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Screening of Dialkoxybenzenes and Disubstituted Cyclopentene Derivatives against the Cabbage Looper, *Trichoplusia ni*, for the Discovery of New Feeding and Oviposition Deterrents

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The antifeedant, oviposition deterrent, and toxic effects of dialkoxybenzene minilibraries and of disubstituted cyclopentene minilibraries (i.e., consisting of four to five compounds) along with their pure constituent compounds were assessed against third instar larvae and adults of the cabbage looper, *Trichoplusia ni*, in laboratory bioassays in a search for new insect control agents. These compounds mimic naturally occurring bioactive odorants and tastants and are relatively easily prepared from commodity chemicals. Most of these libraries strongly deterred larval feeding, with some exhibiting strong toxic and oviposition deterrent effects as well. Our results suggest some structure–function relationships within these libraries. Replacement of a methyl group with larger alkyl substituents increased the feeding deterrent effects in most cases. The presence of a free hydroxyl group, irrespective of the carbon framework or alkyl substituent, served to reduce feeding deterrent effects in all series of compounds. Further, exceeding a certain group size also generally had a detrimental effect. This information will be useful in designing new insect control agents for agriculture. Some of these libraries and compounds may have potential for development as commercial insecticides.

KEYWORDS: *Trichoplusia ni*; feeding deterrents; oviposition deterrents; toxicity; dialkoxybenzenes; disubstituted cyclopentenes

INTRODUCTION

Our knowledge of insect−plant chemical interactions indicates that chemical signals are important behavioral guides for insects, enabling them to find appropriate host plants for feeding and oviposition, locate mates, sense the presence of predators, and even assess the suitability of a host. Insect−plant chemical interactions in nature are usually very subtle. Most plant defensive chemicals discourage insect herbivory, either by deterring feeding and oviposition or by impairing larval growth, rather than by killing insects outright (*1*).

Antifeedants are described as substances that reduce feeding by an insect acting either peripherally (on gustatory chemoreceptors) or centrally. They can be found among all of the major classes of secondary metabolites: alkaloids, phenolics, and terpenoids (*2*). It is in the last category that the greatest number and diversity of antifeedants and the most potent ones have been found (*1*).

All phytophagous insects that have been investigated respond behaviorally to some of these compounds, most of which produce a deterrent response in the insects (*3*). Reduction or complete inhibition of feeding has been demonstrated in Orthoptera, Hemiptera, Coleoptera, larval Lepidoptera, and larval Hymenoptera (*4, 5*).

Among polyphagous insects, the balance of phagostimulatory and deterrent inputs is probably the sole determinant of acceptance or rejection of food, as shown clearly in many investigations (*6, 7*) and demonstrated in simplified models (*8, 9*).

Plant compounds also constitute important sensory cues mediating oviposition in phytophagous insects. Plant compounds may act as oviposition stimulants (*10*) or deterrents (*11, 12*). Host plant acceptance by an ovipositing female is mediated by a balance of sensory inputs from both positive and negative stimuli received from these compounds (*13*). The relative balance between these opposing cues is weighted by the internal physiological state of the insect, such as egg load (*14*).

The search for insect control agents that have potential use as crop protectants (insecticides, antifeedants, and growth inhibitors) often begins with the screening of compounds. Initially, the test insects can be fed the compound either

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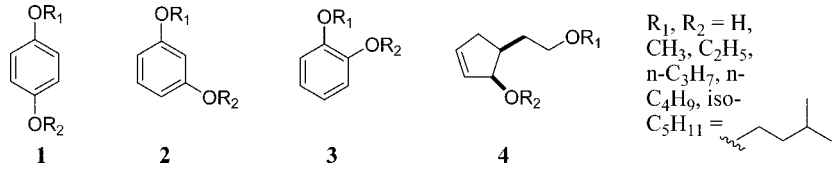
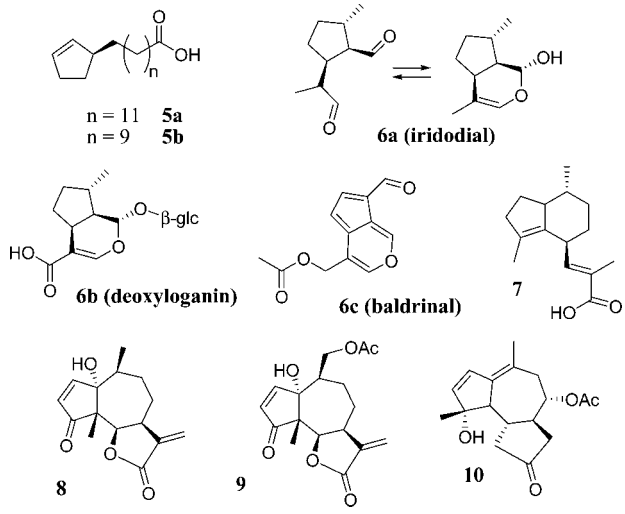
Scheme 1. Structures of the Target Compounds Tested in This Study



Scheme 2. Natural Products That Contain Cyclopentane or Cyclopentene Units



incorporated into an artificial diet or sprayed on a host plant, and the effects on insect behavior and development can be monitored. Once a promising compound has been discovered, the next step is often to investigate the mode of action. This kind of information is needed to ensure safety to nontarget organisms (humans, wildlife, beneficial insects).

