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Ovipositional Responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) to Natural Products from Leaves of Two Maize (*Zea mays* L.) Cultivars

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Ovipositional responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) to hexane extracts of leaves of two maize (*Zea mays* L.) cultivars, one resistant (Kisan) and one susceptible (Basilocal), were studied in two-choice bioassays. Gravid females laid a significantly higher percentage of eggs on substrates smeared with extract of Basilocal leaves (HEBL) (69%) than on those smeared with extracts of Kisan leaves (HEKL) (31%). Several chemicals were isolated from HEKL, three of which were characterized as dotriacontanol, heptadecanol, and nonadecanol. These chemicals were either absent or were present in very small amounts in HEBL, but in HEKL they were detected in much larger amounts. Each isolated chemical was tested for its effect on *C. partellus* oviposition in two-choice bioassays. Maximum ovipositional deterrence (90%) was observed for the compound MR-22a, followed in decreasing order by nonadecanol, MR-7, and heptadecanol. The identity of the remaining compounds is being investigated. The results indicate that the relative resistance of Kisan maize compared to Basilocal is partly due to the presence of certain ovipositional deterrents in its leaves.

KEYWORDS: *Chilo partellus*; Ovipositional deterrence; *Zea mays*; dotriacontanol; heptadecanol; nonadecanol

INTRODUCTION

Maize, *Zea mays* (L.) is one of the most important cereal and fodder crops of the world. One of the major constraints to the production of maize is the attack by various insect pests, especially stem borers. The spotted stem borer, *Chilo partellus* (Swinhoe) is an important insect pest of maize and sorghum in various parts of Asia and Africa (*1*), one whose larvae cause extensive damage. It has been estimated that, in India, the loss in maize yield due to stem borers may exceed Rs. 1100 million (=US $22 million) annually (*2*). The infestation of maize plants by the *C. partellus* larvae causes about 50.3% loss of maize annually in India (*3*).

Maize genotypes differ in their susceptibility and resistance to *C. partellus* (*4*−*6*), and the degree of susceptibility of a plant to an insect species depends on the degree of its suitability for its colonization by the insect. Selection of plant surface for oviposition by females is one of the factors that determine the level of infestation and subsequent damage to plants by its progeny. *C. partellus*, being an oligophagous insect, is capable of assessing the suitability of a plant as its host by recognizing the physical and chemical characteristics of the phylloplane.

Several workers have attempted to measure relative susceptibility and resistance of maize genotypes to *C. partellus* by comparing its oviposition on these genotypes (*7*−*10*). One such cultivar of maize, Kisan, has been reported to be resistant to *C. partellus* as compared to Basilocal (*11*, *12*). Significant differences in oviposition by stem borer on the foliar surface of these cultivars have been observed in one of our previous studies (unpublished work) and the same may be due to differences in their texture and/or contact chemical stimuli. The present work continues research to investigate the causes for the quantitative difference in oviposition by *C. partellus* on Kisan and Basilocal foliage surface, to better understand their relative resistance.

MATERIALS AND METHODS

Insects and Plant Material. Specimens of *C. partellus* were obtained from laboratory culture reared until pupation on the artificial diet formulated according to previous description (*13*). The insectary was maintained at a temperature of 27 ± 4 °C, a lighting schedule of 16h light/8h dark, and relative humidity ranging between 50 and 70%. Pupae were removed from the rearing containers and kept in the ratio of 4 males/3 females in individual eclosion jars (12 cm ht × 10 cm diam); the bottom surface was lined with Whatman filter paper. After eclosion, a pair of male and female moths was kept in separate jars (7 cm ht × 7 cm diam) for oviposition. A cotton swab, soaked in distilled water, was placed in each jar to enable drinking by the moths. The

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next morning, females that had laid eggs were selected for oviposition bioassays to be used in the evening. Each test was replicated five times, and each replicate consisted of the average number of eggs laid by three females. Only those bioassays in which the eggs attained the "blackhead stage" were considered as valid replicates and were selected for statistical analysis.

