Toxicity of Four Dibenzoylhydrazine Correlates with Evagination–Induction in the Cotton Leafworm

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Treatment of last-instars of the cotton leafworm, Spodoptera littoralis, with four dibenzoylhydrazines, RH-5849, tebufenozide (RH-5992), halofenozide (RH-0345), and methoxyfenozide (RH-2485), revealed premature molting leading to death. Methoxyfenozide was the most toxic, followed by tebufenozide, halofenozide, and RH-5849. The potency of the four ecdysone agonists to induce evagination in cultured imaginal discs excised from last-instars was measured and compared with the natural insect molting hormone, 20-hydroxyecdysone (20E). In parallel, competition percentages for binding were analyzed with whole imaginal wing discs cultured with 3H-labeled ponasterone A and different concentrations of ecdysone agonist. We found that the four compounds tested caused the effect as agonists of 20E in vitro. The order of toxicity of the four ecdysone agonists corresponded with that for evagination–induction and binding competition with whole imaginal discs.

Key Words: ecdysone agonist; methoxyfenozide; tebufenozide; halofenozide; RH-5849; Spodoptera littoralis; larvicidal insecticide; imaginal disc; evagination; binding.

INTRODUCTION

In insects, the presence of a rigid exoskeleton requires growth through molting, which is a process regulated by a cue of endocrinological events with as chief the steroid molting hormone, 20-hydroxyecdysone (20E). At apolysis, the ecdysteroids peak manifesting their effect on ecdysteroid responsive genes via interaction with the ecdysteroid receptors (EcRs). Typically, the interaction of the ligand with EcRs may take place in accord with a lock-and-key principle with EcR sequence/structure differing among insects (1–5). So, interference in these phases of the ecdysteroid activities may allow development of new, specific insecticidal actions for selective pest control.

In the past decade, various investigations revealed that synthetic substituted 1,2-dibenzoylhydrazines, such as RH-5849 and tebufenozide, work as nonsteroidal ecdysone agonists that induce, especially in Lepidoptera, precocious molting, leading to death (6–12). Nowadays, tebufenozide is used as an insect growth regulator (IGR) to control caterpillar pests in vegetables, fruits, ornamentals, and forest that has no adverse effects on various predators and beneficials like honeybees (11, 13, 14). Recently, two new structural analogs, methoxyfenozide (RH-2485) and halofenozide (RH-0345), are under development by Rohm and Haas for control of, e.g., Lepidoptera pests in cotton and scarabid larvae and cutworms in turfgrass and ornamentals, respectively (11, 15).

The present study was undertaken to determine the relative activities of the four ecdysone agonists against the cotton leafworm, Spodoptera littoralis. These polyphagous caterpillars are of worldwide importance and cause high damages in, e.g., cotton, vegetables, and ornamentals. In addition, we determined the relative potencies of these agonists of 20E in inducing the development of cultured imaginal wing discs. Imaginal discs are masses of cells which give rise to adult organs during metamorphosis in holometabolous insects by an increase in ecdysteroids. In vitro culture of these tissues may allow direct comparison between larvicidal
toxicity and the activity in terms of the ability to induce evagination under conditions without a source of ecdysteroids and freed from metabolism. A parallel competition binding assay with \(^3\)H-labeled ponasterone A (PonA) in individual imaginal discs made it possible to evaluate the affinity of ecdysone agonist to bind on the EcRs in whole imaginal discs. In addition, we tested in this study also the importance of uptake and efflux of ecdysone agonist from imaginal disc with ouabain, an inhibitor of Na\(^+\),K\(^+\)-ATPase that mediates active transport. A good correlation between biological activity and evagination—induction suggests that the latter might be useful for a rapid assay system for screening for new ecdysone agonists and for target site (EcRs) alternations in other insect species/strains.

**MATERIALS AND METHODS**

**Chemicals**

20E was purchased from Sigma (Bornem, Belgium), and technical samples (99%) of ecdysone agonists were kindly provided by Dr. G. R. Carlson (Rohm and Haas Research Laboratories, Spring House, PA). A sample of tritium-radiolabeled PonA (sp act 180 Ci/mmol) was a generous gift of Prof. P. Maroy (Szeged University, Hungary), and Prof. R. Lafont (Ecole Normale Supérieure, Paris, France) kindly supplied a sample of PonA as standard for RP-HPLC purification using a Varian system with a Waters F-Bondapak C\(_{18}\)-column at methanol:water (6:4, 1 mL/min) and spectrophotometrically determined at 242 nm (9).

