

Life History and Host Range of Hawaii's Endangered Blackburn's Sphinx Moth (*Manduca blackburni* Butler)

Daniel Rubinoff and Michael San Jose

Department of Plant and Environmental Protection Sciences, 310 Gilmore Hall, 3050 Maile Way, University of Hawaii, Honolulu, HI 96822 USA; e-mail: rubinoff@hawaii.edu, tel. 808-956-8432

Abstract. *Manduca blackburni*, Blackburn's sphinx moth (BSM), is a federally listed endangered species endemic to Hawaii. Originally found throughout the main Hawaiian Islands, it has disappeared from most of its range, and is now restricted to small localized populations on the islands of Maui, Hawaii, and Kahoolawe. While the adult and last instar larvae are described, the early life stages of BSM have not previously been figured. Further, the host range of the moth is not known, though it is suspected to feed on a wide range of solanaceous plants. Eggs and larvae of *M. blackburni* were collected from East Maui and reared on both native and invasive solanaceous hosts to examine host specificity in the context of habitat restoration and the importance of new invasive hosts to BSM populations. Larvae developed on several native and introduced solanaceous species. We present the first published descriptions of the early larval instars, and confirmation of several suitable hosts, both native and exotic. Low rates of parasitism in our field collections suggest that, at least in some areas, parasitoids may not severely impact populations, and these areas could be important refugia for BSM. Rearing efforts confirm that at least two species of native *Solanum*, in addition to *Nothoestrum*, can be used in site restoration as potential host plants.

Key words: conservation, Lepidoptera, Maui, Hawaiian Islands, *Solanum*, *Nicotiana*

Introduction

Manduca blackburni, the Blackburn's sphinx moth (BSM), is Hawaii's largest endemic insect. Historically, it was found on all of the main Hawaiian Islands and was mentioned as an occasional pest of eggplant and other solanaceous crops (Zimmerman 1958), much like the mainland species *M. quinquemaculatus*, for which it was frequently confused (Riotte 1986). However, BSM was never common, and by the 1970s the moth had only been recorded a handful of times in the preceding thirty years (USFWS 2005). Bishop Museum (Honolulu, HI) entomologists conducted extensive surveys in the mid 1970s but failed to find BSM and the moth was presumed extinct (Gagné and Howarth 1982). The species was later rediscovered persisting in a few isolated populations on east Maui (Riotte 1986) and is now also known from parts of Hawaii Island and Kahoolawe but it is extinct on the islands of Kauai, Oahu, Lanai and Molokai. Because of BSM's disappearance from several islands and its decline across the remaining islands, it was declared an endangered species in 2000, one of only two moths to ever receive federal protection (USFWS 2005).

Prior to human contact, BSM is thought to have depended largely on endemic solanaceous trees in the genus *Nothoestrum*, all of which have declined and some of which are endangered as a result of development and introduced ungulate-based degradation of Hawaiian dry forest habitats (Riotte 1986). Oddly, since Blackburn's first description (Butler 1881), BSM larvae are known to readily feed on the widespread, invasive tobacco weed, *Nicotiana glauca*. This weed would seem to provide an abundant and resilient host, and in some areas, it may be the main host plant used by BSM larvae. Yet, the range of *N. glauca*

across Hawaii far exceeds that of the moth and includes all the islands from which BSM has disappeared, suggesting the influence of other factors, besides host plant availability, in BSM's decline. Introduced biological control agents attack BSM eggs and probably larvae, further hastening the decline of the moth (Riotte 1986), and these may be important factors endangering the remaining BSM populations.

Riotte (1986) presents an excellent treatment of the taxonomic status of BSM, with detailed morphological descriptions of the 5th instar larva, pupa and adult. However, because the early instars of BSM have never been described, and the species is of conservation concern, we present color figures of all the instars and size ranges to assist in field identifications. An additional goal of this project was to test the host plant range of BSM. There is some concern that BSM is becoming wholly dependent, perhaps even specialized, on the invasive weed, *N. glauca*, now that the original hosts, *Nothoestrum* spp. are so rare (USFWS 2005) and BSM is no longer recorded on solanaceous crop plants. Such dependence discourages the removal of the invasive *N. glauca* from dry forest habitat under restoration. We attempted to rear BSM, which we had collected on *N. glauca* as eggs and larvae, on four native or introduced solanaceous hosts. If BSM can successfully develop on indigenous, fast growing *Solanum* spp., then such plants might be used in restoration efforts, and the proposed removal of *N. glauca* from restored areas might not negatively impact BSM populations.