Seeds of maize cultivars, Basilocal (susceptible) and Kisan (resistant), were obtained from the Indian Agricultural Research Institute (IARI), New Delhi. These were sown in experimental plots of the Zoology Department, University of Delhi. The plants were raised in pesticide-free conditions, under standard farming practice.

Extraction of Plant Foliage Chemicals. Fully expanded, freshly excised young leaves, located on the lower half of 3−5 week old maize plants, which is the preferred site for oviposition by *C. partellus* moths (*7*), were used for extraction. From each test cultivar (Basilocal and Kisan), 500 g of leaves were soaked overnight in 2.5 L of hexane. The extracts were then decanted. The leaves and jars were rinsed three times with 500 mL of hexane each time. The pooled extracts were concentrated to near dryness in vacuo and the residue was redissolved in hexane to prepare a solution containing 1gfle (1 g of fresh leaf extract) per mL of the extract. The extracts were stored at 4 °C until workup.

Isolation and Characterization of Active Components from Hexane Extract of Kisan Leaves (HEKL). The solvent free extract from Kisan leaves was found to be a mixture of several components of varying polarity on TLC and was, therefore, further fractionated by column chromatography. The column was prepared in petrol using silica gel (60−80 mesh) as an adsorbent and eluted with petrol/chloroform mixtures of increasing polarity. Thirteen fractions were collected, and the following compounds were isolated.

Compound **1** was obtained from the fractions eluted with petrol/chloroform (70:30). On treatment with acetic anhydride and dry pyridine, it yielded an acetate (compound **1a**). Compounds **2** and **3** were isolated from the crude extract by column chromatography using petrol/chloroform (10:90) as the eluent.

Characterization of Compounds 1 and 1a. Preliminary examination of compound **1** indicated it to be aliphatic in nature. The absence of a molecular ion peak in the mass spectrum and the presence of a peak at *m*/*z* 448 for ($M^+ - H_2O$) indicated that it contained an −OH group and was therefore analyzed for $C_{32}H_{66}O$. The presence of a hydroxyl group was further supported by ^{13}C NMR, which showed a signal at δ 63.04 for the carbon carrying the hydroxyl group, and this was confirmed by its IR spectrum, which showed a broad absorption band at 3446 cm^{-1}. Its ^1H NMR spectrum exhibited signals for the presence of a terminal methyl group at δ 0.90 and a chain of methylene protons at δ 1.26. A triplet at δ 3.63 showed the presence of a methylene linked to oxygen atom. All these spectral data, when coupled together, indicated compound **1** to be a saturated primary alcohol containing C_{32} carbon atoms. On the basis of the above spectral studies, compound **1** was characterized as dotriacontanol (**1**). The proposed structure was confirmed by analyzing its acetate (**1a**).

Dotriacontanol (1). White solid, mp 87−88 °C. IR ν_{max} (KBr): 3446, 2920, 1465, 1360, 1061, 670 cm^{-1}. ^1H NMR (δ, $CDCl_3$, 250 MHz): 0.90 (t, 3H,$-CH_3$), 1.26 (brs, 58H), 1.54 (m, 2H, H − 2), 3.63 (t, 2H, $-CH_2OH$). ^{13}C NMR (δ, $CDCl_3$, 62.89 MHz): 14.01 ($-CH_3$), 25.67−32.76 ($-CH_2-$), 63.04 ($-CH_2OH$). EIMS *m*/*z* (%): 448 (M^+ −18, 36), 421 (8), 365 (5), 153(18), 71(80), 57(100).

Dotriacontanyl Acetate (1a). White solid, mp 76 °C. ^1H NMR (δ, $CDCl_3$, 250 MHz): 0.90 (t, 3H, $-CH_3$), 1.27 (brs, 58H), 1.60 (m, 2H, H-2), 2.25 (s, 3H, $-COCH_3$), 4.10 (t, 2H, $-CH_2O$-). EIMS *m*/*z* (%): 508 (M^+, 75), 507 (48), 479 (56), 449 (58), 446 (82), 421(47), 390 (42), 364 (46), 335 (45), 307 (51), 293 (59), 279 (55), 265 (42), 252 (58), 238 (55), 211 (45), 197 (55), 183 (35), 151 (26), 123 (92), 99 (100), 83 (92), 69 (91), 57 (92).

