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Effects of visible-light-activated hematoporphyrin dimethyl ether on the survival of leafminer *Liriomyza bryoniae*

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Development of new, ecologically safe technologies to control insect pest populations is of great importance. In this context, photoactive compounds might be used as effective pesticide agents with a low impact on the environment, as they are nontoxic and not mutagenic. The point is that a photoactive compound accumulates within the insect body, and exposure to visible light induces lethal photochemical reactions and the death of organism. The aim of this study was to determine whether the polyphagous plant pest *Liriomyza bryoniae* (Diptera, Agromyzidae) is sensitive to hematoporphyrin dimethyl ether (HPde)-based photosensitization. The data obtained indicate that exposure of insects to a bait containing HPde and the following irradiation with visible light result in a total killing of *Liriomyza bryoniae* adults. Moreover, the survival of insects exposed to a bait containing HPde and irradiated with visible light depended on sex: females were more sensitive to photosensitization than males.

Key words: photosensitization, sunlight-activation, survival, Agromyzidae, Diptera

INTRODUCTION

The genus *Liriomyza* (Diptera, Agromyzidae) is distributed widely in temperate areas. Many species within this genus are economically important, causing damage to agricultural and ornamental plants due to their leafmining activity. In addition, many of *Liriomyza* leafminers are dangerous because of their high degree of polyphagy. Smallholder farms, horticultural industries and ornamental flower producers are affected by one or more of these polyphagous leafminers (Murphy, LaSalle, 1999). *Liriomyza* leafminers can impact crops in at least six ways: (1) by vectoring a disease, (2) by destroying young seedlings, (3) by causing reductions in crop yields, (4) by accelerating leaf drop above developing tomatoes, thus causing "sunburning" of the fruit, (5) by reducing the aesthetic value of ornamental plants, (6) by causing some plant species to be quarantined (Parrella, 1987).

Important primary hosts of *L. bryoniae* include cabbages (*Brassica oleracea* var. *capitata*), cucumbers (*Cucumis sativus*), lettuces (*Lactuca sativa*), courgettes (*Cucurbita pepo*), melons (*Cucumis melo*), tomatoes (*Lycopersicon esculentum*) and watermelons (*Citrullus lanatus*) (Lee et al., 1990).

According to Lithuanian State Plant Protection Service data, in 2001–2003 the relative frequency of *Liriomyza bryoniae* occurrence was 30% in greenhouse surroundings and 19% in market places. This species attacked such plants as *Amaranthus*, *Beta*, *Bryonia*, *Cucumis*, *Datura*, *Gypsophila*, *Lycopersicon*, *Nicandra*, *Nicotiana*, *Petunia*, *Physalis*, *Sisymbrium*, *Solanum tuberosum*, *S. nigrum* and *Spinacia*. Around 56 plant genera were revealed to be suitable hosts for dipterous miners of economic importance (Ostrauskas et al., 2003, 2005). Chemical control of *Liriomyza* leafminers by means of insecticides is usually complicated due to the insect's biology, *i.e.* a short development period, small size and high mobility of adults, a relatively long-lasting pupal stage (in the soil), high reproductive capability, eggs and larvae stay within an area protected by leaf tissue, and ability to develop resistance in adults. Moreover, the insecticides are often more toxic to large parasite complexes than the leafminers themselves.

Finally, insect resistance to traditional pesticides is on the rise, and there are public fears about chemical residues in food, workers' safety, and the potential for environmental damage from pesticide sprays. Thus in 1996, the Food Quality Protection Act

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limited the use of many pesticides, particularly those that share common toxicological mechanisms such as organophosphate and carbamate insecticides, and triazine herbicides (Lyon, Newton, 1999).

Thus, it is becoming clear that alternative pest management tools are needed, which will be less hazardous to humans, non-target animals and the environment. In this context, sunlight-activated pesticides represent a possible alternative to traditional chemical compounds. The question arises how?

