Research Article

Received: 19 June 2015 Revised: 14 December 2015 Accepted article published: 18 December 2015 Published online in Wiley Online Library: 18 January 2016

(wileyonlinelibrary.com) DOI 10.1002/ps.4210

Toxicity to *Diaphania hyalinata*, selectivity to non-target species and phytotoxicity of furanones and phthalide analogues

Gabriela C Resende,[a] Elson S Alvarenga,[a]* Tamíris A de Araújo,[b] Júlia ND Campos[b] and Marcelo C Pincanço[b]

Abstract

BACKGROUND: Despite being of great importance to crop protection, the disadvantages of intensive and inappropriate use of pesticides have stimulated the search for more selective and less harmful agrochemicals. Thus, we have evaluated the effectiveness of 16 synthetic molecules (phthalides and precursors) to control the melonworm *Diaphania hyalinata*, a key pest in cucurbit crops of economic importance in Brazil. The selectivity to beneficial organisms *Solenopsis saevissima* and *Tetragonisca angustula* and the phytotoxicity to *Cucumis sativus* of the promising insecticides were also assessed.

RESULTS: In the screening assay, compounds 1 and 6 provided 91 and 88% mortality of the melonworm. Compound 1 presented higher toxicity (median lethal dose LD_{50} = 15.99 µmol g^{-1}) and higher speed on pest control (median survival time LT_{50} = 420 min) than compound 6 (LD_{50} = 44.51 µmol g^{-1} and LT_{50} = 840 min). Both compounds inhibited less than 11% of host-plant growth and caused ≤36 and ≥93% mortality of predator and pollinator respectively.

CONCLUSION: Among the tested compounds, only compounds 1 and 6 were effective in melonworm control. Both compounds presented no considerable phytotoxicity and were selective to predator but non-selective to pollinator, which enables their application for pest control if the exposure of the bees is minimised.

Supporting information may be found in the online version of this article.

Keywords: insecticide; *Solenopsis saevissima*; *Tetragonisca angustula*; *Cucumis sativus*

1 INTRODUCTION

Biotic factors such as animal pests, weeds and pathogens can result in significant losses in crop production.[1] According to Oliveira,[2] about 25 million t of food, fibre and biofuels is lost in the field (preharvest) owing to insect attack on major crops in Brazil, which corresponds to an annual loss of approximately $US 14.7 billion. To avoid these losses, Brazil's spending on insecticides reached $US 2.9 billion in 2011.[2] In the same year, Brazilian vegetable production hit 19.6 million t. Additionally, in 2012, Brazil was the fourth largest world producer of watermelon (*Citrullus lanatus*) and the ninth largest world producer of melon (*Cucumis melo*), which were the main horticultural products exported in 2013.[3]

The melonworm, *Diaphania hyalinata* (Lepidoptera: Crambidae), is an insect of economic importance in crops of watermelon, melon, cucumber (*Cucumis sativus*), pumpkin (*Cucurbita moschata)* and other vegetables of Cucurbitaceae that occurs mainly in South and Central America.[4] *D. hyalinata* larvae feed mainly on leaves, causing indirect damage by reducing the photosynthetic area of the host plant. By feeding on fruit, the larvae create galleries on the pulp that can cause rotting and make the vegetables unusable for consumption (direct damage).[5] McSorley and Waddill[6] verified that attack of the melonworm on yellow squash (*Cucurbita pepo* L.) caused losses of 9% owing to consumption of the fruit and losses of 23% on fruit weight owing to consumption of shoot.[6] Recently, Mohamed and coworkers[7] investigated the level of damage caused by *D. hyalinata* in six species of cucurbits, the greatest damage observed being 24.9% on snake melon leaves (*C. melo* var. *flexuous*) and 46.6% on sweet melon fruits (*C. melo* var. *cantaloupnesis*).[7]

Chemical control has been used as the main method for controlling *D. hyalinata* on vegetable crops. Although commercial insecticides such as oxadiazines, spinosyns and diamides are allowed in some Brazilian cultures,[8] the lack or the limited number of products registered for vegetable protection exposes these crops to irregular use of pesticides.[9] Moreover, the inappropriate and intensive use of pesticides results in damage to the environment, pest resistance to pesticides and lethal or sublethal effects on non-target organisms.[10–13]

* *Correspondence to: ES Alvarenga, Departamento de Química, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, 36570–900, Viçosa, MG, Brazil. E-mail: elson@ufv.br*

a *Departamento de Química, Universidade Federal de Viçosa, Viçosa, MG, Brazil*

b *Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG, Brazil*

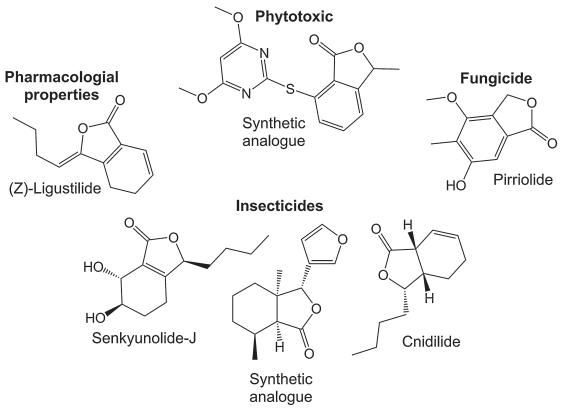

Figure 1. Bioactive benzofuran-1(3*H*)-ones.