Characterization of Compounds 2 and 3. Compound **2** was purified as a white solid having melting point 55−56 °C. Its IR spectrum showed a characteristic absorption at 3445 cm^{-1}, indicating the presence of a hydroxyl group. It gave a molecular ion peak at *m*/*z* 256 along with a peak at 238 ($M^+ - H_2O$), supporting the presence of a hydroxyl group and was assigned the molecular formula $C_{17}H_{36}O$. In its ^1H NMR spectrum, a triplet for two protons at δ 3.58 was assigned for the α-methylene protons and a multiplet at δ 1.52 was assigned for β-methylene protons. Because the remaining methylene groups are similar, the chemical shift appeared as a broad singlet at δ 1.28. A triplet, observed at δ 0.88 for three protons, was assigned for the terminal methyl group. On the basis of the above-mentioned spectral data and comparison of observed melting point with that reported in the literature, compound **2** was identified as heptadecanol. On the basis of similar spectral studies, compound **3** was characterized as nonadecanol.

Heptadecanol (2). White solid, mp 55−56 °C. IR ν_{max} (KBr): 3445, 2924, 1378, 1060, 665 cm^{-1}. ^1H NMR (δ, $CDCl_3$, 250 MHz): 0.88 (t, 3H, $-CH_3$), 1.28 (brs, 28H), 1.52 (m, 2H, $-\mathbf{CH_2}CH_2O-$), 3.58 (t, 2H, $-CH_2OH$). EIMS *m*/*z* (%): 256 (M^+, 8), 238 ($M^+ - H_2O$, 5), 210 (8), 199 (16), 185 (6), 171 (9), 156 (6), 143 (6), 129 (15), 111 (36), 97 (60), 83 (85), 69 (82), 55 (92), 43 (100).

Nonadecanol (3). White solid, mp 58−59 °C. IR ν_{max} (KBr): 3440, 2925, 1380, 1062, 649 cm^{-1}. ^1H NMR (δ, $CDCl_3$, 250 MHz): 0.88 (t, 3H, $-CH_3$), 1.28 (brs, 32H), 1.52 (m, 2H, $-\mathbf{CH_2}CH_2OH$), 3.61 (t, 2H, $-CH_2OH$). EIMS *m*/*z* (%): 284 (M^+, 9), 266 ($M^+ - H_2O$, 6), 241 (16), 227 (18), 213 (6), 199 (16), 185 (26), 171 (16), 157 (14), 143 (3), 129 (42), 95 (42), 83 (63), 73 (96), 71 (100), 69 (42), 61 (88), 57 (66), 55 (76).

The natural compounds **2** and **3** were also synthesized to confirm their proposed structures and to obtain them in sufficient amounts to test the effect of individual compounds on the ovipositional responses of *C. partellus*. Synthetic heptadecanol and nonadecanol were obtained by reducing their corresponding methyl esters with lithium aluminum hydride. Also, heptadecinonitrile (**4**), a model compound, was synthesized by the reaction of 1-bromo hexadecane with potassium cyanide in absolute methanol and fully characterized by its spectral data as given below.

Heptadecinonitrile (4). White solid, mp 34 °C. IR ν_{max} (KBr): 2919, 2852, 2245, 1472, 1421, 1376, 718 cm^{-1}. ^1H NMR (δ, $CDCl_3$, 250 MHz): 0.88 (t, 3H, $-CH_3$), 1.28 (brs, 26H), 1.65 (m, 2H, $-\mathbf{CH_2}CH_2-CN$), 2.40 (t, 2H, $-CH_2CN$). EIMS *m*/*z* (%): 251 (M^+, 14), 222 (25), 208 (35), 194 (31), 180 (26), 166 (23), 152 (28), 138 (40), 124 (67), 110 (100), 97 (92), 83 (74), 71(86), 69 (81), 57 (96).

