

Rapid evasive behaviors of diamondback moth larvae against ants

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Abstract

Larvae of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) wriggle vigorously backward when physically stimulated. To explore whether this behavior is effective against ant attacks on their host plants with three-dimensional structures, we experimentally observed DBM larval movements and consequences thereof when attacked by *Formica japonica* (Hymenoptera: Formicidae) ants on the upper and lower surfaces of host plant leaves. On the upper surfaces of leaves, DBM larvae wriggled backward in response to ant attacks, and most larvae hung from leaf edges by spinning silk threads. On the lower surfaces of leaves, DBM larvae did not wriggle backward but immediately hung from the lower surface in response to attacks. No ants were able to pursue hanging larvae. We conclude that wriggling backward by DBM larvae belongs to a sequence of evasive movements against ant attacks on upper leaf surfaces. To our knowledge, this is the first report of combined wriggling and hanging behaviors by Lepidopteran larvae that is effective against ants in three-dimensional habitats.

Keywords

predator avoidance, agricultural pest, predation by ants

Introduction

Small arthropods have developed various strategies for avoiding ant predation, such as living in defensive shelters (Sendoya and Oliveira 2017), conciliating ants with honeydew (Way 1963), producing deterrent chemicals (Uemura et al. 2017) or chemically simulating host-plant surfaces to prevent ants from recognizing them (Akino et al. 2004). These strategies may reflect the strong predation pressure exerted by ants, which as a group have a biomass greater than that of all mammals and birds combined (Schultheiss et al. 2022).

The diamondback moth (DBM) is one of the most problematic agricultural pests worldwide (Zalucki et al. 2012), and the importance of ants for DBM biocontrol has been previously established (Agarwal et al. 2007). Therefore, understanding defensive behaviors of DBM against ants is crucial. DBM larvae wriggle vigorously when physically disturbed (Wang and Keller 2002). Although previous studies (Nofemela and Kfir 2008, Nofemela 2017; Roger et al. 2000; Lankin-Vega et al. 2022) reported that this behavior is effective against parasitoid wasps and predators other than ants, no studies to date have investigated whether the behavior is effective against ants.

In general, the effectiveness of antipredator defensive behaviors that take place on three-dimensional structures is underestimated in two-dimensional space. For example, dropping and freezing behavior by small insects in response to predator attacks allows escape in three-dimensional habitats (Fabre 1900, Edmunds 1974). However, such behaviors are often interpreted as a simple death-feigning in two-dimensional space (e.g., Miyatake et al. 2004). Similarly, wriggling DBM larvae are always ultimately captured by ants in two-dimensional space (e.g., on the bottom plane of a laboratory container). For example, we preliminarily placed DBM fourth instars one by one on the bottom of a container with *F. japonica* ants and observed all 23 larvae were captured within 3 minutes, indicating that wriggling behavior is ineffective against ants under these circumstances. However, we hypothesized that this defensive behavior may be effective in three-dimensional space, i.e., on host plants.

Although Sendoya and Oliveira (2017) reported that wriggling and hanging escape behaviors of other caterpillar species are each effective against ant attacks on host plants, the relationship between these defensive behaviors is poorly understood. In the present study, we experimentally examined for the first time the combined effects of wriggling and hanging movements by Lepidopteran larvae against ants with respect to positions on host plant leaves.

Material & methods

Insects

DBM feed on wild and cultivated cruciferous plants and are considered one of the most difficult pests to control (Zalucki et al. 2012). Approximately 40–50 DBM larvae were collected from leaf mustard (*Brassica juncea* Czern) in May 2020 in Osaka, Japan. The population was then maintained on leaves of Japanese mustard spinach (*Brassica rapa* var. *pervidiris*) inserted into 10-mL glass tubes filled with water. All larvae were reared at a constant temperature of 25°C, with 50% relative humidity and a L16:D8 photoperiod.

Formica japonica is one of the most abundant ant species in western Japan. It is a generalist predator that often co-occurs with DBM in the wild.

Sequence of DBM larva reactions in response to mechanical stimuli

To describe a sequence of wriggling reactions, DBM fourth instars were introduced onto the upper surface of *B. rapa* leaves, and allowed to establish feeding sites. A fine brush was used to induce the wriggling reaction. The reaction was recorded using a video camera (Sony NEX-VG30H) equipped with a 30-mm focal length lens. After recording, we drew illustrations of the reaction by tracing still images of caterpillars on a screen.