The presence of beneficial organisms as natural enemies and pollinators in agricultural ecosystems is essential for integrated pest management. Ants of the genus *Solenopsis*, including *S. saevissima* (Hymenoptera: Formicidae), are predators of phytophagous arthropods and play an important role in biological control of populations of insect pests in these ecosystems.[14,15] Bees are of great importance to agricultural crops in terms of plant reproduction and therefore the formation of fruits and seeds.[16]

Despite showing great potential for use in agriculture, insecticides can interfere in plant germination and development.[17,18] A simple and accessible method for evaluating the phytotoxicity of these pesticides comprises cucumber seed germination and root/shoot elongation tests,[19] cucumber being among the most recommended plants for this kind of bioassay[20–22] and also a host plant of *D. hyalinata*.

In attempts to reduce the negative effects of insecticides, the search for more efficient, more selective and less persistent active compounds was encouraged. In addition to their use as biopesticides, natural products have been used as models for the development of synthetic pesticides.[23,24] In this context, phthalides and derivatives have shown great potential.

Isobenzofuran-1(3*H*)-ones, or phthalides, are members of a group of secondary metabolites comprising a benzene ring fused to a γ-lactone. Until 2005, approximately 180 naturally occurring phthalides had been isolated, most of them from the Apiaceae family.[25,26] Compounds containing γ-lactone, particularly naturally occurring phthalides and their synthetic analogues, exhibit not only a variety of pharmacological[27] but also phytotoxic,[28] fungicidal[29] and insecticidal activities.[30–33] Some of these molecules are presented in Fig. 1.

Considering the susceptibility of cucurbit vegetables to pest attack, the need for new insecticide chemicals and the promising results of our previous experiments,[33] we have been encouraged to synthesise novel isobenzofuran-1(3*H*)-one analogues and evaluate their insecticidal activity against the key pest *D. hyalinata*. The selectivity of these compounds in favour of pollinator *T. angustula* and predator *S. saevissima* and their phytotoxicity to *C. sativus* have been evaluated.

2 MATERIALS AND METHODS

2.1 Chemicals

2.1.1 General

Chemical structures of the evaluated compounds can be found in supporting information Fig. S1. The most active compounds **1** and **6** are illustrated in Fig. 2.

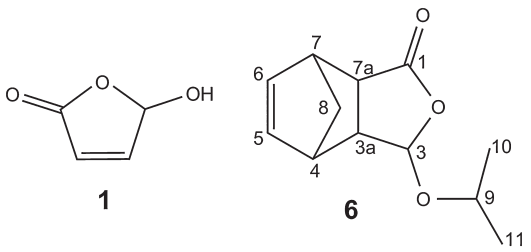

Figure 2. Structures of the most active compounds.

The progress of the reactions was monitored by visualising thin-layer chromatography (TLC) plates in an ultraviolet chamber with a lamp irradiating at 254 nm.[34] All compounds were purified by column chromatography on silica gel (60–230 mesh). Melting points were obtained on an MQAPF-301 melting point apparatus and were not corrected. Infrared (IR) spectra were acquired using a Varian 660-IR spectrometer (equipped with GladiATR), using the thin-film solid method. Nuclear magnetic resonance (NMR) experiments were recorded on a Varian Mercury 300 spectrometer with $CDCl_3$ as solvent. The ^1H NMR chemical shifts were reported using the signal from residual $CHCl_3$ as reference ($\delta = 7.27$ ppm). ^{13}C NMR chemical shifts were reported using the signal from $CDCl_3$ as reference ($\delta = 77.0$ ppm). Electron impact (70 eV) mass spectra were recorded using a Shimadzu GC-MS-QP5050A. 5-Hydroxyfuran-2(5*H*)-one **1** was prepared as previously described.[35]

2.1.2 (±)-(3β,3aβ,4β,7β,7aβ)-3-Isopropoxy-3a,4,7,7a-tetrahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**6**)

