An Oviposition Stimulant for the Carrot Rust Fly from Carrot Leaves

JOSEF BERÜTER and ERICH STÄDLER

Federal Research Station, Section Plant Protection, CH-8820 Wädenswil (Switzerland)

(Z. Naturforsch. 26 b, 339—340 [1971]; received November 12, revised December 16, 1970)

A compound which plays an important role in the stimulation of oviposition in the carrot rust fly was isolated from carrot leaves. The active component has been identified as trans-1,2-dimethoxy-4-propenylbenzene (methyl-iso-eugenol).

The carrot rust fly (Psila rosae F.) is a common pest of various cultivated Umbelliferae. Oviposition takes place in the soil near the host plant. The larvae emerging from the eggs mine in the roots of the food plant. The earlier observation that the fly is stimulated to oviposition through contact with the host leaves was further confirmed by behavioural studies on this insect. This suggested the presence of a chemical oviposition stimulant in carrot leaves. Such oviposition stimulants have been reported for some other phytophagous insects.

An attempt was made to isolate the pure active principle from the leaves of carrots by progressively purifying fractions found active by bioassay. The following procedure was successfully applied. Freshly harvested carrot leaves were frozen at −20 °C and ground in a corn mill. The 17 kg of ground material was steam-extracted with petrol ether (b. p. 35 — 45 °C). The extract was concentrated in a N₂ atmosphere as described. This compound showed the same retention time of 11.5 min; m. p.: 17.5—18.5 °C; n\textsubscript{D}²⁰: 1.5675.

The molecular composition of the active substance, as determined by high resolution mass spectrometry, corresponds to C\textsubscript{14}H\textsubscript{14}O\textsubscript{2}. The following prominent peaks were observed: m/e 178 (100 per cent) M, 179 (11 per cent) M+1, 163 (39 per cent) M—CH\textsubscript{3}, 151 (2 per cent) M—C\textsubscript{2}H\textsubscript{5}, 147 (10 per cent) M—OCH\textsubscript{3}, 135 (7 per cent) 163—CO. Fragment peaks indicated an aromatic derivative: 105 ± 2, 91, 79, 77, 65, 63, 51, 39 and C\textsubscript{6}H\textsubscript{5}: 41. The infrared spectrum (liquid in NaCl) showed no absorption characteristics of hydroxyl or carbonyl groups. Strong absorption occurred in the C—O stretching regions between 1020 — 1050 and 1220 — 1270 cm\textsuperscript{-1}. The nuclear magnetic resonance spectrum (CDCl\textsubscript{3} relative to Si(CH\textsubscript{3})\textsubscript{4} in ppm) showed: 1.86 (3 protons, doublet), 3.90 (3 protons, singlet), 3.93 (3 protons, singlet), 6.01 (1 proton, multiplet), 6.39 (1 proton, doublet, J = 16 Hz), 6.88 (3 protons, multiplet). From these spectral data the active compound was identified as trans-1,2-dimethoxy-4-propenylbenzene (methyl-iso-eugenol).

Synthetic methyl-iso-eugenol was purified by GLC using the chromatographic conditions described. This compound showed the same retention time (30 min) in the analytical GLC (5 m × 2.5 mm column of 4 per cent Carbowax 20 M on 80/100 mesh. "Chrom-G" (AWDMCS) at 190 °C; helium flow rate was 40 cm\textsuperscript{3}/min. The active compound (45 mg) had a retention time of 11.5 min; m. p.: 17.5—18.5 °C; n\textsubscript{D}²⁰: 1.5675.

The active compound (45 mg) had a retention time of 11.5 min; m. p.: 17.5—18.5 °C; n\textsubscript{D}²⁰: 1.5675.

Received November 12, revised December 16, 1970.

Reprints request to E. STÄDLER, Federal Research Station, Section Plant Protection, CH-8820 Wädenswil (Switzerland).

The carrot rust fly (Psila rosae F.) is a common pest of various cultivated Umbelliferae. Oviposition takes place in the soil near the host plant. The larvae emerging from the eggs mine in the roots of the food plant. The earlier observation 3 that the fly is stimulated to oviposition through contact with the host leaves was further confirmed by behavioural studies on this insect. This suggested the presence of a chemical oviposition stimulant in carrot leaves. Such oviposition stimulants have been reported for some other phytophagous insects.

An attempt was made to isolate the pure active principle from the leaves of carrots by progressively purifying fractions found active by bioassay. The following procedure was successfully applied. Freshly harvested carrot leaves were frozen at −20 °C and ground in a corn mill. The 17 kg of ground material was steam-extracted with petrol ether (b. p. 35 — 45 °C). The extract was concentrated in a N₂ atmosphere as described. This compound showed the same retention time of 11.5 min; m. p.: 17.5—18.5 °C; n\textsubscript{D}²⁰: 1.5675.

