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Repellency of Rosemary Oil and Its Components against the Onion Aphid, *Neotoxoptera formosana* (TAKAHASHI) (Homoptera, Aphididae)

Masatoshi HORI and Hiroaki KOMATSU

Leaf Tobacco Research Laboratory, Japan Tobacco Inc., Idei, Oyama, Tochigi 323, Japan

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Olfactory behaviors of *Neotoxoptera formosana*, an aphid pest of allium crops, to host and non-host plant odors were investigated with a linear track olfactometer. Aphids were significantly attracted to odors of host plants, *Allium fistulosum* and *A. tuberosum*. On the contrary, they were repelled by odors of non-host plants, rosemary and pennyroyal. The odor of pennyroyal masked the attractiveness of host plant odor and the odor of rosemary repelled aphids even in the presence of host plant odor. Rosemary oil also had a repellency effect against aphids, and repelled them even in the presence of host plant odor. Six components of rosemary oil identified by GC-MS analysis, 1,8-cineole, *d,l*-camphor, α-pinene, etc., showed this repellency as well. 1,8-Cineole and *d,l*-camphor repelled aphids even in the presence of host plant odor and α-pinene masked host plant attractancy. 1,8-Cineole is the main component of rosemary oil and is thought to be main factor responsible for the repellency of rosemary. It was concluded that *N. formosana* may use host plant odors for host selection and be repelled by certain non-host plant odors. Rosemary volatiles may play an important role in defense of the plants from attack by parasites.

Key words: *Neotoxoptera formosana*, repellent, olfactometer, 1,8-cineole, rosemary oil

INTRODUCTION

Early studies (e.g., KENNEDY et al., 1959) suggested that olfactory cues play no part in host plant selection prior to landing by aphids. But recent studies have revealed that host plant location and selection behaviors by aphids are influenced by volatiles from host and non-host plants (PICKETT et al., 1992). *Cavariella aegopodii* (SCOPOLI) is induced to land on traps by carvone, its host plant volatiles, and reduced by linalool (CHAPMAN et al., 1981). *Aphis fabae* SCOPOLI is attracted by the volatiles of host plants, Sutton dwarf bean and tick bean, but is repelled by the volatiles of non-host plants, such as summer savory and tansy (NOTTINGHAM et al., 1991; NOTTINGHAM and HARDIE, 1993), and some volatile compounds, e.g. isothiocyanates (ISAACS et al., 1993), methyl salicylate and (−)-(1*R*,5*S*)-myrtenal (HARDIE et al., 1994). These repellents mask host plant attractiveness to aphids. Methyl salicylate, a volatile component of *Prunus padus*, the winter host of *Rhopalosiphum padi* (L.), repels spring morphs of this aphid, and appears to play a role in host alternation of this aphid (PETTERSSON et al., 1994). It significantly decreased colonization on field cereal plants by *R. padi*, *Sitobion avenae* (FAB.) and *Metopolophium dirhodum* (WALK.) (PETTERSSON et al., 1994).

Neotoxoptera formosana (TAKAHASHI) is an oligophagous aphid pest of *Allium* species and sometimes forms large colonies on the leaves. In this study, the olfactory behaviors of *N. formosana* to host and non-host plant odors were investigated with a linear tract olfactometer. It was found that aphids were attracted to host plant odors and repelled by rosemary and pennyroyal odor. The repellency of rosemary was traced to a few components in its oil.

MATERIALS AND METHODS

Aphids. Adult apterae of *N. formosana* derived from a stock culture which was maintained on *Allium fistulosum* L. in a greenhouse at 20°C and 16L:8D were used for all experiments. Crowding *N. formosana* under these conditions did not produce alate virginoparae, so that all the experiments were made only with apterae.

Plant materials. All the tested host and non-host plants were grown in a greenhouse. The plant leaves were freshly picked and weighed prior to tests.

Chemicals. Rosemary oil was supplied by Soda Aromatic Co. (Tokyo, Japan). 1,8-Cineole, α-pinene, β-pinene, *d*-(+)-limonene, α-terpineol, borneol, *p*-cymene and bornyl acetate were supplied from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *d,l*-Camphor, myrcene and linalool were supplied by Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan. (+)-Camphene was supplied by Aldrich Chemical Co., Inc. (Milwaukee, Wis., USA). (−)-*trans*-Caryophyllene was supplied by Sigma Chemical Co. (St. Louis, Mo., USA).