5-Isopropyloxyfuran-2-(5*H*)-one **3**[35] (507.9 mg, 3.58 mmol) and freshly distilled cyclopentadiene (1.5 mL, 18.38 mmol) were added to a round-bottom flask (25 mL). The reactants were stirred at room temperature for 96 h, and the excess cyclopentadiene was removed under reduced pressure. The crude material was purified by flash chromatography via silica gel (hexane/ethyl acetate 5:1) to afford adduct **6** as a white solid (mp 63.2–65.0 °C) in 83% yield. IR (thin-film solid, cm^{-1}): 3062, 2974, 2938, 2873, 1763, 1466, 1335, 1215, 1171, 1113, 1083, 938, 876, 829, 722, 637, 593. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.17 (d, 3H, H11); 1.19 (d, 3H, H10); 1.42 (dm, 1H, H8′); 1.61 (dt, 1H, H8); 2.89 (ddd, 1H, H3a); 3.14–3.21 (m, 1H, H4); 3.27–3.33 (m, 1H, H7); 3.33 (dd, 1H, H7a); 3.90 (sept, 1H, H9); 4.97 (d, 1H, H3); 6.19 (dd, 1H, H5); 6.24 (dd, 1H, H6).^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 21.5 (C11); 23.3 (C10); 44.7 (C4); 45.6 (C7); 47.8 (C7a); 48.5 (C3a); 51.8 (C8); 71.5 (C9); 104.1 (C3); 134.3 (C5); 136.3 (C6); 177.6 (C1). MS (EI, 70 eV) *m/z* (%): 208 (0.28, $C_{12}H_{16}O_3$), 166 (15), 149 (25), 148 (17), 122 (24), 121 (19), 101 (16), 93 (23), 91 (40), 83 (33), 66 (100), 65 (19), 55 (18), 43 (36), 41 (33), 40 (13), 39 (38).

2.2 Insecticidal bioassays

2.2.1 General procedures

Second-instar larvae of *D. hyalinata* were obtained from a laboratory rearing located in the Departamento de Biologia Animal of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. Adults of *S. saevissima* and *T. angustula* were collected from nests located on the University campus.

All doses were prepared in µmol compound g^{-1} insect (µmol g^{-1}). The average weight of each insect was estimated by measuring the mass of ten insects on an analytical balance. Bioassays were conducted by topical application. Each solution

was prepared in acetone and applied (0.5 µL) on the abdominal tergum of each individual insect with the aid of a Hamilton microsyringe (10 µL). For negative control, the insects were treated with an equal volume of acetone. As positive control, commercial insecticide chlorantraniliprole was employed at the recommended field dosage (0.015 mg active ingredient mL^{-1}).

After application, the insects were supplied with the appropriate food as follows: discs of chayote leaf to *D. hyalinata* and small pieces of cotton moistened with pure water and honey mixture (honey and water in a proportion of 1:1) placed in plastic containers (1.5 cm diameter × 1.0 cm height) to *S. saevissima* and *T. angustula*.

In all bioassays, mortality included dead individuals as well as those without movement.

2.2.2 *Screening bioassay*

The screening bioassay was performed with a dose of 107 µmol g^{-1} to assess the efficacy of compounds to *D. hyalinata*. The experimental design was completely randomised, with six replicates per treatment. Each experimental unit consisted of ten insects of *D. hyalinata* kept on a glass petri dish (9 cm diameter × 2 cm height) covered with organza.

Mortality was evaluated after 48 h of exposure to treatments, and only values equal to or greater than 80% were considered to be satisfactory.[36] Mortality data were subjected to analysis of variance,[37] and the averages were compared by the Scott–Knott grouping analysis test ($P < 0.05$).

2.2.3 *Dose–mortality*

The most active compounds were subjected to toxicity bioassays against *D. hyalinata*. The experimental design was completely randomised, with six replicates. Each experimental unit consisted of ten insects kept on a glass petri dish (9 cm diameter × 2 cm height) covered with organza. The dose–mortality curves for compounds **1** and **6** were constructed with six and five doses respectively. For the commercial insecticide chlorantraniliprole, six doses were used. These doses were established through preliminary bioassays with four concentrations for each compound to identify the concentration range leading to mortality greater than zero and less than 100%. Mortality was evaluated after 48 h of treatment according to previous analysis.

Dose–mortality data were corrected by Abbott's method[38] and then subjected to probit analysis[39] using the PROC PROBIT procedure in SAS[37] to estimate dose–mortality curves. The curves that presented probabilities greater than 0.05 by the χ^2 test[40] were accepted. The lethal doses that caused 50 and 90% mortality (LD_{50} and LD_{90}) were also estimated.

2.2.4 *Time–mortality*

D. hyalinata was subjected to time–mortality bioassays when exposed to LD_{90} of the most active compounds. The experimental design was completely randomised, with 12 replicates. Each experimental unit consisted of ten insects kept on a glass petri dish (9 cm diameter × 2 cm height) covered with organza. Larval mortality was recorded after 48 h. The intervals between assessments for each treatment (ranging from 30 min to 3 h) were determined previously.

Time–mortality data were subjected to survival analysis ($P < 0.05$) with a non-parametric Kaplan–Meier estimator[41] using the LIFETEST procedure.[37] The survival curves constructed were compared by log-rank test ($P < 0.05$), and the median survival times (LT_{50}) of the larvae were estimated.

2.2.5 *Selectivity*

S. saevissima and *T. angustula* were exposed to LD_{90} of the most active compounds against *D. hyalinata* in order to evaluate their selectivity. The experimental design was completely randomised, with six replicates. Each experimental unit consisted of ten insects of *S. saevissima* and *T. angustula* kept on a glass petri dish (9 cm diameter × 2 cm height) covered with organza. Insect mortality was recorded after 48 h of treatment.

