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Authors: Marti, Orville G., and Carpenter, James E.

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A CHARACTER DEMONSTRATING THE OCCURRENCE OF MATING IN MALE *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

ORVILLE G. MARTI AND JAMES E. CARPENTER
United States Department of Agriculture, Agricultural Research Service
Crop Protection and Management Research Service, P.O. Box 748,Tifton, GA 31793

The arrival of the cactus moth, *Cactoblastis cactorum* (Berg), in the southeastern United States in 1989 (Mahr 2001), and its steady progress westward to the *Opuntia*-growing regions of Mexico and the southwestern United States have resulted in research on the development of control measures to halt or slow the movement of the insect. The most promising control measure against *C. cactorum* is the sterile insect technique (SIT), in which large numbers of male moths are exposed to a sub-sterilizing dosage of ionizing radiation and then released in or in front of the leading edge of the geographic range of the pest. Irradiated males then find wild females, mate with them, and produce progeny which are delayed in development from oviposition to eclosion, fewer in number than in a normal mating, and sterile (Carpenter et al. 2001).

Irradiated male *C. cactorum* released in the SIT program are marked with colored dye to allow their identification as released males upon recapture in pheromone traps (Hight et al. 2005). In order to compare mating success of wild and irradiated males, an anatomic characteristic that indicated the mating status of males was sought.

A number of published reports mention that differences in color of secretions in the *ductus ejaculatorius simplex* in various Lepidoptera species allow determination of mating status of males. Proshold (1991) found that the *simplex* in unmated males of a noctuid, *Heliothis virescens,* the tobacco budworm, varied from light yellow to dark brown. The *simplex* in mated males was clear, cloudy, or white. In *Spodoptera frugiperda*, the fall armyworm, also a noctuid, Snow & Carlysle (1967) reported that the color of the primary *simplex* (Callahan 1958) was light brown to black in unmated males and transparent to yellow in mated males. Furthermore, they reported that a dark spermatophore is transferred to the female at the first mating and that subsequent spermatophores are lighter in color. These authors also reported that mated males held for several days and prevented from mating regenerated only a yellow or light brown color in the *simplex*, and that this color was never as intense as the brown to black color indicative of unmated males. Similar results were reported by Haines (1981) for the noctuid, *Spodoptera littoralis,* by Henneberry & Clayton (1984) for the noctuid *H. virescens*, and by Bergh & Seabrook (1986) for the tortricid*, Choristoneura fumiferana*. These latter authors also summarized results from the above 4 species. Except in *H. virescens*, the color of the *simplex* was regenerated within several days after mating.

Callahan & Cascio (1963) described the male reproductive system of the noctuid, *Heliothis zea*, and reported that the *simplex* consisted of a posterior or cuticular portion, and an anterior or primary portion. They identified two secretory areas of the primary *simplex* and found that the posterior, or first secretory area, of the primary *simplex* was variously colored in different noctuid species and, because of the easily traceable color, was the first secretion to enter the female *bursa* during mating. Outram (1971) stated that the *bulla seminalis* of the female contained a fluid similar in color to the secretion of the primary *simplex* in *C. fumiferana*, the spruce budworm, but that the function of the fluid is not known. Outram (1970) also described and illustrated the male reproductive system of *C. fumiferana* and reported that 7 regions, distinguishable on the basis of differences in secretions and constrictions in the duct, could be identified in the primary *simplex* of this species. However, Callahan & Chapin (1960) found little morphological variation in the primary *simplex* in several different noctuid species, but were able to use characteristics of the cuticular *simplex* to separate individual moths by generic groups.

Newly emerged adults of *C. cactorum* obtained from the colony in the insect rearing facility at USDA, CPMRU, Tifton, GA, were paired in individual containers and allowed to mate. At various times (1-7 d) after pairing, the moths were dissected in saline in a petri dish. Females were examined for the presence of a spermatophore in the *bursa copulatrix*. For males, the thorax was held with a pair of forceps and the *simplex* was pulled out by grasping the abdomen anterior to the claspers with a second pair of forceps and pulling until most of the length of the *simplex* was exposed. This procedure frees the *simplex* for examination without the presence of adhering fat body and tracheal tubules. The forceps can then be used to dissect the remaining portions of the male reproductive system. The posterior portion of the primary *simplex* was measured, the color was noted as "clear and pale" or "opaque and yellow", and the contents were examined with Differential Interference Contrast illumination on a Nikon Optiphot compound microscope at 400×.