Sunlight-activated compounds for insect control were first explored by Barbieri (Barbieri, 1928) who used some xanthene derivatives against *Anopheles* and *Aedes* larvae. More recently Rebeiz has proposed the use of porphyrins as photopesticides (Rebeiz et al., 1987). Moreover, phloxin B, a polyhalogenated fluorescein, has been developed for commercial use as a pesticide (Ben Amor, Jori, 2000). The point is that a photoactive compound accumulates within the insect and, following exposition to visible light, induces damage of its cuticle, Malpighian tubes, midgut wall, followed by feeding inhibition and eventual death (Ben Amor et al., 1998). At the cellular level, most photosensitizers are able to induce apoptotic cell death, which by no means is more acceptable than necrotic (Lukšienë, 2003; Eggen et al., 2005).

The present study is focused on the possible usage of hematoporphyrin dimethyl ether (HPde) as a photopesticide and on the analysis of its potential photoinsecticidal activity on the polyphagous leafmining plant pest *Liriomyza bryoniae.*

MATERIALS AND METHODS

Insects. *Liriomyza bryoniae* culture was established in the laboratory on bitter-sweet *Solanum dulcamara* L. 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, which emerged from the collected larvae, were transferred into plastic oviposition cages with bottoms and tops covered with a 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 the plants were put into plastic bags for collecting puparia. Adult flies as well as larvae/puparia were kept at 22 ± 1 °C and a photoperiod of 15:9 (L:D). Light was provided by an 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 a separate glass vial. Each vial was supplied with a small piece of wet filter paper to maintain humidity. Adult flies were collected daily.

Photosensitizers. The stock solution of hematoporphyrin dimethyl ether (HPde) (the gift from Prof. G. V. Ponomarev, Russia) was prepared in physiological saline (2.5×10^{-3} M) and was stored in the dark below 10 °C.

Evaluation of the uptake of photosensitizer. Feeding of adult flies was estimated by the time the insects spent at feeding places. After emergence, adults were sexed and not allowed to feed for at least 8 h. For feeding, flies were supplied with small sponge pieces containing sucrose solution (0.2 g of sugar in 1 ml distilled water). Control insects were fed with sucrose solution and those used for testing were fed the same solution plus HPde (150 µl $2.5 \cdot 10^{-2}$ M HPde in 1 ml of the solution). Both control and test insects were fed for 15 h in the dark and 5 h under the red light illumination at 22 ± 1 °C. For evaluation of HPde toxicity, the insects were fed with sucrose solution plus HPde (concentration and feeding duration the same as mentioned above) without irradiation.

Irradiation source. The light source used for irradiation of insects consisted of a tungsten lamp (500 W), an optical system for light focusing and an optical filter for UV and infrared light elimination (370 nm $< \lambda <$ 680 nm) (Luksiene et al., 2004a). Light intensity at the position of the cells was 30 mW/cm^2. The irradiation time reached 30 min and the total irradiation dose did not exceed 54 J.

Evaluation of photopesticidal efficiency of the photosensitizer. Curves of survived objects (insects) as functions of post-treatment time (days) were calculated to evaluate the photopesticidal efficiency of the photosensitizer (detailed description in Luksiene, Vaitkuviene, 2004).

Statistical analysis. In order to obtain statistically significant data, groups of 10 insects per experiment were used. Each experiment was repeated three times. Standard error was estimated for every experimental point and marked in Figure as a bar.

RESULTS

A number of photosensitizers have been recently proposed for investigators. Nevertheless, porphyrin-type photosensitizers appear to be especially promising photoinsecticidal agents, since these compounds absorb essentially all the UV-visible wavelengths, *i.e.* these molecules can be efficiently excited by natural sunlight (Luksiene, 2003). We chose the photosensitizer HPde as a representative of dicarboxylic porphyrins, effective and much more chemically homogeneous in comparison with the clinically established agent photofrin (PII) which consists of different porphyrins (Luksiene et al., 2004 d). The chemical structure of HPde is presented in Fig. 1. Thus, in the first step we tried to find evidences of the effective accumulation of HPde by *Liriomyza bryoniae.* To this end, insects were fed with a bait containing 150 μl ($2.5 \cdot 10^{-2}$ M) of HPde in 1 ml. According to our observations, flies accumulate HPde in significant amounts if supplied with a suitable bait containing HPde. It is of importance to note that a clear difference was observed between males and females in feeding the bait containing HPde: females were ac-