Olfactometer. A linear track olfactometer (Fig. 1), basically designed by SAKUMA and FUKAMI (1985), was modified for aphid-test and used in all experiments. It was made of transparent acrylic tubings and steel rods. The rods formed a T-junction at the point where the airstreams carrying treatment and control odors met from two side arms in the olfactometer. An airflow of 0.8 *l*/min was maintained in all experiments. Thirty adult apterous virginoparae were placed in the pot installed at the base of the central vertical tube (Fig. 1). The inner walls of all three vertical tubes at the center and both sides, including the pot, were previously plastered with talcum powder (Wako Pure Chemical Industries) to prevent aphids from climbing the wall and to ensure that they climbed the rod only. All experiments were carried out in a darkroom at 22–24°C to remove the influence of light. The number of aphids in traps on the treatment and control sides was counted 2 h after the start of the

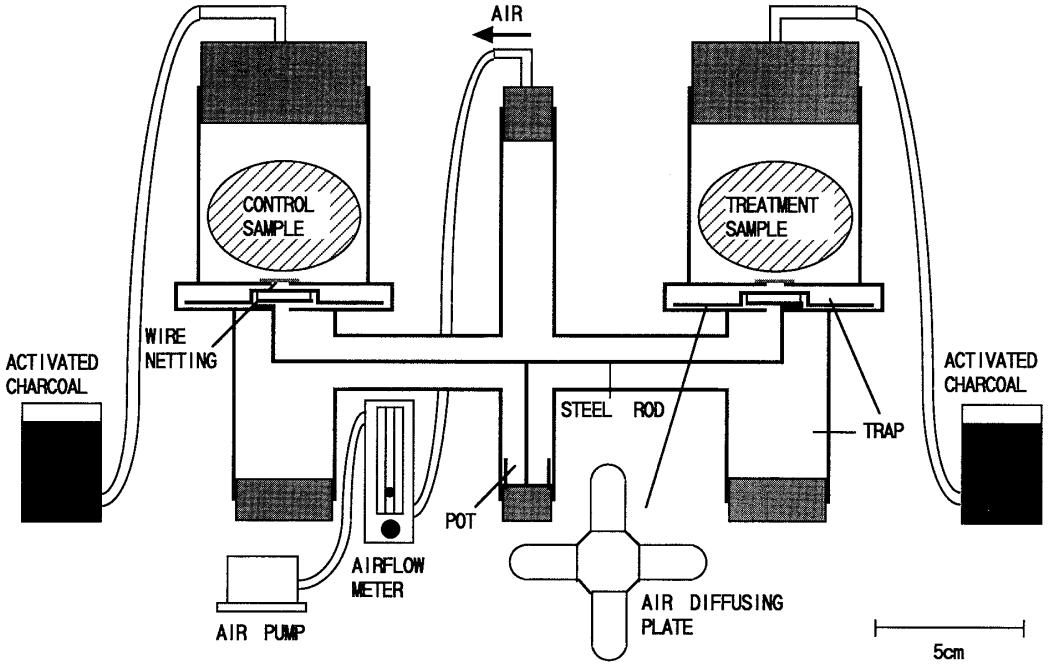

Fig. 1. Linear track olfactometer (side view).

Table 1. Response of apterous virginoparae of *Neotoxoptera formosana* to host and non-host plant leaf odors

Plant leaves	Treatment	Control
A. Host plants		
Allium fistulosum	13.5±1.76	8.6±0.88*
Allium tuberosum	18.3±1.29	7.4±0.67**
B. Non-host plants		
Pennyroyal (*Mentha pulegium*)	5.8±0.65	15.4±1.47**
Rosemary (*Rosmarinus officinalis*)	5.3±0.90	11.9±1.44**
C. Combinations of host and non-host plant		
Pennyroyal and *Allium fistulosum*	10.8±1.83	7.9±1.42 NS
Pennyroyal and *Allium tuberosum*	11.5±1.31	13.5±1.76 NS
Rosemary and *Allium fistulosum*	5.3±1.05	16.7±1.42**
Rosemary and *Allium tuberosum*	7.3±0.92	13.2±1.39**

Values are means±standard error.
*, ** Significant difference at $p < 0.05$, 0.01, respectively, and NS, no significant difference in paired *t*-test ($n = 12$).