Through a natural history study and host plant testing we hope to evaluate the importance and direction of conservation actions for the species. The results of this work will facilitate BSM management by establishing developmental parameters for the larval stages including a discussion of suitable native and introduced larval host plants.

Materials and Methods

Fieldwork and life history. We searched host plants in the Kanaio region of East Maui on Friday, October 16, 2009 for eggs and larvae. We spent approximately three hours searching for BSM; the day was sunny, breezy and clear, with temperatures near 30°C.

Larvae and eggs were collected and photographed. Larvae were reared on native and introduced solanaceous plants to evaluate their palatability. Larvae were reared in 0.5 m tall screen cages under constant ambient conditions at the University of Hawaii, Manoa. Larvae were placed in screen cages on potted plants grown for this project. Larvae were reared entirely on the host plant on which they started to confirm developmental suitability. Larvae found on *N. glauca* in the wild were switched to *Solanum americanum* for the duration of their development. Because our sample size was necessarily small, and *S. sandwicense* is endangered, only two larvae were reared entirely on this host. The endemic, and frequently endangered, species in the genus *Nothoestrum* were not used, since larvae have been found on these plants frequently and they are thought to be the original hosts of BSM. In addition to *N. glauca* and *Nothoestrum* spp., Riotte (1986) confirms only two records of BSM on tomato (*Lycopersicon esculentum*) and one on "popolo" (the Hawaiian name for *Solanum* sp.) from a specimen label, and while larvae have never been reared solely on native *Solanum* spp., grey literature reports (Van Gelder and Conant 1998) suggest the host range may include them. For this study we document larval success entirely on alternate host plants in the Solanaceae.

Larval appearance and size were documented at each instar, by using a micrometer and a Leica[™] dissecting scope. Larger larvae were measured directly with a ruler. Whenever possible, life stages were weighed using a Metler AE 163 scale. This was not possible for some life stages due to the activity of the larvae or their fragility. Since BSM is an endangered species, the health of the larvae was a priority. Because BSM is of conservation concern, a chronological format is used to reflect development times and data is reported on the fate

of individual larvae as much as possible. Reared adults were either frozen for DNA analysis for a concurrent study, or returned to the location of collection on Maui, and released back into the population from which they were collected as eggs.

Results

Field work. We found two life stages in our one-day survey in east Maui. A total of six live eggs were found on both the upper and lower side of *Nicotiana glauca* leaves, as well as one that appeared to have been predated (Fig. 1-A), possibly by a parasitoid, based on the damage to the eggshell. We also found two later instar larvae on leaves and stems at the same time as the eggs, suggesting broad flight periods or overlap of generations. One was a 5th instar larva which thrashed violently and clicked its jaws when disturbed, kindly making it easier to find amongst the foliage. The other larva was a 2nd instar larva, which quickly molted to 3rd without feeding. The two larvae collected as 2nd and 5th instar switched easily from *N. glauca* to the Hawaiian endemic, *Solanum sandwicense*. None of the eggs or larvae collected were parasitized. The following life history description is based on the eight individuals collected as larvae and eggs on *N. glauca* from east Maui on October 16, 2009.

Developmental phenology and natural history. Egg: (Fig. 1-B) Egg is ovoid and light green, turning pale beige as the larva develops. The pronounced caudal horn is easily visible at least 24 hours before larva hatches. Most eggs hatched too quickly after collection for measurements, and larvae almost always consumed the eggshell immediately after eclosion. Length of two eggs were 1.79 mm and 1.89 mm.

