Journal of Nematology 39(3):275–279. 2007.

Nematicidal Activity of Plant Essential Oils and Components From Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*) and Litsea (*Litsea cubeba*) Essential Oils Against Pine Wood Nematode (*Bursaphelenchus Xylophilus*)

Il-Kwon Park, Junheon Kim, Sang-Gil Lee, Sang-Chul Shin

Abstract: Commercial plant essential oils from 26 plant species were tested for their nematicidal activities against the pinewood nematode, *Bursaphelenchus xylophilus.* Good nematicidal activity against *B. xylophilus* was achieved with essential oils of ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and litsea (*Litsea cubeba*). Analysis by gas chromatography-mass spectrometry led to identification of 12, 6 and 16 major compounds from ajowan, allspice and litsea oils, respectively. These compounds from three plant essential oils were tested individually for their nematicidal activities against the pinewood nematode. LC_{50} values of geranial, isoeugenol, methyl isoeugenol, eugenol, methyl eugenol and neral against pine wood nematodes were 0.120, 0.200, 0.210, 0.480, 0.517 and 0.525 mg/ml, respectively. The essential oils described herein merit further study as potential nematicides against the pinewood nematode.
Key words: ajowan, allspice, litsea, nematicidal activity, pine wood nematode, plant essential oils

Pine wilt disease, caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the most serious problem in Korean forests (Park et al., 2005). This disease was first reported in Busan, Gyeongsangnam-do province, in 1988 (Yi et al., 1989) and has spread to several areas of the Korean peninsula. As *Pinus densiflora* and *P. thunbergii* are predominant tree species in Korean forests and are very susceptible to the pine wood nematode, ecological and economical damage is substantial (Korea Forest Service, 2004). Recently, infected *Pinus koreansis* has been found for the first time in Korea.

Control of this disease depends primarily on fumigation of disease-infected trees with metham-sodium, aerial application of synthetic pesticides, such as fenitrothion and thiacloprid, against *Monochamus alternatus*, the insect vector of the pine wood nematode, or injection of nematicides, such as morantel tartrate, emamectin benzoate and levamisole hydrochloride (Kishi, 1995; Korea Forest Service, 2003; Lee et al., 2003). However, there are environmental and human health concerns with synthetic pesticides. To avoid these environmental and health problems, there is a trend to search for naturally occurring toxicants from plants. Plant essential oils may provide potential alternatives to currently used PWN control agents because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages (Isman, 2006). Furthermore, plant essential oils and their components have been reported to have nematicidal activity against PWN (Park et al., 2005; Kong et al., 2006; Choi et al., 2007a, 2007b, 2007c).

In this study, we investigated the nematicidal activity of commercial plant essential oils and their components against PWN to find potential alternatives to currently used PWN control agents or model compounds for the development of chemically synthesized derivatives with enhanced activity or environmental safety.

Materials and Methods

Collection of PWN: Bursaphelenchus xylophilus was isolated from chips of infected pine wood collected in Haman area (in March 2004), Gyoungsangnam-do province, Korea, and extracted by the Baermann funnel method (Chawla and Prasad, 1975). Details of isolation and culture of PWN are well described by Park et al. (2005).

Chemicals: Plant essential oils were purchased from Oshadhi Ltd. (Cambridge, UK) (Table 1). Isoeugenol (purity 98%), 1,8-cineole (purity 99%), (+)-limonene (purity 97%), verbenol (purity 95%) and myrcene (purity 95%) were purchased from Sigma-Aldrich (Milwaukee, WI). Eugenol (purity 99%), *p*-cymene (purity 95%), α;-humulene (purity 98%), γ-terpinene (purity 97%), terpinen-4-ol (purity 99%), geraniol (purity 96%), α;-terpinene (purity 85%) and thymol (purity 99%) were purchased from Fluka (Buchs, Switzerland). Methyl isoeugenol (purity 98%), α;-pinene (purity 95%), camphene (purity 80%), α;-terpineol (purity 95%), β;-caryophyllene (purity 90%), carvacrol (purity 95%) and β;-pinene (purity 94%) were purchased from Tokyo Kasei (Tokyo, Japan). Acetyl eugenol (purity 97%), methyl eugenol (purity 98%) and linalool (purity 98%) were purchased from Wako (Osaka, Japan). Triton X-100 was purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade. Nematicidal activities of isoeugenol, methyl isoeugenol and acetyl eugenol were tested in this study to learn the relationship between nematicidal activity and chemical structure.