**Insects**

All stages of a continuous laboratory colony of the cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae), were maintained under standard conditions of 23 ± 1°C, 70 ± 5% relative humidity, and a photoperiod of 16:8 h light and darkness. Larvae were fed on an artificial diet and adults a 15% honey water solution (7).

**In Vivo Bioassay for Larvicidal Toxicity**

Newly molted (0–12 h) last-instar (6th) larvae of *S. littoralis* were treated orally via artificial diet in 2-cm\(^2\) cylindrical wells of 24-well tissue culture plates (Castor, Belgium) in a manner similar to that described previously (16). A minimum of five different concentrations per compound was prepared in distilled water, and 24 larvae were minimally tested per concentration. Mortality counts were made at 7 days after treatment. After this period, control larvae were metamorphosed into 1-day-old pupae. The larval toxicity in terms of the LC\(_{50}\), the concentration (mg AI/L) required to kill 50% of the larvae, was estimated by probit analysis using POLO-PC (17).

**In Vitro Evagination—Induction Assay on Imaginal Wing**

Individual wing discs were hand-dissected in a laminar flow cabinet from 3-day-old (0–1 h) last instars, which is the moment at the end of the feeding period, and just before the appearance of the natural pulse of ecdysteroids. RIA measurements by Prof. K. Richter (Sächsische Akademie der Wissenschaften zu Leipzig, Jena Universität, Jena, Germany; 18,19) of hemolymph samples at different moments in the last instar indicated that between days 3 and 4 the endogenous titer of molting hormone is rising, reaching a peak at day 5 (Fig. 1). After the collection of 3-day-old last-instar larvae of *S. littoralis*, they could be kept in the refrigerator at 4°C for a maximum period of 1 week. Under these conditions both good synchronization and interruption

![FIG. 1. Changes in molting hormone titer during last-instar development of *Spodoptera littoralis*. Results are expressed as ng 20E equivalents/mL hemolymph.](image)
of pupal development were realized. In all series of experiments, fresh larval weight was evaluated, and subsequently larvae were water-anesthetized and briefly surface-sterilized in 70% ethanol for 15 min prior to dissection. Then, cultivation was performed in modified Grace’s medium, with heat-inactivated fetal calf serum (9%, Sigma), 30% bovine serum albumin (BSA) solution (3%, Sigma) and gentamycine sulfate (50 μg/ml medium, Sigma)] at 25 ± 1°C and 97 ± 2% relative humidity to prevent evaporation (9). Ten discs were kept in 1 mL culture medium in a 35- by 10-mm plastic tissue culture plate (Falcon 3001, Beckton Dickinson and Co., Belgium). For each compound at least three different concentrations of 20E and ecdysone agonist were tested; at least 30 imaginal discs were used per concentration. Suitable concentrations of 20E were prepared in methanol, and those of the four ecdysone agonists were made in dimethyl sulfoxide (DMSO). Not more than 1 μL of the suitable dilution was added to 1 mL medium. An equivalent volume of solvent was added to control cultures. After 72 h of cultivation, the wing discs were inspected for treatment-induced evagination. The minimum requirement for a positive result was completion of the first phase of evagination (20, 21) in which epidermal folds are developed inside the epidermal sac. Dose–response curves were plotted and EC_{50}s (in nM) calculated by regression analysis (22).