1st instar: (Fig. 1-C). October 17, 17:50: eggs collected on October 16 began to eclose and larvae immediately consumed their eggshells. Right after eclosion, larvae are light beige, rapidly becoming light green after feeding. Caudal horn is narrow, light brown and between $\frac{1}{3}$ and $\frac{1}{2}$ the length of the body, becoming red as larva feeds, sometimes recurved or bent (in subsequent molts it always curves posteriorly and thickens). Thin, light setae are sparse across body, right after eclosion, becoming more prominent raised white tubercles through instar. Late first instar larvae have faint dorso-lateral white lines from behind head capsule to caudal horn. Length 5.4 mm (prefeeding 1st instar larvae) to 10.5 mm prior to molt to second instar, n=4. 1st instar larvae that had just hatched weighed 0.001 g and 0.002 g each (n=2).

The two first 1st instar larvae that were placed on *S. sandwicense*, a woody endangered wet forest species endemic to Kauai and Oahu with relatively thick leaves, seemed reluctant to take it, though the larvae successfully developed entirely on it. All other larvae readily accepted *S. americanum*, an indigenous, thin-leafed herbaceous species.

2nd instar: (Fig. 1-D) The first larva that eclosed from an egg on October 17 molted into the 2nd instar on October 20, thus the 1st instar lasted about 3 days. Larva is a dark green color, and intersegmental cuticle is yellow gold towards head. Abundant thick, white setae on raised tubercles give the head and thorax a spiny rough appearance, with setae and tubercles much reduced distally, to abundant white spots. Spiracles are faintly red surrounded by light green, caudal horn is red. The oblique white lines above spiracles become more pronounced as larva proceeds through instar. Length 8.2-15 mm prior to molt to third instar, n=4.

3rd instar: (Fig. 1-E) Third instar lasted about 3 days. Third instar larvae are lighter green than 2nd instar, spiracles are prominent, red circled by yellow. White raised setae are still prominent on head and thorax, but now also raised across rest of body and caudal horn, though less so than head and thorax where a very spiny appearance is maintained. Oblique white lines down body are present but still relatively faint. Caudal horn is white and green basally become light red distally. Length 19.7-26 mm, n=4.