Synthesis of neral, geranial and 6-Methyl-5-hepten-2-one: 6-Methyl-5-hepten-2-one, geranial and neral were ob-

Received for publication 3 August, 2007.
Division of Forest Insect Pests and Diseases, Korea Forest Research Institute, Seoul 130–712, Republic of Korea
E-mail: parkil1@forest.go.kr
This paper was edited by Ed Lewis.

TABLE 1. List of plant essential oils tested.

Oil	Source of plant	Part	Origin
Ajowan	*Trachyspermum ammi*	Seeds	India
Allspice	*Pimenta dioica*	Berries	Jamaica
Amyris	*Amyris balsamifera*	Wood	Caribbean
Artemisia afra	*Artemisia afra*	Flowering plant	South Africa
Cabreuva	*Myrocarpus fastigiatus*	Wood	Brazil
Cajeput	*Melaleuca cajuputii*	Leaves	Indonesia
Cananga	*Cananga odorata*	Blossoms	Indonesia
Cardamom	*Elettaria cardamomum*	Seeds	Equador
Carrot Seeds	*Daucus carota*	Seeds	France
Carvi	*Carum carvi*	Seeds	Egypt
Clementine	*Citrus clementina*	Rind	South Africa
Copaiva	*Copaifera reticulata*	Resin	Brazil
Coriander	*Coriandrum sativum*	Fruits	Argenitina
Davana	*Artemisia pallens*	Leaves	India
Dill	*Anethum graveolens*	Seeds	Bulgaria
Elemi	*Canarium luzonicum*	Resin	Phillipines
Fokienia	*Fokienia hodgensii*	Wood	Vietnam
Frankincense	*Boswellia carterii*	Resin	Ethiopia
Galbanum	*Ferula galbaniflua*	Resin	Iran
Geranium	*Pelargonium graveolens*	Leaves	Reunion
Gurjum	*Dipterocarpus turbinatus*	Resin	Indonesia
Hyssop	*Hyssopus officinalis*	Flowering plant	France
Larh	*Larix europea*	Resin	Austria
Lavandin	*Lavandula hybrida*	Flowering plant	France
Litsea	*Litsea cubeba*	Fruits	Vietnam
Patchouli	*Pogostemon patchouli*	Whole plant	Indonesia

tained from corresponding alcohols by Pyridinium dichromate (PDC) oxidation (Corey and Schmidt, 1979). The products were confirmed by comparison of the MS spectral data and the retention time with those of authenticated samples. PDC oxidation of 6-methyl-5-hepten-2-ol gave the corresponding ketone in >99.9% purity. However, oxidation of geraniol and nerol gave 85.8% pure geranial (main impurity was neral at 12.1%) and 75.4% pure neral (main impurity was geranial at 21.8%), respectively. Because geraniol and nerol are allyl alcohols, PDC oxidation of those alcohols gave geometric isomers, as reported by Corey and Schmidt (1979). Synthesized geranial and neral were used in bioassay without further purification.

Nematicidal activity: Concentrations of plant essential oils and their components were prepared by serial dilution with distilled water containing Triton X-100 (5,000 ppm). Test solutions were introduced into in wells of 96-well plates. In each well, the concentration of nematodes was between 50 and 150 nematodes (mixtures of juvenile and adult nematodes, male:female: juvenile ≈ 1:1:2) per 100 μl of water. Controls received a distilled water-Triton X-100 solution. Treated and control nematodes were held under the same conditions as used for colony maintenance. Mortality of nematodes was recorded after 24 hr under a microscope. Nematodes were defined as dead if their bodies were straight and they did not move, even after transferral to clean water.