In Vitro Receptor Binding Assay in Competition with [³H]PonA

Imaginal wing discs were dissected from 3-day-old last instars of S. littoralis as described above. Competition binding studies were performed by applying 6 nM [³H]PonA with increasing concentrations of unlabeled competitor, 20E, or ecdysone agonist (9). [³H]PonA and unlabeled competitor were transferred in a small Eppendorf tube and freeze–dried with a Savant lyophilizator (Ankersmit, Belgium). Further, aliquots of 100 μL medium containing 20 imaginal wing discs were added and incubated at 25 ± 1°C for 2.5 h. The discs were homogeneously spread in the solution through very careful vortexing of the Eppendorf at each 30 min of incubation. For each treatment at least two replicates were performed. After incubation, bound and free [³H]PonA were separated by placing the imaginal discs on a Whatman GF/A glass fiber filter (Belgium) and washing five times for 3 min with 1 mL cold medium. Then, the medium was sucked through the filter by a water aspirator. The filter (+imaginal discs) was placed in a glass 20-mL liquid scintillation vial with a fine forceps and shaken overnight with 1 mL tissue solubilizer “LumaSolve” and 10 mL “LumaSafe” (both Lumac LSC, Mechelen, Belgium). The amount of radioactivity in the vial was determined in a Kontron liquid scintillation counter (LKB). The [³H]PonA binding competition curves were drawn and IC_{50}s (in nM) estimated by regression analysis (22). Maximal binding is shown as 0% competition, and total competition (=unspecific binding only) is expressed as 100%.

In an optimization assay with 6 nM [³H]PonA (in 100 μL culture medium) and 20 imaginal wing discs from 3-day-old last instars of S. littoralis, equilibrium of binding was observed after 2.5 h of cultivation, and the maximal amount of specifically bound radioactivity yielded 1780 ± 191 cpm. The total amount (specific + nonspecific binding) of bound [³H]PonA yielded 2699 cpm, whereas this was 919 cpm when incubating 6 nM [³H]PonA + 1 μM 20E that indicates the amount of nonspecific binding.

For receptor identification, a Scatchard plot experiment was done using 20 imaginal wing discs in 100 μL culture medium with different concentrations of [³H]-labeled PonA (0.6, 6, 60, and 600 nM). After 2.5 h of incubation at 25°C, the amount of bound radioactivity was determined as described above. The equilibrium binding constant K_D and the theoretical number of receptors (β_max) were calculated as the negative reciprocal value of the regression coefficient of the Scatchard line and the extrapolated X value when Y = 0, respectively (23).

In a separate series of assays, four freshly dissected imaginal wing discs from 3-day-old last-instar larvae of S. littoralis were incubated for 1, 4, and 24 h in 390 μL culture medium (in sterile Eppendorf tubes). At time 0 h, 10 μL
of a \(^{14}\)C-labeled ecdysone agonist concentration in methanol was added at a level of 39,225 and 37,137 cpm for tebufenozide and methoxyfenozide, respectively. Three replicates were performed per treatment. Radiochemically pure \([^{14}\text{C-t-butyl}]\)tebufenozide and \([^{14}\text{C-t-butyl}]\)methoxyfenozide with specific activities of 23.06 and 20.30 mCi/g, respectively, were kind gifts from Rohm and Haas research laboratories (Spring House, PA). Similarly, four imaginal wing discs were treated after being preincubated for 24 h with 10 μM of ouabain, an ATP-dependent transport inhibitor (Sigma), in 390 μL culture medium in sterile Eppendorf tubes to evaluate active transport out of the disc tissues to the culture medium. The amount of radioactivity retained in the disc tissues was separated as described above for labeled PonA and quantified with 1 mL “LumaSolve” and 10 mL “LipoLuma” (both Lumac LSC, Belgium) using a Kontron β-counter. Treated imaginal disc tissues showed no change in cell viability during the course of the assay (24).

RESULTS

Larvicidal Toxicity

To evaluate the relative toxicities of the four ecdysone agonists, newly molted last-instar caterpillars of *S. littoralis* were fed artificial diet with various concentrations of each ecdysone agonist. Intoxicated larvae underwent precocious larval apolysis that was observed within the first 48 h of treatment. Further on, at day 3–4, such larvae died while still within their old cuticle, showing inhibition of ecdysis. Finally at day 7, pupation in treatments was significantly reduced, and this reduction was proportional to the increase in concentration. Concentrations as low as 0.5 mg/L of methoxyfenozide caused no effects in reduction in formation of pupae, whereas 1 and 2 mg/L resulted in only 54 and 10% pupal development. The same respective levels of mortality were observed only with higher concentrations of the other ecdysone agonists: 10 and 15 mg/L of tebufenozide, 50 and 90 mg/L of halofenozide, and 70 and 300 mg/L of RH-5849. The calculated LC₅₀s by probit analysis showed that methoxyfenozide caused 50% mortality at 1.15 mg/L, while for tebufenozide, halofenozide, and RH-5849 a respective concentration of 7.47, 44.8, and 105 mg/L was required. Concentration–mortality curves of the four ecdysone agonists showed that methoxyfenozide was the most toxic; it was about 7, 39, and 92 times more potent than tebufenozide, halofenozide, and RH-5849, respectively (Fig. 2; Table 1).