Reactions of DBM larvae when contacted by ants on the host plant

To examine whether the wriggling reactions of DBM larvae are effective against ants on the host plant, we introduced 5–10 DBM fourth instars onto a leaf of *B. rapa* (ca. 200 mm length and 100 mm width) inserted into a 10-mL glass tube filled with water. We placed the tube next to a nest hole of *F. japonica*, and inserted an iron stake into the ground to orient the leaf surfaces horizontally $\pm 20^\circ$. Ants were able to easily access the leaves by walking across the iron stake and glass tube (Fig. 1). This experiment was conducted on the campus of Kyoto University during 30 September to 14 October 2020.

We recorded reactions of larvae on the upper and lower surfaces in response to intruding ants. In total, we recorded 26 observations of DBM on the upper surfaces of leaves and 35 on the lower

surfaces. No larvae on the same plant touched one another during observations. The frequencies of successful predation and of larvae wriggling backward were compared using Fisher's exact test (SAS 9.22).

Results

Sequence of DBM larva reactions in response to mechanical stimuli

Figure 2 presents a sequence of wriggling reactions on the upper leaf surface. Figure 2A shows a DBM larva feeding. Subsequently, the larva contracts in response to mechanical stimuli (2B), bends (2C), jumps by kicking the leaf surface (2D), and lands with its body still bent (2E). The larva then stretches its body (2F) to return to an outstretched position. The distance of jumps was approximately equal to its body length. The larva repeated the movements shown in 2B–2F several times while spinning the silk thread before it reached the leaf edge (wriggling). Each wriggling took approximately 0.1 seconds.

Reactions of DBM larvae when contacted by ants on the host plant

The frequency of successful predation did not differ between the upper and lower leaf surfaces ($P > 0.99$, Fisher's exact test, Table 1). However, defensive behaviors of larvae in response to attack clearly differed between locations on the host plant ($P < 0.0001$, Fisher's exact test, Table 1). On the upper surface of leaves, all larvae jumped backward, and 81.0% then hung from leaf edges by spinning silk threads. In contrast, no larvae jumped backward on the lower surfaces of leaves; however, 96.6% of them immediately hung from the lower surface (supplement video). Moreover, no ants ($n = 50$) pursued the hanging larvae.

Discussion

It has been pointed out that particular antipredator behaviors in insects are triggered only under particular prey behavioral contexts (Hoy et al. 1989; Cinel et al. 2020). Although wriggling and hanging behaviors of many Lepidopteran larvae in response to enemy attacks have been reported (Roger et al. 2000; Sendoya and Oliveira 2017; Lankin-Vega et al. 2022), to our knowledge this is the first report that systemically demonstrated reasons why Lepidopteran larvae use different evasive movement depending on their position on host plant leaves. On upper leaf surfaces, DBM larvae

wriggled backward in response to ant attacks, and hung from leaf edges. On lower leaf surfaces, DBM larvae did not wriggle backward, but immediately hung from the lower surface in response to attacks. DBM larvae apparently cannot wriggle backward on the lower surfaces of leaves, likely because they must leave the leaf surface while jumping (Fig. 2D). Otherwise, jumping backward on lower leaf surfaces may have automatically resulted in hanging.

Some of the observed DBM larvae (19.0%) remained on the upper leaf surfaces and did not hang from the leaf edge, which may be due to the large size of the leaves (ca. 200 × 100 mm) used in the experiment. Because DBM larvae prefer younger (i.e. smaller) leaves to older ones (Moreira et al. 2016), most DBM larvae should hang from leaves under natural conditions.

Although some slow-moving Lepidopteran larvae show vigorous evasive behavior such as thrashing and twisting that sometimes lead them to drop from the plant (Mcclure 2011, Sendoya and Oliveira 2017), these behaviors usually provoke additional attacks by ants (Malicky 1971). In contrast, DBM larvae wriggled backward so rapidly that no ants were able to reattack them. Thus, this is the first report of combined wriggling and hanging behaviors that is effective against ant attacks on their host plants with three-dimensional structures. Another evasive behavior of slow-moving Lepidopteran larvae is to simply drop from the plants (Pierce and Eastal 1986). Compared to such escape behavior, the escape behavior of hanging seems advantageous in that it ensures return to the appropriate food plant and to avoid the risk of being attacked by ants and other ground predators (Yamazaki 2010).

