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IDENTIFICATION OF 1-OCTACOSANAL AND 6-METHOXY-2-BENZOXAZOLINONE FROM WHEAT AS OVIPOSITIONAL STIMULANTS FOR HESSIAN FLY, *Mayetiola destructor*

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Abstract—Bioassay-guided fractionations of a dichloromethane extract of wheat epicuticular wax allowed the identification of 1-octacosanal and 6-methoxy-2-benzoxazolinone (MBOA) as the major components that stimulate oviposition by the Hessian fly, *Mayetiola destructor*. These compounds were identified by their mass spectral fragmentation patterns and by comparison of their gas chromatographic retention times with synthetic samples. Synthetic samples of 1-octacosanal or MBOA stimulated significant oviposition when compared with solvent controls. In combination, these compounds elicited a synergistic effect on the number of eggs laid by females compared to when they were presented alone. In a choice bioassay, a mixture of synthetic 1-octacosanal and MBOA in the approximate concentrations determined to be present in one plant equivalent of crude extract stimulated the same amount of oviposition as one plant equivalent of extract. This showed that together these two compounds appear to be responsible for the major proportion of the ovipositional stimulatory activity of the wheat epicuticular wax extract. Comparison of the activity of five straight-chain primary aldehydes with chain lengths from C_{22} to C_{30} revealed a relationship between chain length and the number of eggs laid by female Hessian flies, with 1-hexacosanal and 1-heptacosanal the most active of the aldehydes tested.

Key Words—Cecidomyiidae, insect behavior, egg-laying, chemoreception, MBOA, Gramineae.

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INTRODUCTION

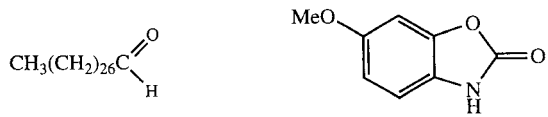
The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a major pest of wheat (*Triticum aestivum* L.) in North America and North Africa and, to a lesser extent, in Europe and New Zealand (Barnes, 1956; Ratcliffe and Hatchett, 1997). Hessian fly females oviposit on the surface of wheat leaves and the larvae move down to feed near the crown of the plant. In addition to wheat, Hessian flies are known to oviposit and develop on rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), and a number of wild grasses in the genera *Elymus*, *Aegilops*, *Hordeum*, and *Agropyrum* (Barnes, 1956; Harris et al., 1996). However, females oviposit less on oat (*Avena sativa* L.) and larvae do not survive on this grass (Morrill, 1982). As Hessian fly larvae have limited mobility and are incapable of moving between plants, the choice of oviposition site by the adult female is critical to larval survival.

Previous studies have shown that the selection of oviposition sites by Hessian flies is influenced by visual and tactile cues and by epicuticular leaf waxes (Harris and Rose, 1990; Foster and Harris, 1992). In particular, preference for the host grass wheat over the nonhost grass oat can be largely explained by the oviposition preferences towards their epicuticular wax extracts. Fractionation of wheat extracts on silica gel indicated that there were at least two different compounds in the epicuticular wax of wheat that stimulated oviposition by Hessian fly females (Foster and Harris, 1992).

In this paper we report the identification of two compounds, 1-octacosanal (**1**) and 6-methoxy-2-benzoxazolinone (MBOA) (**2**) (Figure 1), from wheat that together appear to be responsible for most of the ovipositional stimulatory activity of wheat leaf wax extract on the Hessian fly.

METHODS AND MATERIALS

Insects. Hessian fly females used in experiments were from a culture in Auckland maintained on wheat plants. This culture originated from pupae collected in 1994 from a wheat field in Palmerston North, New Zealand. Adults emerging from these pupae were left to mate, and females subsequently were



1 **2**

FIG. 1. Chemical structures of 1-octacosanal (**1**) and 6-methoxy-2-benzoxazolinone (**2**).

allowed to oviposit on wheat plants (cultivar Otane) in the two- to three-leaf stage. Infested plants were held in a glasshouse (temperatures from 15 to 25°C) where they were watered and fertilized (Hoagland's solution) until Hessian fly puparia were observed (30–50 days after infestation). Plants were then moved to a controlled environment chamber (21 ± 0.5°C, 50–70% relative humidity, 14L : 10D, with lights on at 07 : 00 hr) until adult flies started to emerge some 2–10 days later. This Hessian fly culture was reared in this manner for four years, at a rate of five to seven generations a year.