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The mating status of the males, based on the presence or absence of a spermatophore in the female with which they had been paired, was compared with the color characteristics of the posterior primary *simplex* and with the presence or absence of numerous hyaline granules that had also been observed in the posterior *simplex*. The granules were 3-5 µm by 5-10 µm. Most granules, particularly the larger ones, were prolate spheroids resembling small footballs, although many were irregular in shape. The granules were highly reflective and were not responsible for the yellow color observed in the posterior *simplex* of the unmated males. Several authors who described color differences in the *simplex* also commented on the presence of globules in the *simplex* without mentioning whether granules were also present (Snow & Carlysle 1967; Haines 1981; Callahan & Cascio 1963). Musgrave (1937) mentioned the presence of ovoid, hyaline, granules in the accessory glands and large angular lumps in the *simplex* of *Ephestia kuhniella* (Pyralidae), but did not state how these were related.

Complete information on the mating status, character of the posterior primary *simplex* and presence or absence of granules was obtained in 71 (n = 25 once-mated, n = 46 unmated) males from 80 pairs. Comparison of mating status of the males with the character of the posterior *simplex* showed that all 46 unmated males had a yellow, opaque simplex and none had a clear, pale simplex (Fig. 1). In contrast, 24 of 25 mated males had a clear, pale simplex and 1 had an opaque, yellow simplex. The difference was significant (χ^2 = 66.71, df = 1, P = 0.00001).

In comparing mating status of the males with the presence or absence of granules in the posterior primary simplex, granules were present in 44 of 46 unmated males and were absent in 2 of 46 unmated males (Fig. 2). In contrast, granules were absent in all 25 mated males. The difference was significant (χ^2 = 62.88, df = 1, P = 0.00001). Although a faint yellow color may be regenerated after mating, no granules were found in males that were examined up to 96 h after mating.