Chemicals that inhibit feeding of phytophagous insects may be an integral part of plant defense itself, conferring some measure of resistance to insect attack, or they may be applied to the plant in the same way as other agricultural chemicals (*15*), serving as exogenous crop protectants (*16*). Interest in the feeding and oviposition deterrent properties of compounds has arisen both because deterrence is an important mediator of plant−insect interactions and because it is potentially useful for manipulating the behavior of crop pests (*17*).

One of the limitations of using plant-derived botanicals is resource availability. In the present study we have used synthetic libraries of compounds that can be generated rapidly, in large amounts and in high purity.

The purpose of the present study was to assess the antifeedant, oviposition deterrent, and toxic effects of dialkoxybenzene minilibraries (**Scheme 1**, **1**, **2**, and **3**) and of disubstituted cyclopentenes (**Scheme 1**, **4**) against the cabbage looper, *Trichoplusia ni*. Low molecular weight phenol derivatives are important constituents of smoke (*18*, *19*), which is known to have insect repellent and insecticidal properties (*20*). Substituted cyclopentenes can be found in plants, in several contexts. For example, chaulmoogric acid (**Scheme 2**, **5a**) and its homologue hydnocarpic acid (**5b**) are antibacterials from the seeds of *Hydnocarpus wightiana*. Iridanes (**Scheme 2**, **6a**), iridoids (**6b**), and secoiridoids (**6c**) feature cyclopentane and cyclopentene units prominently. Also, many plant sesquiterpenes, such as valerenic acid (**Scheme 2**, **7**), parthenin (*8*), tetraneurin-A (*9*), and matricin (*10*) contain substituted cyclopentene units (*21*). Some of these compounds possess insecticidal properties. For example, at a dietary concentration of 3.0 mM/kg fresh weight, parthenin reduced larval growth of *Heliothis zea* by 59% relative to controls in a chronic feeding bioassay (*22*) and the related compound, tetraneurin-A, acted as an antifeedant against sixth instar larvae of *Spodoptera litura* (*23*).

This study will serve as the foundation for more detailed investigations of the effects of individual lead compounds on feeding and oviposition behaviors as well as the toxic effects against *T. ni* and other agricultural pests. The cabbage looper is an important pest of cruciferous plants but also attacks several other crops including lettuce, beets, peas, celery, tomatoes, certain ornamentals, and many weedy plants (*24*). Because *T. ni* has evolved resistance against many synthetic insecticides (*25*) and the microbial insecticide *Bacillus thuringiensis* (*26*, *27*), it is very important to develop new methods that could be used to protect crops in integrated pest management schemes. Here we have chosen to investigate compounds that mimic naturally occurring bioactive odorants and tastants and that are relatively easily prepared from commodity chemicals.

MATERIALS AND METHODS

Plant Material. Cabbage plants (*Brassica oleraceae* var. Stonehead) used in the bioassays were routinely grown in plastic pots with a mixture of sandy loam soil and peat moss (4:1) in a greenhouse at the University of British Columbia, Vancouver, BC, Canada. Leaves were collected from cabbage plants that were 5–6 weeks old.

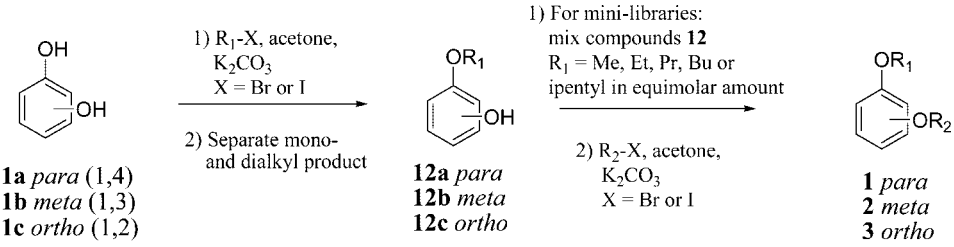
Test Insects. *T. ni* larvae and moths were obtained from a long established colony (>50 generations) maintained on an artificial diet, Velvetbean Caterpillar Diet No. F9796 [Bio-Serv Inc. (Frenchtown, NJ.)] in the insectary of the University of British Columbia (UBC). The diet was supplemented with finely ground alfalfa, to improve acceptability, and vitamins [No. 8045; Bioserv Inc. (Frenchtown, NJ.)].

Test Compounds. Dialkoxybenzene minilibraries (consisting of four to five compounds) and pure compounds were synthesized as described elsewhere (*28*). Briefly, dialkoxybenzenes were synthesized from the corresponding dihydroxybenzenes (**11a**, **11b**, or **11c**) by monoalkylation (**Scheme 3**). The pure monoalkylated compounds were mixed in equimolar amounts, for the synthesis of minilibraries, and subjected to a second round of alkylation. Thus, the minilibraries have one alkyl group constant and the other one variable. Testing the minilibraries of increasing constant group size and the pure compounds for which $R_1 = R_2$, we can infer which general group size and regiochemistry (*para*, a; *meta*, b; *ortho*, c) is best for a particular bioactivity.

