

COMPARISON BETWEEN EGGSTICKS OF TWO CACTOPHAGOUS MOTHS, *CACTOBLASTIS CACTORUM* AND *MELITARA PRODENIALIS* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The invasive cactus borer *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) now co-occurs with a species of borer native to North America, *Melitara prodenialis* Walker (Lepidoptera: Pyralidae), in the southeastern United States. Because *C. cactorum* damages native species of pricklypear (*Opuntia*) often through population outbreaks, it is necessary to distinguish this species from the native cactus borer that has reduced impact on pricklypear populations. *Cactoblastis cactorum* and *M. prodenialis* eggs are laid one on top of another to form eggsticks that resemble cactus spines. Both moth species utilize the same host plant species in the *Opuntia* genus, and during larval life stages it is easy to distinguish the two species. Determining species identification based on eggs alone, however, has been difficult. Yet, it is impractical to wait for eggs to hatch and larvae to develop to determine species identification for research or monitoring purposes. Several objective, quantitative differences were found between the two species that can be used in laboratory identification to train research assistants or invasive management personnel for the field. Based on the data, we provide a dichotomous key to distinguish the eggsticks of these two species.

Key Words: cactus, borer, cactus moth, dichotomous key, field identification, *Opuntia*, species delineation

RESUMEN

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae), una especie de barrenador de cactus invasiva, ahora co-existe en el Sureste de los Estados Unidos de América con *Melitara prodenialis* Walker (Lepidoptera: Pyralidae), una especie de barrenador de cactus nativa de Norteamérica. Dado que *C. cactorum* causa daño a las especies de cactus (*Opuntia*) nativas, comúnmente como resultado de un brote en la población, es necesario distinguir esta especie invasiva de la especie nativa que produce impactos reducidos en las poblaciones de cactus. *Cactoblastis cactorum* y *M. prodenialis* ovipositan huevos uno sobre otro formando hileras que a simple vista son parecidas a las espinas de los cactus. Ambas especies de palomillas utilizan como hospedero a las plantas del género *Opuntia*, y durante los estadios larvarios es fácil distinguir entre especies. Sin embargo, la identificación de estas especies basada en la apariencia de los huevecillos ha sido difícil. Con fines de investigación y monitoreo, además, es impráctico esperar a que los neonatos eclosionen y las larvas se desarrollen. Varias diferencias objetivas y cuantitativas se encontraron entre los huevecillos de estas dos especies que pueden ser usadas para identificarlas en el laboratorio y para entrenar a los asistentes de investigación y al personal que hace el manejo de especies invasivas en el campo. Con base en estos datos, aquí presentamos una clave dicotómica para distinguir a las hileras de huevos en forma de bastón de estas dos especies.

Palabras Clave: barrenador del nopal, palomilla del nopal, clave dicotómica, identificación en el campo, *Opuntia*, identificación de especies

Several species of cactophagous moths exist in North America (Neunzig 1997), including the invasive *Cactoblastis cactorum* (Berg) and the native *Melitara prodenialis* Walker. Both of these latter 2 species infest members of *Opuntia*, where they co-occur in the southeastern United States (Baker & Stiling 2009). Because *C. cactorum* is rapidly spreading and poses a threat to rare *Opuntia* species in Florida and the deserts of North America (Stiling & Moon 2001, Marsico et al. 2011), efforts have been made to control the dispersal of

C. cactorum. One method of *C. cactorum* control, utilized in South Africa and suggested for the United States, involves collecting and destroying eggs from impacted cladodes (Zimmermann et al. 2000). Though egg removal is not currently a strategy employed for *C. cactorum* control in the U.S., all research efforts aimed at controlling damage and spread of *C. cactorum* require the collection of moths for laboratory experimentation and rearing, and often include the collection of eggs. Moreover, increasingly the similar, native, co-occurring *M.*

prodenialis is being studied in comparative experiments with *C. cactorum* (e.g., Woodard et al. 2012). Therefore, it is important for researchers to be able to distinguish eggs laid by these 2 species.

Cactoblastis cactorum and *M. prodenialis* are now sympatric and select the same host plants (Baker & Stiling 2009). Therefore, it is possible that using indiscriminant techniques to remove eggs and cladodes will result in the destruction of native *M. prodenialis* individuals, negatively impacting their populations. As a best practice, invasive species management should negatively impact native species as little as possible. Additionally, resources should not be spent removing moths or moth-damaged plants from populations comprised entirely of *M. prodenialis*. As such, it is necessary to distinguish between the 2 insect species at all life stages. Larvae can easily be identified at middle and late instars based on their color; however, identification of early instar larvae is more difficult. Another means of identification at any life-stage is the sequence obtained from the *COI* gene (Marsico et al. 2011; Sauby et al. 2012), though this is impractical for rapid laboratory or field use. Here we report a quantifiable method that trained researchers and invasive species managers can use to distinguish between the eggsticks of *C. cactorum* and *M. prodenialis*.