Gas chromatography (GC-FID): Gas chromatography analysis was performed on the Agilent 6890N equipped with flame ionization detector. Retention times for comparison with authentic compounds were measured with a DB-1MS and a DB-FFAP column (30m × 0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as: isothermal at 40°C for 1 min, then raised to 250°C at 6°C/min and held at this temperature for 4 min. Helium was used as the carrier gas at the rate of 1.5 ml/min.

Gas chromatography-mass spectrometry: The essential oils of ajowan, allspice and litsea were analyzed on a gas chromatograph (Agilent 6890N)-mass spectrometer (Agilent 5973N MSD) (GC-MS) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.5 ml/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (280°C). Ionization was obtained by electron impact (70eV, source temperature 230°C). Scan range was 25–800 amu. Compounds were tentatively identified by comparison of mass spectra of each peak with those of authentic samples in the NIST MS library.

Statistical analysis: Proportional nematode mortality was transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test, and LC_{50} values were calculated by probit analysis (SAS, 1999). Six concentrations for ajowan (1, 0.7, 0.6, 0.5, 0.4 and 0.3 mg/ml) and litsea oil (2, 1, 0.7, 0.5, 0.3 and 0.2 mg/ml) and five concentrations for allspice (0.9, 0.7, 0.6, 0.5 and 0.4 mg/ml) were used to obtain the LC_{50} values. Four concentration for geranial (0.6, 0.4, 0.2 and 0.1 mg/ml), five concentrations for neral (1, 0.8, 0.6, 0.4 and 0.2 mg/ml), isoeugenol (0.4, 0.3, 0.2, 0.15 and 0.1 mg/ml) and methyl isoeugenol (0.4, 0.3, 0.2, 0.15 and 0.1 mg/ml) and six concentrations for eugenol (0.8, 0.6, 0.5, 0.4, 0.3 and 0.2 mg/ml) were used to obtain the LC_{50} values. Three replicates were completed for each concentration.

RESULTS

Nematicidal activity of plant essential oils: Nematicidal activity of plant essential oils are shown in Table 2. LC_{50} values of ajowan, allspice and litsea were 0.431, 0.609 and 0.504 mg/ml, respectively. LC_{50} values of the other plant essential oils were >2.0 mg/ml.

Chemical components of plant essential oils: Total ion chromatograms and chemical compositions of three active essential oils, ajowan, allspice and litsea, are shown in Table 3. Retention indices were obtained with an equation proposed by van Den Dool and Kratz (1963).

Nematicidal activity of individual compounds: Nemati-

TABLE 2. Nematicidal activity of ajowan, allspice and litsea essential oils.

Essential oils	Slope(± SE)	LC_{50} (mg/ml)	95% CL[1]	df	χ^2
Ajowan	4.3 ± 0.4	0.431	0.400–0.460	4	5.58
Allspice	10.1 ± 0.8	0.609	0.589–0.630	3	4.26
Litsea	2.2 ± 0.2	0.504	0.444–0.566	4	4.81

[1] denotes confidence limit.

cidal activity of compounds identified in ajowan, allspice and litsea are shown in Table 4. Geranial, eugenol, isoeugenol and methyl isoeugenol showed 100% nematicidal activity against PWN at 1.0 mg/ml concentration. Neral and methyl eugenol produced moderate activity. Nematicidal activity of other compounds was less than 10%. LC_{50} values of active compounds are shown in Table 5. Geranial was the most active (LC_{50} = 0.120 mg/ml), followed by isoeugenol (LC_{50} = 0.200 mg/ml), methyl isoeugenol (LC_{50} = 0.210 mg/ml), eugenol (LC_{50} = 0.480 mg/ml), methyl eugenol (LC_{50} = 0.517 mg/ml) and neral (LC_{50} = 0.525 mg/ml).

