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The effect of food source on survival and development of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of air potato (Dioscoreales: Dioscoreaceae)

Koushal Rao¹, Veronica Manrique², and William A. Overholt^{2,*}

Air potato (*Dioscorea bulbifera* L.; Dioscoreales: Dioscoreaceae) is a noxious vine in Florida, where it grows over and smothers other vegetation (Schultz 1993; Schmitz et al. 1997). It is native to Asia and Africa and was introduced into Florida in 1905 (Nehrling 1944). Molecular studies revealed that air potato in Florida is of Asian origin (Croxtan et al. 2011). The vine has now spread throughout Florida and occurs sporadically in Georgia, Mississippi, Alabama, Louisiana, and Texas (EDMapS 2015). Air potato reproduces vegetatively through the production of aerial tubers, also referred to as bulbils. In Florida, bulbils occur in 2 forms; dark brown with a warty texture, and light tan with a relatively smoother skin (Hammer 1998; Overholt et al. 2014). *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae), a beetle native to China, was introduced and released as a biological control agent for air potato in Florida beginning in 2012 (Center et al. 2013). This beetle feeds primarily on air potato leaves but is also known to feed on bulbils (Pemberton & Witkus 2010). The objective of the present study was to determine the effect of food source on beetle development and survival. We hypothesized that beetles would perform better when fed air potato leaves compared with bulbils because leaves are the typical tissue fed upon in nature (Center et al. 2013).

Freshly picked young air potato leaves and freshly picked brown bulbils and tan bulbils (4–7 cm in diameter) were collected as needed from healthy air potato plants grown in a greenhouse. In a preliminary non-replicated experiment, five 1st, 2nd, or 3rd instar larvae were placed in plastic containers with 1 of the 3 food sources and held at 25 °C. Survival was recorded daily for 1 wk. No 1st or 2nd instars survived on bulbils of either type, and there was no evidence of feeding damage, whereas 40% of 3rd instars survived on each type of bulbil. Survival on leaves was higher, with 60% of 1st and 2nd instars and 80% of 3rd instars alive after 1 wk.

Based on these results, only 3rd instars were used in a 2nd experiment in which larvae were placed on leaves, brown bulbils, and tan bulbils (6 replicates each) and reared to adult emergence. As in the 1st experiment, young leaves and bulbils (4–7 cm) were freshly picked from air potato plants grown in a greenhouse. Each replicate consisted of 5 larvae placed on each food source, and survival was recorded twice a week. After 1 wk, vermiculate was added to the containers as a substrate for pupation. Digital images were taken of each adult, and ImageJ software (National Institutes of Health, Bethesda, Maryland) (Abramoff et al. 2004) was used to measure adult length and color.

Color was analyzed in a 2.5 mm diameter circle located in the center of the right elytron of each insect by using the histogram function of ImageJ. The values were recorded in the red, green, and blue spectra and used a range from 0 to 255. After measurement, each adult was dissected under a microscope to determine sex. The toughness of bulbils and leaves was estimated with a leaf penetrometer constructed from a scale (Pesola Light Line Spring Scale, Pesola AG, Baar, Switzerland) attached to a steel probe with a blunt 0.5 mm diameter point. The amount of pressure in grams required to penetrate the epidermis of bulbils or penetrate a leaf was measured on 10 brown bulbils, 10 tan bulbils, and 46 leaves. Analysis of variance was used for all statistical comparisons (PROC GLM, SAS Institute, 2013) and proportions were arcsin, square root transformed prior to analysis.

Percentage survival to the adult stage was higher on air potato leaves and brown bulbils (45–55%) compared with survival on tan bulbils (10%) ($F = 4.63$; $df = 2, 15$; $P = 0.027$) (Fig. 1). Because very few individuals survived to the adult stage on tan bulbils, they were excluded from the analysis of development time. Development time was nearly 2 d shorter when larvae were fed leaves (23.2 ± 0.6 d) compared with brown bulbils (25.1 ± 0.6 d) ($F = 5.03$; $df = 1, 17$; $P = 0.039$) (Fig. 2). There were no significant differences in adult size among sexes ($F = 1.61$; $df = 1, 17$; $P = 0.225$) or food sources

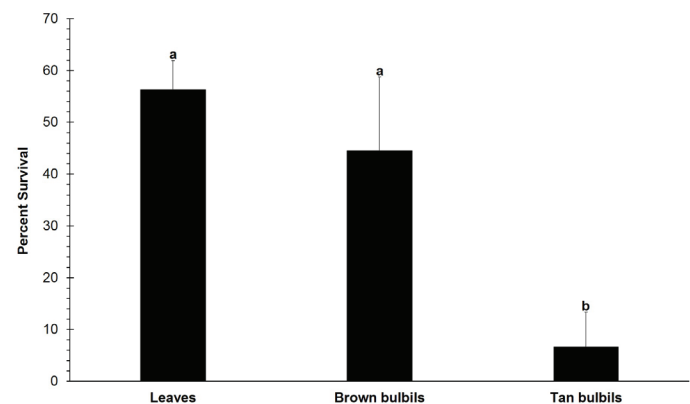


Fig. 1. Percentage survival (mean ± SE) of *Lilioceris cheni* from 3rd instar to adult when reared on leaves, brown bulbils, or tan bulbils of air potato.