Other compounds isolated from HEKL but not yet identified are coded as MR-1, MR-7, MR-22a, MR-22b, and MR-27. Fractions obtained from silica gel chromatography, in the case of hexane extract of Basilocal leaves (HEBL), were analyzed along with those from HEKL by TLC. It was observed that, while the spots of compounds from HEKL were very prominent, the corresponding spots could not be seen in HEBL on TLC, using solvent systems $CHCl_3$/petrol (1:1, v/v) and benzene/EtOAc (9:1, v/v) and spraying with 5% alcoholic solution of polymolybdic acid and heating the plate as well as exposing the plate to iodine vapors. Therefore, further investigation of HEBL was not carried out.

Bioassay Equipment. The oviposition chamber was made of a vertical emery paper cylinder (6 cm ht × 6.8 cm diam) closed at both ends by glass Petri dishes (1 cm ht × 7 cm diam). Rough surface of emery paper provided a contrast to the smooth surface of glass Petri dishes, which is preferred by *Chilo* females for egg laying (*14*). Moreover, test stimuli were presented on the bottom Petri dish, because preliminary studies showed that the stem borer females, on the second night of emergence, laid a higher percentage of eggs on the bottom surface, compared to the top surface.

Bioassay Protocol. The ovipositional responses to hexane extracts of foliage of test cultivars and to chemicals extracted from HEKL were studied in two-choice bioassays. The test solution was smeared in one-half of the bottom Petri dish, so as to deposit 100 μg of residue per cm^2 of the substrate, and the other half (control) was smeared with an equal volume of hexane.

In bioassays where hexane extracts of Basilocal as well as those of Kisan foliage were tested simultaneously, one-half of the bottom Petri dish was smeared with HEBL and with HEKL in the other. The solvent was allowed to evaporate for nearly 30 min before releasing adults into the oviposition chamber. The test chambers were arranged randomly, so as to eliminate positional bias.

A pair of mated male and female moths was released in the oviposition chamber at 6 p.m. Moths were removed the next morning,

Table 1. Ovipositional Responses of *Chilo partellus* (Swinhoe) Females[a] to Hexane Extracts of Leaves of Two Maize Cultivars, Basilocal and Kisan

stimulus[b]		mean[c] no. eggs laid per female on		% eggs laid per female on		Chi-sq	
A	B	A	B	A	B	(pooled)	% ODI[d]
hexane	HEBL	13 ± 4	110 ± 6	10 ± 3	90 ± 3	384.39	−80 ± 5
hexane	HEKL	64 ± 5	46 ± 3	58 ± 2	42 ± 2	14.78	+16 ± 3
HEBL	HEKL	86 ± 8	39 ± 5	69 ± 1	31 ± 1	13.49	+39 ± 3

[b] The hexane extract of Basilocal leaves (HEBL) or of Kisan leaves (HEKL) was smeared on one-half (B) and solvent hexane on the other half (A) of the oviposition substrate. In other tests, HEBL was smeared on one half (A) and HEKL on the other half (B). [c] Mean ± S. E. Data were subjected to Wilcoxon signed rank test for statistical significance in the number of eggs laid on the two sides of the oviposition substate. This test was followed by Chi-square test with heterogeneity to test the heterogeneity of the test individuals, if any. The level of statistical significance in all cases was 1%. The chi-square (heterogeneity) was nonsignificant in all tests. [d] % Oviposition Deterrence Index ± S. E. % ODI is calculated as the % difference in the no. of eggs laid on A and B halves. No. of replicates in each test was 5.

and the Petri dishes containing the egg masses were stored in a glass jar, which was capped tightly. When the eggs had developed up to the "blackhead stage", they were counted following the method of Room et al. (*15*).

The efficacy of the test stimuli was compared with the solvent control on the basis of oviposition deterrence index (ODI). The ODI of females for the test stimuli was calculated as $100(A - B)/(A + B)$, with A and B being the number of eggs laid on the control- and stimulus-treated halves of the oviposition substrate, respectively. In case of two-choice tests, A represents the other test stimulus. Positive ODI values indicate deterrence, whereas negative values indicate stimulation of oviposition.