For experiments, mated females were collected daily by aspirator shortly after the hours of peak female eclosion and mating (07 : 00–10 : 00 hr). Mated females were distinguished from virgin females by observing whether or not they exhibited the characteristic calling posture (Bergh et al., 1992). Mated females were transferred to experimental cages before they began to oviposit (before 11 : 00 hr) (Harris and Rose, 1991).

Wheat Extract. Wheat plants (cultivar Otane) were grown in a glasshouse (ambient temperature 15–25°C) during September 1998. Plants (approx. 5000) at the two- to three-leaf stage (11 days after planting) were cut at the base to give 645 g of fresh material. Extracts were prepared by dipping the leaves into cool (10°C) dichloromethane (500 ml for 1000 plants) for 60 sec after the method of Bianchi and Figini (1986). The solvent was removed from the combined crude extracts by rotary evaporation at 37°C in a Buchi Rotavapor-R rotary evaporator, to give 0.524 g of residue.

Trimethylsilylation of Wheat Extract. Wheat plants (cultivar Otane) were grown as above during August 1999. Plants (25, 7.0 g fresh weight) were extracted by dipping the leaves into ethyl acetate (20 ml) for 60 sec. The solvent was removed by rotary evaporator at 37°C. Dichloromethane (0.5 ml) was added and evaporated (repeated twice) to remove residual water. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (Sigma Chemical Co., St. Louis, Missouri) (50 μl) was added to the residue (within 15 min of extraction) and allowed to react for 30 min at 65°C. This solution was analyzed directly by gas chromatography–mass spectrometry (GC-MS).

Fractionation. The wheat dichloromethane extract was separated by flash column chromatography on silica gel (120 g; Merck Kieselgel 60 Å, 230–400 mesh) with the following solvent gradient: 240 ml petroleum spirit (fraction 1), 2 × 240 ml petroleum spirit–diethyl ether (9 : 1) (fractions 2 and 3), 2 × 240 ml petroleum spirit–diethyl ether (4 : 1) (fractions 4 and 5), 2 × 240 ml petroleum spirit–diethyl ether (1 : 1) (fractions 6 and 7), 240 ml petroleum spirit–diethyl ether (2 : 3) (fraction 8), 240 ml petroleum spirit–diethyl ether (1 : 4) (fraction 9), 2 × 240 ml diethyl ether (fractions 10 and 11), and 240 ml ethanol + 1% acetic acid (fraction 12). Fraction 10 (28 mg dry weight) from this column was further separated by gel permeation chromatography on Sephadex LH-20 (45 g;

Pharmacia Biotech) with methanol–dichloromethane (1 : 1) as the mobile phase to give 14 fractions, one each of 50 ml (fraction 1), 30 ml (fraction 2) and 20 ml (fraction 3) and 11 of 10 ml (fractions 4–14). Solvents were analytical grade (Analar, BDH Laboratory Supplies) and were distilled prior to use.

Synthetic Compounds. Synthetic samples of MBOA (**2**), 1-docosanol, 1-hexacosanol, 1-heptacosanol, 1-octacosanol, and 1-triacontanol were obtained from Sigma Chemical Co. The straight-chain primary alcohols (7–10 mg of each) were oxidized with pyridinium chlorochromate by the method of Corey and Suggs (1975) to give the corresponding aldehydes: 1-docosanal, 1-hexacosanal, 1-heptacosanal, 1-octacosanal (**1**), and 1-triacontanal. These were purified by passage through a silica gel (1 g) column with a petroleum spirit–diethyl ether (9 : 1) eluent.

Analytical Methods. Gas chromatography (GC) was performed with a Hewlett Packard 6890 gas chromatograph with splitless injection and a flame ionization detector. The column used was a Zebron ZB-1 capillary column (15 m × 0.32 mm ID × 0.10-μm film thickness) (Phenomenex, Torrance, California). Helium was the carrier gas at a linear velocity of 30 cm/sec. The oven was programmed from 80 to 280°C at 20°C/min with a 1-min initial delay and a 10-min final hold time. The retention times of compounds in fractions were compared with the retention times of standard compounds, including long-chain *n*-alkanes, alcohols, and aldehydes. A Hewlett Packard 5890 gas chromatograph coupled to a VG 70-SE mass spectrometer with the same temperature program and column as above was used for GC-MS analysis of extracts. The mass spectrometer was run in the electron ionization mode.

Merck silica gel 60 F_{254} glass-backed plates (E. Merck, Darmstadt, Germany) were used for analytical thin-layer chromatography (TLC). TLC plates were developed with dichloromethane–ethanol (99 : 1) (Tulloch and Hoffman, 1971). They were sprayed with rhodamine G (Sigma Chemical Co.) and visualized under 254 nm UV light.