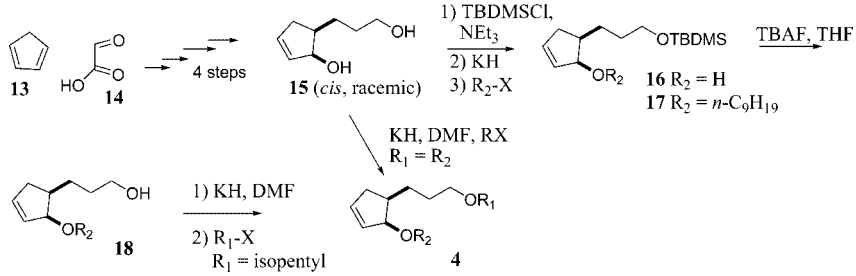
The cyclopentene compounds were synthesized as shown in **Scheme 4** and as described in detail elsewhere (*29*). Briefly, diol **15** can be obtained from cyclopentadiene **13** and glyoxylic acid **14** in a four-step procedure. For compounds with $R_1 = R_2$, the diol is dialkylated by treatment with KH in dimethylformamide, followed by the appropriate alkyl halide. Production of compounds with different R groups necessitated protection of the primary alcohol with a *tert*-butyldimethylsilyl (TBDMS) group. The resulting secondary alcohol **16** was alkylated to **17**, which was deprotected to furnish alcohol **18**. This compound was then alkylated to give a variant of **4**, with R_1 = isopentyl and R_2 = *n*-nonyl. This higher molecular mass compound was included in the present study as a representative of a more hydrophobic, sterically hindered and less volatile compound.

General Testing Procedure. Initially, all compounds were tested at 50 μg/cm^2 in feeding deterrent bioassays. Those compounds that

Scheme 3. Optimized Synthesis of Dialkoxybenzenes



Scheme 4. Overview of the Synthesis of Cis-Substituted Racemic Cyclopentene Odorants for This Study[a]



[a] Abbreviations in order of appearance: TBDMSCl = *tert*-butyl dimethyldiyl chloride; NEt_3 = triethylamine; TBAF = *tert*-butylammonium fluoride; THF = tetrahydrofuran; R−X = alkyl halide (bromide or iodide); DMF = dimethylformamide.

exhibited >50% feeding deterrence at this concentration were subjected to further testing for oviposition deterrent effects and contact toxicity at 0.25% of the test substance. For compounds exhibiting ≥50% values for feeding deterrence and ≥70% mortality by contact, DC_{50} (concentration causing 50% feeding deterrence compared with the control) and LC_{50} (concentration causing 50% mortality compared with the control) were determined, respectively, based on bioassays involving a minimum of four concentrations (3.12–25 $\mu g/cm^2$).

Feeding Deterrent Bioassays. Leaf disk choice bioassays (*30*, *31*) were conducted to determine feeding deterrent effects of the synthetic compounds using freshly molted third instar larvae starved for 4–5 h prior to each bioassay. Larvae were given the choice of feeding on two leaf disks, one treated with 10 μL of a solution of the test substance painted on each side and the other treated with a carrier solvent alone. The number of larvae was 25 per treatment. Bioassays were terminated when ∼50% of the control disk had been eaten (normally 3–5 h).

Areas of control and treated leaf disks consumed by the larvae were measured using Scion Image software, and feeding deterrence was calculated (*31*) using the formula $[(C - T)/(C + T)] \times 100$, where C and T are areas consumed of the control and treated leaf disks, respectively.

Oviposition Deterrent Bioassays. Oviposition response of *T. ni* moths was measured according to our previously described oviposition choice bioassay (*32*, *33*). *T. ni* larvae were reared on normal diet from neonates (<24 h old) until pupation. Pupae were sexed and put in separate plastic containers until emergence. After eclosion, pairs of moths (one male and one female) were introduced into each cage with a control and a treated cabbage leaf. Pairs of moths ($n = 25$) were used per treatment. Each leaf (approximately 100–110 cm^2) was sprayed with 0.5 mL of MeOH or a methanolic solution of the test chemical on each side. Eggs were counted on each cabbage leaf after 48 h. ODI (oviposition deterrence index) was calculated using the formula ODI $= [(C - T)/(C + T)] \times 100$, where C and T are the numbers of eggs laid on the control and treated leaf disks, respectively (*32*, *33*).

Contact Toxicity Bioassays. Mortality was determined 24 h after spraying larvae directly with test solutions (*34*). Third instar *T. ni* larvae were sprayed in 90 mm × 15 mm Petri dishes (Falcon) lined with Fisher Scientific filter paper (90 mm diameter). Small plastic hand spraying bottles (50 mL capacity) were used. Larvae were then transferred to Petri dishes (90 mm × 15 mm) with a small piece of artificial diet. Each Petri dish contained 10 larvae. Three replicates, each consisting of 10 larvae, were used per treatment.

Comparison of Toxicity, Oviposition, and Feeding Deterrence Values. The mortality of each test material was plotted against its respective oviposition deterrence value (determined at 0.25%) to explore the relationship between the two bioassays using correlation analysis. Similarly, feeding deterrence was plotted against oviposition deterrence and mortality.

Data Analysis. Feeding deterrence data (percent) for initial screening concentration were analyzed by analysis of variance (ANOVA) after arcsin transformation using statistics software (*35*). Where significant F values were found, Tukey's HSD multiple comparison tests were used to test for significant differences between individual treatments.

RESULTS

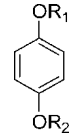
***p*-Dialkoxybenzene Libraries, Pure Compounds, and 1-Hydroxy-4-alkoxy Compounds.** All six of the *p*-dialkoxybenzene libraries and five individual compounds exhibited >50% feeding deterrence at the initial screening concentration (50 $\mu g/cm^2$) and, therefore, were subjected to further testing (**Table 1**) against third instar *T. ni* larvae for toxic and oviposition deterrent effects. The response of the larvae to the initial screening concentration varied significantly in most cases (one-way ANOVA; $F_{16405} = 9.6$, $p < 0.0001$).

