Journal of Insect Physiology 96 (2017) 93–97

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Searching for particular traits of sawfly (Hymenoptera: Tenthredinidae) larvae that emit hemolymph as a defence against predators

Jean-Luc Boevé [a,*], Tina E. Trenczek [b], Sergio Angeli [b,1]

[a] OD Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, 1000 Brussels, Belgium
[b] Institute of Zoology, Justus-Liebig-University Giessen, Stephanstreet 24, 35392 Giessen, Germany

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form 18 October 2016
Accepted 19 October 2016
Available online 20 October 2016

Keywords:
Tenthredinidae
Sawfly larvae
Easy bleeding
Hemolymph
Antibacterial activity

ABSTRACT

Easy bleeding is a defence strategy that allows the larvae of some Tenthredinidae sawfly species to emit deterrent hemolymph when attacked by a predator. However, a drawback of this defence is that hemolymph is frequently in contact with the exterior, thus potentially subjected to multiple microbial infections at any body's integumental spot. Here we aimed to identify physiological traits that are linked to easy bleeding. First, larvae of several sawfly species were subjected to daily experimental losses of hemolymph equivalent to 10% of their body weight, and changes in body weight and survival were recorded. Easy bleeders' survival rates were better compared to non-easy bleeders. Second, testing hemolymph melanisation revealed that nearly all sawfly hemolymph samples did not melanise over a 24 h period. Third, inhibition zone tests against live *Escherichia coli* were conducted using hemolymph collected 24–48 h after a sterile wounding and an infection with *Micrococcus luteus*, as well as from control, untouched individuals. Sterile wounding induced similar antibacterial activities compared to those detected in the control group. However, the activity was significantly enhanced upon infection in some species, similarly to other insects. Thus, easy bleeders have a tendency to compensate for hemolymph loss resulting from predator-prey interactions, whereas a non-melanising hemolymph is probably a characteristic of sawflies, and the antimicrobial activity can be high but is comparable in easy bleeders versus other insects.

1. Introduction

Hemolymph fills the hemocoel of insects, and normally has no contact with the external environment due to the integument that acts as a physical barrier. However, organisms from outside may enter the host insect when parasitoids lay eggs or when entomopathogenic fungal hyphae penetrate through the integument (Augustyniuk-Kram and Kram, 2012; Binnington and Retnakaran, 1991). The hemolymph is also directly exposed to the exterior when a seizing/biting predator injures the integument. Hence, it can be expected that an insect's survival depends strongly on its ability to induce a prompt, effective immunological response.

The defence of insects against pathogens (e.g. bacteria, viruses, fungi) relies on an innate immune system that includes wound healing, hemolymph coagulation, a cellular response by hemocyte activities such as phagocytosis and encapsulation, a humoral response by the production of soluble effector molecules such as antimicrobial peptides (AMPs), and components of the prophenoloxidase activating cascade that finally lead to melanisation (e.g. Siva-Jothy et al., 2005; Strand, 2008; Theopold et al., 2002). From an evolutionary point of view, we may expect that physiological responses induced by pathogenic attacks co-evolved with other life history traits of the insect taxa. Larvae of some sawflies (Hymenoptera, Tenthredinidae) defend themselves against attacking predators by a mechanism referred to as easy bleeding (Boevé and Schaffner, 2003). These insects are plant-feeders at larval stage, and easy bleeders sequester in their hemolymph deleterious secondary metabolites from the food plant (Prieto et al., 2007 and literature therein). The whole body integument of easy bleeders is easily wounded under mechanical stress such as a bite from a predator (Boevé et al., 2004). The larva becomes immobile and immediately releases a droplet of the distasteful hemolymph at the wound site, which in most cases stops the predator's attack (Boevé, 2009). Afterwards and within several minutes, the larva can suck back the released drop of hemolymph, provided that the droplet does not spread and is not too large. Furthermore, easy

* Corresponding author.
E-mail addresses: jean-luc.boeve@naturalsciences.be (J.-L. Boevé), tina.e.trenczek@allzool.bio.uni-giessen.de (T.E. Trenczek), sergio.angeli@unibz.it (S. Angeli).
[1] Present address: Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bolzano, Italy.

http://dx.doi.org/10.1016/j.jinsphys.2016.10.012
0022-1910/

bleeders have an integument that efficiently heals after wounding due to local hemolymph clotting, scab development, followed by melanisation; this repair mechanism is completed with the next moult (Jakubowska, 2002). Easy bleeding differs from reflex bleeding where hemolymph is only released from specific weak integumental points and can become sticky rapidly after its release (e.g. Wallace and Blum, 1971).

The adaptive significance of easy bleeding is to shorten the predator-prey interaction, which may occur several times during a prey's life. However, a potential cost of easy bleeding is that hemolymph comes in contact with the external environment and, hence, increases the infection probability with microbial agents. Moreover, the risk of microbial infection is expected to increase even further when the hemolymph is retracted into the hemocoel. We therefore hypothesize that easy bleeders evolved physiological and immunological adaptations to resist direct and repeated invasions by pathogenic microorganisms.

