg chorions, and neonate larvae of *C. crinicornis*.

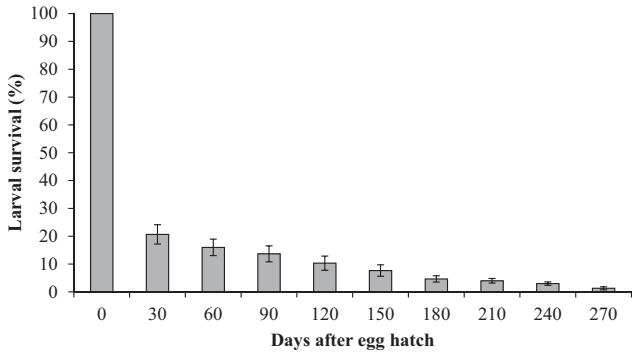

Fig. 5. Mean percentage ± SEM survival of *C. crinicornis* larvae obtained from eggs deposited by field-collected adults in the laboratory during 2011. In each of 10 larval rearing containers filled with peat moss and corn seedlings, 30 neonate larvae were placed on the days eggs hatched. None of the larvae reached the adult stage.

Description of Full-Sized Larvae. Head capsule well developed, orange, with no ocelli. Antenna three segmented: first segment longest; second much shorter than first; third segment bilobed with one lobe broad and other slender with setae at the tip. Widest point of labrum approximately the same width as the distal edge of the clypeus; labrum partly covering mandibles. Clypeus near-trapezoid, the proximal edge twice as wide as the distal edge; top of clypeus two thirds the width of the widest point of the cranium. Mandibles well sclerotized, brown, tridentate; medial tooth longer and wider than the other two. Maxillae with four segmented palpus and mala; apical segment of palpus entirely amber and the other segments amber only basally; mala longer than wide and half of palpal length, bearing sturdy setae directed inside. Prementum with two unsegmented labial palpi. Thorax near white; a pair of large spiracles on the prothorax. Abdomen mostly white to pale yellow with some amber-colored portions in the latter half; pairs of spiracles on first through eighth abdominal segments, similar in size, much smaller and less visible than thoracic spiracles. Legs well-developed with coxa, trochanter, femur, tibia, and tarsus; four basal segments near-white; long tarsal claws brown.

Mature Larvae, Pupae, and New Adults. Some larvae collected in the field during the early summer of 2012 were successfully reared to the adult stage in the laboratory. Larvae became slightly more yellow than younger individuals before constructing ovoid-shaped pupal cells. Prepupal bodies became straighter than the typical C-shaped larval body form, and thoracic legs were reduced in size (Fig. 7). Mature larvae and prepupae remained in pupal cells for 7.6 ± 0.7 d (range: 6–10 d; $n = 5$) before pupation. Pupae were exarate (Fig. 8a). The whole body was bright white shortly after pupation (Fig. 8b), but it later became slightly yellow. Certain parts of the body also changed color with time. Eyes and large setae became brown first (Fig. 8a), and mandibles and legs turned brown later. The mean body length of pupae was 5.30 ± 0.21 mm (range: 4.81–5.68 mm, $n = 4$). The pupal stage lasted 9.6 ± 0.5 d (range: 8–11 d; $n = 5$). Pupae were normally motionless. However, when disturbed, they quickly twisted their bodies multiple

