Search for photoinsecticides: effect of hematoporphyrin dimethyl ether on leafmining pest *Liriomyza bryoniae* (Diptera: Agromyzidae)

V. Būda[1], Ž. Lukšienė[2], S. Radžiutė[1] N. Kurilčik[2] and S. Juršėnas[2]

[1]Institute of Ecology, Vilnius University, Akademijos 2, Vilnius-21, LT-08412, Lithuania; e-mail: vinbuda@ekoi.lt
[2]Institute of Materials Science and Applied research, Vilnius University, Saulėtekio 9, Vilnius, LT-10223, Lithuania; e-mail: zivile.luksiene@mtmi.vu.lt

Abstract. Photoactive compounds might be used as effective pesticide agents, with almost no impact on the environment, being nontoxic and not mutagenic. The point is, that nontoxic photoactive compound accumulates within the insect body and, following exposition to visible light, induces lethal photochemical reactions and death of target pest. The aim of this study was to determine, whether hematoporphyrin dimethyl ether (HPde) might be effective as photoinsecticide. Leafmining fly *Liriomyza bryoniae* (Diptera, Agromyzidae) was selected as the target species. Results obtained indicate, that exposition of insects to the bait, containing HPde and following irradiation with visible light resulted in total killing of *Liriomyza bryoniae* adults. Moreover, survival of insects, exposed to the bait, containing HPde and irradiated with visible light depended on sex: females were more sensitive to photosensitization compare to males. Females survived less than 1 day after the treatment and males survived up to 3 days. We assume this was predetermined by difference in feeding intensity.

Key words: photoinsecticide, survival, sexual dimorphism, fly

INTRODUCTION

Nontoxic and not mutagenic photoactive compounds may be used as effective pesticide agents, with almost no impact on the environment. The point is, that photoactive compound accumulates within the insect body and, following exposition to visible light, induces lethal photochemical reactions and death of target pest. The very first sunlight-activated compound for insect control was tested by Barbieri in 1928. Xanthene derivatives, porphyrins were suggested as potential photopesticides and phloxin B, a polyhalogenated fluorescein, has been developed for commercial usage (review in: Luksiene, 2003).

Many flies within genus *Liriomyza* (Diptera, Agromyzidae) are economically important pests, causing damage of agricultural and ornamental plants. *Liriomyza* leafminers can impact crops at least in six ways: 1) by vectoring 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. For *L. bryoniae* important primary hosts are: cabbage (*Brassica oleracea* var. *capitata*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), courgette (*Cucurbita pepo*), melon (*Cucumis melo*), tomato (*Lycopersicon esculentum*) and watermelon (*Citrullus lanatus*). In Lithuania the flies attacked: *Amaranthus, Beta, Bryonia, Cucumis, Datura, Gypsophila, Lycopersicon, Nicandra, Nicotiana, Petunia, Physalis, Sisymbrium, Solanum tuberosum, S. nigrum* and *Spinacia* (Ostrauskas et al., 2003). Chemical control of *Liriomyza* by means of traditional insecticides is complicated due to the insect's biology, i.e. 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 protected area inside the leaf tissue; and ability to develop resistance in adults. Moreover, the insecticides are often more toxic to large parasite complex comparing to the leafminers themselves.

It is clear that alternative pest management tools are needed. Sunlight-activated pesticides can be developed as alternative to traditional pesticides to control the pest.
The present study is focused on the possible application of hematoporphyrin dimethyl ether (HPde) as potential photopesticide for polyphagous leafmining pest *Liriomyza bryoniae* control**.**

MATERIALS AND METHODS

Insects. *Liriomyza bryoniae* culture was established in the laboratory on bitter-sweet (*Solanum dulcamara* L). Adult flies as well as larvae/puparia were maintained at 22 ± 1°C and photoperiod of 15 : 9 (L : D). To prevent mating after adult eclosion, each puparium was placed into separate glass vial.

Photosensitizers. The stock solution of hematoporphyrin dimethyl ether (HPde) (the gift from prof. G.V. Ponomarev, Russia) was prepared in physiological saline (2,5 x 10^{-3} M) and was stored in the dark below 10˚C.