Bioassays. The bioassay used was that described by Harris and Rose (1990). Briefly, an extract, fraction, or synthetic chemical was dissolved in dichloromethane, and 60 μl of the solution was applied to the top two thirds of a grass leaf model consisting of 10-cm × 0.5-cm Whatman No. 2 filter paper strips treated with Hansell's yellow food coloring (Hansells Ltd., Masterton, New Zealand). A leaf model treated with 60 μl of dichloromethane served as a control. Each filter paper strip was attached by its untreated end to a metal clip and positioned vertically in cages (20 cm diameter × 13 cm high, blue card sides, screen top, damp sand floor). Treated leaf models were placed in the cage in random order in a grid formation that maximized the distances between strips and between strips and the cage wall. Cages were held in a temperature-controlled room (22 ± 0.5°C) under a light source consisting of eight fluorescent tubes (Biolux-B, NEC). After models were set up (11 : 00–12 : 00 hr), 15 mated

females were placed in each cage. At 15 : 00 hr, the females were removed and the number of eggs on each model counted by examination under a microscope. Extracts or fractions from column chromatographic separations were tested at 4 plant equivalents (PE).

Statistical Analysis. Data from each experiment were first checked for homogeneity of variance and normality by O'Briens test. If they failed to meet these constraints, data were log-transformed, which in all cases allowed them to meet the requirements for analysis by ANOVA. Data were analyzed by ANOVA, and means compared either by Dunnett's test for comparison with the solvent control, or by Student's *t* test, unless otherwise stated. For the experiments with a factorial design, the data were log-transformed and analyzed by the computer program JMP (SAS Institute, 1995), with amounts of the two compounds as the main effects.

RESULTS

Fractionation of Wheat Extract and Identification of Compounds in Active Fractions. The wheat leaf extract was separated by flash column chromatography on silica gel to give 12 fractions. These were tested in a choice bioassay to determine the ovipositional stimulatory activity of each. When the log-transformed data were compared with the solvent control by Dunnett's test, five fractions had significantly greater numbers of eggs laid on them than the control. These fractions were clustered into two groups: one at fraction 3, and the other consisting of fractions 8–11. In the second group, fraction 10 received the greatest number of eggs (Figure 2). GC analysis of fraction 3 (130 mg dry weight) showed one major peak with a retention time similar to that of 1-octacosanal (**1**), and a series of minor peaks corresponding to odd-numbered, C_{23}–C_{33} straight-chain hydrocarbons. The identity of compound **1** was confirmed by GC-MS analysis and comparison of its GC retention time with that of the synthetic compound as 1-octacosanal (diagnostic ions, m/z 408, 390, 380).

GC analysis of fractions 9, 10, and 11 showed them to have similar chromatographic profiles, with a large peak with a retention time similar to that of 1-octacosanol, along with an unidentified peak at 6.6 min. Fractions 6, 7, and 8 also contained a peak corresponding to 1-octacosanol and a smaller peak corresponding to 1-hexacosanol, but no peak at 6.6 min. As the greatest number of eggs were laid on models treated with fraction 10 out of this group, further chemical analysis was confined to this fraction. TLC analysis of fraction 10 showed several spots; one at R_f = 0.14 was visible under UV light.

Fraction 10 from the silica gel column separation was further separated by gel permeation chromatography on Sephadex LH-20 to give 14 fractions. These were tested in the bioassay, which showed that the activity had been concentrated

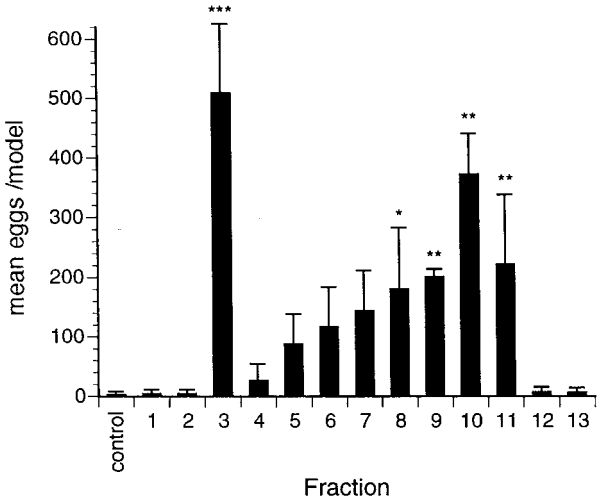


FIG. 2. Mean numbers of eggs laid by female Hessian flies on filter paper leaf models treated with one of fractions 1–12 from a silica gel column chromatographic separation of a dichloromethane extract of wheat leaves (4 PE/model). Models treated with dichloromethane only were the control. Means with asterisks are significantly different from the control (Dunnett's test on log-transformed data, $^{*}0.05 > P > 0.01$, $^{**}0.01 > P > 0.001$, $^{***}P < 0.001$). Each mean was calculated from four replicates. Bars are standard errors of the means. Total eggs over four replicates = 7521.