To evaluate selectivity, mortality of non-target species was compared with pest mortality by Student's *t*-test for independent samples ($P < 0.05$).

2.3 Phytotoxicity bioassays

The phytogrowth activities of the insecticide compounds were evaluated at 250, 125 and 50 µM on seeds of cucumber (*C. sativus*), with three replicates of each concentration in a completely randomised design. Each experimental unit consisted of 20 seeds kept on a petri dish (9 cm diameter × 2 cm height) containing 5 mL of test solution. The petri dishes were sealed with polyvinyl chloride (PVC) film and stored in the dark at 25 °C for 5 days. An appropriate amount of compound was weighed, dissolved in dimethyl sulphoxide (DMSO) and diluted with distilled water to prepare 30 mL of an aqueous solution containing DMSO 0.3% (v/v). One-half of this solution was used in bioassays, and the other half was diluted with aqueous DMSO 0.3% (v/v) to prepare a less concentrated solution. Seeds were treated with 5 mL of aqueous DMSO 0.3% (v/v) for negative control and with the pre-emergence commercial herbicide *S*-metolachlor (Dual™) for positive control. After the germination period, seeds were digitally photographed and measured.

Phytogrowth data were presented as percentage differences from the control in a bar graph with standard deviation error bars. Thus, zero represents the control, positive values represent growth stimulation and negative values represent growth inhibition.

3 RESULTS

3.1 Toxicity to the insect pest *D. hyalinata*

Significant differences in the mortality data of *D. hyalinata* ($F_{17,90} = 65.596$; $P = 0.000$) were observed after 48 h of exposure to the treatments, and the evaluated compounds could be separated into five main groups (Fig. 3). The most active compounds **1** and **6** provided 91 and 88% mortality respectively, being statistically equivalent to chlorantraniliprole (Cap) at the recommended field dose. Compound **2** was slightly active, causing 60% mortality. In turn, compound **9** caused just 26% mortality. Finally, mortality afforded by compounds **3**, **5**, **7**, **10**, **11** and **16** ranged from 7 to 13%, and compounds **4**, **8** and **12** to **15** were as active as the negative control (0–4%).

The most active compounds (**1** and **6**) were subjected to further analysis to find the response of the insects by varying their dose. The resulting dose–mortality curves show low χ^2 values (<5.94) and high *P* values (>0.11), indicating data adequacy to the PROBIT model used to estimate the mortality curves (Fig. 4 and Table 1). The doses required to kill 50% (LD_{50}) and 90% (LD_{90}) of the larvae were also estimated. Compound **1** ($LD_{50} = 15.99$ µmol g^{-1}) is almost 3 times more toxic than compound **6** ($LD_{50} = 44.51$ µmol g^{-1}) and exhibits the lowest slope (1.92) of the dose–mortality curves (Table 1). Under the same conditions, the LD_{50} and LD_{90} values found for chlorantraniliprole were 0.176 and 47.998 nmol g^{-1} respectively.

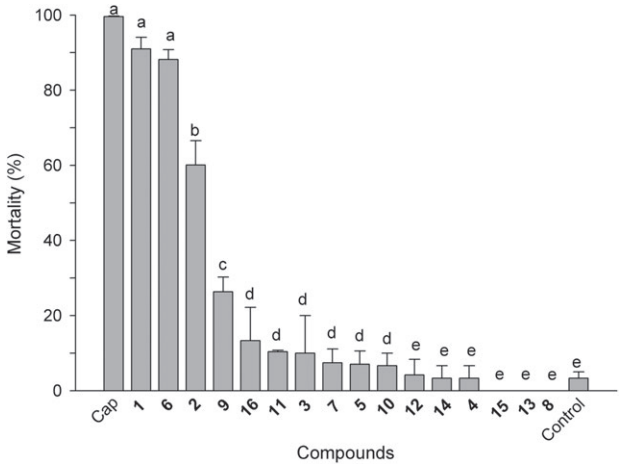

Figure 3. Mortality (mean ± standard error) of second-instar larvae of *Diaphania hyalinata* after 48 h of exposure to the compounds tested (**1** to **16**) at 107.5 μmol g^{-1}. Histograms followed by the same letter show no significant differences by the Scott–Knott test at $P < 0.05$. Control = acetone; Cap = chlorantraniliprole.

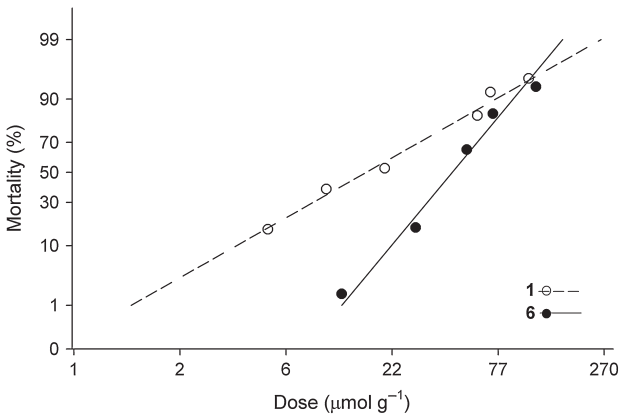

Figure 4. Dose–mortality curves of compounds **1** and **6** for second-instar larvae of *Diaphania hyalinata*.