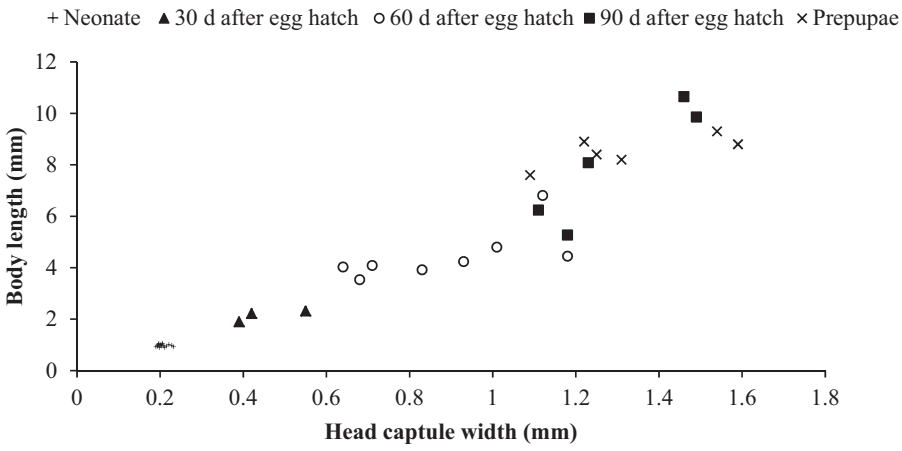

Fig. 6. The head capsule width and body length of each immature *C. crinicornis* (day of egg hatch, 30 d after egg hatch, 60 d after egg hatch, 90 d after hatch, and prepupal stage) in the laboratory. All larvae were obtained from eggs deposited by field-collected adults in 2011, while prepupae were collected as overwintering larvae in the field in 2012 and reared through the adult stage for identification.

times to reposition themselves in the pupal cells. Newly eclosed adults stayed in pupal cells for 4.4 ± 0.5 d (range: 3–6 d; $n = 5$). New adults initiated feeding on corn leaves 1.2 ± 0.4 d (range: 0–2 d; $n = 5$) after emerging from pupal cells.

Female Fecundity on Corn. Beetles readily fed on corn leaves placed in oviposition containers during 2010 and 2011 experiments. In addition to feeding or motionlessly staying on the surface of containers, both males and females sometimes partially or completely buried themselves among soil clumps. Beetles were observed to mate multiple times. Females oviposited in the soil as close as 5 mm from the surface, while some eggs were found at the bottom of the oviposition containers (~15 mm from the soil surface). Eggs were found inside soil clumps or in spaces around clumps, and they were deposited in one or two clusters in the life time of each female (Fig. 2). The second egg mass was deposited at least 3 or 4 d after the first. Results of *t*-tests indicate that there were no significant differences in mean total eggs laid per female ($t = -0.73$, df = 27.06; $P = 0.4742$) or percentage egg hatch ($t = 0.52$; df = 30; $P = 0.6059$) when 2010 data were compared with 2011 data. Similarly, the mean percentage of females that oviposited was not significantly different between years ($X^2 = 1.0737$; $P = 0.3001$). Therefore, when data were pooled over years, mean total eggs laid per female was 80.7 ± 4.4 ($n = 38$), mean proportion of females that oviposited was 55.9 ± 6.3 ($n = 68$), and mean percentage egg hatch was 95.3 ± 0.8 ($n = 32$) under laboratory conditions. There was no significant year ($F = 0.30$; df = 1,16; $P = 0.5901$), or year × egg cluster interaction ($F = 2.14$; df = 1,16; $P = 0.1630$), but the mean eggs per cluster was significantly greater in the first cluster than the second ($F = 68.21$; df = 1,16; $P < 0001$; mean eggs per cluster: first cluster: 79.9 ± 4.3, $n = 10$; second cluster: 35.1 ± 8.3, $n = 10$). All larvae from the same cluster usually eclosed within a few hours.

Fig. 7. *C. crinicornis* prepupa.

Adult Feeding Experiments

Adult Feeding Preference: Corn Versus Soybean. In 2012, adult *C. crinicornis* readily fed on both corn and soybean leaves when presented in the choice and no-choice feeding experiments. Adults produced characteristic feeding patterns on leaves (Figs. 9a and b). Feeding could begin on any part of a leaf, including the edge and interior. Beetles sometimes expanded and elongated initial holes, but size and shape of the holes were irregular. During each experiment, all beetles fed and survived to the termination of each replication. The choice test analyses indicated a strong trend, but no significant difference in leaf area consumed between sexes ($F = 3.79$; df = 1, 39.35; $P = 0.0588$) or leaf area consumed between food treatments ($F = 0.05$; df = 1, 39.14; $P = 0.8313$), and no significant sex × food interaction ($F = 2.25$; df = 1, 39.14; $P = 0.1413$) was detected (Table 1). The mean total leaf area consumed per beetle during the choice tests was 233.0 ± 28.7 mm^2. The no-choice tests between corn and soybean leaves also revealed no significant differences in leaf area consumed between sexes ($F = 0.40$; df = 1, 73.27; $P = 0.5311$), in leaf area consumed between food treatments ($F = 0.38$; df = 1, 72.74; $P = 0.5410$), or in the sex × food interaction ($F = 0.66$; df = 1, 73.55; $P = 0.4204$) (Table 1). The mean leaf area consumed per beetle was 224.45 ± 21.5 mm^2 during the no-choice tests.