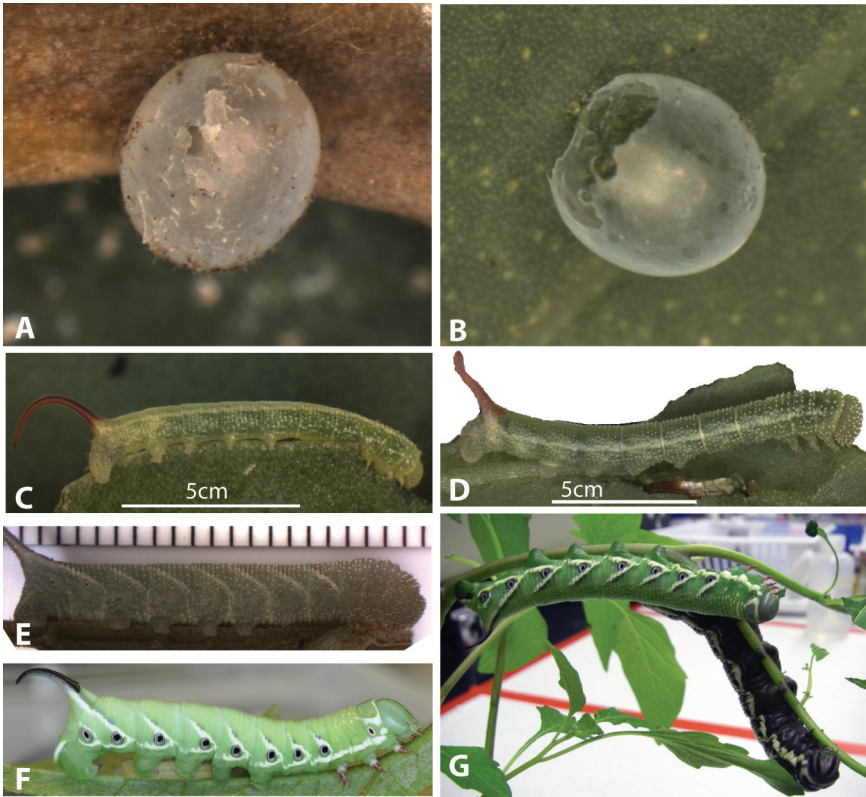


Figure 1. *Manduca blackburni*. (A) Unhatched *M. blackburni* egg, eaten or parasitized based on damage to egg shell. Found on *N. glauca*. (B) Hatched egg. Most larvae quickly ate the entire eggshell after hatching; in this case, it was only partially eaten. Consumption of the eggshell, in part or whole, can be indicative of a successful hatch. (C) Late first instar larva; note the red color and prominence of the horn. (D) Second instar larva, ruler in cm, shed skin in foreground. (E) Third instar larva; note the prominence of the red spiracles. (F) Early fifth instar larva. (G) Fifth instar larvae, both color morphs (green and purple).

4th instar: (not figured) The fourth instar appeared much as the third, but with the lateral oblique white lines becoming more pronounced. In the early 4th instar, one larva was 30 mm in length. In the late 4th instar, larvae measured 36 mm, 55 mm, 40 mm, 42 mm, 42 mm in length. On October 21 the larva found as a second instar, and which molted to 3rd on October 18th, molted to 4th, thus 3 days between 3rd and 4th instar.

5th instar: (Figs. 1-F, 1-G) As is typical of many Sphingidae, the 5th instar represents a remarkable change from the preceding four instars in coloration and shape (Tuttle 2007). In BSM, there are two color forms, green and purple (sometimes referred to as gray). The 5th instar has been described in great detail by Riotte (1986); we offer only a brief description, the interested reader should refer to the cited literature, and figure (Figs. 1-F, 1-G). Both color forms are present and have very smooth skin, with none of the white setae so prominent as in earlier instars. The green form has striking black spiracles ringed in yellow

and then blue. The caudal horn is black, strong and smooth, with a whitish sheen ventrally. There are small indistinct areas of purplish coloration above white oblique lines of white chevrons, more prominent distally (this is not visible on the purple form). The thoracic shield is as described by Riotte (1986). The purple form has a purple background color, but is otherwise remarkably similar to the green form. The purple form differs in two other obvious ways: it has two prominent dark stripes down the head capsule, which are much harder to see, but present, on the green form, and in the green form, the last three spiracles toward the horn become increasingly circled in yellow, before the blue coloration typical of all the spiracles takes over. In the purple form there is no yellow at all, just blue around a black spiracle.

Fifth instar larvae were up to 90 mm long and Head capsule measurements were: 5.5 mm, 6 mm, 7 mm, 7 mm, 8 mm, average: 6.7 mm. Larval weights during 5th instar just prior to pupation were: 16.075 g, 12.024 g, 12.10 g, 12.21 g, 13.38 g, 14.45 g, average weight: 13.37 g.

Following individual larva, on October 20th the larva found as a 5th instar finished feeding and was searched for a pupation site. It weighed 12.024 g, its pupa (when dug up later) weighed 6.458 g. Resulting male moth weighed 1.93 g, and emerged on November, 27 2009 at 13:15. By 13:45 wings fully expanded and dried. Adult was quiescent until dark, when it began to fly actively.

The adult moth that resulted from the larva collected as a late 2nd instar emerged December 1, 2009 at 1630. This male weighed 2.22 g.

On November 1st all the 6 larvae found as eggs molted out of 4th instar into 5th; these larvae all hatched on October 17, meaning 2 weeks between hatching from the egg and entering the 5th instar.

On November 9, the last of the six larvae in this cohort, consisting of three purple and two green color forms, went down to pupate. We weighed this last larva in the wandering stage to contrast with its pupal weight, it weighed 14.45 g when searching, and the resulting pupa weighed 7.87 g.

Pupation: Pupal weights: (n=8, average, 6.718 g) 7.874 g, 7.186 g, 5.294 g, 6.042 g, 7.494 g, 6.355 g, 6.743 g, 6.762 g. Males: n=5, average=6.529 g, Females n=3, average=7.034 g.