The molecular composition of the active substance, as determined by high resolution mass spectrometry, corresponds to C\textsubscript{14}H\textsubscript{14}O\textsubscript{2}. The following prominent peaks were observed: m/e 178 (100 per cent) M, 179 (11 per cent) M+1, 163 (39 per cent) M—CH\textsubscript{3}, 151 (2 per cent) M—C\textsubscript{2}H\textsubscript{5}, 147 (10 per cent) M—OCH\textsubscript{3}, 135 (7 per cent) 163—CO. Fragment peaks indicated an aromatic derivative: 105 ± 2, 91, 79, 77, 65, 63, 51, 39 and C\textsubscript{6}H\textsubscript{5}: 41. The infrared spectrum (liquid in NaCl) showed no absorption characteristics of hydroxyl or carbonyl groups. Strong absorption occurred in the C—O stretching regions between 1020 — 1050 and 1220 — 1270 cm\textsuperscript{-1}. The nuclear magnetic resonance spectrum (CDCl\textsubscript{3} relative to Si(CH\textsubscript{3})\textsubscript{4} in ppm) showed: 1.86 (3 protons, doublet), 3.90 (3 protons, singlet), 3.93 (3 protons, singlet), 6.01 (1 proton, multiplet), 6.39 (1 proton, doublet, J = 16 Hz), 6.88 (3 protons, multiplet). From these spectral data the active compound was identified as trans-1,2-dimethoxy-4-propenylbenzene (methyl-iso-eugenol).

Synthetic methyl-iso-eugenol was purified by GLC using the chromatographic conditions described. This compound showed the same retention time (30 min) in the analytical GLC (5 m × 2.5 mm column of 4 per cent Carbowax 20 M on 80/100 mesh. "Chrom-G" (AWDMCS) at 190 °C; helium flow rate was 40 cm\textsuperscript{3}/min. The active compound (45 mg) had a retention time of 11.5 min; m. p.: 17.5—18.5 °C; n\textsubscript{D}²⁰: 1.5675.

We thank Dr. J. SEIBL and Dr. E. PRETSCH from the Swiss Federal Institute of Technology, Zürich for the carrying-out and interpretation of mass and NMR spectra respectively, and H. GUTEKUNST for technical assistance in gas chromatographic procedures.

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz. Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.
column of 3 per cent Carbowax 20 M) at 190 °C as the isolated active substance. The identity of the active component was further substantiated by bioassay and by the identical infrared spectra of the synthetic and the isolated substances.

A bioassay was devised to evaluate the potency of the isolated fractions and to compare the activity of the isolated and the synthetic methyl-iso-eugenol. Insects which had no contact with natural host plants were reared as described elsewhere. For the bioassay about 150—300 pairs of flies were caged and provided with water in form of 1% agar and with food consisting of sugar and yeast hydrolisate. The fraction or chemical to be tested was dissolved in 10 ml diethyl ether and applied in equal amounts on 2 paraffin-coated leaf devices, consisting of a plastic celery leaf inserted in a 100 ml bottle, which was also coated with paraffin (surface area of each device: 800 cm²). The solvent was removed by evaporation. The control leaves were treated with ether alone. Both control and treated leaves were placed in duplicate in the cage on the oviposition devices consisting of a petri-dish with a wetted foam rubber lining, covered with blade cloth. The eggs were laid through two black nylon nets (mesh 1 mm) on to the cloth. Oviposition was allowed to take place during 24 hours (light/dark regime: 16 h/6 h). The number of eggs laid when treated leaves were used was compared to the number laid in the control. The results were statistically analysed by transformation \((\sqrt{y} + x)\) and the t-test.

As can be seen from Table 1 similar oviposition ratios were obtained for the isolated and the synthetic methyl-iso-eugenol. The tested amount of 1.0 mg which corresponds to a concentration of 0.64 \(\mu g/cm^2\) leaf surface was found to be the optimal concentration for stimulating oviposition.

From these results it would appear that methyl-iso-eugenol plays an important role in host selection possibly by stimulating contact chemoreceptors of the female carrot rust fly before oviposition. Further investigations are in progress to elucidate the possible role of various environmental factors in host selection behaviour.

Methyl-iso-eugenol has also proved to be a good attractant for males of the oriental fruit fly \(D\). dorsalis. It is of interest that the same chemical can act as a male attractant for \(D\). dorsalis and as an oviposition stimulant for \(P\). rosae.

\[
\begin{array}{ccccccc}
\text{Methyl-iso-} & \text{Amount applied} & \text{Total number of} & \text{Oviposition} & \text{Number of} \\
\text{eugenol} & \text{on 2 leaf-devices} & \text{eggs} & \text{ratio} & \text{repetitions} \\
\text{mg calculated} & \text{treated} & \text{treated/} & \text{control} & \\
\text{}\mu g/cm^2 & \text{un-} & \text{un-} & \text{treated/} & \text{treated} \\
\text{calculated} & \text{treated} & \text{treated} & \text{treated} & \text{treated} \\
\hline
\text{I Isolated} & 0.1 & 0.064 & 549 & 437 & 1.3 & * & 3 \\
\text{from carrot leaves} & 0.5 & 0.32 & 1933 & 1679 & 1.2 & * & 8 \\
\text{1.0} & 0.64 & 591 & 166 & 3.6 & ** & 9 \\
\text{II Synthetic} & 0.1 & 0.064 & 1000 & 910 & 1.1 & 10 \\
 & 1.0 & 0.64 & 371 & 81 & 4.6 & *** & 4 \\
 & 2.0 & 1.28 & 1406 & 621 & 2.3 & * & 10 \\
 & 5.0 & 3.21 & 1390 & 621 & 2.1 & * & 10 \\
 & 10.0 & 6.40 & 2500 & 1005 & 2.5 & *** & 12 \\
\end{array}
\]

Table 1. Oviposition in the carrot rust fly stimulated by isolated and by synthetic methyl-iso-eugenol. Level of significance: * \(P=0.05\), ** \(P=0.01\), *** \(P=0.001\).

5 Fluka AG, Buchs, Switzerland.

7 L. F. Steiner, J. econ. Ent. 45, 341 [1954].