Feeding Deterrent Effects. 1-Isopentyloxy-4-alkoxybenzene had the lowest DC_{50} value (8.5 $\mu g/cm^2$) followed by 1-butyloxy-4-alkoxybenzene (14.5 $\mu g/cm^2$) and 1-allyloxy-4-isopentoxybenzene (15.7 $\mu g/cm^2$) (**Table 1**). 1-Hydroxy-4-methoxybenzene and 1-hydroxy-4-propoxybenzene acted as feeding stimulants at the screening concentration. 1-Hydroxy-4-ethoxybenzene (a precursor to diethyl and the ethyl minilibrary) was a weak feeding deterrent.

Toxic Effects. 1,4-Diethoxybenzene and the 1-ethoxy-4-alkoxybenzene library were the most toxic (**Table 1**) at 0.25% (LC_{50} values were 0.03% for both) followed by 1-butyloxy-4-alkoxybenzene and 1-propoxy-4-alkoxybenzene. This high specificity for the ethyl-substituted compounds is remarkable, because mortality was <25% for other members in the *p*-dialkoxybenzene series.

Oviposition Deterrent Effects. 1,4-Diethoxybenzene, the 1-ethoxy-4-alkoxybenzene library, and the 1-butyloxy-4-alkoxybenzene library showed the strongest oviposition deterrent

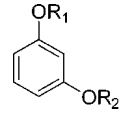
Table 1. Bioactivities of 1,4-Dialkoxybenzene Libraries and Analogues against Third Instar *T. ni* Larvae[a]



compound	R_1	R_2	FD (%), mean ± SE (n = 25)	DC_{50}, μg/cm^2 (r^2)[b] (n = 25)	mortality (%) (n = 3 × 10)	OD (%), mean ± SE (n = 25–33)
1,4-dimethoxybenzene	CH_3	CH_3	9.9 ± 18.0[cd]	–[c]	–	–
1,4-diethoxybenzene	C_2H_5	C_2H_5	80.8 ± 11.3[b]	25.9 (0.91)	100.0[d]	74.7 ± 13.3[a]
1,4-dipropoxybenzene	C_3H_7	C_3H_7	96.0 ± 2.8[a]	20.3 (0.99)	22.6	11.8 ± 13.1[b]
1,4-diisopentoxybenzene	C_5H_{11}	C_5H_{11}	44.0 ± 18.3[abc]	–	–	–
1,4-diallyloxybenzene	C_3H_5	C_3H_5	96.9 ± 3.6[a]	23.9 (0.97)	16.0	14.1 ± 13.1[b]
Me library (1-methoxy-4-alkoxybenzene)	CH_3	CH_3, C_2H_5, C_3H_7, C_5H_{11}	80.2 ± 9.8[b]	34.6 (0.90)	23.3	6.0 ± 5.1[b]
Et library (1-ethoxy-4-alkoxybenzene)	C_2H_5	CH_3, C_2H_5, C_3H_7, C_5H_{11}	90.7 ± 10.2[a]	23.4 (0.99)	96.8[e]	56.6 ± 16.9[ab]
Pr library (1-propoxy-4-alkoxybenzene)	C_3H_7	CH_3, C_2H_5, C_3H_7, C_5H_{11}	53.4 ± 15.8[abc]	39.7 (0.94)	53.1	9.6 ± 13.8[b]
Bu library (1-butyloxy-4-alkoxybenzene)	C_4H_9	CH_3, C_2H_5, C_3H_7, C_5H_{11}	83.4 ± 9.7[b]	14.5 (0.83)	58.1	50.1 ± 14.5[ab]
iPent library (1-isopentyloxy-4-alkoxybenzene	C_5H_{11}	CH_3, C_2H_5, C_3H_7, C_5H_{11}	100.0 ± 0.0[a]	5.8 (0.85)	18.8	22.9 ± 14.2[ab]
allyl small library (1-allyloxy-4-alkoxybenzene)	C_3H_7	CH_3, $C_2H_5C_3H_7$	82.4 ± 10.7[b]	27.9 (0.93)	10.0	5.4 ± 13.9[b]
1-allyloxy-4-butoxybenzene	C_3H_7	C_4H_9	84.0 ± 10.7[b]	22.6 (0.90)	10.0	16.8 ± 13.8[ab]
1-allyloxy-4-isopentoxybenzene	C_3H_7	C_5H_{11}	75.1 ± 12.7[abc]	15.7 (0.90)	7.0	18.8 ± 14.3[ab]
1-hydroxy-4-methoxybenzene	H	CH_3	−26.5 ± 18.6[d]	–	–	–
1-hydroxy-4-ethoxybenzene[f]	H	C_2H_5	29.8 ± 18.2[bcd]	–	–	–
1-hydroxy-4-propoxybenzene	H	C_3H_7	−28.0 ± 18.4[d]	–	–	–
1-hydroxy-4-isopentoxybenzene	H	C_5H_{11}	29.5 ± 16.6[bcd]	–	–	–

[a] Feeding deterrent (FD) effects (mean ± SE) at 50 μg/cm^2 are expressed in %. DC_{50}s (concentrations causing 50% feeding deterrence compared with the control) were calculated for samples showing >50% feeding deterrence in initial screening (≥50 μg/cm^2) using Excel; linear regression analysis was conducted for all dose−response experimental data. Mortality and oviposition deterrent effects were determined at 0.25% for samples showing >50% feeding deterrence in initial screening. LC_{50} (concentrations causing 50% mortality compared with the control) was calculated for test compounds exhibiting ≥70% mortality at 0.25%. Means followed by the same letters within a column do not differ significantly (Tukey's test, $p < 0.05$). [b] Coefficient of determination. [c] Not tested. [d] LC_{50} = 0.03%. [e] LC_{50} = 0.03%. [f] Precursor to diethyl and the ethyl minilibrary.