Table 2. Response of apterous virginoparae of *Neotoxoptera formosana* to rosemary oil and combinations of host plant odor

Test substances	Treatment	Control
Rosemary oil	5.1±1.12	19.1±1.26**
Rosemary oil and *Allium tuberosum* leaf	5.1±0.80	14.7±1.82**

Values are means±standard error.
** Significant difference at $p < 0.01$, in paired *t*-test ($n = 12$).

test. The olfactometer was washed with soapy water and the treatment and control sides were alternated after every trial. Twelve replicates were made in each test. The mean number of aphids in the treatment trap was compared with that in the control trap by a paired *t*-test analysis. When the behavioral activity of aphids in the olfactometer was low, the test was abandoned and the aphids were replaced with fresh insects.

Response of aphids to host and non-host plants. Fresh leaves (4 g) of a test plant and a sheet of filter paper (ADVANTEC, No. 2, ϕ90 mm) moistened with distl. water (1.5 ml) were placed in the treatment side, while three sheets of filter paper moistened with distl. water (1.5 ml) were placed in the control side. In the tests with combinations of host and non-host plants, 4.0 g leaves of both plants were set together in the treatment side.

Response of aphids to chemicals. In the tests with liquid samples, a 10 μl sample applied to a piece of filter paper (ADVANTEC, No. 2, 10×20 mm) was placed in the treatment side and filter paper was placed in the control side. In the tests with solid samples, 10 mg samples in 40 μl ethanol applied to filter paper were compared with ethanol controls. Both treatment and control filter papers were dried out to remove ethanol before being placed in the olfactometer.

Response of aphids to combinations of chemicals and host plants. Ten ethyl vinyl acetate beads (EVAFREX 250C®, Soda Aromatic Co., Tokyo, Japan) soaked in each test chemical

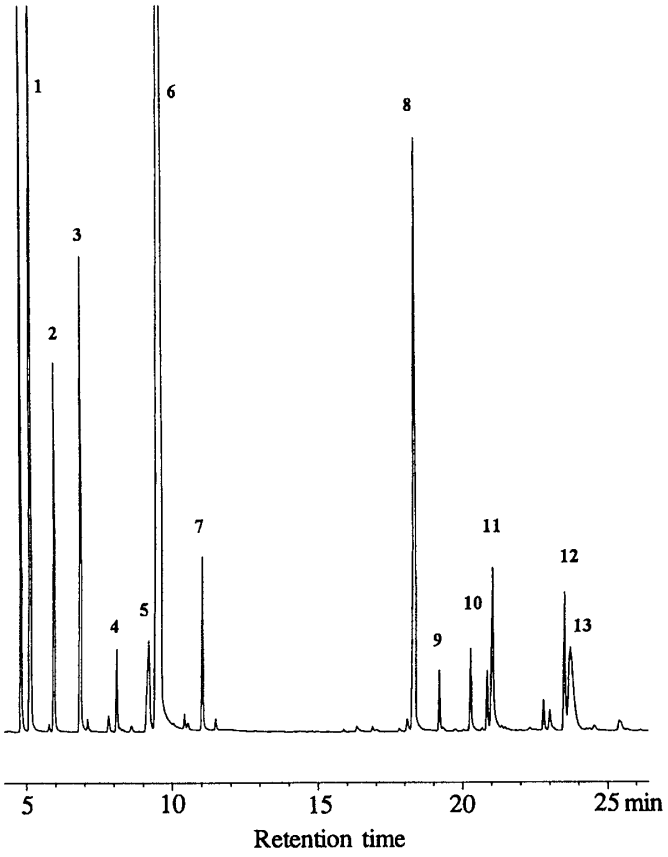

Fig. 2. GC profile of rosemary oil.

except for *d,l*-camphor, 4 g fresh leaves of *Allium tuberosum* and a piece of filter paper (ADVANTEC, No. 2, ϕ90 mm) moistened with distl. water (1.5 ml) were set together in the treatment side. In the control side, ten untreated ethyl vinyl acetate beads and three sheets of filter paper moistened with distl. water (1.5 ml) were placed. In the case of *d,l*-camphor, 0.5 g wrapped in thin paper (Kimwipe®, Kimberly-Clark Co., Tokyo, Japan), 4 g fresh leaves of *A. tuberosum* and filter paper moistened with distl. water (1.5 ml) were set together in the treatment side. In the control side, the thin paper and three sheets of filter paper moistened with distl. water (1.5 ml) were placed.

Chemical analysis. Compounds of rosemary oil were identified by comparison of GC retention times to those of the standards and by GC-MS data.