Uptake of photosensitizer. After emergence adults were sexed and not allowed to feed at least for 8 h. For feeding the flies were supplied with sponge pieces containing sucrose solution (0.2 g of sugar in 1 ml distilled water). Control insects were fed with the sucrose solution and those used for testing were fed the same solution plus HPde (150 µl 2.5· 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.

Evaluation of HPde in the bait. Fluorescence spectra of HPde was recorded of the solution effective against *L. bryoniae* adults (150 µl HPde ml^{-1} sucrose solution in water, the latter contained 0.2 g of sucrose). Luminescence spectrometer was used with light emitting diode (394 nm), double monochromator (Jobin Yvon Model HRD-1), cooled photomultiplier (Hamamatsu R1463P) connected to a photon counting system. Measurements were performed at room temperature.

Insect irradiation source. The light source used consisted of tungsten lamp (500 W), optical system for light focusing and optical filter for UV and infrared light elimination (370 nm < λ < 680 nm) (Luksiene et al., 2004a). Light intensity at the position of the cells was 30 $mWcm^{-2}$. The irradiation time reached 30 min., total irradiation dose not exceeded 54 J.

Evaluation of toxic and photoinsecticidal efficiency. To check if there is any toxic effect of HPde, control group of insects and group fed with HPde were not irradiated.

For evaluation of photoinsecticidal efficiency insects (both control and fed with HPde) were irradiated as indicated above. Survival of the insects was recorded daily.

Statistical analysis. Every experiment was repeated 3 times. Three groups of 10 insects per experiment were used. Standard error was estimated for every experimental point and marked in figure as bar.

RESULTS

We choose photosensitizer HPde as representative of dicarboxylic porphyrins which appear to be especially promising as photoinsecticidal agents, since they absorb essentially all the UV-visible wave-lengths, i.e. can be efficiently excited by natural sunlight (Luksiene et al., 2004b). In the first step we tried to find evidence on the effective accumulation of HPde in *Liriomyza bryoniae*. For this aim, insects were fed with bait containing HPde 150 µl ($2.5 \cdot 10^{-2}$ M) in 1 ml bait. In sucrose solution HPde appeares in aggregated form, which is not fluorescenting and possess no pesticidal activity. Within the organism HPde disaggregates (becomes monomeric) and starts fluorescenting. The solution which was used for feeding of the flies HPde was checked both with admixture of triton X-100 and without it (Fig. 1). Triton X-100 cause disaggregation of HPde the same way as target organism.

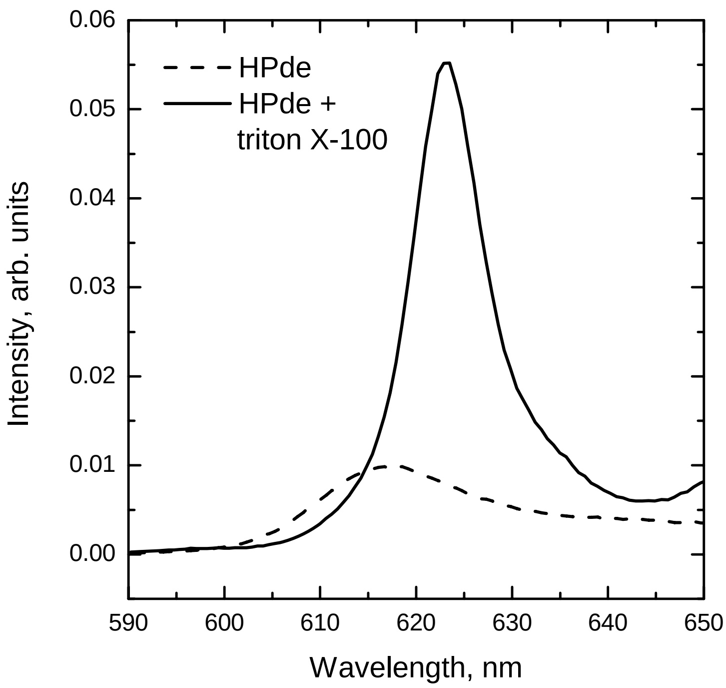

Fig. 1. Fluorescence spectra of sucrose solution bait containing HPde in nonactive aggregated form (with no triton X-100, dashed line) and in active monomeric form (with admixture of triton X-100, solid line).