Survival analysis of *D. hyalinata* exposed to the control (acetone) and to more active compounds (**1** and **6**) indicated significant differences among treatments (log-rank test: $\chi^2 = 90.591$; df = 2; $P < 0.001$). The survival of *D. hyalinata* was 95% in the control after 2600 min of exposure, while compounds **1** and **6** led to 80% mortality in the same time (Fig. 5). Despite this, the median survival time (LT_{50}) observed for compound **6** (840 min) is twice the observed time for compound **1** (420 min).

3.2 Selectivity to non-target organisms

The mortality of S. saevissima exposed to compounds 1 and 6 was 26 and 36% respectively. These values are lower than the values observed for D. hyalinata (80 and 83% for compounds 1 and 6 respectively) and are also significantly different, indicating that compound 1 ($t_{10} = 12.28$; $P < 0.001$) and compound 6 ($t_{10} = 7.68$; $P < 0.001$) are selective in favour of the predator (Fig. 6). On the other hand, compounds **1** and **6** are non-selective to *T. angustula*, as pollinator mortality was 100 and 93% respectively (Fig. 6).

3.3 Phytotoxicity

As expected, *S*-metolachlor (Dual™) was the most efficient against root development at all evaluated concentrations, providing inhibition of 70, 70 and 65% of the cucumber radicle at concentrations of 250, 125 and 50 μM respectively (Fig. 7). In the aerial parts, the commercial herbicide provided inhibitions of 25, 24 and 7% at 250, 125 and 50 μM respectively. On the other hand, compounds **1** and **6** afforded a maximum of 10% inhibition at all concentrations.

Compound **1** inhibited growth of shoots at 125 μM (7.3%) and 50 μM (9.1%) but stimulated the growth at 250 μM (2.3%). In the roots, this compound stimulated a small growth at all concentrations (1.7–2.5%). In turn, compound **6** inhibited the root (3.8–10%) and stimulated the shoot (0.2– 6.2%) at all concentrations.

4 DISCUSSION

Although compound **2** caused reasonable mortality (60%) during screening bioassays, only values of 90 ± 10% are considered to be satisfactory according to the recommendation of the Brazilian Health Surveillance Agency for tests of efficacy on pest control products.[36] The high mortality values provided by compound **1** (93%) and compound **6** (88%) make them lead compounds for controlling the insect pest *D. hyalinata*.

The slope of the dose–mortality curve generated for compound **6** (4.10) was higher than that observed for compound **1** (1.92), indicating a more homogeneous response of the *D. hyalinata* population exposed to compound **6**.[42] This means that a small variation in the dose of compound **6** promotes wide variations in pest mortality, increasing the risk of failures in the control. Beyond that, compound **1** was more toxic than compound **6** (smaller LD_{50}), so the cost of pest control employing compound **1** tends to be lower. Both compounds showed fast-acting control (less than 24 h) in the survival analysis, a desirable quality for chemical control of large infestations.

An insecticide can be physiologically or ecologically selective.[43] It is physiologically selective if the insecticide is more toxic to the pest than to a non-target organism, depending on physiological factors such as penetration, sequestration, excretion and detoxification and the target site of these pesticides in each organism. Ecological selectivity is characterised by minimisation of the contact between the insecticide and non-target organism. Risk assessment to beneficial species was the same for both compounds: physiologically selective in favour of the predator *S. saevissima* and non-selective to the pollinator *T. angustula*. Once bees are common pollinators of cucurbits,[44–46] treatment with

Table 1. Results of probit analysis on mortality of *Diaphania hyalinata* after 48 h of exposure to compounds **1** and **6**[a]

Compound	*y*	χ^2	df	*P*	LD_{50} (μmol g^{-1})	LD_{90} (μmol g^{-1})
1	2.68 + 1.92*x*	2.91	4	0.57	15.99 (13.69–18.49)	73.94 (60.55–94.93)
6	−3.06 + 4.10*x*	5.94	3	0.11	44.51 (40.86–48.26)	89.98 (81.82–105.21)

[a] *y* = curve equation; χ^2 = chi-square test; df = degrees of freedom; *P* = probability; LD = lethal dose with 95% fiducial limits.