1) GC. A HP 6890 gas chromatograph equipped with a flame ionization detector was used. The GC conditions were as follows: column, DB-WAX (J&W) 30 m×0.25 mm I.D. 0.25 μm; He carrier gas flow rate, 1.0 ml/min; split ratio, 50 : 1; temperature program, isotherm 1 min at 50°C, 4°C/min gradient to 220°C, isotherm 15 min; injection temperature, 250°C.

2) GC-MS. A HP 5890 Series II gas chromatograph equipped with a HP 5971 mass spectrometer was used. The mass data were analyzed by a HP MS chemstation system. The GC conditions were as follows: column, DB-WAX (J&W) 60 m×0.25 mm I.D. 0.25 μm; He carrier gas flow rate, 0.25 ml/min; splitless; temperature program, isotherm 3 min at

Table 3. Identification and contents of rosemary oil components

Peak No.	Components	Contents of components (%)
1	α-Pinene	12.3
2	(+)-Camphene	4.5
3	β-Pinene	6.5
4	Myrcene	1.0
5	(+)-Limonene	2.3
6	1,8-Cineole	48.3
7	*p*-Cymene	2.1
8	*d,l*-Camphor	11.6
9	Linalool	1.2
10	Bornyl acetate	0.8
11	(−)-*trans*-Caryophylene	3.0
12	α-Terpineol	2.2
13	Borneol	3.5

50°C, 2°C/min gradient to 250°C, isotherm 60 min; injection temperature, 250°C; detector temperature, 250°C.

RESULTS

Response of aphids to combinations of host and non-host plant odors

Aphids were attracted to odors of their host, *A. fistulosum* and *A. tuberosum*, leaves. Significant differences were obtained between the mean numbers of aphids trapped in the treatment and control sides, specifically, 13.5 vs. 8.6 in the test with *A. fistulosum*, and 18.3 vs. 7.4 in the test with *A. tuberosum* (Table 1, A).

Odors from pennyroyal and rosemary leaves, non-host plants of the aphid, repelled aphids, showing highly significant differences between the mean numbers of aphids trapped in the treatment and control sides, namely, as 5.8 vs. 15.4 in the test with pennyroyal leaves, and 5.3 vs. 11.9 in the test with rosemary leaves (Table 1, B).

The odor of pennyroyal leaves masked the attractiveness of both host plant leaf odors. A combination of pennyroyal and *A. fistulosum* or *A. tuberosum* leaf odors was neither attractive nor repellent. Rosemary odor significantly repelled aphids even in the presence of host plant odor. The mean number of aphids trapped in the side containing the rosemary and *A. fistulosum* combination was 5.3, while that in the control side was 16.7. For the combination of rosemary and *A. tuberosum*, the mean number of aphids in the treatment side was 7.3, while that in the control side was 13.2 (Table 1, C).

Response of aphids to rosemary oil

Aphids were significantly repelled by rosemary oil (Table 2). The mean number of aphids in the treatment side was 5.1, while that in the control side was 19.1. A combination of rosemary oil and *A. tuberosum* also significantly repelled aphids. The mean number of aphids in the treatment sides was 5.1, while that in the control side was 14.7.

Chemical analysis

Thirteen main components of rosemary oil were identified by comparison of GC

Table 4. Response of apterous virginoparae of *Neotoxoptera formosana* to rosemary oil components

Chemicals	Treatment	Control
1,8-Cineole	5.0±1.21	16.8±1.31**
d,l-Camphor	7.5±0.76	16.9±1.22**
α-Pinene	6.0±1.02	21.1±1.48**
Borneol	6.2±0.61	9.3±1.03*
Bornyl acetate	5.8±0.86	10.0±1.06*
α-Terpineol	7.8±0.84	11.3±1.24*
(+)-Camphene	8.8±1.03	8.0±0.89 NS
p-Cymene	9.3±1.38	12.7±1.44 NS
d-(+)-Limonene	8.7±1.05	8.6±1.12 NS
Linalool	6.8±1.14	9.6±1.31 NS
Myrcene	8.7±0.78	11.3±1.61 NS
β-Pinene	6.9±1.07	11.5±1.33 NS
(−)-*trans*-Caryophyllene	7.0±1.03	9.3±1.09 NS

Values are means±standard error.
*,** Significant difference at $p < 0.05$, 0.01, respectively, and NS, no significant difference in paired *t*-test ($n = 12$).