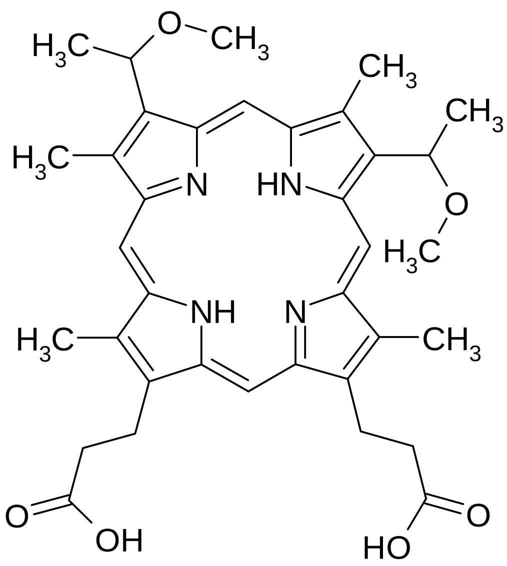

Fig. 1. Chemical structure of selected photoactive compound hematoporphyrine dimethyl ether (HPde)

cumulating HPde more intensively than males. This might be due to the body size and biological activity of females.

In general, the photosensitivity of porphyrin-loaded insects persists for about 48 h after the administration of the photosensitizer with bait (Ben Amor, Jori, 2000).

The survival of *Liriomyza bryoniae* in the control, untreated group reached 25 days. Data presented in Fig. 2 show a 9-day period only. In all cases no detectable decrease of survival was observed among flies exposed to light under identical conditions, but in the absence of porphyrin. A certain rather insignificant trend (3.5%) towards reduction in the survival of males was observed after feeding with the bait containing HPde in the absence of irradiation. In contrast, a sharp difference as compared with control (in all cases 100% mortality) was observed within one day after irradiation of male flies fed with the bait containing HPde. 50% of male died immediately following the irradiation.

Liriomyza bryoniae female susceptibility to HPde-based photosensitization is presented in Fig. 3. The survival of the control, untreated group lasted 28 days. An insignificant decrease in the survival of flies was observed after feeding them with bait containing HPde (3%). Moreover, no lethal effect was induced by irradiation of *Liriomyza bryoniae* with visible light (54J) in the absence of HPde, indicating that light

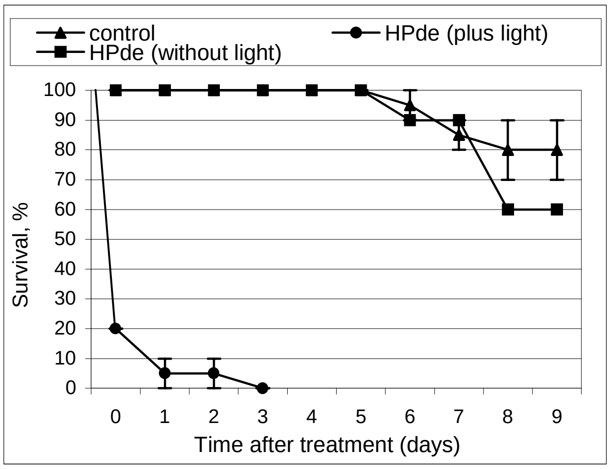

Fig. 2. Survival of male *Liriomyza bryoniae* after 30 min irradiation with broad-spectrum visible light at fluence rate 30 mW/cm^2. The insects have been exposed to a bait containing 150 μl 2.5 10^{-2} M hematoporphyrin dimethyl ether (HPde)/ml bait