DISCUSSION

Many plant essential oils and phytochemicals are known to possess nematicidal activity (Chitwood, 2002). Essential oils of *Carum carvi, Foeniculum vulgare, Mentha rotundifolia* and *Mentha spicata* (Oka et al., 2000), three Lamiaceae (*Ocimum basilicum*, *O. sanctum* and *Mentha piperatum*) and two Myrtaceae (*Callistemon lanceolatus* and *Eugenia caryophyllata*) (Sangwan et al., 1990) have been reported to show nematicidal activity. Nematicidal activity of plant essential oils and their components against PWN has also been reported (Park et al., 2005; Choi et al., 2007a, 2007b, 2007c). In our study, a total of three plant essential oils, ajowan, allspice and litsea, showed nematicidal activity at 2 mg/ml. Among essential oils tested, ajowan oil was the most toxic, followed by litsea and allspice.

Various compounds, including alcohols, aldehydes, fatty acid derivatives, terpenoids and phenolics exist in plant essential oils. Jointly or independently, they contribute to insecticidal or nematicidal activity. In this study, the nematicidal constituents of oils were identified by GC-MS analysis. Among identified components of ajowan oil, nematicidal activity of α;-pinene, camphene, β;-pinene, myrcene, limonene, γ-terpinene, terpinen-4-ol, thymol and carvacrol against PWN have been reported in a previous study (Choi et al., 2007a). Thymol and carvacrol were very effective against PWN. The present and previous studies confirm that nematicidal activity of ajowan oil was mainly attributed to the activity of thymol and carvacrol. Eugenol was the most abundant component, followed by β;-caryophyllene, methyl eugenol, α;-humulene, limonene and 1,8-cineole in allspice oil. To investigate the structure-

TABLE 3. Chemical composition of ajowan, allspice and litsea essential oils.

No.	Compound	RI[1] DB-1	RI[1] FFAP	Composition ratio (%) Ajowan	Composition ratio (%) Allspice	Composition ratio (%) Litsea
1	α-Pinene	928	1,014	0.87	—	1.22
2	Camphene	940	1,054	0.10	—	0.27
3	Sabinene	962	1,147	—	—	1.51
4	6-Methyl-5-hepten-2-one	964	1,330	—	—	1.34
5	β-Pinene	967	1,098	1.26	—	1.06
6	Myrcene	981	1,155	0.48	—	0.73
7	α-Terpinene	1,007	1,169	0.13	—	—
8	*p*-Cymene	1,012	1,261	24.40	—	—
9	1,8-Cineole	1,018	1,196	0.32	0.10	1.21
10	Limonene	1,020	1,189	0.44	0.18	14.64
11	γ-Terpinene	1,050	1,237	27.77	—	—
12	Linalool	1,084	1,536	—	—	1.38
13	Verbenol	1,121	1,642	—	—	0.35
14	Citronellal	1,130	1,467	—	—	0.57
15	Terpine-4-ol	1,159	1,590	0.32	—	—
16	α-Terpineol	1,170	1,685	—	—	0.44
17	Neral	1,216	1,670	—	—	30.27
18	Geraniol	1,236	1,834	—	—	0.50
19	Geranial	1,245	1,721	—	—	39.23
20	Thymol	1,273	2,167	41.77	—	—
21	Carvacrol	1,278	2,193	0.55	—	—
22	Eugenol	1,334	2,151	—	86.44	—
23	Methyl eugenol	1,370	2,006	—	3.87	—
24	β-Caryophyllene	1,414	1,583	—	7.7	0.60
25	α-Humulene	1,447	1,656	—	0.99	—
Total				98.42	99.28	95.32

[1] Retention indices = van Den Dool and Kratz retention index (van Den Dool and Kratz, 1963) on DB-1 and FFAP columns, according to *n*-alkanes (C9–C16; DB-1, C7–C20; FFAP). Components were identified by co-injection with authentic standard on two columns.

TABLE 4. Nematicidal activity of components from ajowan, allspice and litsea esstial oils.