Survival of *Liriomyza bryoniae* in control, not treated group reached 29 days. In Fig. 2 there are presented data for shorter period, namely for 9 days 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. Some, but not significant trend (3.5%) towards reduction in survival of males was observed after feeding with the bait containing HPde in the absence of irradiation. In contrary, sharp difference compare with control (in all cases 100% mortality) was observed within 1 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 the HPde-based photosensitization is presented in Fig. 2. The survival of control, not treated fly group lasted for 34 days (in Fig. 2 data was presented for shorter period, namely for 9 days only). No significant decrease in the survival of the 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 (54 J) in the absence of HPde, indicating, that light alone had no effect on survival of female as well as male 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.

Our feeding experiments clearly demonstrate that the flies accumulate HPde in significant amounts if supplied with suitable bait containing HPde. Of importance to note, that clear difference was obtained between males and females in accumulation of HPde while feeding: females accumulated HPde more intensively compare to males (Fig 2). This might be due to clear difference in feeding activity (much higher in females) and due to some difference in body size.

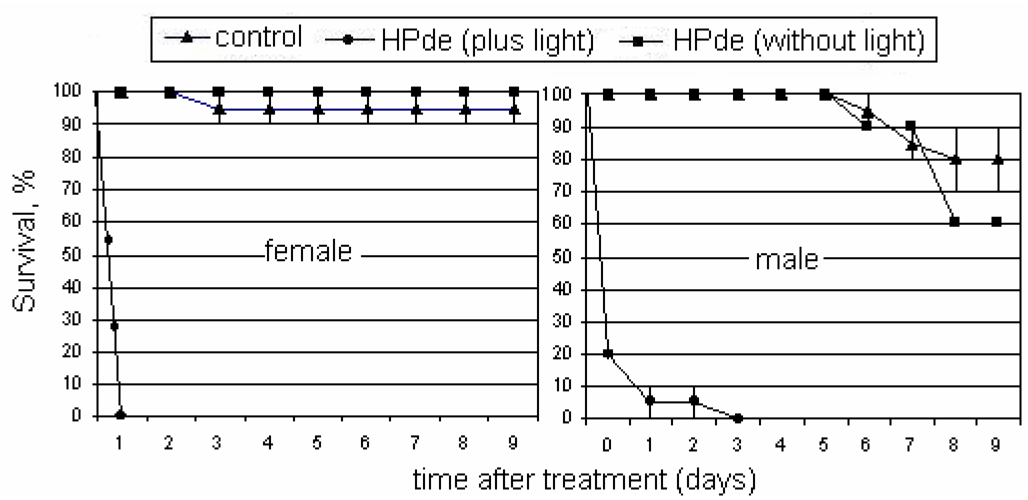

Fig 2. Survival of females and males of *Liriomyza bryoniae* after 30 min irradiation with broad-spectrum visible light at fluence rate 30 Mw·cm^{-2}. The insects have been supplied with bait containing sucrose solution with hematoporphyrin dimethyl ether (HPde) ($2.5 \cdot 10^{-2}$ M). Control insects have been exposed to the sucrose solution only.

DISCUSSION

During 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 few different insect species (Ben Amor & Jori, 2000). It seems reasonable to propose, that any photoactive compound, added in suitable bait to facilitate its uptake by the target insect and avoid its uptake by non-target one, has the potential for acting as an insecticide. Due to the tremendous variety of insect species, differential uptake of the dye, size, morphology and cuticle structure, the different composition of baits might guarantee high specificity of this new methodology. 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 those photosensitizers, which are already registered as food additives or phototherapeutic agents (Ben Amor et al., 1998). Consequently, these studies could open the way for the definition 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 photosensitizer used. For instance, according to our unpublished data, phenothiazine class compound methylene blue exerts extremely low photopesticidal activity against *Liriomyza bryoniae*. On contrary, this compound was very effective against yellow mealworms (*Tenebrio* larvae) and cabbage butterfly (*Pieris* sp.). This might be explained by the fact, that attractiveness of food, containing HPde is specific to insect species. According to the present our results, flies *Liriomyza bryoniae* were readily attracted by sugar bait, containing HPde. Moreover, they appear to consume enough of the bait to trigger phototoxic porphyrin action, when the flies were exposed to visible light. A drastic drop in the number of surviving flies (especially female) was observed even 1 hour following exposure to light. It looks like, that HPde has a good potential as a 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 sensitive to other porphyrin-type compounds when exposed to light (Ben Amor et al., 1998, 2000; Kassab et al., 2002). This might be related with porphyrins favorable feature to absorb essentially all the wavelengths including 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., 2005). In addition, the structure of porphyrins might be easily modified in accordance with special needs, it is not expensive (1–2 US$ g^{-1}) and has high accumulation capacity in insects (Ben Amor & Jori, 2000).