In Vitro Molting Hormone Activity as Measured with the Evagination in Imaginal Discs

If the toxicity of ecdysone agonists correlates well with the induction of evagination in cultured imaginal discs, then it should be possible...
TABLE 1

Relative Toxicities of Four Ecdysone Agonists Fed via Artificial Diet to Last-Instar Larvae of Spodoptera littoralis

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of larvae used</th>
<th>LC_{10} (95% FL)</th>
<th>LC_{50} (95% FL)</th>
<th>LC_{90} (95% FL)</th>
<th>Slope ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxyfenozide</td>
<td>346</td>
<td>0.56 (0.22–0.81)</td>
<td>1.15 (0.79–1.50)</td>
<td>2.36 (1.77–4.38)</td>
<td>4.10 ± 0.48</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>464</td>
<td>3.80 (2.43–4.82)</td>
<td>7.47 (6.29–8.63)</td>
<td>14.67 (12.06–20.89)</td>
<td>4.37 ± 0.42</td>
</tr>
<tr>
<td>Halofenozide</td>
<td>300</td>
<td>21.77 (11.27–28.90)</td>
<td>44.78 (36.11–53.75)</td>
<td>92.13 (71.82–161.00)</td>
<td>4.09 ± 0.50</td>
</tr>
<tr>
<td>RH-5849</td>
<td>278</td>
<td>31.51 (15.04–45.92)</td>
<td>105.02 (72.92–196.56)</td>
<td>377.20 (261.31–689.66)</td>
<td>2.38 ± 0.30</td>
</tr>
</tbody>
</table>

To use cultured imaginal discs as a bioassay to screen for new agonists. Concentration–response curves of the different compounds were parallel and showed that the relative order of activity of the four ecdysone agonists is methoxyfenozide > 20E > tebufenozide ≈ halofenozide > RH-5849 (Fig. 3). This order of activity for ecdysone agonists showed a trend that agrees with the order observed for their larval toxicity. The EC_{50}s calculated by regression analysis showed that methoxyfenozide caused 50% evagination–induction at 10.9 nM, whereas to cause the same effect with tebufenozide, halofenozide, and RH-5849 a concentration of as high as 403 nM, 472 nM, and 44.5 μM, respectively, was required. For 20E, evagination was induced in 50% of the discs cultured at 291 nM, which corresponds well with the endogenous ecdysteroid peak titer at day 5 (82.1 ng equiv/mL) of the respective last instars, that was measured by RIA (Fig. 1).

In Table 2, we listed the biological activity in vitro for the four ecdysone agonists and 20E in terms of their pEC_{50}s, that is the log value of the reciprocal of the concentrations that induce evagination in 50% of imaginal discs tested. The evagination–induction assay revealed that the potency range of these compounds in terms of pEC_{50}s corresponded with that for toxicity pLC_{50}s (log of the reciprocal of LC_{50}s) as follows:

\[
(pLC_{50}) = 0.5668 \times (pEC_{50}) + 0.0735, \text{ with } R = 0.912. \quad [1]
\]

**Competitive Binding Activity with \[^3\text{H}]\text{PonA} Receptor in Imaginal Discs**

Concentration-dependent competition curves for binding (Fig. 4) of the different compounds to bind receptors in competition with 6 nM \[^3\text{H}]\text{PonA} showed a trend that these curves were
TABLE 2

*In Vitro* Molting Hormone Activity of Four Ecdysone Agonists and 20E on Evagination-Induction (EC50 in nM) and Binding Activities (IC50 in nM) in Imaginal Wing Discs from 3-Day-Old Last-Instar Larvae of *Spodoptera littoralis*