Table 5. Response of apterous virginoparae of *Neotoxoptera formosana* to combinations of rosemary oil components and host plant odor

Combinations of chemical and host plant	Treatment	Control
1,8-Cineole and *Allium tuberosum*	3.8±0.45	15.1±2.03**
d,l-Camphor and *Allium tuberosum*	4.4±0.89	9.0±1.04*
α-Pinene and *Allium tuberosum*	7.8±1.42	11.4±0.80 NS

Values are means±standard error.
*,** Significant difference at $p < 0.05$, 0.01, respectively, and NS, no significant difference in paired *t*-test ($n = 12$).

retention times to those of the standards and by GC-MS data (Fig. 2, Table 3). The content of 1,8-cineole was ca. 48%, the highest of the components. Contents of α-pinene and *d,l*-camphor were ca. 12% and other components were less than 10%.

Response of aphids to rosemary oil components

1,8-Cineole, *d,l*-camphor and α-pinene had high repellency against aphids (significant difference at $p < 0.01$; Table 4). The repellencies of borneol, bornyl acetate and α-terpineol were somewhat lower than those of 1,8-cineole, *d,l*-camphor and α-pinene. No significant repellency of (+)-camphene, *p*-cymene, *d*-(+)-limonene, linalool, myrcene, β-pinene and (−)-*trans*-caryophyllene was observed.

Response of aphids to combinations of rosemary oil components and host plant odor

A combination of *A. tuberosum* and 1,8-cineole or *d,l*-camphor repelled aphids significantly (Table 5). Repellency of *d,l*-camphor was lower than that of 1,8-cineole (in 1,8-cineole, with a significant difference at $p < 0.01$; in *d,l*-camphor, there was a significant

difference at $p < 0.05$). α-Pinene didn't repell the aphids in the presence of A tuberosum but masked the attractiveness of *A. tuberosum* odors.

DISCUSSION

The aphid *N. formosana* was proved to be attracted by leaf odors of its host plants, *A. fistulosum* and *A. tuberosum*. These results support the conclusions offered by CHAPMAN et al. (1981), NOTTINGHAM et al. (1991) and PICKETT et al. (1992) that aphids are able to recognize and use host-plant volatiles as token stimuli in host detection. In these experiments, only the apterous virginoparae were used, because we were unable to establish alate aphid production. However, it is reasonable that the alate virginoparae of this species are also attracted by host plant odors, because oligophagous aphids such as *N. formosana* may be unable to find a host plant with only visual responses in the absence of olfactory responses. NOTTINGHAM et al. (1991) reported that both alatae and apterae of *A. fabae* virginoparae were attracted by host plant odors. Their results show that behavioral responses of alate and apterous virginoparae of the aphid to host plant odor may be similar.

Contrary to the attractiveness of host plant odors, rosemary and pennyroyal leaf odors repelled aphids. NOTTINGHAM et al. (1991) reported that odors of some labiate non-host plants belonging to the same family as pennyroyal and rosemary repelled certain aphids even with host plant odors or masked host plant attractancy. Labiate herbs may be a promising source of repellents against aphids. Rosemary oil also had high repellency against *N. formosana*. Rosemary oil repels not only *N. formosana* but also *Myzus persicae* (SULZER) and *Aphis gossypii* GLOVER (HORI, unpublished). Six rosemary oil components repelled *N. formosana*. In particular, 1,8-cineole had high activity against aphids and repelled them even in the presence of host plant odor. Since 1,8-cineole is the main component of rosemary oil, it may be the main factor of repellency in rosemary oil.

These repellents, released from a vessel placed among plants, may inhibit aphid landing on plants through influences on aphid olfactory behaviors. In a field study by PETTERSSON et al. (1994), over a 50% reduction in the population of *R. padi* on barley was obtained using methyl salicylate applied from slow-release vials. Synthetic organic pesticides cannot prevent primary infections of nonpersistent viruses, while these repellents may be able to prevent them through inhibition of aphid landing. The repellents, which do not kill aphids directly, will probably not cause the aphids to develop insecticide resistance. Non-host plant repellents are natural products and direct applications to plants is unnecessary. Consequently, they will have less influence on the environment and man itself.

Use of synthetic organic pesticides has many problems such as influence on the environment, risks to man and development of insecticide resistance. Therefore, it is necessary to establish novel aphid control agents to replace present agricultural pesticides. As mentioned above, pest control using repellents has many advantages and great potential to resolve these serious problems.

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