By comparing easy bleeding sawflies with non-easy bleeding ones, we here explore and determine whether easy bleeding affects the capability to recover hemolymph, the process of hemolymph melanisation and the immune response to a bacterial infection. The results are discussed whether or not traits are peculiar in easy bleeders as compared to other sawflies and insects in general.

2. Material and methods

2.1. Insect species

Sawfly larvae were mainly collected in the field (Table 1) and maintained in the laboratory on leaves of their host plant. They were identified following Lorenz and Kraus (1957) as well as by comparative collection material. Voucher specimens are kept at the Royal Belgian Institute of Natural Sciences (J.-L. Boevé collection). Indoor rearing is virtually impossible since most used species are specialists (i.e. larvae feeding and females laying eggs on a single plant genus or species) and monovoltine, needing a winter diapause period.

2.2. Hemolymph recovery and survival of larvae after repeated loss of hemolymph

Several days before eonymph moulting, sawfly larvae were kept individually in 5 cm diameter Petri dishes and provided daily with a piece of fresh leaf of their host plant and moistened filter paper. At Day 0, larvae were divided randomly into test or control larvae. From Day 1 onwards, they were all weighed on a daily basis and their health status (healthy, moribund, or dead) was recorded. Hemolymph was then collected from the test group only. The integument was gently pierced and a given amount of oozing hemolymph collected with a glass capillary. The amount taken from each larva was 10% of its present body weight (assuming a hemolymh density of 1 g/ml). Six sawfly species, 2 non-easy and 4 easy bleeders, were tested (see Table 1).

To differentiate between the impact of piercing the integument versus collecting hemolymph, larvae of one of the non-easy bleeders (*Tomostethus nigritus*) were segregated randomly in four groups and treated individually as described above, except that the treatment was performed only once. From two groups, hemolymph was collected as an equivalent of 5% and 10% of their body weight (n = 20 and n = 18 individuals, respectively). Larvae of a third group were injured only, without collecting hemolymph (n = 17), whereas those of a fourth group served as control and were left untreated (n = 18). The number of larvae reaching the eonymph stage (i.e. able to moult) was recorded at regular intervals during 96 h.

Table 1
Host plant and occurrence of defence by easy bleeding in the studied sawflies.

Species	Field host plant	Defence	Investigations
Tenthredinidae, Allantinae			
Allantus rufocinctus (Retzius)	*Rosa canina*	EB	b
Eriocampa ovata (L.)	*Alnus glutinosa*	–	b
Monostegia abdominalis (Fabricius)	*Lysimachia* sp.	–	b
Tenthredinidae, Athaliinae			
Athalia rosae (L.)	(indoor population)	EB	a, b
Tenthredinidae, Blennocampinae			
Monophadnus sp.	*Helleborus viridis*	EB	b, c
Phymatocera aterrima (Klug)	*Polygonatum multiflorum*	EB	a, b, c
Rhadinoceraea bensoni Beneš	*Lilium martagon*	EB	c
Rhadinoceraea micans (Klug)	*Iris pseudacorus*	EB	a, b, c
Rhadinoceraea nodicornis Konow	*Veratrum album*	EB	c
Tomostethus nigritus (Fabricius)	*Fraxinus excelsior*	–	a, b
Tenthredinidae, Nematinae			
Hemichroa australis (Lepeletier)	*Alnus glutinosa*	–	b
Nematus miliaris (Panzer)	*Salix* sp.	–	b
Nematus pavidus Lepeletier	*Salix* sp.	–	b
Nematus ribesii (Scopoli)	*Ribes* sp.	–	b
Pristiphora sp. cf *cincta*	*Lonicera caerulea*	–	b
Susana cupressi Rohwer & Middleton	*Cupressus sempervirens*	–	b
Tenthredinidae, Selandriinae			
Aneugmenus padi (L.)	*Pteridium aquilinum*	EB	a, c
Dolerus sp.	Poaceae	–	b
Strongylogaster multifasciata-group	*Pteridium aquilinum*	–	a, b, c
Tenthredinidae, Tenthredininae			
Tenthredo scrophulariae L.	*Verbascum* sp.	–	b
Argidae			
Arge berberidis Schrank	*Berberis vulgaris*	–	b
Arge ochropus (Gmelin)	*Rosa* sp.	–	b
Arge pagana (Panzer)	*Rosa* sp.	–	b, c
Diprionidae			
Gilpinia hercyniae (Hartig)	(indoor population)	–	b
Pergidae			
Lophyrotoma zonalis (Rohwer)	*Melaleuca leucadendra*	–	b

Larvae are defended by easy bleeding (EB) or not (–), following Boevé and Schaffner (2003) and own observations. Investigations: hemolymph recovery (a); hemolymph melanisation (b); hemolymph antibacterial activity (c).

2.3. Hemolymph melanisation

Four to 5 µl hemolymph was collected from a larva, using 2 or 3 individuals per species (Table 1). The hemolymph droplet was placed on a piece of Parafilm® in a humid chamber, at room temperature (20–25 °C). Changes in appearance were recorded within a 24 h period.

2.4. Antibacterial activity of hemolymph

Infection assays were performed on seven sawfly species (Table 1). Since larvae were collected in the field, it appeared difficult to obtain enough larvae from the same instar for each species.