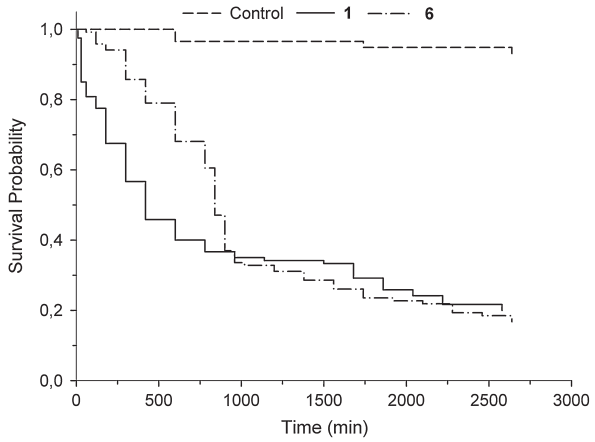

Figure 5. Survival curves of second-instar larvae of *Diaphania hyalinata* exposed to LD_{90} of compounds **1** and **6**. Control = acetone.

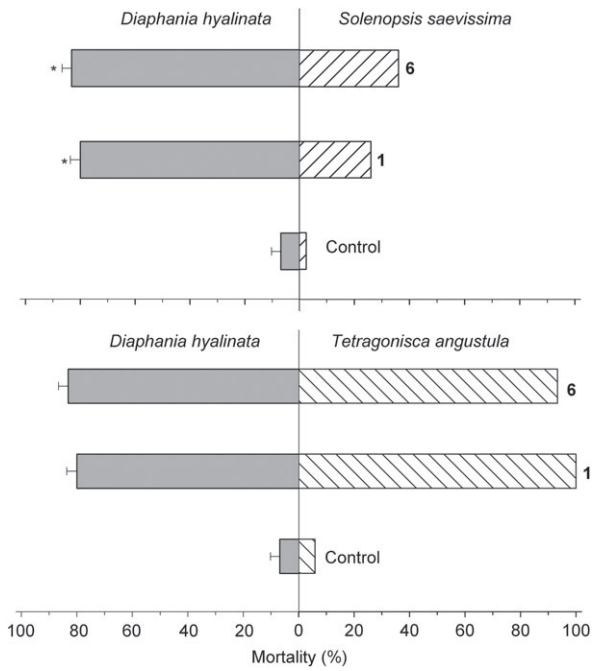

Figure 6. Mortality (mean ± standard error) of the insect pest *Diaphania hyalinata* and the non-target organisms *Solenopsis saevissima* and *Tetragonisca angustula* after 48 h of exposure to LD_{90} of compounds **1** and **6**. * These treatments caused higher mortality to the pest than to non-target organisms according to the *t*-test ($P < 0.05$). Control = acetone.

insecticides can be performed provided that such insects are not active at the time of application.

Lastly, both compounds have presented no considerable phytotoxicity to *C. sativus*, even in concentrations higher than their LD_{90} estimated for *D. hyalinata*, which indicates that they can be used in cucurbit crops.

The toxicity level of an insecticide depends on its physicochemical properties and factors such as rate of penetration, mechanism of action, decomposition and excretion.[47,48] Owing to affinity, non-polar molecules tend to penetrate into the insect's lipophilic cuticle at higher rates,[48] but, for this work, the polarity cannot be regarded as decisive for the activity of compounds, as butenolide **1** is more polar and more active than phthalide **6**. Although any generalisation based on the functionality and

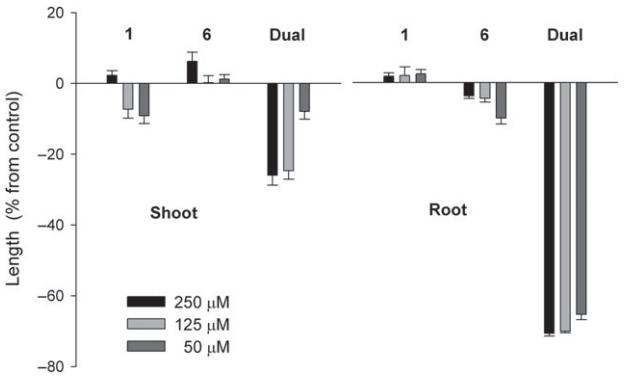

Figure 7. Growth effects (mean ± standard deviation) of compounds **1** and **6** on *Cucumis sativus*. Values are expressed as percentage difference from control. Dual = *S*-metolachlor.

skeletal type of these compounds is difficult to make, the highest activity of molecule **1** can be related to the presence of the double bond conjugated with the carbonyl group, as the unsaturated lactone is an electrophilic centre of biological targets such as enzymes.[49,50] Flupyradifurone is the only butenolide insecticide ever registered.[51] It acts reversibly as an agonist on insect nicotinic acetylcholine receptors (nAChRs) and has a pharmacophore system containing a nitrogen atom attached to the double bond, similarly to other nAChR agonists and in contrast to compound **1**.

5 CONCLUSIONS

We have evaluated the efficacy of 13 synthetic phthalides and three precursors to control the melonworm, *D. hyalinata*, and assessed the toxicity of the most active compounds to the predator *S. saevissima*, the pollinator *T. angustula* and the host plant *C. sativus*. The results have indicated that butenolide **1** and phthalide **6** are effective in controlling the melonworm, especially when quickness is required. The compounds also exhibited low toxicity to the predator and to the host-plant seeds, which are important attributes when considering potential agricultural defences for pest control. However, exposure of the bees to these compounds should be avoided.