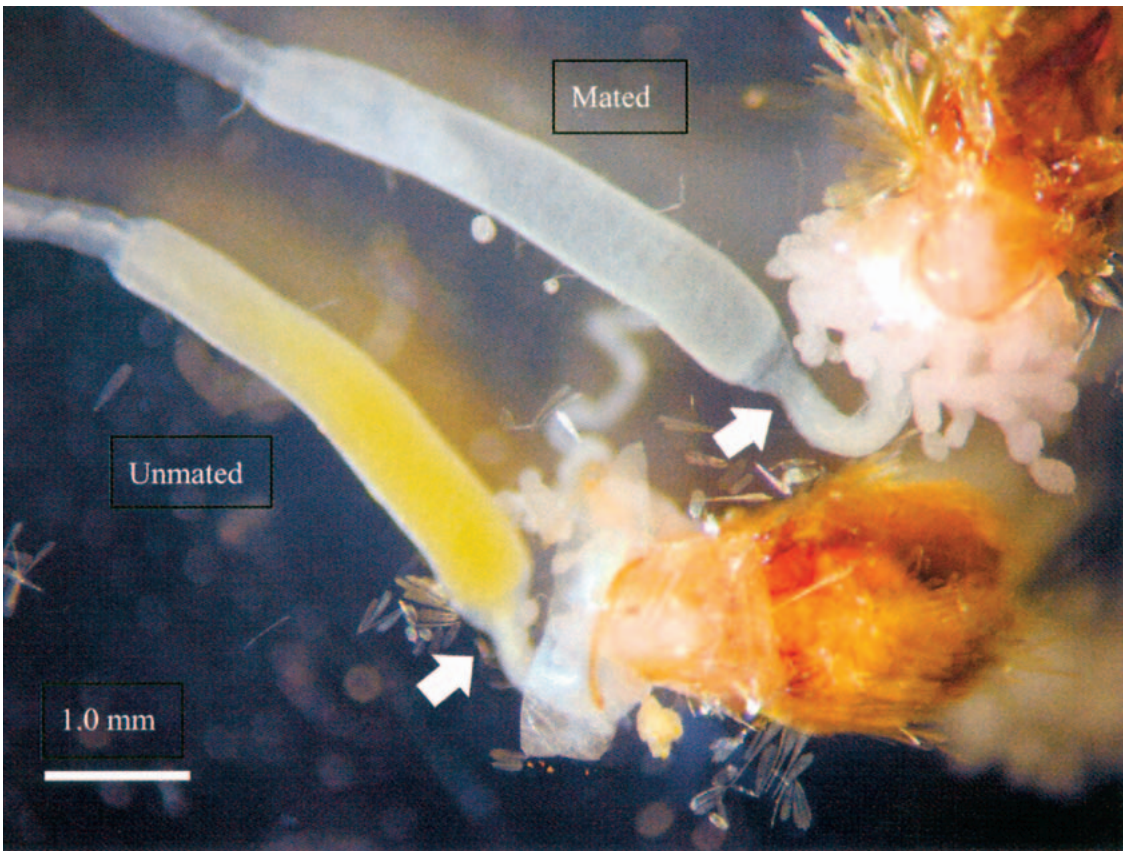


Fig. 1. Posterior primary *simplex* of mated (top) and unmated (bottom) male *Cactoblastis cactorum*. A constriction (white arrow) marks the posterior end of the primary *simplex* and the anterior end of the cuticular *simplex*, here partly obscured by fat body and genital structures, including claspers. Scale = 1.0 mm

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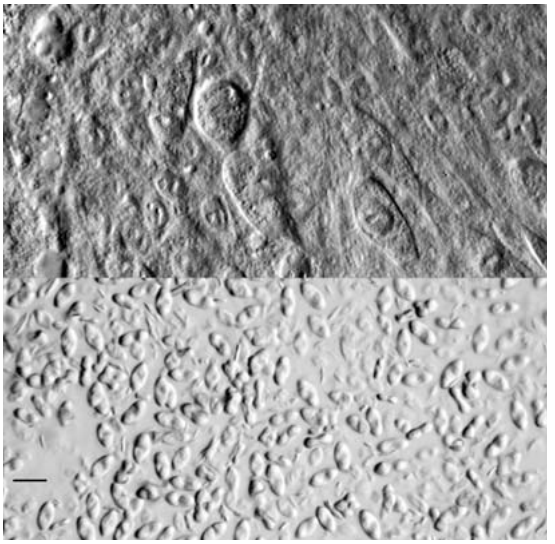


Fig. 2. Contents of posterior primary *simplex* of mated (top) and unmated (bottom) male *Cactoblastis cactorum*. Scale = 10 µm

In comparing the character of the posterior primary *simplex* with the presence or absence of granules, granules were absent from the posterior primary *simplex* of all 24 males with a clear, pale posterior primary *simplex*. In contrast, granules were present in 44 of 47 males with an opaque, yellow posterior *simplex* and absent from 3 of 47 males with a clear, pale posterior primary *simplex*. The difference was significant (χ^2= 59.08, df = 1, P=0.00001).

The posterior primary *simplex* of 64 of the above 71 males was measured to determine whether there was a difference in length in mated versus unmated males. In mated (n = 24) males, the posterior primary *simplex* was 2.94 ± 0.74 mm in length (mean ± SE). In unmated males (n = 40), the posterior primary *simplex* was 3.46 ± 0.63 mm in length. These means were significantly different (t = -3.004, df = 63, P = 0.004).

An additional 86 unmated male *C. cactorum* which emerged from cocoons collected in the wild on Little Dauphin Island, AL, in Sep, 2006, were dissected and the simplex was examined for color and granules. The simplex was yellow in 85 of these males and granules were present in 83 (χ^2= 0.01, df = 1, P = 0.91), showing that the yellow color and the presence of granules were closely associated in unmated male *C. cactorum*.

The presence of an opaque yellow color and small granules in the posterior primary *simplex* of adult male *C. cactorum* are reliable indicators that the male has not mated. Conversely, the clear pale posterior primary *simplex* and the absence of granules is a reliable indicator that the male has mated.

Summary

The reproductive system of adult male *Cactoblastis cactorum*, the cactus moth, was examined to determine whether the mating status of males could be ascertained. In unmated males, the posterior portion of the primary *ductus ejaculatorius simplex* is opaque yellow in color and contains many small football-shaped hyaline granules 3-5 × 5-10 µm in size. In mated males, the posterior *simplex* is clear and contains no granules. The presence or absence of these characters was found to be highly reliable and should be of value in determining mating status in marked-recaptured males of this species in a sterile insect release program directed against *Cactoblastis*.

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