in fractions 12 and 13 from this column (2.7 mg combined dry weight). When analyzed by GC, these fractions contained the compound with a retention time of 6.6 min and again showed a TLC spot at R_f = 0.14. Analysis by GC-MS of fractions 12 and 13 from the Sephadex LH-20 separation showed that the compound with a retention time of 6.6. min was 6-methoxy-2-benzoxazolinone (MBOA) (**2**). The mass spectrum gave ions at m/z (%) 165 (100), 150 (48), 122 (9), 106 (30), 80 (11), 52 (16), and 39 (12), in agreement with the data of Lyons et al. (1988). This was confirmed by comparison of the GC retention time and TLC R_f value with those of a synthetic sample of this compound.

Trimethylsilylated Wheat Extract. The GC-MS chromatogram of the trimethylsilyl (TMS)-derivatized wheat extract contained prominent peaks for MBOA–TMS. The mass spectrum showed ions at m/z (%) 237 (72), 194 (100), 73 (40), and the TMS ether of 1-octacosanol, along with several minor peaks for trimethylsilylated long-chain carboxylic acids, alcohols, and furanosides. There were no detectable peaks present for underivatized MBOA or for the TMS derivative of 2,4-dihydroxy 7-methoxy-2*H*-1,4-benzoxazin-3-one (DIMBOA-TMS_2) [mass spectrum in Atkinson et al. (1991)].

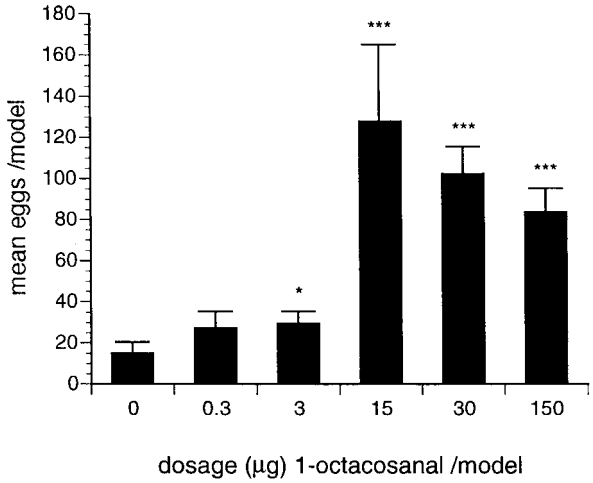


FIG. 3. Dose–response data for numbers of eggs laid by female Hessian fly on filter paper leaf models treated with one of five different amounts of 1-octacosanal (**1**). Models treated with dichloromethane only were the control. Means with asterisks are significantly different from the control (Dunnett's test on log-transformed data, $^{*}0.05 > P > 0.01$, $^{***}P < 0.001$). Each mean was calculated from 10 replicates. Bars are the standard error of the mean. Total eggs over 10 replicates = 3860.

*Dose–Response Profiles for Compounds **1** and **2**.* Compound **1** was bioassayed at 0.3, 3, 15, 30, and 150 μg/model (Figure 3). Comparison of log-transformed data with the control by Dunnett's test revealed that the 3-, 15-, 30-, and 150-μg treatments all had significantly ($P < 0.05$) more eggs laid on them than the control. The number of eggs laid on models treated with 15, 30 and 150 μg/model were not significantly different from each other ($P < 0.05$, Student's t test). This dose–response pattern showed a leveling off of activity above a concentration around 15 μg/model.

Compound **2** was bioassayed at 0.3, 1, 3, 10, and 30 μg/model. The data showed a trend of increasing numbers of eggs laid on treatments with higher dosages of compound **2** (Figure 4), with no apparent plateau over the range tested. Use of Dunnett's test showed that the mean numbers of eggs laid on the 3-, 10-, and 30-μg treatments were all significantly ($P < 0.05$) greater than that for the solvent control. A linear regression analysis showed a linear relationship between the mean number of eggs laid (y) and the amount of compound **2** in micrograms (x) applied to each treatment [$y = 5.64\ (\pm 0.1)\ x + 21.3\ (\pm 1.4)$; $R^2 = 0.999$; $F_{1,4} = 2764.1$, $P < 0.0001$].