ACKNOWLEDGEMENTS

We are grateful to the Brazilian Agencies CAPES, FAPEMIG and CNPq for financial support. We would also like to thank Anna Weichert for proofreading the manuscript.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Oerke EC, Crop losses to pests. *J Agric Sci* **144**:31–43 (2006).
2 Oliveira CM, Auad AM, Mendes SM and Frizzas MR, Crop losses and the economic impact of insect pests on Brazilian agriculture. *Crop Prot* **56**:50–54 (2014).
3 Reetz ER, Kist BB, Santos CE, Carvalho C and Drum M, *Anuário Brasileiro de Hortaliças*. Gazeta Santa Cruz, Santa Cruz do Sul, RS, Brazil (2014).
4 Capinera JL, Melonworm, Diaphania hyalinata Linnaeus (Lepidoptera: Pyralidae). in *Encyclopedia of Entomology*, ed. by Capinera JL. Springer, Dordrecht, The Netherlands, pp. 2337–2339 (2008).

5 Guimarães JA, Filho MM and Liz RS, Manejo de pragas em campos de produção de hortaliças in *Embrapa Hortaliças*. Technical Circular 94, Embrapa, Brasília, Brazil, p. 12 (2011).
6 McSorley R and Waddill VH, Partitioning yield loss on yellow squash into nematode and insect components. *J Nematol* **14**:110–118 (1982).
7 Mohamed MAM, Mohamed HZE and Suliman AIA, Host preference of the melonworm, *Diaphania hyalinata* L. (Lepidoptera: Pyralidae), on cucurbits in Gezira state, Sudan. *Persian Gulf Crop Prot* **2**:55–63 (2013).
8 *Monografias Autorizadas de Agrotóxicos*. [Online]. Agência Nacional de Vigilância Sanitária (2015). Available: http://s.anvisa.gov.br/wps/s/r/i [18 June 2015].
9 Cruz D, As hortaliças e o registro de agrotóxicos. *Hortic Bras* **31**, DOI: 10.1590/S0102-05362013000200028 (2013).
10 Aktar MW, Sengupta D and Chowdhury A, Impact of pesticides use in agriculture: their benefits and hazards. *Interdisc Toxicol* **2**:1–12 (2009).
11 Chagnon M, Kreutzweiser D, Mitchell EAD, Morrissey CA, Noome DA and Sluijs JPV, Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res* **22**:119–13429 (2015).
12 Desneux N, Decourtye A and Delpuech JM, The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* **52**:81–106 (2007).
13 Pimentel D, Environmental and economic costs of the application of pesticides primarily in the United States. *Environ Dev Sustain* **7**:229–252 (2005).
14 Way MJ and Khoo KC, Role of ants in pest management. *Annu Rev Entomol* **37**:479–503 (1992).
15 Queiroz JM, Almeida FS and Pereira MPS, Conservação da biodiversidade e o papel das formigas (Hymenoptera: Formicidae) em agroecossistemas. *Floresta Ambiente* **13**:37–45 (2006).
16 Giannini TC, Boff S, Cordeiro GD, Cartolano EA, Jr, Veiga AK, Imperatriz-Fonseca VL *et al.*, Crop pollinators in Brazil: a review of reported interactions. *Apidologie* **46**:209–223 (2015).
17 Lichtenstein EP, Millington WF and Cowley GT, Effect of various insecticides on growth and respiration of plants. *J Agric Food Chem* **18**:251–256 (1963).
18 Tavares WS, Cruz I, Petacci F, Freitas SS, Serrão JE and Zanuncio JC, Insecticide activity of piperine: toxicity to eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Pyralidae) and phytotoxicity on several vegetables. *J Med Plant Res* **5**:5301–5306 (2011).
19 Wang X, Sun CS, Gao S, Wang L and Shuokui H, Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. *Chemosphere* **44**:1711–1721 (2001).
20 Seed germination/root elongation toxicity tests EC12. Office of Toxic Substances, US Environmental Protection Agency, Washington, DC (1982).
21 Terrestrial plants: growth test. OECD guidelines for testing of chemicals, document 208. Organisation for Economic Cooperation and Development, Paris, France (1984).
22 Seed germination and root elongation, environmental assessment technical assistance, document 406. US Food and Drug Administration, Washington, DC (1987).
23 Cantrell CL, Dayan FE and Duke SO, Natural products as sources for new pesticides. *J Nat Prod* **75**:1231–1242 (2012).
24 Gerwick BC and Sparks TC, Natural products for pest control: an analysis of their role, value and future. *Pest Manag Sci* **70**:1169–1185 (2014).
25 Lin G, Chan SSK, Chuang HS and Li SG, Chemistry and biological activities of naturally occurring phthalides, in *Studies in Natural Products Chemistry, Vol.* **32**, ed. by Rahman A. Elsevier, London, UK, pp. 611–669 (2005).