Compounds[1]	Mortality (Mean ± SE, %)
α-Terpinene	3.3 ± 1.9c[2]
p-Cymene	6.7 ± 2.8
1,8-Cineole	3.4 ± 0.9
β-Caryophyllene	1.2 ± 0.5c
α-Humulene	2.2 ± 0.7c
6-Methyl-5-hepten-2-one	1.8 ± 1.4c
Verbenol	5.2 ± 1.3c
Neral	82.3 ± 3.8b
Geranial	100a
Eugenol	100a
Methyl eugenol	73.9 ± 2.0b
Isoeugenol	100a
Methyl isoeugenol	100a
Acetyl eugenol	12.5 ± 1.6c
Control	1.1 ± 0.5c

[1] 1.0 mg/ml concentration was applied.
[2] Means within a column followed by same letters are not significantly different ($P = 0.05$, Scheffes' test).

activity relationships, isoeugenol, methyl isoeugenol and acetyl eugenol were also tested in this study. Among the test compounds, acetyl eugenol showed the weakest activity against PWN at 1.0 mg/ml concentration. This result indicates that the functional group at the C1 position of the benzene ring is very important for nematicidal activity. Nematicidal activity of compounds with hydroxyl (-OH) or methoxy (OCH_3) groups were stronger than those with an acetyl group. Among active compounds, there were differences in nematicidal activities. Nematicidal activities of isoeugenol and methyl isoeugenol were stronger than eugenol and methyl eugenol. This result indicates that the position of the double bond of the propenyl group is very important for nematicidal activity. Nematicidal activity of citral (a mixture of neral and geranial, 3:7) against PWN has been reported in a previous study (Choi et al., 2007a). In this study, we synthesized two isomers to determine the activity of each compound. Purities of synthesized geranial and neral were 85.8% and 75.4%, respectively. Nematicidal activity of geranial was about 4.3 times greater than neral. Structure-activity relationships of *cis-* and *trans-* isomers of various compounds have been well studied. Park (2000) reported that the insecticidal activity of *cis*-asarone was more pronounced against adults of *Sitophilus oryzae* (Coleoptera: Rhynchophoridae), *Callosobruchus chinensis* (Coleoptera: Bruchidae) and *Lasioderma serricorne* (Coleoptera: Anobiidae) than that of *trans*-asarone. Lee et al. (2002) also reported that there was a difference in insecticidal activity between *cis-* and *trans*-asarone. These and our results indicate that the position of the substituent in geometrical isomer is very important for insecticidal or nematicidal activity.

TABLE 5. Nematicidal activity of active compounds from ajowan, all spice and litsea essential oils.

Compounds	Slope (± SE)	LC_{50} (mg/ml)	95% CL[1]	df	χ^2
Neral	3.1 ± 0.1	0.525	0.503–0.548	3	4.50
Geranial	2.2 ± 0.1	0.120	0.107–0.132	2	3.59
Eugenol	5.6 ± 0.2	0.480	0.468–0.491	4	5.13
Methyl eugenol	2.3 ± 9.2	0.517	0.490–0.546	4	2.97
Isoeugenol	5.7 ± 0.2	0.200	0.194–0.205	3	2.26
Methyl isoeugenol	4.9 ± 0.1	0.210	0.204–0.216	3	1.72

[1] denotes confidence limit.

Elucidation of the mode of action of oils and their constituents is of practical importance for nematode control, because it may give useful information on the most appropriate formulation and delivery means. In the current study with *B. xylophilus*, the dead nematode body was generally extended and without movement. Kong et al. (2006) reported that PWN bodies treated with the muscle activity blockers levamisole hydrochloride and morantal tatrate usually exhibited semicircular and coiling shapes, respectively. These results suggest that the nematicidal mode of action between the essential oils and commercial nematicides might be different. Amino and hydroxyl groups have been hypothesized as target sites of methyl isothiocyanate in nematodes (Wright, 1981). Some essential oils have been reported to interfere with the neuromodulator octopamine (Kostyukovsky et al., 2002) or GABA-gated chloride channels of insect pests (Priestley et al., 2003). However, the exact mode of action of essential oils and its components against PWN is unclear.

In conclusion, ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and litsea (*Litsea cubeba*) essential oils and their components described appear to be useful as natural nematicides for *B. xylophilus*. For the practical use of the three essential oils and their components as novel nematicides to proceed, further study is necessary on systemic action, phytotoxicity and formulation for improving nematicidal potency and stability and reducing cost.