*Combinations of Compounds **1** and **2**.* It was estimated from the GC peak areas and the weights of fractions containing compounds **1** and **2** that 1 PE of

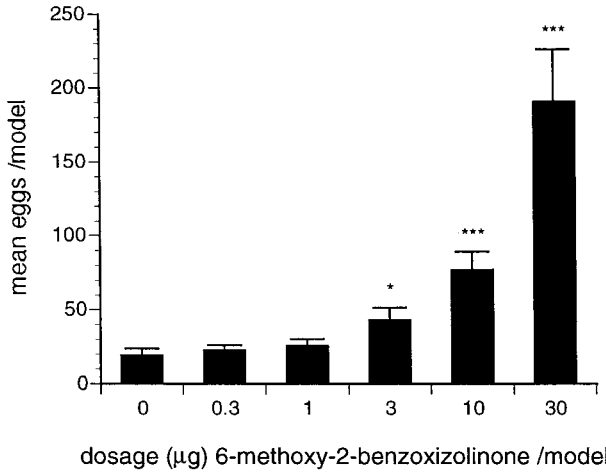


FIG. 4. Dose–response data for number of eggs laid by female Hessian fly on filter paper leaf models treated with one of five different amounts of 6-methoxy-2-benzoxazolinone (**2**). Models treated with dichloromethane only were the control. Means with asterisks are significantly different from the control (Dunnett's test on log-transformed data, $^{*}0.05 > P > 0.01$, $^{***}P < 0.001$). Each mean was calculated from 10 replicates. Bars are the standard error of the mean. Total eggs over 10 replicates = 3779.

crude extract contained approximately 35 μg of compound **1** and 1 μg of compound **2**. In a three-choice bioassay with a solvent control, a mixture of synthetic **1** and **2** in these amounts, and 1 PE of extract, there was no significant difference in the mean number of eggs laid on either of the two treatments (Figure 5). Both had significantly greater numbers of eggs laid on them than the control.

*Combinations of Compounds **1** and **2**—Factorial.* The first factorial-design bioassay tested combinations with or without compound **1** (15 μg or 0 μg) or compound **2** (1 or 0 μg) (Figure 6a). The main effect of compound **1** was highly significant ($F = 73.8$, $P < 0.0001$), as was the main effect of compound **2** ($F = 30.5$, $P < 0.0001$). There was a significant interaction between the two main effects ($F = 8.08$, $P = 0.007$). The second experiment, in which the amount of compound **1** was the same as in the first experiment (15 or 0 μg) and the amount of compound **2** was increased (10 or 0 μg), gave a similar result, with both main effects being highly significant ($F = 126.6$, $P < 0.0001$ and $F = 52.3$, $P < 0.0001$, respectively, for compounds **1** and **2**), and again a significant interaction between the two effects ($F = 11.2$, $P = 0.002$) (Figure 6b). Finally, the third experiment, which had treatments with the higher dosage of compound **2** (10 or 0 μg) and lower dosages of compound **1** (3 or 0 μg), showed a significant effect for compound **1** ($F = 19.9$, $P < 0.0001$) and for compound **2** ($F = 9.45$,

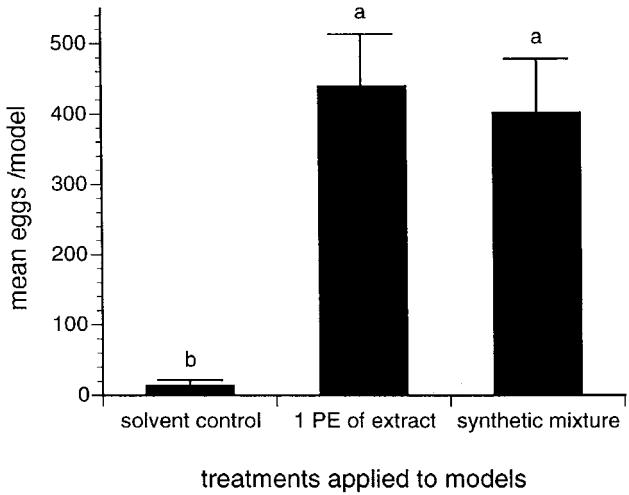


FIG. 5. Mean numbers of eggs laid by female Hessian flies on filter paper leaf models treated with 1 PE of a dichloromethane wheat extract, a mixture of synthetic compounds **1** (35 μg) and **2** (1 μg) or a solvent control. Means with different letters are significantly different at $P < 0.001$ when compared using Student's t test on log-transformed data. Each mean was calculated from four replicates. Bars show standard errors of the means. Total eggs over four replicates = 3419.