In contrast, the defensive reactions of DBM larvae are not effective against parasitoid wasps. The parasitoid wasp *Diadegma semiclausum* waits for hanging larvae to climb back to the leaf, while another parasitoid wasp *Cotesia vestalis* pursues the host down the silk thread (Wang and Keller 2002). These examples suggest that the parasitoids have counter-adapted against the defensive reactions of DBM larvae. This may potentially explain why some lepidopteran larvae drop from plants instead of hanging. Alternatively, although parasitism has significant effect on DBM population (Furlong et al. 2004, Wang et al. 2004), they may not have developed effective defenses against parasitoids, as the latter pose less of a mortality risk compared to ants and spiders (Farias 2020). Although the frequency of successful predation per individual ant attack was less than 20% (Table 1), ants may serve as a

major mortality risk because of their enormous population size and consequent high frequency of attacks. Because ants along with parasitoids always briefly inspect a prey item with antennae before capturing it, wriggling backward and consequently hanging in response to mechanical stimuli should reduce mortality of DBM larvae by these enemies. Therefore, it seems reasonable to propose that the sequence of defensive reactions in response to stimuli may have evolved in DBM larvae at least in part to avoid predation by ants. However, DBM larvae exhibit wriggling and hanging behaviors not only against parasitoids and predators but also in response to stimuli caused by raindrops (Eziah et al. 2010) and conspecifics (Salinas 1972). Therefore, we cannot rule out the possibility that wriggling and hanging in DBM have evolved for functions other than predator avoidance and the ability of those behaviors to avoid predation is a byproduct. Further research is needed to answer this question.

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Author contributions

KI: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Validation; Visualization; Writing—original draft; Writing—review & editing. SY: Conceptualization; Funding acquisition; Methodology; Supervision; Writing—review & editing.

Declaration of interests

The authors declare no competing interests.

Data availability

All data can be found in Table 1.

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Captions

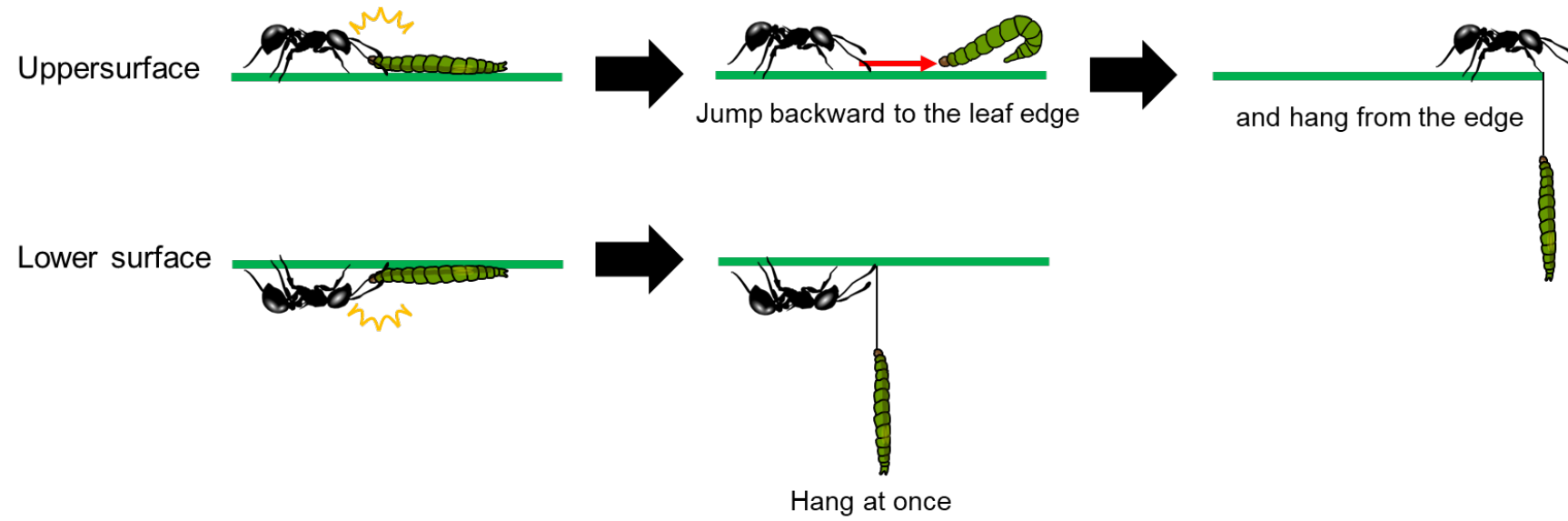
Table 1. Reactions of DBM larvae when attacked by *Formica japonica* on the host plant.