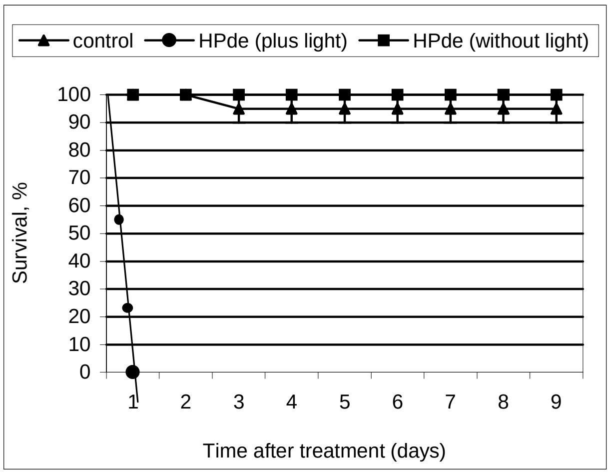

Fig. 3. Survival of female *Liriomyza bryoniae* after 30 min irradiation with broad spectrum visible light at fluence rate 30 mW/cm^2. The insects have been exposed to a bait containing 150 μl 2.5 10^{-2}M hematoporphyrin dimethyl ether (Hpde)/ml bait

alone had no effect on the survival of female as well as male flies under our experimental conditions (data not shown). However, complete and very fast (0.25 h) mortality of *Liriomyza bryoniae* females could be achieved by applying light and HPde-containing bait. Some mortality of these flies was observed already during the irradiation.

DISCUSSION

Over the last decade, several independent investigators demonstrated that near-UV- or visible light-absorbing dyes belonging to different categories of organic compounds possessed a photopesticidal effect towards a few insect species (Ben Amor, Jori, 2000). It seems reasonable to propose that any photoactive compound, added to a suitable bait to facilitate its uptake by the target insect and avoid its uptake by non-target one, has a potential for acting as an insecticide. Due to the tremendous variety of insect species, differences in the uptake of the dye, size, morphology and cuticle structure, baits of different composition might guarantee a high specificity of this new method. Sunlight-activated compounds are usually characterized by a low environmental impact and negligible toxicological risk for humans, plants or animals. Of special interest would be the photosensitizers that are already registered as food additives or phototherapeutic agents (Ben Amor et al., 1998). Consequently, these studies could open the way for the formulation of phototreatment protocols tailored to specific insects and environmental conditions.

The results described in this paper point out that plant pest *Liriomyza bryoniae* (Diptera, Agromyzidae) is sensitive to HPde-based photosensitization. The point is that treatment efficiency strongly depends on the photosensitizer used. For instance, according to our unpublished data, the phenothiazine class compound methylene blue exerts an extremely low photopesticidal activity on *Liriomyza bryoniae.* On the contrary, this compound was very effective against yellow mealworms (*Tenebrio* larvae) and cabbage butterfly (*Pieris* sp.) (Lavialle, Dumortier, 1978). This might be explained by the fact that attractiveness of food containing HPde is specific to insect species. According to our present results, flies *Liriomyza bryoniae* were readily attracted by sugar bait containing HPde. Moreover, they appeared to consume enough of the bait to realize phototoxic porphyrin action when exposed to visible light. A drastic drop in the number of surviving flies (especially female) was observed even one hour following exposure to light. HPde seems to be a very promising photopesticide against *Liriomyza bryoniae.*

Actually, in recent years, increasing attention has been focused on the photosensitizing properties of porphyrin class compounds. Several insects (*Ceratitis capitata, Bactrocera oleae, Stomoxys calcitrans, Colpoda inflata*) have been shown to be sensitive to other porphyrin-type compounds when exposed to light (Ben Amor et al., 1998; Ben Amor et al., 2000; Kassab et al., 2002). This might be related with a favorable feature of porphyrins to absorb essentially all the wavelengths in the near-UV and visible sunlight. Moreover, porphyrins are natural compounds usually devoid of any appreciable intrinsic cytotoxicity in the absence of light (Luksiene et al., 2004 c, d; Luksiene et al., 2005 a, b). In addition, the structure of porphyrins might be easily modified in accordance with special needs, it is not expensive (1–2 US $/g) and shows a high accumulation capacity in insects (Ben Amor, Jori, 2000).