Table 2. Bioactivities of 1,3-Dialkoxybenzene Libraries and Analogues against Third Instar *T. ni* Larvae[a]



compound	R_1	R_2	FD (%), mean ± SE (n = 25)	DC_{50}, μg/cm^2 (r^2)[b] (n = 25)	mortality (%) (n = 3 × 10)	OD (%), mean ± SE (n = 25–33)
1,3-dimethoxybenzene	CH_3	CH_3	20.4 ± 19.1[bcde]	–[c]	–	–
1,3-diethoxybenzene	C_2H_5	C_2H_5	89.4 ± 8.3[ab]	28.7 (0.85)	36.7	3.4 ± 14.1[b]
1,3-dipropoxybenzene	C_3H_7	C_3H_7	96.9 ± 3.1[a]	26.8 (0.79)	76.7[d]	33.0 ± 14.6[ab]
1,3-diisopentoxybenzene	C_5H_{11}	C_5H_{11}	−12.2 ± 18.8[de]	–	–	–
Me library (1-methoxy-3-alkoxybenzene)	CH_3	CH_3, C_2H_5, C_3H_7, C_5H_{11}	54.8 ± 16.6[abcd]	61.5 (0.94)	16.7	70.2 ± 17.3[a]
Et library (1-ethoxy-3-alkoxybenzene)	C_2H_5	CH_3, C_2H_5, C_3H_7, C_5H_{11}	69.8 ± 13.8[abc]	26.5 (0.98)	20.0	14.3 ± 13.5[ab]
Pr library (1-propoxy-3-alkoxybenzene)	C_3H_7,	CH_3, C_2H_5, C_3H_7, C_5H_{11}	98.0 ± 1.5[a]	21.5 (0.96)	50.0	2.7 ± 14.2[b]
Bu library (1-butyloxy-3-alkoxybenzene)	C_4H_9,	CH_3, C_2H_5, C_3H_7, C_5H_{11}	84.1 ± 8.9[ab]	14.4 (0.85)	30.0	25.9 ± 13.9[ab]
iPent library (1-isopentyloxy-3-alkoxybenzene	C_5H_{11}	CH_3, C_2H_5, C_3H_7, C_5H_{11}	82.6 ± 12.0[ab]	19.8 (0.96)	20.8	35.7 ± 13.4[ab]
1-hydroxy-3-methoxybenzene	H	CH_3	44.7 ± 17.5[abcde]	–	–	–
1-hydroxy-3-ethoxybenzene	H	C_2H_5	−18.7 ± 17.8[e]	–	–	–
1-hydroxy-3-propoxybenzene	H	C_3H_7	4.0 ± 20.4[cde]	–	–	–
1-hydroxy-3-isopentoxybenzene	H	C_5H_{11}	−5.8 ± 18.7[de]	–	–	–

[a] Feeding deterrent (FD) effects (mean ± SE) at 50 μg/cm^2 are expressed in %. DC_{50}s (concentrations causing 50% feeding deterrence compared with the control) were calculated for samples showing >50% feeding deterrence in initial screening concentration (≥50 μg/cm^2) using Excel; linear regression analysis was conducted for all dose−response experimental data. Mortality and oviposition deterrent effects were determined at 0.25% for samples showing >50% feeding deterrence in initial screening. LC_{50} (concentrations causing 50% mortality compared with the control) was calculated for test compounds exhibiting ≥70% mortality at 0.25%. Means followed by the same letters within a column do not differ significantly (Tukey's test, $p < 0.05$). [b] Coefficient of determination. [c] Not tested. [d] LC_{50} = 0.16%.

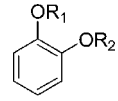
effects (74.7%, 56.6%, and 50.1%, respectively) when tested at 0.25% (**Table 1**). Other members in the group demonstrated weak oviposition deterrent effects (<23%). Responses of moths varied significantly in most cases (one-way ANOVA; $F_{10304} = 2.8$, $p < 0.003$).

***m*-Dialkoxybenzene Libraries, Pure Compounds, and 1-Hydroxy-3-alkoxy Compounds.** All five of the *m*-dialkoxybenzene libraries and two pure compounds exhibited >50% feeding deterrence in initial screening (50 μg/cm^2) and therefore were subjected to further testing (**Table 2**). The response of the larvae to initial screening concentration varied significantly in most cases (one-way ANOVA; $F_{12307} = 8.6$, $p < 0.0001$).

Feeding Deterrent Effects. The 1-butoxy-3-alkoxybenzene library had the lowest DC_{50} value (14.4 μg/cm^2) followed by the 1-isopentoxy-3-alkoxybenzene library (DC_{50} = 19.8 μg/cm^2). Three compounds acted as feeding stimulants to third instar *T. ni* larvae.