Data Analysis. Because the data did not conform to the assumptions of the normal distribution, we used the nonparametric Wilcoxon Signed Rank Test for making comparisons among means for statistical significance. The hypothesis was that number of eggs on significantly stimulating substrates would be greater than those on the control substrate, and conversely, they would be lesser yet on deterrent substrates. The data were further subjected to Chi-square test with heterogeneity with ovipositional preference of 50:50 for the two choices, whereby we could assess the heterogeneity of the test individuals, if any. All statistical analyses were performed with the computer software program SigmaStat 2.0 (*16*).

RESULTS AND DISCUSSION

Observations on the number of eggs laid in tests with extracts of the two cultivars are given in **Table 1**. A portion of eggs (90%) was laid on the surface smeared with the HEBL as compared to only 10% on the contralateral half treated with the solvent (hexane) only. The difference is highly significant ($P < 0.01$). However, oviposition on the surface smeared with the HEKL was significantly lower (42%) compared to that on the control surface (58%). These observations clearly indicate the role of hexane-extractable chemicals from the foliage surface of Basilocal and Kisan for the quantitative difference in oviposition. These chemicals of Basilocal foliage surface promote oviposition by *C. partellus*, whereas those from Kisan deter it. Significant differences in oviposition by stem borer on foliar surface of these cultivars have been observed in one of our previous studies (unpublished work), and the same may be due to differences in their texture and/or contact chemical stimuli. Similar observations about the role of leaf surface waxes on oviposition by phytophagous insects have also been reported earlier (*17*).

When the females were offered a choice between oviposition substrates smeared with HEBL and HEKL, over twice as many eggs (69%) were laid on the former than the latter (31%). The ovipositional preference of females for HEBL was significantly higher than that for HEKL (**Table 1**). Because the extract was prepared by dipping the leaves in hexane at room temperature, it is most likely that the lipid-soluble components present on the surface of the leaves were extracted, and as such, the response of the females was due to the hexane-soluble leaf surface chemicals. Therefore, attempts were made to isolate, characterize, and synthesize the chemicals present in the extracts. Sequential fractionation of HEKL yielded eight compounds, three of which were characterized as dotriacontanol (**1**), heptadecanol (**2**), and nonadecanol (**3**). Bioassays were then carried out to evaluate the effect of individual compounds on oviposition. Compounds **2** and **3** have been synthesized along with the fourth, heptadecinonitrile (**4**). The remaining compounds have not yet been identified and are being investigated further.

Results of tests to study the effect of these isolated compounds on ovipositional responses of stem borer females are presented in **Table 2**. Maximum oviposition deterrence index (90%) was observed in the case of the compound MR-22a, followed by

Table 2. Ovipositional Responses of *Chilo partellus* (Swinhoe) Females[a] to Chemicals Isolated from Hexane Extracts of Leaves of Maize Cultivar, Kisan (HEKL)

stimulus[b]		mean[c] no. eggs laid per female on		% eggs laid per female on		Chi-sq	
A	B	A	B	A	B	(pooled)	% ODI[d]
hexane	MR-1[e]	58 ± 3	53 ± 9	53 ± 5	47 ± 5	1.75	+7 ± 10
hexane	heptadecinonitrile	26 ± 5	80 ± 12	26 ± 5	74 ± 5	142.88	−51 ± 10
hexane	heptadecanol	84 ± 11	22 ± 4	78 ± 5	22 ± 5	182.97	+56 ± 10
hexane	MR-7[e]	86 ± 15	17 ± 8	82 ± 10	18 ± 10	188.49	+65 ± 20
hexane	nonadecanol	88 ± 8	18 ± 6	84 ± 4	16 ± 4	225.0	+68 ± 7
hexane	dotriacontanol	31 ± 8	71 ± 14	31 ± 8	69 ± 8	41.9	−38 ± 15
hexane	MR-22a[e]	106 ± 6	6 ± 6	95 ± 5	5 ± 5	265.2	+90 ± 10
hexane	MR-22b[e]	34 ± 8	66 ± 2	32 ± 6	68 ± 6	49.79	−36 ± 13
hexane	MR-27[e]	47 ± 12	63 ± 15	44 ± 9	56 ± 9	1.49	−13 ± 18