The efficacy of photoactive compounds as pesticides certainly depends also on feeding intensity and ingestion of the dye by a target insect (Lavialle, Dumortier, 1978, Mangan, Moreno, 2001). In this context, we consider the presence of sexual difference in sensitivity to this treatment to be of great importance, because females are responsible both for pest infestation spread by laying eggs and for damaging the host-plants physically by penetrating leaves during feeding. In contrast, males are unable to penetrate leaves because of a different morphology of their proboscis, and for feeding they search for and use leaves already damaged by females. Of course, we must consider the performance of the HPde/bait under actual field or greenhouse conditions in order to reveal the specificity of this methodology regarding the target flies and safety for beneficial insects.

Summarizing, our data obtained show HPde to possess several positive features enough to warrant further investigations on its use as a photopesticide. Perhaps this methodology will open a new avenue for development of new generation pesticides, which would be human-safe, environmentally friendly, low-cost and not mutagenic for flies.

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References

1. Barbieri A. Sensibilizadores fluorescents como larvicidas. Action fotodynamica de la luz. *Riv Malariol.* 1928. Vol. 7. P. 456–463.
2. Ben Amor T., Trochin M., Bortolotto L., Verdiglione R., Jori G. Porphyrins and related compounds as photoactivatable insecticides. 1. Phototoxic activity of hematoporphyrin toward *Ceratitis capitata* and *Bactrocera oleae*. *Photochem. Photobiol.* 1998. Vol. 67. P. 206–211.
3. Ben Amor T., Jori G. Sunlight- activated insecticides: historical background and mechanisms of phototoxic activity. *Insect Biochem. Mol. Biol.* 2000. Vol. 30. P. 915–925.
4. Ben Amor T., Bortolotto L., Jori G. Porphyrins and related compounds as photoactivatable insecticides. 3. Laboratory and field studies. *Photochem. Photobiol.* 2000. Vol. 71. P. 124–128.
5. Eggen Furre I., Shahzidi S., Luksiene Z., Moller M. T. N., Borgen E., Morgan J., Nesland J. M., Peng Q. Specific target of peripheral benzodiazepine receptor by hexaminolevulinate-mediated photodynamic therapy induces an apoptosis-inducing factor dependent pathway of apoptosis in human leukemia Reh cells. *Cancer Res.* 2005 (*in press*).

