Note

Behavioral Responses of the Pine Wood Nematode to Terpenes

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Received April 13, 1983

We have tested various chemicals for their modifying effects on the behavior of the pine wood nematode. Several compounds containing the oleyl group, i.e., ethylene glycol monooleate, 1-monoolein, oleic acid and oleyl alcohol were attractive.¹⁾ Allyl isothiocyanate, naringenin, 1-tyrosine, 1-tryptophan and calcium chloride were also attractive, while capsaicin and magnesium chloride exhibited repellency.²⁾

As a continuation of these works, we tested behavioral responses of the nematode to terpenes. The results are presented in this paper.

MATERIALS AND METHODS

Test chemicals. Commercially available terpenes (analytical grade) were used without further purification.

Bioassay. The procedure for behavioral tests was described in detail in the preceding paper.²⁾ Agar plates in petri dishes were perforated to make a pair of holes, and filled with agar containing the test chemical and untreated agar respectively. A pad of absorbent cotton impregnated with a suspension of the nematodes reared on colonies of *Botrytis cinerea* was placed in the center of the agar plates. After incubating in darkness at 25°C for 3 days, the number of nematodes in the treated and untreated agar discs were counted and analysed statistically. All the test compounds had been previously dissolved in ethanol and water was added to provide ethanol concentration below 6%. A preliminary check showed that the effect of ethanol was negligible below 10%. The test was not performed for concentrations higher than 10⁻³ M, since the chemicals could not be maintained in suspension.

RESULTS

All the chemicals were first tested at 10⁻³ M. If a

significant activity was noted, the concentration was lowered successively by an order of magnitude. The results obtained with 26 terpenes are presented in Table I.

Abietic acid and phytol were attractive at the order of 10⁻⁵ M, followed by β -myrcene and geraniol, which were active at 10⁻⁴ M. Nerol was attractive at 10⁻³ M. On the other hand, β -pinene, citronellol, isoborneol, *p*-cymene and *d*-camphoric acid exhibited repellent activity, with threshold levels of 10⁻³ M. Between stereoisomeric pairs, geraniol and isoborneol were respectively more active than nerol and borneol. Farnesol was lethal at 10⁻³ M. No significant effects could be detected for other terpenes.

DISCUSSION

Watanabe tested attraction of volatile terpenes contained in *Pinus densiflora* and found that β -myrcene was highly attractive to the nematode, while other terpenes, i.e., α -pinene, β -pinene, limonene, β -phellandrene, 3-carene and camphene, were weakly attractive.³⁾ Our results coincided with his concerning the high attractancy of β -myrcene, but β -pinene was repellent in our test system.

Behavioral effects of monoterpenes on insects have been frequently reported. In particular, ipsenol, a monoterpene structurally related to β -myrcene, was found to be an aggregation pheromone of *Ips proconfusus*, one of the bark beetles which infest pine.⁴⁾ Reports on the behavioral activity of diterpenes have not often appeared, but the present work disclosed the remarkable attractiveness of phytol and abietic acid to the nematode. Recently, the latter compound was found to be a feeding stimulant to the larch sawfly.⁵⁾ Abietic acid is contained in the pine resin, and this might be connected with infestation of the pine by the nematode.

REFERENCES

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TABLE I. RESPONSE RATIO OF THE PINE WOOD NEMATODE TO TERPENES

Compounds	Response ratio (%) ^a			
	Concentration (M)			
	1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
Abietic acid	49.8 ± 4.5	58.2 ± 6.8	60.2 ± 4.5	64.4 ± 5.1
Borneol				57.7 ± 8.6
Iso-borneol			45.5 ± 6.1	42.0 ± 4.7
Camphene				50.2 ± 4.7
DL-Camphor				44.8 ± 8.4
D-Camphoric acid			51.2 ± 5.4	42.7 ± 3.1
(+)-3-Carene				55.2 ± 6.0
Citral				49.0 ± 7.2
Citronellal				49.6 ± 8.0
Citronellol			44.7 ± 7.5	39.4 ± 4.8
<i>p</i> -Cymene			45.1 ± 5.1	40.8 ± 6.1
Eucalyptol (Cineole)				47.8 ± 4.2
Farnesol		49.6 ± 3.8	50.4 ± 6.4	lethal
Geraniol		54.1 ± 7.7	57.8 ± 6.6	64.4 ± 3.6
D-Limonene				53.4 ± 6.6
Linalool				46.2 ± 6.5
L-Menthol				48.5 ± 3.5
β -Myrcene		52.8 ± 4.6	58.7 ± 3.8	64.8 ± 3.8
Nerol			53.3 ± 6.3	62.4 ± 5.3
Phytol	44.9 ± 7.8	57.6 ± 5.3	63.6 ± 8.4	58.1 ± 4.9
α -Pinene				53.5 ± 7.4
β -Pinene			48.7 ± 7.5	43.1 ± 4.9
(+)-Pulegone				43.7 ± 10.3
Terpin hydrate				51.9 ± 6.5
α -Terpineol				52.9 ± 4.0
Terpinolene				52.5 ± 5.3

^a 15 replicates each. Values are means $\pm$ 95% in confidence limits. This ratio was calculated by the following equation: $(A/(A+B)) \times 100$, where A and B are the numbers of the nematodes in the treated and untreated agar discs respectively. The lower limits greater than 50 indicate attraction and the upper limits less than 50 indicate repulsion.