LITERATURE CITED

Chawla, M. L., and Prasad, S. K. 1975. Techniques in nematology. II. Comparative efficiency of sampling tools and nematode extraction methods. Indian Journal of Nematology 4:115–123.

Chitwood, D. J. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology 40:221–249.

Choi, I. H., Kim, J. H., Shin, S. C., and Park, I. K. 2007a. Nematicidal activity of monoterpenoids against the pine wood nematode (*Bursaphelenchus xylophilus*). Russian Journal of Nematology 15:35–40.

Choi, I. H., Park, J. Y., Shin, S. C., Kim, J. H., and Park, I. K. 2007b. Nematicidal activity of medicinal plant essential oils against the pine wood nematode (*Bursaphelenchus xylophilus*). Applied Entomology and Zoology 42:397–401.

Choi, I. H., Shin, S. C., and Park, I. K. 2007c. Nematicidal activity of onion (*Allium cepa*) oil and its components against the pine wood nematode (*Bursaphelenchus xylophilus*). Nematology 9:231–235.

Corey, E. J., and Schmidt, G. 1979. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in approtic media. Tetrahedron Letters 20:399–402.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51:45–66.

Kishi, Y. 1995. The pine wood nematode and the Japanese pine sawyer. Tokyo: Thomas Company Limited.

Kong, J. O., Lee, S. M., Moon, Y. S., Lee, S. G., and Ahn, Y. J. 2006. Nematicidal activity of plant essential oils against *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). Journal of Asia-Pacific Entomology 9:173–178.

Korea Forest Service. 2003. Guideline for the control of forest diseases and insect pests. Daejeon: Korea Forest Service.

Korea Forest Service. 2004. Statistical yearbook of forestry. Daejeon: Korea Forest Service.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N., and Shaaya, E. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests. Pest Management Science 58:1101–1106.

Lee, S. M., Chung, Y. J., Moon, Y. S., Lee, S. G., Lee, D. W., Choo, H. Y., and Lee, C. K. 2003. Insecticidal activity and fumigation conditions of several insecticides against Japanese pine sawyer (*Monochamus alternatus*) larvae. Journal of Korean Forestry Society 92:191–198.

Lee, H. K., Park, C., and Ahn, Y. J. 2002. Insecticidal activities of asarones identified in *Acorus gramineus* rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutoidae). Applied Entomology and Zoology 37:459–464.

Oka, Y., Nacar, S., Putievsky, E., Ravid, U., Yaniv, Z., and Spiegel, Y., 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology 90:710–715.

Park, C. 2000. Insecticidal activity of β;-asarone derived from *Acorus gramineus* rhizome against insect pests. MS thesis, Seoul National University, Suwon.

Park, I. K., Park, J. Y., Kim, K. H., Choi, K. S., Choi, I. H., Kim, C. S., and Shin, S. C. 2005. Nematicidal activity of plant essential oils and components from garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) oils against the pine wood nematode (*Bursaphelenchus xylophilus*). Nematology 7:767–774.

Priestley, C. M., Williamson, E. M., Wafford, K. A., and Sattelle, D. B. 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human $GABA_A$ receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. British Journal of Pharmacology 140:1363–1372.

Sangwan, N. K., Verma, B. S., Verma, K. K., and Dhindsa, K. S. 1990. Nematicidal activity of some essential plant oils. Pesticide Science 28:331–335.

SAS INSTITUTE. 1999. SAS/STAT User's Guide, release 8.0, SAS Institute: Cary, North Carolina.

van Den Dool, H., and Kratz, P. D. 1963. Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A 11:463–471.

Wright, D. J. 1981. Nematicides: Mode of action and new approaches to chemical control. Pp 421–449 *in* B. M. Zukerman and R. A. Rhode, eds. Plant Parasitic Nematodes, Vol. III, New York: Academic Press.

Yi, C. K., Byun, B. H., Park, J. D., Yang, S. I., and Chang, K. H. 1989. First finding of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, and its insect vector in Korea. Research Reports of Forestry Research Institute 38:141–149.