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>pEC50 (nM)</th>
<th>IC50 (nM)</th>
<th>pIC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxyfenozide</td>
<td>10.9</td>
<td>1.037</td>
<td>24.3</td>
<td>1.386</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>403</td>
<td>2.605</td>
<td>86.7</td>
<td>1.938</td>
</tr>
<tr>
<td>Halofenozide</td>
<td>472</td>
<td>2.674</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RH-5849</td>
<td>44500</td>
<td>4.648</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20E</td>
<td>291</td>
<td>2.464</td>
<td>158</td>
<td>2.199</td>
</tr>
</tbody>
</table>

approximately parallel to one another (Fig. 4). The calculated IC50s in Table 2 represent the concentration of unlabeled competitor causing 50% competition with the maximal amount of specifically bound [3H]PonA and show that methoxyfenozide caused 50% competition for binding at 24.3 nM, whereas for tebufenozide 86.7 nM and for 20E 158 nM was necessary. The potency range of ecdysone agonists in terms of their IC50s corresponded with that of the activity *in vitro* by EC50s. The molting activity *in vitro* expressed by pEC50 (nM) was compared with the competitive receptor binding activity by pIC50 (nM; log of the reciprocal of IC50s) to formulate

\[(\text{pEC}_{50}) = 1.9234 \times (\text{pIC}_{50}) - 1.5049,\]

with \(R = 0.9207\). [2]

For identification of EcRs in whole imaginal wing discs, Scatchard plot analysis with different concentrations of tritiated PonA (Fig. 4, inset) demonstrated an equilibrium binding constant \(K_D\) of 6.211 nM and a theoretical number of receptors \(\beta_{\text{max}}\) of 18.1 fmol per 20 imaginal discs.

Incubation of imaginal discs with labeled tebufenozide and methoxyfenozide revealed that about 400 cpm retained in the disc tissues within 1 h of incubation with no further significant

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**FIG. 4.** [3H]PonA binding competition curves of ecdysone agonists, tebufenozide (RH-5992) and methoxyfenozide (RH-2485), and 20E with cultured imaginal wing discs of *Spodoptera littoralis*. Zero % refers to maximal binding of [3H]PonA; 100% refers to total competition. (Inset) Scatchard plot analysis when incubating imaginal wing discs of *Spodoptera littoralis* with different concentrations of [3H]PonA.
(P = 0.05) increase in uptake (Fig. 5). Preincubation of discs with 10 μM ouabain to inhibit ATP-dependent transport specifically, indicated no significant impact of active transport out of the cultured wing disc tissues for both tebufenozide and methoxyfenozide.

**DISCUSSION**

Nowadays environmentally friendly insecticides gain favor for insect control, especially in the frame of integrated pest management programs in which selective chemicals are used in combination with natural enemies and beneficial insects. In consequence, and as Williams already speculated in 1968 (25), chemicals that mimic insect hormone actions and/or interact with their receptors provide new possibilities to discover new modes of action and to overcome resistance problems with neurotoxins. Tebufenozide was the first commercial example of such a highly target-selective insecticide that has been produced by rational design and chemical synthesis acting as a mimic of 20E in caterpillars (14). At present, methoxyfenozide and halofenozide are two new structural analogs of tebufenozide, both with a specific activity spectrum (11, 15).

In this paper we have described that the relative toxicities of the four ecdysone agonists, tebufenozide, methoxyfenozide, halofenozide, and RH-5849, to larvae of *S. littoralis* were similar to their potencies in inducing evagination that was mediated through binding on EcRs in cultured imaginal wing discs. For *S. littoralis* we found a positive correlation between larval mortality and evagination induction as shown in Eq. [1].

Imaginal disc evagination induction has previously been observed for several ecdysteroids. In the current assays, we showed that the four ecdysone agonists, as can the natural molting hormone 20E, could initiate and sustain the evagination of isolated wing discs. Based on earlier *in vitro* work with a wide variety of insects (3, 20, 21, 26–28), we found a reliable activity profile of 20E to induce evagination in imaginal discs of *S. littoralis*. For methoxyfenozide, its effectiveness is greater than that of tebufenozide which is in return more active than 20E acting as a mimic of 20E in caterpillars (14). At present, methoxyfenozide and halofenozide are two new structural analogs of tebufenozide, both with a specific activity spectrum (11, 15).