[a] Two day old mated females that had laid eggs on the succeeding night of emergence were used for oviposition bioassays. [b] The chemical solution was smeared on one-half (B) and solvent hexane on the other half (A) of the oviposition substrate in two-choice tests. [c] Mean ± S. E. Data were subjected to Wilcoxon signed rank test for statistical significance in the number of eggs laid on the two sides of the oviposition substate. This test was followed by Chi-square test with heterogeneity to test the heterogeneity of the test individuals, if any. The level of statistical significance in all cases was 1%. The chi-square (heterogeneity) was nonsignificant in all tests. [d] % Oviposition Deterrence Index ± S. E. % ODI is calculated as the % difference in the no. of eggs laid on A and B halves. No. of replicates in each test was 5. [e] Code nos. refer to compounds that are unidentified.

nonadecanol (**3**) (68%), MR-7 (65%), and heptadecanol (**2**) (56%). On the other hand, heptadecinonitrile (**4**) and dotriacontanol (**1**) stimulated oviposition, with ODIs being −51 and −38%, respectively.

A study of the chemical composition of normal epicuticular waxes of glossy mutants of maize indicated the presence of n-dotriacontanol as the principal constituent (*18*). Epicuticular waxes of *Panicum miliaceum*, *P. texanum*, and *Setaria italica* have also been reported to contain dotriacontanol as the major free alcohol (*19*). The presence of this compound in the crude alkaloid fraction of aerial portion of *Malvastrum tricuspidatum*, a plant used for its hypotensive properties, has also been reported (*20*). It has also been found in significant quantity in petroleum and alcoholic extracts of *Euphorbia granulata*, a species known to possess blood-purifying properties (*21*) and in the ethyl acetate-extractable fraction of *E. cauducifolia*, which is believed to possess antitumor properties (*22*). Dotriacontanol, which is found to possess insecticidal, antibacterial, antifungal, and antipyretic properties, has been isolated and characterized from the shoots of *Leucas aspera* (*23*). On the other hand, heptadecanol-1 has also been reported to be one of the essential oil constituents in the rinds of Eastern Mediterranean sour oranges (*Citrus aurantium* L.) (*24*).

The ovipositional responses of the females may be influenced by visual, volatile, and contact stimuli. Visual and volatile stimuli are distance perceivable, eliciting orientational responses of the insect. However, in the present case, the influence of these stimuli on the ovipositional responses is ruled out, because the females were directly released on the stimulus surface. The difference in egg laying is thus only due to the contact stimuli provided by the chemical constituents of the oviposition surface. The role of contact phytochemicals in eliciting oviposition of phytophagous insects has also been reported in other studies (*25*−*28*). Chemical cues of corn plants stimulate oviposition in *Ostrinia nubilalis* (Hubner) (*29*); i.e., pentane leaf extracts of corn containing *n*-alkanes (*26*, *30*). Conversely, corn methanol extracts were deterrent to ovipositing *O. nubilalis* (*26*). Pentane and steam distillation extracts of eight corn hybrids contain chemicals that elicit oviposition response in *Sesamia nonagrioides* (*31*). Also, their methanol extracts may contain chemicals that deter or repel females from oviposition.

The present study shows that hexane-soluble chemicals of the two maize culitvars, Basilocal and Kisan, significantly influence the egg delivery responses of *Chilo* females, as evidenced from two-choice tests in which hexane extract of Basilocal foliage elicited significantly higher responses compared to Kisan foliage extract. These observations are further supported by tests with individual isolated compounds, as stated above. The present findings clearly indicate that susceptibility/resistance of Basilocal and Kisan, against attack by *C. partellus*, is due inter alia to differences in their foliage chemicals. These chemicals, extractable with hexane, account for differences, in part, in the ovipositional responses of the maize stem borer. Identification of plant chemicals that affect oviposition may be useful in developing cultivars resistant to attack by spotted stem borer. Stimulatory phytochemicals may also have potential for use in disrupting oviposition behavior of *C. partellus* in the field, thereby reducing population levels.

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