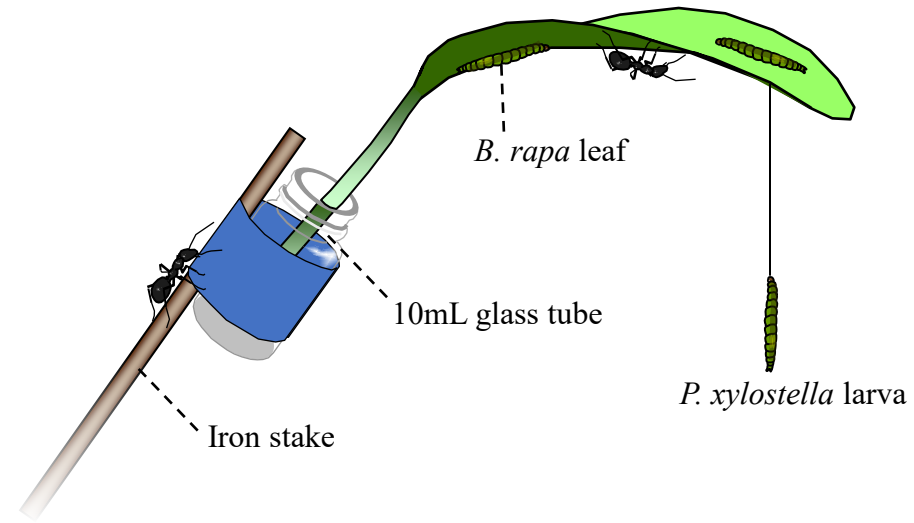
Fig. 1 Experimental design used to investigate the defensive reactions of diamondback moth (DBM) larvae attacked by *F. japonica* ants on host plants.

Fig. 2 Sequence of wriggling reactions performed by a DBM caterpillar.

Graphical abstract: Defensive behaviors of DBM larvae on upper and lower leaf surfaces.

Supplement video: *P. xylostella* larvae falling one after another from the lower leaf surface when contacted by a *F. japonica* ant. (This is a preliminary video. We can prepare a higher resolution one without water mark.)





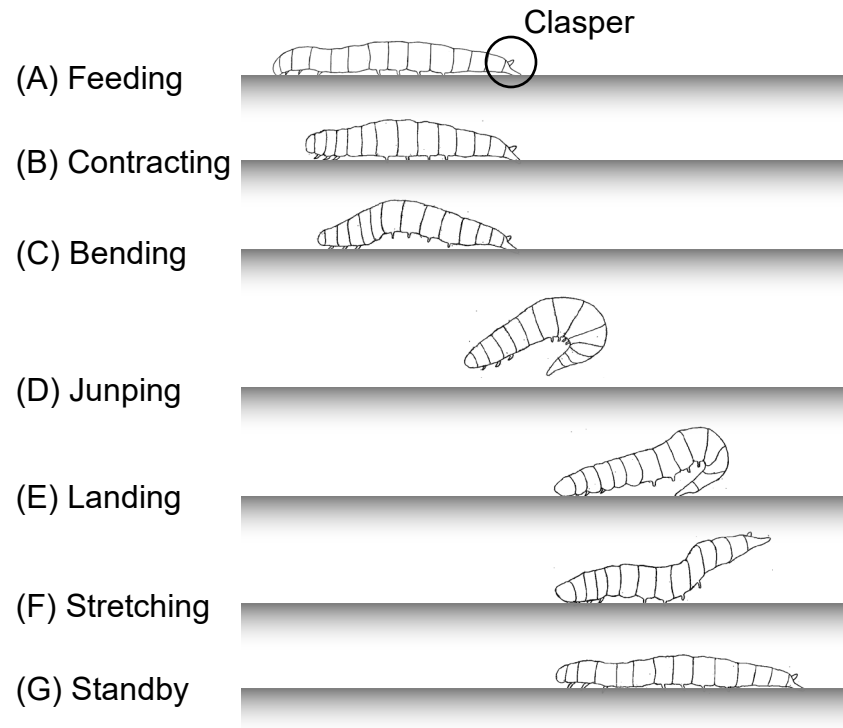


Table 1. Reactions of DBM larvae when attacked by *Formica japonica* on the host plant.

	% Successful predation	% Jumped backward	% Hung at once	% Hung from leaf edge
Upper surface	19.2 (n = 26)	100 (n = 21)	-	81.0 (n = 21)
Lower surface	17.1 (n = 35)	0 (n = 29)	96.6 (n = 29)	-
P	> 0.99	< 0.0001	-	-