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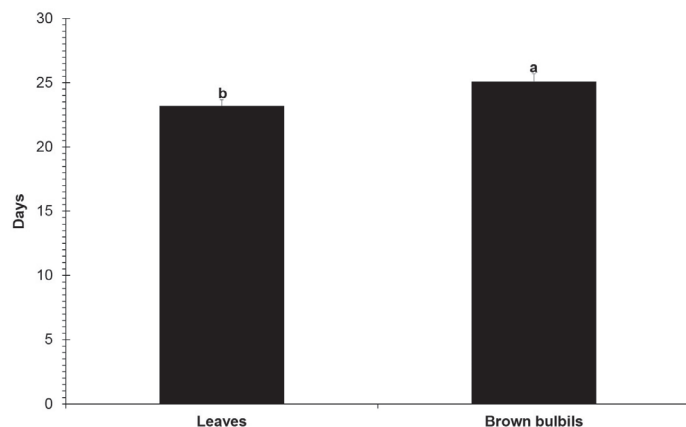


Fig. 2. Development time (mean \pm SE) of *Lilioceris cheni* from 3rd instar to adult when reared on leaves or brown bulbils of air potato.

($F = 0.13$; $df = 1, 17$; $P = 0.722$). Elytral coloration of adults reared on leaves and brown bulbils was not different in the red spectrum ($F = 2.62$; $df = 1, 17$; $P = 0.124$) or blue ($F = 1.46$; $df = 1, 17$; $P = 0.243$), but was different in the green spectrum ($F = 10.45$; $df = 1, 17$; $P = 0.005$). Visually, beetles reared on leaves appeared a darker red than beetles reared on brown bulbils. The amount of pressure required to penetrate brown and tan bulbils was not different (700 ± 47 g and 677 ± 23 g, respectively), but was much higher than the pressure required to penetrate leaves (7.7 ± 1.7 g) ($F = 799$; $df = 2, 63$; $P < 0.0001$).

Several dietary factors may have affected the performance of *L. cheni*. Food toughness is known to influence the size of meals, the speed at which food passes through the gut, and the efficiency of nutrient assimilation in insects (Clissold et al. 2009). The epidermal layer of bulbils was much tougher than leaves, which may explain why only older larvae, with larger mandibles, were able to survive on bulbils. Another influential factor may have been the nutritional value of the food sources. The protein-to-carbohydrate ratio, although not quantified, was undoubtedly much higher for leaves than bulbils, which are composed almost entirely of starch (23–33%) and water (63–66%) (Coursey 1967). A number of studies have shown a relationship between plant nutritional quality and development time, with increased nitrogen often resulting in decreased development time (Scriber 1984). Finally, the concentration of defensive compounds may have varied between leaves and bulbils, and between brown and tan bulbils, which may explain the poor survival on tan bulbils.

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Summary

This study demonstrated that early instars of *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae) larvae survived on air potato (*Dioscorea bulbifera* L.; Dioscoreales: Dioscoreaceae) leaves, whereas older larvae successfully developed on leaves and bulbils. However, leaves provided a superior diet to bulbils for older larvae based on a shorter development time. Survival from 3rd instar to adult

was not different on leaves and brown bulbils, but for unknown reasons, survival was very poor on tan bulbils.

Key Words: weed biological control; insect diet; insect color

Sumario

Este estudio demostró que los primeros estadios de larvas de *Lilioceris cheni* Gressitt y Kimoto (Coleoptera: Chrysomelidae) sobrevivieron en las hojas de papa trepadora (*Dioscorea bulbifera* L.; Dioscoreales: Dioscoreaceae) de aire, mientras que las larvas mayores desarrollaron con éxito en las hojas y bulbillos. Sin embargo, las hojas siempre proveyeron una dieta superior a los bulbillos para larvas de más edad en base a un tiempo de desarrollo más corto. La sobrevivencia del 3° instar hasta el adulto no fue diferente en las hojas y bulbillos de color café, pero era por razones desconocidas, la sobrevivencia fue muy pobre en los bulbillos bronceados.

Palabras Clave: control biológico de malezas; dieta del insecto; color de insectos

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