Toxic Effects. 1,3-Dipropoxybenzene was the most toxic (**Table 2**) at 0.25% and had a LC_{50} value of 0.16% followed by the 1-propoxy-3-alkoxybenzene library (50% mortality).

Table 3. Bioactivities of 1,2-Dialkoxybenzene Libraries and Analogues against Third Instar *T. ni* Larvae[a]



compound	R_1	R_2	FD (%), mean ± SE (n = 25)	DC_{50}, $\mu g/cm^2$ (r^2)[b] (n = 25)	mortality (%) (n = 3 × 10)	OD (%), mean ± SE (n = 25–33)
1,2-dimethoxybenzene	CH_3	CH_3	26.0 ± 17.6[abcd]	—[c]	—	—
1,2-diethoxybenzene	C_2H_5	C_2H_5	−2.1 ± 18.6[bcd]	—	—	—
1,2-dipropoxybenzene	C_3H_7	C_3H_7	26.2 ± 17.6[abcd]			
1,2-dibutoxybenzene	C_4H_9	C_4H_9	56.4 ± 13.8[abcd]	43.8 (0.90)	10.0	19.6 ± 14.4[a]
1,2-diisopentoxybenzene	C_5H_{11}	C_5H_{11}	69.9 ± 11.5[abc]	19.6 (0.96)	40.0	11.5 ± 13.8[a]
1,2-diallyloxybenzene	C_3H_5	C_3H_5	70.4 ± 13.2[abc]	22.4 (0.99)	20.0	15.0 ± 16.6[a]
Me library (1-methoxy-2-alkoxybenzene)	CH_3	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	23.5 ± 15.9[abcd]	—	—	—
Et library (1-ethoxy-2-alkoxybenzene)	C_2H_5	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	71.0 ± 10.6[ab]	24.1 (0.95)	6.7	28.7 ± 16.4[a]
Pr library (1-propoxy-2-alkoxybenzene)	C_3H_7	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	100.0 ± 0.0[a]	19.4 (0.89)	13.8	31.7 ± 17.4[a]
Bu library (1-butoxy-2-alkoxybenzene)	C_4H_9	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	98.0 ± 1.9[a]	16.8 (0.90)	6.7	28.7 ± 16.7[a]
iPent library (1-isopentoxy-2-alkoxybenzene)	C_5H_{11}	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	66.9 ± 12.7[abc]	32.5 (0.90)	10.0	29.4 ± 16.7[a]
allyl library (1-allyloxy-2-alkoxybenzene)	C_3H_7	CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}	67.8 ± 13.5[abc]	30.0 (0.90)	23.3	66.7 ± 16.9[a]
1-hydroxy-2-allyloxybenzene	H		31.1 ± 15.5[abcd]	—	—	—
1-hydroxy-2-methoxybenzene	H	CH_3	−7.5 ± 19.8[cd]	—	—	—
1-hydroxy-2-butoxybenzene	H	C_4H_9	12.0 ± 20.3[bcd]	—	—	—
1-hydroxy-2-ethoxybenzene	H	C_2H_5	−5.3 ± 19.8[bcd]	—	—	—
1-hydroxy-2-propoxybenzene	H	C_3H_7	−12.0 ± 20.0[e]	—	—	—

[a] Feeding deterrent (FD) effects (mean ± SE) at 50 $\mu g/cm^2$ are expressed in %. DC_{50}s (concentrations causing 50% feeding deterrence compared with the control) were calculated for samples showing >50% feeding deterrency in initial screening (≥50 $\mu g/cm^2$) using Excel; linear regression analysis was conducted for all dose−response experimental data. Mortality and oviposition deterrent effects were determined at 0.25% for samples showing >50% feeding deterrency in initial screening. Means followed by the same letters within a column do not differ significantly (Tukey's test, $p < 0.05$). [b] Coefficient of determination. [c] Not tested.

Oviposition Deterrent Effects. The 1-methoxy-3-alkoxybenzene library demonstrated the strongest oviposition deterrent effect (70.2%) followed by the 1-isopentyl-3-alkoxybenzene library (35.7%) (**Table 2**) when tested at 0.25%. Responses of moths varied significantly in most cases (one-way ANOVA; $F_{6199} = 2.19$, $p < 0.04$).

***o*-Dialkoxybenzene Libraries, Pure Compounds, and 1-Hydroxy-2-alkoxy Compounds.** Six *o*-dialkoxybenzene libraries and three individual compounds exhibited >50% feeding deterrence in initial testing and therefore were subjected to further testing (**Table 3**) as explained above. The response of the larvae to initial screening concentration varied significantly in most cases (one-way ANOVA; $F_{16401} = 5.4$, $p < 0.0001$).

Feeding Deterrent Effects. The 1-butoxy-2-alkoxybenzene library had the lowest DC_{50} value (16.8 $\mu g/cm^2$) followed by the 1-propoxy-2-alkoxybenzene library (DC_{50} = 19.4 $\mu g/cm^2$).

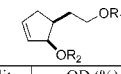
Toxic Effects. None of the *o*-dialkoxybenzene libraries or pure compounds caused >40% mortality at 0.25% (**Table 3**).

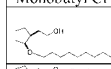
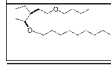
Oviposition Deterrent Effects. The 1-allyloxy-2-alkoxybenzene library demonstrated strong oviposition deterrent activity (66.7%) at 0.25%. All other libraries and compounds had only modest oviposition deterrent activities (**Table 3**) that were not statistically significant (one-way ANOVA; $F_{7205} = 1.07$, $p = 0.38$).