Both species lay eggs one on top of the other to form eggsticks that are superficially similar in appearance (Hubbard 1895; Hoffmann & Zimmermann 1989). Close inspection of *C. cactorum* and *M. prodenialis* eggsticks indicate distinct differences between the two. Despite needs for a reliable method of distinguishing between the eggsticks of these species, differences have not been quantified (Rose et al. 2011). To make the distinction between moth

species, photographs of field-collected *C. cactorum* and *M. prodenialis* eggsticks were used to measure several aspects of the eggstick morphology.

MATERIALS AND METHODS

Eggsticks of *C. cactorum* were collected from 5 locations in the Florida Panhandle, U.S.A., in May 2009, Jul 2009, and May 2010. Eggsticks of *M. prodenialis* were collected from 4 locations in the Florida Panhandle, U.S.A., in May 2009, Jun 2009, and May 2010. Measurements were taken on photographs of the field-collected eggsticks using Adobe Photoshop CS5. Photographs of 163 *C. cactorum* and 141 *M. prodenialis* eggsticks were taken with a metric ruler included as a size reference. For each eggstick, the number of eggs was counted, and the lengths and widths of the terminal and middle eggs were measured using the Photoshop CS5 measuring tool. Egg length was calculated in the same direction of eggstick length, and egg width was calculated perpendicular to length. If the eggstick was straight, the measure tool was used to directly take the length of the entire eggstick. For eggsticks that were curved, a path was created with the pen tool down the length of the stick. The path was then filled with text (i.e., periods), which was copied and pasted on a straight path and measured with the measuring tool. Curvature was measured as change in angle per change in unit length. The length of the sharpest curve was taken, and the angle of that curve was taken by the measuring tool. A Student's t-test was used to test for significant differences between species for number of eggs, terminal egg length, and terminal egg width. Due to a violation of assumptions of the

TABLE 1. COMPARISON BETWEEN CHARACTERISTICS OF *CACTOBLASTIS CACTORUM* AND *MELITARA PRODENIALIS* EGGSTICKS.

Characteristic	Species	
	<i>C. cactorum</i>	<i>M. prodenialis</i>
Number of eggs	63 ± 19.9 ^a	23 ± 10.2 ^a
range	17-99	6-53
Terminal egg length (mm)	0.53 ± 0.09	0.58 ± 0.08
range	0.335-0.974	0.332-0.783
Terminal egg width (mm)	0.92 ± 0.10	1.18 ± 0.09
range	0.631-1.144	0.911-1.402
Middle egg length (mm)	0.39 ± 0.04	0.44 ± 0.04
range	0.325-0.56	0.329-0.511
Middle egg width (mm)	0.96 ± 0.08	1.20 ± 0.09
range	0.716-1.166	0.898-1.396
Eggstick length (mm)	26 ± 8.2	10 ± 4.3
range	7.712-46.957	3.00-23.536
Curvature (°/mm)	11 ± 2.4	5 ± 4.4
range	0.0-32.342	0-18.143

^aMean values are followed by standard deviation.

parametric test, middle egg length and width, eggstick length, and curvature were analyzed using a Mann-Whitney U-test.

RESULTS

Qualitatively, there are differences in egg color between species as the eggs reach maturity. *Cactoblastis cactorum* eggs become gray, silvery, and darken to black as the embryos mature. *Melita-*

ra prodenialis eggs become increasingly darker brown as the embryos mature. There were significant differences between the species ($P < 0.001$ for Student's t or Mann-Whitney U tests) in the average and ranked values for all the characteristics measured (Table 1; Fig. 1; Fig. 2). Based on data associated with variable means, in addition to data ranges, the following dichotomous key for species delineation was developed using interquartile values.

