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Towards environmentally and human friendly insect pest control technologies: Photosensitization of leafminer flies *Liriomyza bryoniae*

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Abstract

Development of new, ecologically safe technologies to control insect pest populations is of great importance. Photoactive compounds usually used for photosensitization might be effective as pesticide agents, with low impact on the environment, being non-toxic and not mutagenic. Phosensitizer accumulates within the insect body and, following exposure to visible light, induces lethal photochemical reactions and death. The aim of this study is to evaluate the possible usage of several photosensitizers (acridine orange, aminolevulinic acid, hematoporphyrin dimethyl ether, methylene blue) as photopesticides to control population of polyphagous plant pest *Liriomyza bryoniae* (Kaltenbach, 1858) (Diptera, Agromyzidae). Fluorescence measurements of intact cooled insects indicate that insect feeding with bait containing HPde and sugar induces remarkable accumulation of this compound in the body of insect. This accumulation is strongly dependent on sex and feeding duration. The highest HPde amount in the body of insect was detected 16 h after feeding, whereas no significant photosensitizer amount was detected in the same insect following 48 h. Following irradiation with visible light results in fast death of *L. bryoniae*. Of importance to note that survival of insects after feeding and irradiation depends on sex: female insect died much faster than males.

Keywords: Photosensitization; Environmentally friendly photoinsecticides; Diptera; Agromyzidae

1. Introduction

There is considerable interest at present in the application of environmentally friendly tools to control insect pests. Despite plethora of conventional insecticides have been successfully used, they often show undesirable side-effects. For instance, significant toxicity was observed to non-target organisms, such as useful insects, fishes or mammals. Human health is also related to wide application of conventional insecticides, because their residues are found in water, different kinds of food and might induce various illnesses.

In addition, insect resistance to traditional pesticides is on the rise, and there are public fears about chemical residues in food and environment. Thus, in 1996, the Food Quality Protection Act limited the use of many pesticides, particularly those that share common toxicological mechanisms such as organophosphate and carbamate insecticides, and triazine herbicides [1].

Despite it, insecticides are still widely used to control leafminer pests, including *Liriomyza bryoniae* (Diptera, Agromyzidae). This insect species as well as many others within the genus *Liriomyza* is economically important: due to their leafmining activity plethora of agricultural

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and ornamental plants are damaged. *L. bryoniae* leafminers can impact crops in at least six ways: (1) vector disease, (2) destroy young seedlings, (3) cause reductions in crop yields, (4) accelerate leaf drop above developing tomatoes, thus cause "sunburning" of the fruit, (5) reduce the aesthetic value of ornamental plants, (6) cause some plant species to be quarantined [2].

Around 56 plant genus were revealed to be suitable hosts for dipterous miners of economic importance [2–4]. Main primary hosts of *L. bryoniae* include: cabbages, *Brassica oleracea* (L.) (Brassicales, Brassicaceae), cucumbers, *Cucumis sativus* (L.) (Cucurbitales, Cucurbitaceae), lettuces (*Lactuca sativa* (L.) (Asterales, Asteraceae), courgettes, *Cucurbita pepo* (L.) (Violales, Cucurbitaceae), melons, *Cucumis melo* (L.) (Cucurbitales, Cucurbitaceae), tomatoes, *Lycopersicon esculentum* Mill. (Solanales, Solanaceae) and watermelons, *Citrullus lanatus* (Thunb) (Cucurbitales, Cucurbitaceae) [5].

Moreover, chemical control of *L. bryoniae* population is usually complicated due to the insect's biology, i.e. short generation period (besides, eggs and larvae are protected by leaf tissue as they stay inside a leaf); a relatively long lasting pupal stage (in the soil); high reproductive capability; and adult resistance towards insecticides. In addition, the insecticides are often more toxic to large parasite complex than to the leafminers themselves [1].

So it is becoming clear that alternative pest management tools are needed, less hazardous both to humans, non-target animals, and the environment. In this context, sunlight-activated pesticides represent a possible alternative to traditional chemical compounds [6,7].

The aim of this study is to evaluate the possible efficiency of several photopesticides taking into account their feeding deterrent properties, accumulation capacity and analysis of their photoinsecticidal activity on polyphagous leafmining plant pest *L. bryoniae.*

2. Materials and methods

2.1. Insects

L. bryoniae (Kaltenbach, 1858) (Diptera, Agromyzidae) culture was established in the laboratory on bitter-sweet, *Solanum dulcamara* L. (Solanales, Solanaceae). To initiate the culture, leaves containing *L. bryoniae* mines were collected from naturally infested bitter-sweet plants in Vilnius district, Lithuania. Adult flies of both sexes emerged from the collected larvae were transferred into plastic oviposition cages with bottoms and tops covered by nylon screen. Six to eight normally flushed leaves from the top of the bitter-sweet seedlings were provided to the flies for oviposition. Seedlings were replaced at 3- to 4-day intervals. Once the plants were infested with leafminer eggs, the oviposition cages were removed and plants were put in plastic bags for collecting puparia. Adult flies as well as larvae/puparia were maintained at 22 ± 1 °C and photoperiod of 15:9 (L:D). Light was provided by incandescent 400 W lamp (type DRLF, for greenhouses). Bitter-sweet seedlings were grown in commercial potting peat substratum.

To prevent mating after adult eclosion, each puparium was placed into separate glass vial. Each vial was supplied with small piece of wet filter paper to maintain humidity. Adult flies were collected daily.

2.2. Feeding behaviour registration

Feeding activity of adult flies was estimated by the duration the insect spent demonstrating feeding behaviour during 30 min period. Feeding behaviour was clearly different from that of non-feeding by insect position in a vial and its body posture: when feeding fly was at the feeding bait with head lowered down and proboscis extended. Such behaviour was recorded as feeding behaviour. Any other kind of behaviour (sitting quite or moving) was recorded as non-feeding behaviour. Observer recorded behaviour of a fly as feeding/non-feeding by pushing/releasing button on a keyboard, one button for each fly. Duration of feeding/non-feeding behaviour was counted using original computer programme, which allowed recording behaviour of five flies simultaneously, each fly in a separate channel. Accuracy of each behavioural record was few milliseconds. After automatic calculation of total feeding duration, precision of measurement was 0.5 s. Feeding behaviour of adult flies was registered under the red light illumination at 22 ± 1 °C. Red light allowed observer to register feeding behaviour of the flies and alongside ensured stable conditions for photosensitizer in the bait. The flies were fed in individual glass vials supplied with little sponge moistured with feeding bait solution (1 mL sugar/water solution at the concentration of 0.2 g/mL and 150 μL photosensitizer/physiological saline solution at the concentration of 2.5×10^{-2} M). In every control and test group behaviour of 15 insects was recorded and analyzed.

2.3. Photosensitizers

The stock solution of hematoporphyrin dimethyl ether (HPde) (the gift from prof. G.V. Ponomarev, Russia), acridine orange (AO), methylene blue (MB), aminolevulinic acid (ALA) (precursor of endogenous protoporphyrin PpIX) ("MERCK") were prepared in physiological saline (2.5×10^{-2} M) and were stored in the dark below 10 °C.

2.4. Evaluation of the photosensitizer pharmacokinetics in the body of insect

After emergence adults were sexed and not allowed to feed at least for 8 h. For feeding flies were supplied with small sponge pieces containing either sucrose solution (control) or bait solution (test). Control insects were allowed to feed with the sucrose solution at the concentration 0.2 g of sugar in 1 mL distilled water. Test insects were allowed to feed bait solution (1 mL sugar/water solution at the concentration of 0.2 g/mL and 150 μL HPde/physiological saline