$P = 0.004$) (Figure 6c). However, in this experiment there was no significant interaction between the two main effects ($F = 1.65$, $P = 0.2$). Thus, in the first two experiments, the combination of compounds **1** and **2** had a synergistic effect on egg laying, while in the third experiment, the effect was additive.

Structure–Activity Relationship of Straight Chain Primary Aldehydes. The primary aldehydes, 1-docosanal, 1-hexacosanal, 1-heptacosanal, 1-octacosanal (**1**), and 1-triacontanal were compared in a choice bioassay at a dosage of 30 μg (Figure 7). Relative to the solvent control, all of these compounds elicited significant numbers of eggs to be laid by females (Student's t test, $P < 0.05$). In this series, the numbers of eggs laid on leaf models treated with 1-hexacosanal or 1-heptacosanal were greater than the numbers laid on models treated with (**1**) or 1-triacontanal. This indicated that the numbers of eggs laid by females was dependent on aldehyde chain length, with maximum oviposition induced by aldehydes with chain lengths of C_{26} and C_{27}.

Activity of 1-Octacosanol. 1-Octacosanol was the most abundant component in our wheat leaf wax extract, with each plant containing approximately 70 μg. In a choice bioassay, consisting of models treated with 0 (solvent control), 3, 30, 150, or 300 μg of this compound, there was no significant difference (Dun-

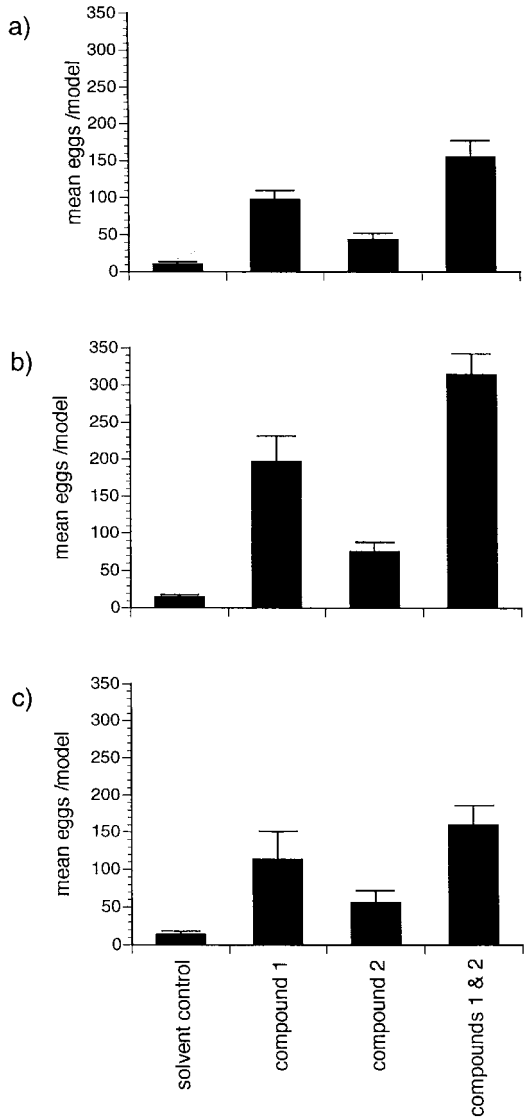


FIG. 6. Mean numbers of eggs laid by female Hessian flies in factorial-design experiments with filter paper leaf models treated with compound **1**, compound **2**, a mixture of compounds **1** and **2**, or dichloromethane-only: (a) 15 or 0 μg of **1**, 1 or 0 μg of **2**; (b) 15 or 0 μg of **1**, 10 or 0 μg of **2**; (c) 3 or 0 μg of **1**, 10 or 0 μg of **2**. The analysis of the data is given in the text. Each mean was calculated from 10 replicates. Bars show standard errors of the means. Total eggs over 10 replicates: (a) = 3074, (b) = 6013, (c) = 3431.