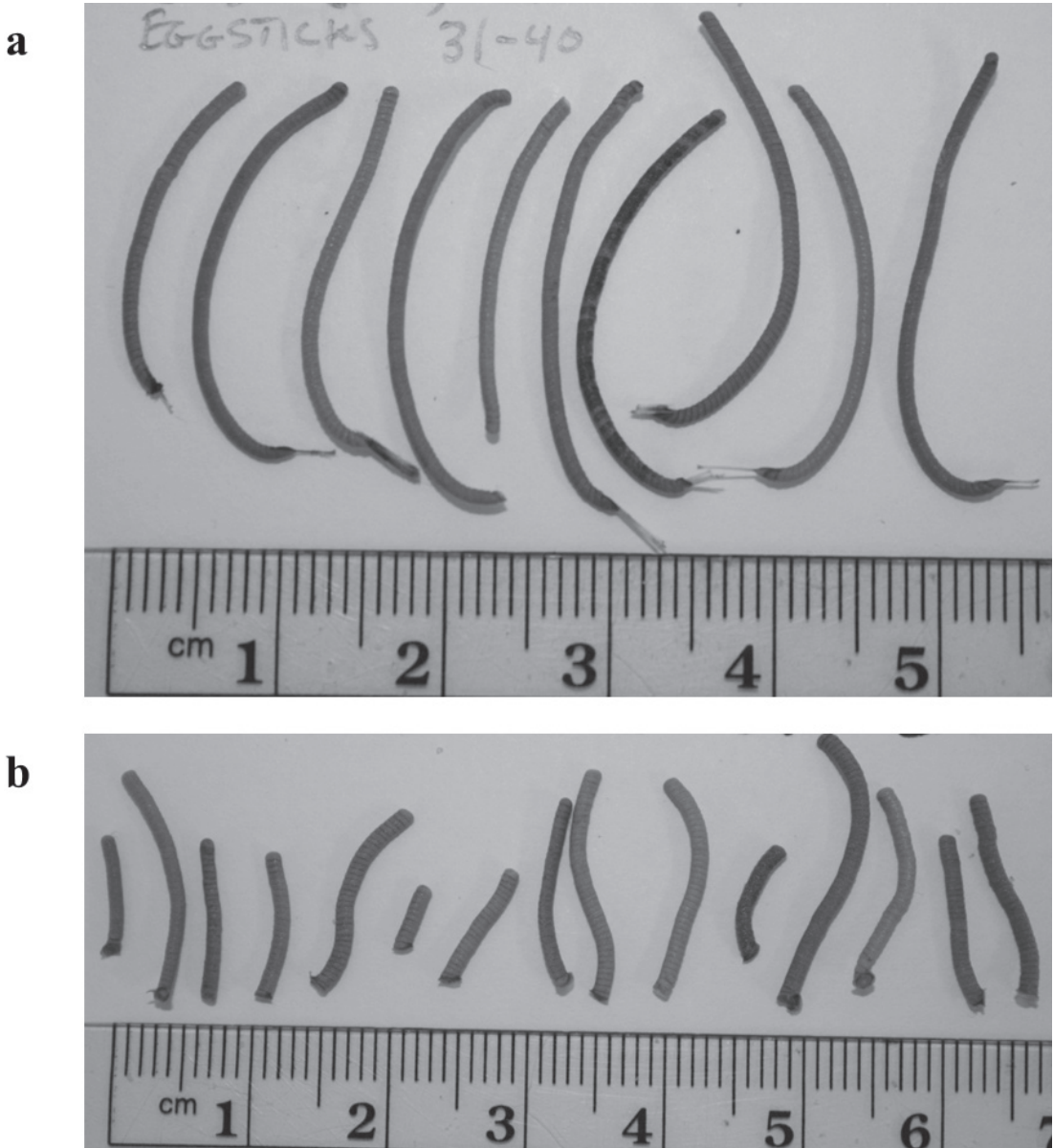
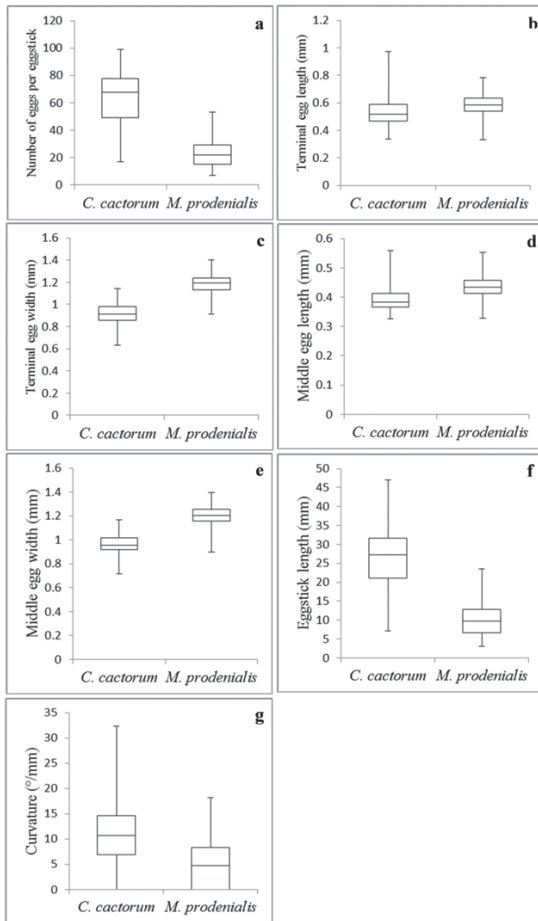