Effect of Food Plant on Adult Longevity. The effect of food treatment on mean longevity of adult *C. crinicornis* was significant ($F = 52.74$; df = 3, 121; $P < 0.0001$). Beetles without food had significantly reduced lifespans compared with individuals fed leaf tissues (Table 2). The mean longevity across all food treatments was significantly longer for females than males ($F = 11.45$; df = 1, 121; $P = 0.0010$) (Table 2). In general, females lived about 2 d longer than males (Table 2). The sex × food interaction was not significant ($F = 0.25$; df = 3, 121; $P = 0.8613$).

Effect of Food Plant on Female Fecundity. During the fecundity test, the mean percentage ovipositing females was significantly affected by food treatment ($F = 5.56$; df = 3, 11; $P = 0.0144$). The percentage ovipositing females was not significantly different among leaf tissue treatments, but the percentage ovipositing beetles with no food was significantly reduced (Table 3). Among ovipositing females, food treatment did not significantly affect the mean number of eggs per female ($F = 0.28$; df = 3, 40; $P = 0.8428$), which was 81.04 ± 5.63. Eggs from beetles that received no food did not hatch, while a high percentage egg hatch was observed from females in leaf tissue treatments (Table 3).

Discussion

The general biology of *C. crinicornis* appears to be similar to that of other temperate *Colaspis* species that have been studied. The grape

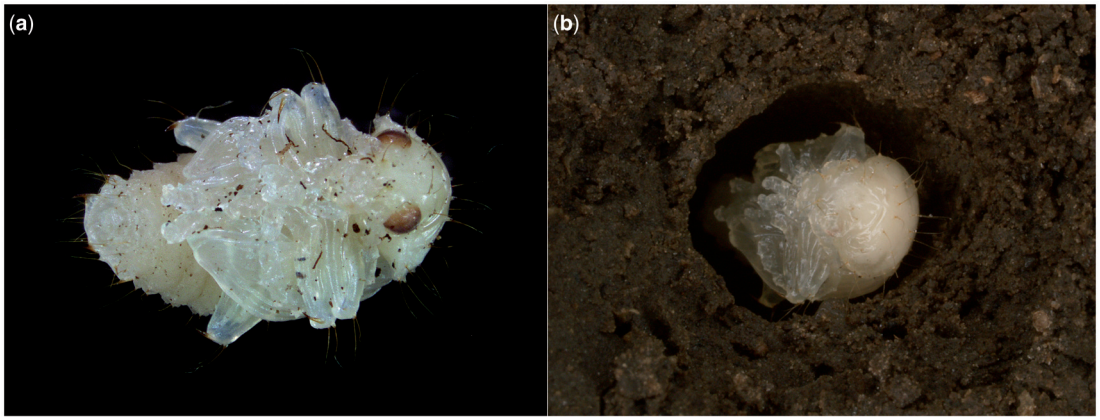

Fig. 8. (a) A *C. crinicornis* pupa a few days after pupation and (b) a newly formed pupa in a pupal cell.