6. Kassab K., Ben Amor T., Jori G., Coppelloti O. Photosensitization of *Colpoda inflat*a cysts by meso-substituted cationic porphyrins. *Photochem. Photobiol*. 2002. Vol. 1. P. 560–564.
7. Lavialle M., Dumortier B. Effects photodynamique du bleu de methylene sur les larves de *Pieris brassicae*. *CR Acad Sci.* 1978. Vol. 287. P. 875–878.
8. Lee H. S., Wen H. C., Lu F. M. The occurrence of *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) in Taiwan. *Chinese Journal of Entomology.* 1990. Vol. 10. P. 133–142.
9. Lyon G. D., Newton A. C. Implementation of elicitor mediated induced resistance in agriculture. In: A. A. Agrawal, S. Tuzun, and E. Bent (eds.). *Induced Plant Defenses against Pathogens and Herbivores*. 1999. APS Press, St. Paul, MN. P. 299–318.
10. Luksiene Þ. Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment. *Medicina.* 2003. Vol. 12. P. 1137–1150.
11. Luksiene Þ., Astrauskas J., Kabbara I. LED-based light source for photodynamic inactivation of leukemia cells *in vitro. SPIE*. 2004a. Vol. 5610. P. 306–311.
12. Luksiene Z., Vaitkuviene A. Hypericin-based photodynamic therapy: antitumor activity, accumulation potential and induced cell death pathway. *SPIE*. 2004b. Vol. 5610. P. 75–82.
13. Luksiene Z., Maroziene A., Kliukiene R., Firantas H., Cenas N. Effects of photosensitized and ionizing irradiation on the activities of antioxidant and phase II enzymes in EAT cells. *Biology.* 2004c. Vol. 1. P. 23–25.
14. Luksiene Z., Peciulyte D., Lugauskas A. Inactivation of fungi by photosensitization: preliminary results. *Ann. Agric. Environ. Med.* 2004d. Vol. 11. P. 215–220.
15. Luksiene Z., Peciulyte D., Jurkoniene S., Puras R. Inactivation of possible fungal food contaminants by photosensitization. *Food Technol. Biotechnol.* 2005a *(in press).*
16. Luksiene Z., Labeikyte D., Juodka B., Moan J. On the mechanism of radiosensitization by porphyrins. *J. Environ. Toxicol. Pathol. Oncol.* 2005b *(in press).*
17. Mangan R. L., Moreno D. S. Photoactive dye insecticide formulations: adjuvants increase toxicity to Mexican fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 2001. Vol. 94. P. 150–156.
18. Minkenberg O. P. J. M., van Lenteren J. C. The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. *Agricultural University Wageningen Papers*. 1986. Vol. 86(2). 50 p.
19. Murphy S. T., LaSalle J. Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. *Biocontrol. News and information*. 1999. Vol. 20(3). P. 91–104.
20. Ostrauskas H., Pakalniškis S., Taluntytë L. Dipteran leafminers in the vicinity of glasshouses and plant markets in Lithuania. *OEPP/EPPO Bulletin*. 2005. Vol. 35. P. 73–77.
21. Ostrauskas H., Pakalniðkis S., Taluntytë L. The species composition of plant mining dipterous (Insecta: Diptera) of greenhouse surroundings in Lithuania. *Ekologija*. 2003. Nr. 3. P. 3–11.
22. Parrella M. P. Biology of Liriomyza. *Annual Review of Entomology*. 1987. Vol. 32. P. 201–224.
23. Rebeiz C. A., Montazaer-Zouhour A., Mayasich J. M., Tipathy B. C., Wu S. M., Rebeiz C. C. Porphyric insecticides. In: J. M. Heitz, K. R. Downum (eds.). *Light Activated Pesticides* ACS, DC. Washington, 1987. P. 295–328.

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MATOMOS ÐVIESOS AKTYVUOTO HEMATOPORFIRINO DIMETILO ETERIO ÁTAKA AUGALØ LAPØ MINUOTOJØ *Liriomyza bryoniae* IŠGYVENAMUMUI

S a n t r a u k a

Naujø, ekologiðkai saugiø augalø apsaugos priemoniø paieðka iðlieka iki ðiol aktuali. Todël netoksiniø, nemutageniniø ir ekologiðkai neutraliø fotoaktyviø organiniø junginiø (fotosensibilizatoriø) panaudojimas augalø apsaugai galëtø bûti viena realiø iðeièiø. Fotosensibilizacijos esmæ sudaro tai, kad fotoaktyvus organinis junginys, ádëtas á masalà, geba kauptis vabzdyje, po vabzdþio apðvietimo matoma ðviesa jame indukuojamos citotoksinës reakcijos ir vabzdys þûva. Ðio darbo tikslas – nustatyti, ar augalø kenkëjai minamusës *Liriomyza bryoniae* (Diptera, Agromyzidae) yra jautrios fotosensibilizatoriams, konkreèiai – hematoporfirino dimetilo eteriui. Gauti duomenys leidþia daryti iðvadà, kad ðis fotosensibilizatorius gali bûti naudojamas kaip efektyvus fotopesticidas kovoje su augalø kenkëju *Liriomyza bryoniae.* Mûsø atlikto bandymo sàlygomis pavyko sunaikinti 100% ðiø minamusiø. Bûtina paþymëti, kad minamusiø patelës yra daug jautresnës panaudotam fotosensibilizatoriui ir þûva kur kas greièiau negu patinëliai.

Raktaþodþiai: fotosensibilizacija, matomos ðviesos aktyvacija, iðgyvenamumas, Agromyzidae, Diptera