Fig. 1. Eggsticks belonging to *Cactoblastis cactorum* (a) and *Melitara prodenialis* (b). For both species, darker eggsticks are more mature, and lighter eggsticks are less developed. *Cactoblastis cactorum* eggs that are close to hatching are almost black (see for example the 4th eggstick from the right in panel a). *Melitara prodenialis* eggsticks also darken as they near hatching (see for example the 5th eggstick from the right in panel b). A tight curve at the basal end of the longer, thinner *C. cactorum* eggsticks is typical, while *M. prodenialis* sticks are usually straighter.

KEY TO DISTINGUISHING BETWEEN EGGSTICKS OF TWO CACTOPHAGOUS MOTHS, *CACTOBLASTIS CACTORUM* AND *MELITARA PRODENIALIS*

- 1a. Eggsticks maturing to a silver-gray and ultimately black coloration, often with 49-78 eggs; total eggstick length 21-32 mm, often with sharp curve (6.8-14.7°/mm), usually positioned at basal end, though eggstick can be nearly or completely straight, especially if shorter than usual; individual eggs from the middle of the eggstick 0.37-0.41 mm long and 0.92-1.0 mm wide, resulting in a slender eggstick *Cactoblastis cactorum*
- 1b. Eggsticks maturing to a brown coloration, often with 15-29 eggs (occasionally more); total eggstick length 7-13 mm, with little or often no curvature (0-8.3°/mm); individual eggs from the middle of the eggstick 0.41-0.44 mm long and 1.16-1.26 mm wide, resulting in a stocky eggstick. *Melitara prodenialis*



DISCUSSION

Useful characteristics for identification are those that are distinct with minimal overlap between species. Even though there are significant differences for all variables measured for the 2 cactus moth species, the range for all variables overlaps between species (Table 1). For this reason, a single character cannot be used as the sole identifier, and therefore, the characteristics need to be considered together to make reliable positive identifications. Because the length and width of eggs in *C. cactorum* and *M. prodenialis* differ significantly by only fractions of a millimeter, such small differences are not easy to discern outside the laboratory. Yet with practice, individual egg sizes can be distinguished by the observer. *Cactoblastis cactorum* and *M. prodenialis* eggsticks differ significantly in length and number of eggs, both which can be measured and counted easily (Fig. 1). Degree of curvature, particularly for long eggsticks and particularly positioned at the base of the eggstick, also tends to be greater in *C. cactorum*, which adds an important characteristic for identification purposes (Fig. 1).

We have now quantified the observable differences for the important diagnostic characteristics of eggsticks of *C. cactorum* and *M. prodenialis*. As an important invasive species with a large impact, it is critical to be able to distinguish *C. cactorum* from the similar, native, co-occurring species *M. prodenialis*, particularly at phases of the life cycle that are poorly characterized. Additionally, because these organisms are the focus of current and future research, scientists will benefit from the results reported here. Though the quantified differences and dichotomous key presented are useful for scientists and other individuals experienced with this study system and invasive species management system, the similarities between the eggsticks of these moths may make field identification impractical for invasive species control programs based on *C. cactorum* eggstick removal. These programs often rely on volunteers and assistants that may not have the time to become experienced in nuanced identification of the more difficult life stages of these species, and invasive

Fig. 2. Boxplots comparing characteristics of *Cactoblastis cactorum* and *Melitara prodenialis* eggsticks. Boxplots show the means (horizontal lines within boxes), interquartile values (horizontal boundaries of the boxes that represent 25% and 75% of values that fall within the boxes), and the ranges (minimum and maximum values depicted as hatches at the end of vertical lines) for each variable measured. (a) Number of eggs, (b) terminal egg length, (c) terminal egg width, (d) middle egg length, (e) middle egg width, (f) eggstick length, and (g) eggstick curvature, measured as change in angle over length (i.e., °/mm). All lengths were measured in millimeters.

species managers would not want inexperienced workers to leave *C. cactorum* eggsticks in the field based on the mistaken identification. Still, prudence in invasive species control strategies must be exercised with respect to similar native species, and populations of *M. prodenialis* that do not contain *C. cactorum* should not be put at risk of removal. We hope that utilizing these characteristics will add to the practical arsenal used by invasive species managers in their attempts to minimize invasive species impact of *C. cactorum* while simultaneously working to protect native populations of *M. prodenialis*.

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