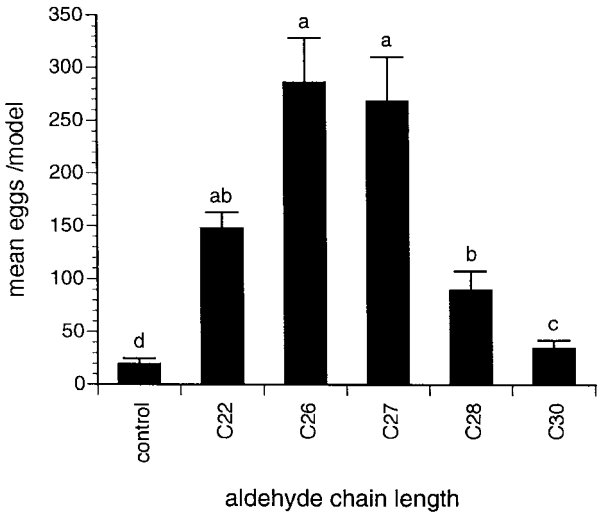


FIG. 7. Mean numbers of eggs laid by female Hessian flies on filter paper leaf models treated with one of five straight-chain primary aldehydes. Models treated with dichloromethane only were the control. Means with different letters are significantly different at $P < 0.05$ when compared by Student's t test on log-transformed data. Each mean was calculated from 10 replicates. Bars show standard errors of the means. Total eggs over 10 replicates = 8457.

nett's test, $P < 0.05$) between egg numbers on the solvent control model and treated models (Figure 8).

DISCUSSION

In this study we identified two compounds from the epicuticular wax of wheat leaves that stimulate oviposition by Hessian flies: 1-octacosanal (**1**) and 6-methoxy-2-benzoxazolinone (MBOA) (**2**). When applied to leaf models, a mixture of these compounds, containing approximately the amounts in one PE of a dichloromethane extract of wheat leaves, stimulated female Hessian flies to lay the same number of eggs as did one PE of crude extract. This suggests that these two compounds accounted for a high proportion of the stimulatory activity of the leaf extract.

The leaf waxes of various wheat varieties have been the subject of considerable investigation (Tulloch and Hoffman, 1971, 1973; Bianchi and Corbellini, 1977). In general, alcohols are the most abundant class of compound in wheat leaf waxes, with octacosanol typically the most abundant alcohol (Tulloch and Hoffman, 1973; Bianchi and Corbellini, 1977; Bianchi and Figini, 1986; Bianchi

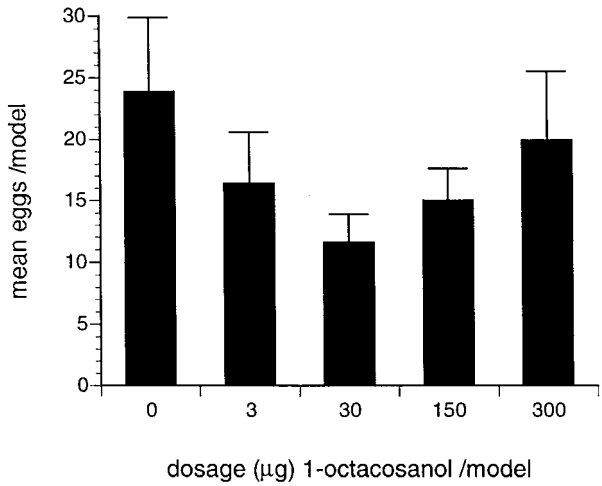


FIG. 8. Dose–response data for numbers of eggs laid by female Hessian fly on filter paper leaf models treated with one of four different amounts of 1-octacosanol. Models treated with solvent only (dichloromethane) served as controls. None of the treatment means were significantly different from the control mean (Dunnett's test on log-transformed data). Each mean was calculated from 10 replicates. Bars show standard errors of the mean. Total eggs over 10 replicates = 860.

et al., 1980). However, octacosanol, at the level found in our wheat extracts did not stimulate oviposition by Hessian fly. Aldehydes, predominantly **1**, are less abundant than alcohols in wheat waxes (Bianchi and Corbellini, 1977; Bianchi and Figini, 1986), with the proportion of aldehyde in the wax decreasing as the plant matures. Moreover, aldehydes are relatively more abundant in younger wheat leaves than in more mature ones (Bianchi et al., 1979). This corresponds with the observed preferences of female Hessian flies (Harris and Rose, 1989), suggesting that levels of **1** in the wax may be a factor in the ovipositional preference of female flies for young over older leaves.