Cyclopentene Ethers 4. Three of six cyclopentene ethers exhibited >50% feeding deterrence in initial screening and therefore were subjected to further testing (**Table 4**). The response of the larvae to initial screening concentration varied significantly in most cases (one-way ANOVA; $F_{8216} = 4.6$, $p < 0.0001$).

Feeding Deterrent Effects. The dibutyl-, dipropyl-, and diisopentylcyclopentene ethers demonstrated similar feeding deterrent effects with DC_{50} values <35 $\mu g/cm^2$. Interestingly, the most volatile (smallest) congeners (dimethyl and diethyl) were not deterrent. Deterrence was high in the medium molecular size range and dropped off for the high molecular mass nonyl/isopentyl ether.

Table 4. Bioactivities of Cyclopentene Ether Libraries and Analogues against Third Instar *T. ni* Larvae[a]



Compound	R_1	R_2	FD (%) Mean ± S. E. (n=25)	DC_{50} µg/cm² (r^2*) (n = 25)	Mortality (%) (n = 3x10)	OD (%) Mean ± S. E (n = 25-33)
Dimethyl CP	CH_3	CH_3	-17.6 ± 16.2[b]	-	-	-
Diethyl CP	C_2H_5	C_2H_5	-17.8 ± 16.8[b]	-	-	-
Dipropyl CP	C_3H_7	C_3H_7	66.7 ± 13.7[a]	32.6 (0.90)	37.5	41.8 ± 15.3[a]
Dibutyl CP	C_4H_9	C_4H_9	76.0 ± 13.3[a]	29.2 (0.89)	30.0	29.5 ± 14.4[a]
Diisopentyl CP	C_5H_{11}	C_5H_{11}	58.6 ± 14.3[a]	34.4 (0.90)	44.8	47.0 ± 15.6[a]
Diol CP	H	H	36.6 ± 17.0[ab]	-	-	-
Monobutyl CP	H	C_4H_9	41.6 + 13.2[ab]	-	-	-
	H	C_9H_{19}	45.2 ± 15.6[ab]	-	-	-
	C_5H_{11}	C_9H_{19}	22.0 ± 19.0[ab]	-	-	-

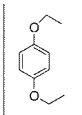
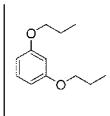
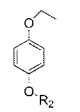
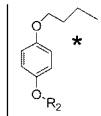
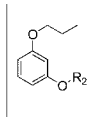
[a] Feeding deterrent (FD) effects (mean ± SE) at 50 $\mu g/cm^2$ are expressed in %. DC_{50}s (concentrations causing 50% feeding deterrence compared with the control) were calculated for samples showing >50% feeding deterrence in initial screening concentration (≥50 $\mu g/cm^2$) using Excel; linear regression analysis was conducted for all dose−response experimental data. Mortality and oviposition deterrent effects were determined at 0.25% for samples showing >50% feeding deterrence in initial screening. Means followed by the same letters within a column do not differ significantly (Tukey's test, $p < 0.05$). *, coefficient of determination. −, not tested.

Toxic Effects. The diisopentylcyclopentene ether was the most toxic, exhibiting 44.8% mortality at 0.25% (**Table 4**).

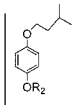
Oviposition Deterrent Effects. Although the diisopentylcyclopentene ether demonstrated the strongest oviposition deterrent effects (47%) followed by the dipropylcyclopentene ether (41.8%) and the dibutylcyclopentene ether (30%) when tested at 0.25% (**Table 4**), they were not statistically significant (one-way ANOVA; $F_{275} = 0.69$, $p = 0.69$).

Comparison of Toxicity, Oviposition, and Feeding Deterrence Values. Toxicity and oviposition deterrence: There was a very slight positive correlation ($y = 0.33x + 18.0$, $R^2 = 0.18$) although there were some strong deterrents that were not toxic in the data set.

Table 5. Summary of the Strongest Feeding and Oviposition Deterrents, Grouped According to Their Contact Toxicity to Third Instar *T. ni* Larvae[a]

Toxic lead compounds and mini libraries					
Feeding deterrency	strong	strong	strong	strong	strong
Oviposition deterrency	strong	moderate	strong	strong	none

R_2 – Me, Et, Pr, isopentyl * moderate toxicity (58% mortality)

Low toxicity lead compounds and mini libraries					
Feeding deterrency	strong	strong	strong	strong	strong
Oviposition deterrency	weak	weak	weak	none	weak
					
Feeding deterrency	strong	strong	strong	moderate	
Oviposition deterrency	moderate	moderate	moderate	strong	

R_2 = Me, Et, Pr, Bu, isopentyl

[a] Compounds with >80% mortality were considered toxic, and compounds with <25% mortality were considered of low toxicity. See Tables 1−3 for the activity data. Strong feeding deterrency, >80%; moderate feeding deterrency, >60%. Strong oviposition deterrency, >50%; moderate, >25%; weak, >10%; none, <10%.

Feeding deterrence and oviposition deterrence: There was no correlation ($y = -0.26x + 48.0$, $R^2 = 0.04$) within the data set.

Feeding deterrence and toxicity: There was no correlation ($y = 0.07x + 77.0$, $R^2 = 0.01$) within the data set.