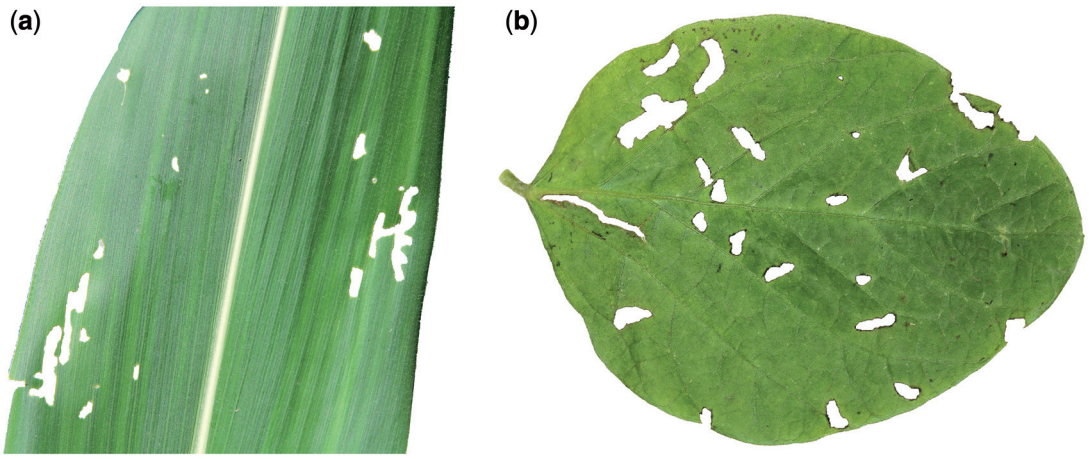

Fig. 9. Feeding injury of adult *C. crinicornis* to (a) a corn leaf and (b) a soybean leaf.

Table 1. Mean ± SEM leaf areas (mm^2) consumed by an adult *C. crinicornis* in 72 h laboratory choice and no-choice tests during 2012

Food plant	Male		Female		Mean	
	n	Leaf area consumed	*n*	Leaf area consumed	*n*	Leaf area consumed
Choice tests						
Corn	17	83.23 ± 10.65Aa	25	96.76 ± 18.72Aa	42	95.85 ± 12.02a
Soybean	17	80.79 ± 18.70Aa	25	152.15 ± 27.53Aa	42	126.27 ± 18.90a
Total[a]	17	164.02 ± 19.89A	25	248.91 ± 33.47A		
No-choice tests						
Corn	20	183.24 ± 27.79Aa	26	223.17 ± 43.62Aa	46	205.80 ± 27.35a
Soybean	17	193.80 ± 30.82Aa	15	315.82 ± 62.33Aa	32	250.99 ± 34.68a
Mean	37	188.09 ± 20.37A	41	257.06 ± 36.08A		

For each test, means in the same row followed by the same upper-case letter and means in the same column followed by the same lower-case letter are not significantly different ($P > 0.05$: LSD).
[a]The mean total leaf area consumed per beetle (corn and soybean combined).

Table 2. Mean ± SEM longevity (days) of adult *C. crinicornis* subjected to different food treatments in the laboratory during 2012

	n	Corn	*n*	Soybean	*n*	Both	*n*	No food	*n*	Sex
Male	23	13.28 ± 0.96Aa	19	12.11 ± 1.03Aa	14	12.67 ± 1.18Aa	14	4.42 ± 0.48Ba	70	11.28 ± 0.63b
Female	19	16.32 ± 0.81Aa	18	14.17 ± 0.89Aa	14	14.17 ± 1.16Aa	14	5.75 ± 0.54Ba	65	13.18 ± 0.65a
Food	42	14.59 ± 0.68A	37	13.11 ± 0.70A	28	13.42 ± 0.83A	28	5.08 ± 0.38B		

Means in the same row followed by the same upper-case letter and means in the same column followed by the same lower-case letter are not significantly different ($P > 0.05$: LSD). Beetles were held individually which prevented mating or oviposition. Longevity was calculated as days elapsed between collection of beetles in emergence cages in the field and beetle death.