![Graph](image-url)  
**FIG. 5.** Time course of the absorption for labeled tebufenozide (RH-5992) and methoxyfenozide (RH-2485) in imaginal wing discs of *Spodoptera littoralis*, and the effect of ouabain, an inhibitor of ATP-dependent transport, indicating no significant differences in impact on active transport out of the disc tissues for both ecdysone agonists.
mellonella; and 20E is more effective than RH-5849 (11, 28, 30). Generally, methoxyfenozide is more potent than tebufenozide against Lepidoptera.

The usefulness of the imaginal disc assay was again confirmed in the experiments to evaluate binding in competition with radiolabeled PonA. Besides, Scatchard plot analysis proved the presence of EcRs in imaginal wing discs showing a $K_D$ of 6.211 nM for PonA that concurs well with values in other species. Similarly, our data on the binding affinity of 20E and ecdysone agonists were in good agreement with the results obtained with other insects. It was clear that the estimated IC$_{50}$ for 20E falls in the range of physiologically relevant concentration of 20E (4). In addition, we could not report differences between tebufenozide and methoxyfenozide in absorption profile of labeled tebufenozide and methoxyfenozide in cultured wing discs of S. littoralis, and in effects of preincubation with ouabain, an inhibitor of ATP-dependent transport. As a consequence, the affinity to bind EcRs of the different ecdysone agonists with cultured imaginal discs can be estimated from their competitive binding curve. In this study, we demonstrated for imaginal discs of S. littoralis that variations in IC$_{50}$s corresponded well with those in EC$_{50}$s as shown in Eq. [2]. This linear relationship ($r = 0.92$) is rather similar to that for the induction of evagination and the 50% competition by either of PonA, muristerone A, inokosterone, and 20E in cultured imaginal discs of Calliphora vicina (28) and in wing discs of G. mellonella, Leptinotarsa decemlineata, and S. exigua (9, 30). Such relationship has also been noted in previous studies when the affinities of ecdysteroids in binding assays using $K_c$ cells and mass-isolated imaginal discs of Drosophila melanogaster correlated well with their biologically active concentration (3). Thus, it is indicated that imaginal disc evagination and the competition binding activity using imaginal discs are a true approximation of the receptor binding affinity for both ecdysteroids and ecdysone agonists. Besides, the level of 20E causing 50% competition binding in imaginal discs of S. littoralis corresponds well with the ecdysteroid peak titer in the hemolymph of the respective last-instar larvae.

On insecticidal activity, methoxyfenozide was found more toxic against larvae of S. exigua (16), which concurs with the current data in S. littoralis. In a previous study, Ishaaya et al. (31) showed that it was about 7–14 times more potent than tebufenozide against a pyrethroid-resistant strain of the cotton leafworm. Similarly, this new ecdysone agonist is 3- to 10-fold more toxic than tebufenozide in Ostrinia nubilalis and Choristoneura fumiferana and about 40-fold more active than halofenozide and over 200 times more potent than RH-5849 (12, 32). Generally, tebuobtained with other insects. It was clear that the estimated IC$_{50}$ for 20E falls in the range of physiologically relevant concentration of 20E (4). In addition, we could not report differences between tebufenozide and methoxyfenozide in absorption profile of labeled tebufenozide and methoxyfenozide in cultured wing discs of S. exigua (9, 30). Such relationship has also been noted in previous studies when the affinities of ecdysteroids in binding assays using $K_c$ cells and mass-isolated imaginal discs of Drosophila melanogaster correlated well with their biologically active concentration (3). Thus, it is indicated that imaginal disc evagination and the competition binding activity using imaginal discs are a true approximation of the receptor binding affinity for both ecdysteroids and ecdysone agonists. Besides, the level of 20E causing 50% competition binding in imaginal discs of S. littoralis corresponds well with the ecdysteroid peak titer in the hemolymph of the respective last-instar larvae.

In the present study, it was promising that variations in concentration required to elicit evagination agreed with variations in larvicidal toxicity, suggesting a good correlation between in vitro activity on imaginal disc development and in vivo toxicity against the larval developmental stages. Besides, it was very striking that comparison between the current data with S. littoralis and those in S. exigua (30) revealed a high similarity between Lepidoptera species. For the three insects species that we have tested so far on toxicity and evaginations, induction, a
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