26 Beck JJ and Chou SC, The structural diversity of phthalides from the Apiaceae. *J Nat Prod* **70**:891–900 (2007).
27 Chen XP, Li W, Xiao XF, Zhang LL and Liu CX, Phytochemical and pharmacological studies on radix *Angelica sinensis*. *Chin J Nat Med* **11**:0577–0587 (2013).
28 Lüthy C, Zondler H, Rapold T, Seifert G, Urwyler B, Heinis T *et al.*, 7-(4,6-Dimethoxypyrimidinyl)oxy- and -thiophthalides as novel herbicides: Part 1. CGA 279 233: a new grass-killer for rice. *Pest Manag Sci* **57**:205–224 (2001).
29 Yang XL, Zhang S, Hu QB, Luo DQ and Zhang Y, Phthalide derivatives with antifungal activities against the plant pathogens isolated from the liquid culture of *Pestalotiopsis photiniae*. *J Antibiot* **64**:723–727 (2011).
30 Momin RA and Nair MG, Mosquitocidal, nematicidal, and antifungal compounds from *Apium graveolens* L. seeds. *J Agric Food Chem* **49**:142–145 (2001).
31 Tsukamoto T, Ishikawa Y and Miyazawa M, Larvicidal and adulticidal activity of alkylphthalide derivatives from rhizome of *Cnidium officinale* against *Drosophila melanogaster*. *J Agric Food Chem* **53**:5549–5553 (2005).
32 Guo Y, Qu H, Zhi X, Yu X, Yang C and Xu H, Synthesis and insecticidal activity of some novel fraxinellone derivatives modified in the B ring. *J Agric Food Chem* **61**:11937–11944 (2013).
33 Alvarenga ES, Teixeira MG, Pimentel MF and Picanço MC, Formulação e uso de ftalidas com atividade inseticida. Brazilian Patent 10 2014 031753 8 (2014).
34 Alvarenga ES, Saliba WA and Milagres BG, Montagem de câmara com lâmpada de ultravioleta de baixo custo. *Quim Nova* **28**:927–928 (2005).
35 Resende GC, Alvarenga ES, Galindo JCG and Macias FA, Synthesis and phytotoxicity of 4,5-functionalized tetrahydrofuran-2-one. *J Braz Chem Soc* **23**:2266–2270 (2012).
36 *Manual de Protocolo para Testes de Eficácia de Produtos Desinfestantes*. Agência Nacional de Vigilância Sanitária, Brasília, Brazil (2004).
37 *SAS/STAT User's Manual, Version 9.4*. SAS Institute, Cary, NC (2013).
38 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
39 Finney DJ, *Probit Analysis*. Cambridge University Press, Cambridge/London, UK, 333 pp. (1971).
40 Young LJ and Young JH, Goodness-of-fit tests. in *Statistical Ecology: a Population Perspective*. Kluwer Academic Publishers, Boston, MA, pp. 42–70 (1998).
41 Kaplan EL and Meier P, Nonparametric estimation from incomplete observations. *J Am Statist Ass* **53**:457–481 (1958).
42 Brown CC, The statistical analysis of dose–effect relationships, in *Principles of Ecotoxicology SCOPE12*, ed. by Butler GC. John Wiley & Sons, Chichester, UK, pp. 115–148 (1978).
43 Wright DJ and Verkerk RHJ, Integration of chemical and biological control systems for arthropods: evaluation in a multitrophic context. *Pestic Sci* **44**:207–218 (1995).
44 Fronk WD and Slater JA, Insect fauna of cucurbit flowers. *J Kans Entomol Soc* **29**:141–145 (1956).
45 Meléndez-Ramirez V, Magaña-Rueda S, Parra-Tabla V, Ayala R and Navarro J, Diversity of native bee visitors of cucurbit crops (Cucurbitaceae) in Yucatán, México. *J Insect Conserv* **6**:135–147 (2002).
46 Souza FF and Malerbo-Souza DT, Entomofauna visitante e produção de frutos em melancia (*Citrullus lanatus* Thunb.) – Cucurbitaceae. *Acta Sci Agron* **27**:449–454 (2005).
47 Wilkinson CF, *Insecticide Biochemistry and Physiology*. Plenum Press, New York, NY (1976).
48 Gerolt P, Insecticides: their route of entry, mechanism of transport and mode of action. *Biol Rev* **58**:233–274 (1983).
49 Cavallito CJ and Haskel TH, The mechanism of action of antibiotics. The reaction of unsaturated lactones with cysteine and related compounds. *J Am Chem Soc* **67**:1991–1994 (1945).
50 Schmidt TJ, Structure–activity relationships of sesquiterpene lactones, in *Studies in Natural Products Chemistry, Vol.* **33**, ed. by Rahman A. Elsevier, London, UK, pp. 309–392 (2006).
51 Nauen R, Jeschke P, Velten R, Beck ME, Kintscher EU, Thielert W *et al.*, Flupyradifurone: a brief profile of a new butenolide insecticide. *Pest Manag Sci* **71**:850–862 (2015).