Table 3. Mean ± SEM percentage ovipositing females, mean ± SEM number of eggs per ovipositing female, and mean ± SEM percentage egg hatch from females subjected to different food treatments in the laboratory during 2012

	n	Corn	*n*	Soybean	*n*	Both	*n*	No Food
Percentage ovipositing females (%)	26	54.76 ± 16.32a	29	73.39 ± 4.29a	20	71.25 ± 3.75a	19	6.25 ± 6.25b
Eggs per ovipositing female	14	78.85 ± 12.89a	21	87.33 ± 8.93a	12	73.58 ± 8.87a	1	74.00a
Percentage egg hatch (%)	9	95.96 ± 1.48a	15	92.52 ± 1.44a	9	91.27 ± 1.66a	1	0b

Means in the same row followed by the same letter are not significantly different ($P > 0.05$: LSD).

colaspis, *Colaspis brunnea* (F.), *C. crinicornis*, and the pine colaspis, *Colaspis pini* Barber, all have a long larval stage, whereas the egg, pupal, and adult stages are relatively short (Lindsay 1943, Echols 1963). These species also feed as adults on above-ground portions of plants, while larvae are root feeders (Lindsay 1943, Echols 1963).

Field collected adults readily oviposited in the soil in the laboratory. Although oviposition behaviors of *C. crinicornis* have not been observed in the field, it is highly likely that eggs are also deposited in soil in the field as documented for other *Colaspis* species (Lindsay 1943, Echols 1963, Lopez et al. 2002). Furthermore, *C. crinicornis* females utilized existing spaces around soil clumps as corridors for movement and as oviposition sites in the laboratory. Because beetles did not make burrows or oviposit on smooth soil in the laboratory, female *C. crinicornis* may use crevices created by soil clumps, soil

cracks, roots, or burrows of other organisms to move through soil to reach oviposition sites in the field. This behavior is exhibited by *Diabrotica* species (Kirk 1979, 1981a,b), which coexist in cornfields with *C. crinicornis* in southeastern Nebraska (Miwa 2014).

The lack of significant differences in mean percentage females that oviposited, mean total number of eggs laid, mean egg viability, and cluster size trends between years suggests that the addition of replacement males in 2011 laboratory oviposition experiments did not significantly impact female reproductive fitness. Follow-up studies would be needed to confirm the frequency of mating needed to produce a full complement of viable eggs and whether sperm precedence takes place when different males mate with an individual female.

Egg viability was high under optimal laboratory conditions, but it is unknown whether viability would be as high under field conditions where eggs can be exposed to various biotic and abiotic stressors. *Colaspis* eggs are fragile and hatch relatively quickly. They do not appear to be structurally designed to survive harsh environments for long periods of time as seen in some chrysomelid species that exhibit long egg diapause periods such as some *Diabrotica* species (Chiang 1973). In addition, eggs of *C. crinicornis* often became moldy when removed from original oviposition sites and placed in egg incubation dishes or on the soil surface in oviposition containers. Substances secreted by females during oviposition as chemical defense for eggs against natural enemies such as fungi and predators have been reported for several chrysomelid species (Howard et al. 1982, Ferguson and Metcalf 1985, Pasteels et al. 1986, Tallamy et al. 1998). It would be interesting to conduct a follow-up study to determine if *Colaspis* eggs may be chemically protected in a similar way and sealed to soil substrate by females during oviposition which when disturbed exposes eggs to various microorganisms.

Survival and establishment of young larvae on host roots was low in this study even though egg viability was high (Fig. 5). Similar observations have been made with other *Colaspis* species. Lindsay (1943) reported that high mortality of *C. brunnea* was observed, especially during the first few weeks of the larval stage. Various methods used by Echols (1963) in an attempt to rear *C. pini* resulted in 100% mortality within a few days after egg hatch. The most successful method allowed only approximately 2% of individuals to survive beyond the first instar and none of them reached maturity (Echols 1963). In a study with *Colaspis bridarollii* (Bechyné), Lopez et al. (2002) reported that no individuals survived beyond the first instar. The causes of larval mortality are unclear. Soils used in this study and by Echols (1963) were autoclaved which may have removed some critical factor that facilitates survival. In this study, most neonate larvae appeared to have died within a few days after eclosion before beginning to feed. Newly eclosed larvae had limited mobility, and they were often found dead after being trapped in root hairs or condensation inside rearing containers. Because most neonate larvae starved to death even when corn roots were present, the artificial environment in the rearing container may not have provided the structure (e.g., root channels) or specific cues needed for larvae to initiate feeding and establish on host plants. Because of the fragility of eggs and low mobility of young larvae, female choice of oviposition sites may be crucial for survival and establishment of the offspring on host plants in the field.