DISCUSSION

Our results have demonstrated that *p*-dialkoxybenzene libraries and related pure compounds were the most active, with 65% of the members exhibiting feeding deterrence in the range of 53–100% at 50 μg/cm^2. This was followed by the *m*-dialkoxybenzene libraries and related individual compounds. Over half of the members exhibited feeding deterrence in the range of 55–98%. Finally, half of the members in the *o*-dialkoxybenzene libraries and related individual compounds exhibited feeding deterrence in the range of 57–100%. The cyclopentene ethers and analogues were the least active group, with one-third of the members exhibiting feeding deterrence in the range of 59–76% at the initial screening concentration. The presence of a free hydroxyl group, irrespective of the carbon framework or alkyl substituent, served to reduce feeding deterrent effects in all series of compounds. Similarly, the presence of methyl groups, irrespective of the carbon framework or position, also reduced feeding deterrent effects in all libraries. Replacement of a methyl group with larger alkyl substituents increased the feeding deterrent effects in most cases. Further, exceeding a certain group size (butyl, isopentyl) also generally had a detrimental effect. Thus, our best oviposition and feeding deterrent lead compounds from this study tend to be compounds with intermediate (propyl) group sizes.

It is interesting to note that most of these libraries have strong feeding deterrent effects, but only a few have strong deterrent as well as toxic properties. This is especially true for four of the *para* libraries and two of the *meta* libraries. There are two phenomena that contribute to the deleterious effects of a compound on an insect. A compound may have adverse effects because it is not acceptable (deterrent) and the insect is essentially deprived of food, or it may have postingestive or toxic effects (*36*). Deterrence is usually associated with toxicity under ecological conditions, meaning that either the deterrent itself is toxic or that it is associated with a toxin (*37*). However, an unequivocal link between deterrence and toxicity has rarely been demonstrated (*38*). Azadirachtin is one example of a compound possessing both deterrent and toxic properties simultaneously. Our study suggests a lack of a lack of correlation between feeding deterrence and toxic properties for the minilibraries and compounds.

In addition to having feeding deterrent and toxic properties some of these libraries (three *para* libraries, one *meta* library, one *ortho* library, and one cyclopentene ether library) also possess strong oviposition deterrent effects against *T. ni* moths. All others possess medium to low oviposition deterrent effects. Our study indicates a lack of correlation between feeding deterrence and oviposition deterrence as reported by others (*39, 40*). However, some plant extracts and pure allelochemicals may possess both feeding and oviposition deterrent effects (*32, 33, 41, 42*). There was a positive correlation between toxicity and oviposition deterrence in the minilibraries and compounds. Seed and leaf extracts of *Virex negundo* possess both toxic and oviposition deterrence effects against *Plutella xylostella* (*41*). Therefore, it can be assumed that toxicity plays an important role in predicting host-plant choice and not the deterrent properties of chemicals in a plant. The presence of toxic compounds usually signals unsuitability of a plant, resulting in rejection behavior that prevents the female moth from ovipositing and protecting her offspring from the toxins that are likely to be encountered in the plant.

Using minilibraries in insect behavioral screening studies has two advantages. First, minilibraries would prevent the insects from developing resistance or habituation as they contain mixtures of compounds (*43*). Plant defense chemicals that exhibit more than one mode of action are most suitable for crop protection (*44*), constituting a "multichemical defense" against a variety of potential herbivores. Second, using some individual compounds and minilibraries that have been systematically varied and have some overlapping compounds, we can obtain some structure–function data, without having to test every compound individually. This approach minimizes assay times while still allowing the exploration of many compounds.

Our results suggest both antifeedant activity, causing a reduction in food consumption, for most of the libraries tested and contact toxicity for some. Both actions can reduce growth and increase development time and likely expose herbivores to increased mortality in the field as a result of biotic and abiotic factors (*45*). Our study strongly suggests that some of these libraries and individual compounds have potential for development as commercial insecticides. However, their impact on beneficial organisms and environmental fates needs to be determined.

Future studies will focus on detailed investigations of the effects of selected libraries and analogues on feeding and oviposition behaviors as well as the toxic effects against other agricultural pests in the laboratory and greenhouse. Selected libraries might also be useful in "push−pull" or stimulodeterrent diversionary strategies (*46*) for crop protection based on their feeding/oviposition deterrent and feeding stimulant properties. In this case the "push" can come from an antifeedant (**Table 5**)

or an oviposition deterrent [1,4-diethoxybenzene (**Table 1**) and many others (**Tables 2**, **3**, and **4**)] applied to the crop needing protection, while the "pull" can come from an attractant [1-hydroxy-4-propoxybenzene, 1,3-diisopentoxybenzene (**Tables 1** and **5**), and many others (**Tables 2**, **3**, and **4**)] applied to an adjacent trap crop or trap rows of the main crop. As a result of this behavioral manipulation, a mobile adult insect would likely abandon an otherwise suitable host plant for feeding and oviposition and move to another potential host plant some distance away.

Since our minilibraries and compounds possess one or more deterrence or toxicity activities, we could eventually utilize them in a more targeted manner than conventional insecticides, rendering our lead compounds more effective as crop protectants. To protect the tender, more valuable upper leaves of a crop, these can be sprayed with an antifeedant (**Table 5**) and the lower parts, where leaf damage can be tolerated, with a toxic compound (**Table 5**) (*47*). Under this treatment regime, the target insect will be driven to the lower leaves and suffer mortality after coming in contact with the toxic compound. We can choose a highly toxic compound or a less toxic compound depending upon the situation.

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