By no means, the efficacy of photoactive compounds as pesticides depends on feeding intensity, ingestion of the dye by the target insect as well (Mangan & Moreno, 2001). In this context, presence of sexual difference in sensitivity to this treatment we have found is of great importance because namely females are responsible both for pest infestation spread by laying their eggs and for damage of host-plants physically by penetrating leaves during feeding. In contrary, males are unable to penetrate the leaves due to different morphology of their proboscis, and for feeding they search for and use leaves already damaged by females. Experiments on HPde/bait should be carried out under natural field or greenhouse conditions in order to reveal the specificity and effectivity of this methodology to the target flies and safety for beneficial ones.

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

CONCLUSIONS

1. Hematoporphyrin dimethyl ether (HPde) is a non-toxic compound effective for pest management of *Liriomyza bryoniae* adult flies.

2. Exposition to visible light adults of *Liriomyza bryoniae*, fed with HPde causes death of the pest, i.e. HPde is effective as photoinsecticide for this species.

3. Effect on adult flies *Liriomyza bryoniae* depended on sex: HPde affected females to greater extent compare to males.

ACKNOWLEDGEMENTS. The authors are thankful Prof. G.V. Ponomariov (Russia) for providing photosensitizer as well as Dr. S. Palalniškis (Lithuania) for rearing consultations and for initial stock collection of flies for rearing. This work was supported by the Lithuanian State Science and Study Foundation, grant No T-06079.

REFERENCES

Ben Amor, T. & Jori, G. 2000. Sunlight-activated insecticides: historical background and mechanisms of phototoxic activity. *Insect Biochem. Mol. Biol.* **30**, 915–925.

Ben Amor, T., Bortolotto, L. & Jori, G. 2000. Porphyrins and related compounds as photoactivatable insecticides.3. Laboratory and field studies. *Photochem. Photobiol.* **71**, 124–128.

Ben Amor, T., Trochin, M., Bortolotto, L., Verdiglione, R. & Jori, G. 1998. Porphyrins and related compounds as photoactivatable insecticides. I. Phototoxic activity of hematoporphyrin toward *Ceratitis capitata* and *Bactrocera oleae*. *Photochem. Photobiol.* **67**, 206–211.

Kassab, K., Ben Amor, T., Jori, G. & Coppelloti, O. 2002. Photosensitization of *Colpoda inflat*a cysts by meso-substituted cationic porphyrins. *Photochem. Photobiol.* **1**, 560–564.

Luksiene, Z. 2003. Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment. *Medicina* **12**, 113–1150.

Luksiene, Z., Astrauskas, J. & Kabbara, I. 2004(a). LED-based light source for photodynamic inactivation of leukemia cells *in vitro*. *SPIE* **5610**, 306–311.

Luksiene, Z., Peciulyte, D., Jurkoniene, S. & Puras, R. 2005. Inactivation of possible fungal food contaminants by photosensitization. *Food Technol. Biotechnol.* **43**, 335–341.

Mangan, R.L. & Moreno, D.S. 2001. Photoactive dye insecticide formulations: adjuvants increase toxicity to Mexican fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* **94**, 150–156.

Murphy, S.T. & LaSalle, J. 1999. Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. *BiocontrolNews and information* **20**(3), 91–104.

Ostrauskas, H., Pakalniškis, S. & Taluntytė, L. 2003. The species composition of plant mining dipterous (Insecta: Diptera) of greenhouse surroundings in Lithuania. *Ekologija* **3**, 3–11.