When we compared the egg-laying responses of females to aldehydes with different chain lengths, their responses were not greatest to **1**, but to 1-hexacosanal and 1-heptacosanal, which we did not detect in our wheat extracts. However, 1-hexacosanal may comprise up to 20% of the aldehyde complement in the leaf wax of certain wheat cultivars (Bianchi and Figini, 1986) and is the major aldehyde of both rye (Bianchi, 1985) and barley (von Wettstein-Knowles, 1974). From our results, it is apparent that the chain length, as well as the abundance of the aldehydes present in a leaf wax, influences Hessian fly oviposition preferences for different wheat varieties and grasses.

Benzoxazolinones are the decomposition products of 1,4-benzoxazin-3-

ones, which have been isolated from wheat, maize (*Zea mays*), rye, and species in several other genera in the Gramineae, but few species outside this family (Niemeyer, 1988). In solution, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) decomposes to give MBOA with release of formic acid (Bravo and Niemeyer, 1985). DIMBOA is the major hydroxamic acid found in wheat tissue (Zúñiga and Massardo, 1991), with MBOA, apparently present in 100-fold lower concentration (Leszczynski and Dixon, 1990). To determine whether the MBOA identified in our dichloromethane extracts originated from wheat leaf wax or from decomposition of DIMBOA during storage and fractionation, we derivatized an ethyl acetate extract with BSTFA to give TMS derivatives. The BSTFA was added within 15 min of extraction, before significant decomposition of DIMBOA to MBOA could occur (Bravo and Niemeyer, 1985). GC-MS analysis of the derivatized extract showed a peak for MBOA–TMS, but lacked a detectable peak for DIMBOA–TMS_2, indicating that MBOA is relatively more abundant in the leaf wax of wheat.

Following the finding that MBOA is not involved in host location by western corn rootworm larvae (Bjostad and Hibbard, 1992; Bernklau and Bjostad, 1998), this appears to be the first report of a benzoxazolinone being used in host identification by an insect.

MBOA (**2**) and DIMBOA, its precursor in plants, are known to be toxic and have antifeedant properties to many species of insects (see examples in Bjostad and Hibbard, 1992). Nothing is known about the toxicity of these compounds to Hessian fly larvae. MBOA also has some, albeit limited, volatility. The host selection behavior of the female Hessian fly is influenced by volatile compounds from wheat leaf wax extracts (Foster and Harris, 1992; Rani and Harris, unpublished). Whether MBOA (**2**) influences host selection by female Hessian flies over a distance or only upon contact will be the subject of behavioral studies.

Our results from the wheat cultivar Otane are in accord with a previous, preliminary investigation into the oviposition stimulants in the epicuticular leaf wax of the wheat cultivar Newton (Foster and Harris, 1992). Fractionation of the leaf waxes of Newton on silica gel also resulted in two active fractions, eluting with solvents of similar polarity to those of the Otane extract. Given that the leaf waxes of different wheat varieties largely contain the same compounds (Bianchi and Corbellini, 1977; Bianchi et al., 1980; Bianchi and Figini, 1986), it is reasonable to assume that the activity in the two fractions of Newton was due, at least in part, to the same two compounds, or class of compounds, as in Otane. However, in Newton, as opposed to Otane, the later-eluting active fraction had approximately equal activity to the earlier fraction. This suggests that Newton wax contained relatively higher amounts of benzoxazolinone(s) compared to aldehyde(s) than did Otane. Such variation could be the basis for a chemical mechanism for the differential preferences of the female Hessian fly toward different wheat varieties (Harris et al., 1996).

In the previous study of the cultivar Newton, the combination of the two most active fractions had a large synergistic effect on egg-laying. In the present study a similar synergistic effect on Hessian fly egg-laying responses was apparent when compounds **1** and **2** were combined. The magnitude of this effect was dependent on the amounts of these two compounds applied to leaf models and was greater for treatments with a larger amount of compound **2** (amount of compound **1** kept constant). However, if the level of compound **1** was below that which was active when tested alone in the dose–response experiment, a synergistic effect was not apparent. The larger synergistic effect observed with the combination of fractions of Newton extract may have been due to differing concentrations of one or both of these compounds, the presence of other, unidentified stimulatory compounds, or to differences in host chemical preferences between the New Zealand and Kansas strains of Hessian fly that were used in the two studies.

The identification of these compounds should be useful for assessing the host preferences of female Hessian flies toward different wheat varieties and could be of value in wheat breeding programs aiming to breed varieties resistant to the Hessian fly. Currently, antibiosis is the major mechanism selected for when breeding Hessian fly resistant wheat. A possible alternative mechanism for resistance to Hessian fly is antixenosis, which could be achieved by selecting for low production of these two compounds. The levels of these compounds in different wheat varieties and hosts will be explored in future studies.

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