Six of the eight pupae were at or near the bottom of the pot in which the larva burrowed (~150 mm deep) suggesting that the larvae might prefer, or at least be able to burrow even deeper into the soil prior to pupation. However, the two other pupae were less than 25 mm below the surface of the soil, and one of these larvae was in a pot with 220 mm of soil into which it could have burrowed. It is perhaps significant that both of these shallow pupation chambers were under the root ball of the host plants in those pots. The rootball provides significant structural support for the pupal chamber and larvae are likely to take advantage of this when they can. When no rootball is available, they may need to burrow deeper to form a pupation chamber.

Adult: The adult has been described and figured repeatedly and is similar to *M. quinquemaculatus*. Zimmerman (1958) suggests that the pupal stage may last a year, but there are no data supporting this suggestion. While we have no reason to doubt that this is possible, we reared eleven moths in the past five years (some of which were not part of the present study) and this was never the case. Moths always emerged within six weeks of pupation. We did not expose the pupae to any additional moisture, or higher than ambient temperatures to encourage eclosion. Pupae were maintained under the same lab conditions as the larvae. It is certainly possible that in natural populations a subset of the pupae hold over for an extended period of time, as this is typical of many sphinx moths (Tuttle 2007).

Emergences and release of reared *Manduca* collected in Kanaio. In the afternoon of December 17th, one male emerged from its pupae. December 18, late morning a female emerged. Both moths were released on December 19 in the Kanaio area of Maui, within 100

yards of where their eggs had been collected. On December 20 and 23 and 24 two females and a male emerged, all in the afternoon. All were released. In sum, two male and three female BSM were released on east Maui near where they were collected as eggs. Given the very low survival rates generally expected for eggs becoming adult moths, the release of five endangered moths represents a positive contribution to the population from which eight eggs were taken.

Discussion

Life history. While *M. quinquemaculatus* has a black form (Tuttle 2007), in addition to the typical green form, BSM purple form is not nearly as dark and, unlike *M. quinquemaculatus*, we have never observed BSM to have intermediate forms besides the purple and green. These two forms are sympatric and thus do not seem to have an environmental basis, occurring in larvae from eggs found on the same plant, and reared together on the same host plant. Out of eight larvae reared on this and prior occasions, three became the purple form, which suggest a much higher rate of dimorphism than is mentioned for *M. quinquemaculatus*. Anecdotal evidence from previous collections also suggests that the purple form is not uncommon. Our egg to pupa development times of approximately 60 days match well with the developmental times that Williams (1947) found when rearing larvae from Molokai.

In an earlier rearing experiment, we collected three BSM eggs from the Kanaio region of Maui and reared the larvae entirely on domestic tomato and eggplant (*Solanum melongena*) varieties, and moved larvae between the species frequently because they consumed the smaller plants entirely (D.R., unpublished data). Those larvae exhibited no ill effects in development time or size. The same hosts were also noted when BSM was still present on Oahu (Zimmerman 1958). These results confirm that BSM larvae can develop on a wide variety of native and introduced solanaceous hosts, which bodes well for BSM reintroduction, but leaves the disappearance of the species a mystery.

Parasitism rates. It is interesting to note that of the six eggs, and the 2nd instar and 5th instar larvae collected, none were parasitized, since introduced parasitoids are thought to be a cause of the species' decline (Gagné and Howarth 1982). This is in contrast to findings from Molokai (Williams 1947), which suggested much higher levels of parasitism, in a population that is now extinct. Obviously our sample sizes are extremely low, but it is unlikely that much larger numbers of wild larvae and eggs of this endangered moth will be available for future parasitism studies. In some persistent populations of other sphinx moth species more than 90% of eggs and larvae can be parasitized (Tuttle 2007). It is possible that BSM persists in the restricted, very localized places where we found it precisely because parasitism is suppressed in these refugia due to local environmental conditions. Our fieldwork suggests a very small role for parasitoids in the mortality of BSM, but our sample sizes may simply have been so small as to not, by chance, pick up on what is normally crippling parasitism rates. Further research on the impacts of parasitoids on BSM larvae will be essential in evaluating this threat and selecting reintroduction sites as part of the species' recovery. We propose that reintroduction efforts first assess the levels of parasitism in BSM by exposing reared eggs and larvae to assess apparent levels of parasitism as was done for other endemic Hawaiian moths (King et al. 2010).

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