Attempts to rear individuals from egg to adult in the laboratory failed as none of the mature larvae pupated. Moreover, larvae collected in the field during the late fall also failed to reach the adult stage in the laboratory (K.M., unpublished data). In contrast, overwintering larvae collected in the field during the spring or summer pupated and reached the adult stage when the standard larval rearing method was used. Therefore, *C. crinicornis* larvae may require a hibernation period or cues such as temperature change to facilitate pupation. *Colaspis* as a genus appears to be difficult to rear in captivity. Establishment of a more effective rearing method would be needed to address population level questions about ecology and management of *Colaspis* species.

Colaspis crinicornis appeared to have at least three instars although the exact number was not determined because of rearing difficulties. A wide range of head capsule sizes were recorded between neonate larval and prepupal stages (Fig. 6), but small sample sizes precluded specific conclusions about the number of instars. For other *Colaspis* species, three instars have been reported for *C. pini* (Echols 1963) and *C. bridarollii* (Lopez et al. 2002) although no morphological measurements or clear supporting evidence were mentioned. In contrast, Lindsay (1943) concluded that *C. brunnea* had 10 larval instars and up to 17 with extra molts after measuring head capsule size and searching for cast skins every 2–3 d in a laboratory study. However, it is questionable whether having 10–17 larval instars is realistic for a *Colaspis* species. Chrysomelid species usually have only three or four larval instars, and few species have more than four (Jolivet and Verma 2002). An attempt was made during this study to apply the methods of Lindsay (1943) to determine the number of larval instars of *C. crinicornis*, but frequent removal of young larvae from rearing containers dramatically increased mortality and most likely stressed survivors. Moreover, it was nearly impossible to find cast skins, especially of small larvae.

Results from a series of adult feeding experiments indicate that leaves of both corn and soybean are suitable adult food sources as beetles readily fed, survived, and reproduced on both. In choice tests, a near significant trend ($P = 0.0588$) suggests that there may be a tendency for females to consume more leaf tissue than males. Nutritional requirements may be different between males and females because females are often larger than males (K.M., unpublished data) and females invest a lot of energy into egg production.

Mean longevity was significantly greater for females than males, but it was uncertain how reproductive behaviors would affect the longevity of each sex because the beetles used in the longevity experiment were not allowed to mate or oviposit. Feeding was required for adults to survive for more than a few days, but longevity and fecundity of beetles that fed was not significantly affected by food plant treatment (Tables 2 and 3). Females did not normally oviposit without feeding (Table 3) perhaps because they did not obtain energy required for reproduction or did not live long enough to reproduce due to starvation. It was unclear why one female oviposited without feeding during the experiment (Table 3). Nevertheless, none of the eggs from that female hatched, while females that received a continuous food supply deposited a high percentage of fertile eggs (Table 3), suggesting that females must feed in order to produce viable eggs.

Being able to feed, survive, and reproduce equally on corn and soybean leaves (Tables 1–3) suggests that *C. crinicornis* is likely a polyphagous species as recorded for *C. brunnea* (Lindsay 1943, Rolston and Rouse 1965). In addition, during the course of this study, adults of *C. crinicornis* have been observed to feed on other types of food, including corn silks, soybean flowers, leaves of *Abutilon theophrasti* Medik, *Amaranthus* species, *Andropogon gerardii* Vitman, *Digitaria* species, *Melilotus officinalis* (L.) Pallas, *Polygonum hydropiperoides* Michaux., *Rumex* species, *Setaria* species, *Sorghastrum nutans* (L.) Nash, *T. pretense* L., and *Vitis* species. An additional study would be needed to evaluate the impact of other food sources on consumption, longevity, and fecundity of *C. crinicornis.* However, the ability to effectively utilize tissues of plant species from very different plant families as adult food sources may be one of the many factors that have allowed *C. crinicornis* to adapt to agroecosystems and become increasingly abundant in corn and soybean fields. The results of this study contribute new biological information that increases our understanding of *Colaspis* and will also serve as a baseline for future studies to better understand the natural history and pest potential of *C. crinicornis.*

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