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D. R. Viglierchio
University of California - Davis

Armand R. Maggenti
University of California - Davis

R. V. Schmitt
University of California - Davis

G. A. Paxman
University of California - Davis

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Nematicidal Injection: Targeted Control of Plant-Parasitic Nematodes of Trees and Vines

D. R. VIGLIERCHIO[1], A. R. MAGGENTI[1], R. V. SCHMITT[2], and G. A. PAXMAN[2]

Abstract: Pressurized injection of nematicidal solutions was effective for control of nematodes within trees and vines. Significant ($P = 0.01$) control of *Pratylenchus vulnus* on grape was attained with four nonfumigants (carbofuran, oxamyl, phenamiphos, and sulfocarb) and one fumigant nematicide (DBCP). *Pratylenchus penetrans* was controlled ($P = 0.05$ and 0.1) in apples and walnuts with sulfocarb and oxamyl. This species also was controlled in apples with carbofuran and phenamiphos. The advantages of pressure injection over traditional methods of nematicide applications are discussed. *Key Words:* grapes, apples, walnuts, *Pratylenchus vulnus*, *P. penetrans*.

The concept and practice of tree injection is not new; Granade (1) in 1158 injected trees for medicinal enhancement of the fruit. Leonardo da Vinci's fifteenth century notebooks contain sketches of equipment and experiments of tree injections (1). Heffernan (1) compiled an extensive review of tree implantation and injection. Tarjan (4) was the first in Nematology to try pressure injection for control of *Radopholus similis* (Cobb) Thorne. He was unsuccessful in his trial with 54 test chemicals, none of which were recognized nematicides.

Among the pressing problems in agricultural nematology is the control of nematodes parasitizing deciduous fruit and nut trees, and vines. Currently, growers use preplant fumigation, clean stock or resistant rootstocks, and, in some instances, postplant-soil applications of fumigant and nonfumigant nematicides. To date, postplant treatments have not been particularly efficient in the control of nematodes within tree roots.

Our objective was to determine the efficacy of injections in the control of nematodes parasitic on trees and vines. A secondary objective concerned possible seasonal differences in the sensitivity of treated plants.

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[1] Nematologists and [2]Staff Research Associates, respectively, Division of Nematology, University of California, Davis, CA 95616.

MATERIALS AND METHODS

Injection equipment: For greenhouse studies, a glass high-pressure chromatography preparative column 15 mm x 1,000 mm (capacity 174 ml) capable of withstanding 21.1 kg/cm^2 was used as a chemical reservoir. Pressure from a nitrogen cylinder was adjusted by means of a pressure regulator. Injection volume was calibrated on the glass reservoir and controlled by a four-way teflon-copolymer-slide valve rated at 35.2 kg/cm^2. Tubing, rated at 35.2 kg/cm^2, was teflon (3.2 mm, o.d.). The terminal coupling was modified to accommodate a female Luer-Lok for a hypodermic needle (No. 20). A "C" clamp (Fig. 1) was modified to hold the hypodermic needle in the tree during injection. To prevent leakage, rubber pads were attached to the expanded faces of the clamp. For field applications, a 40-mm x 700-mm stainless steel tube, capacity 880 ml, was utilized for the chemical reservoir. Pressure supply, tubing and the "C" clamp holding the hypodermic needle were the same as used in greenhouse studies. The volume of solution injected was controlled by an external calibrated sight tube (Type T, 70.3 kg/cm^2) and a four-way teflon-copolymer-slide valve.

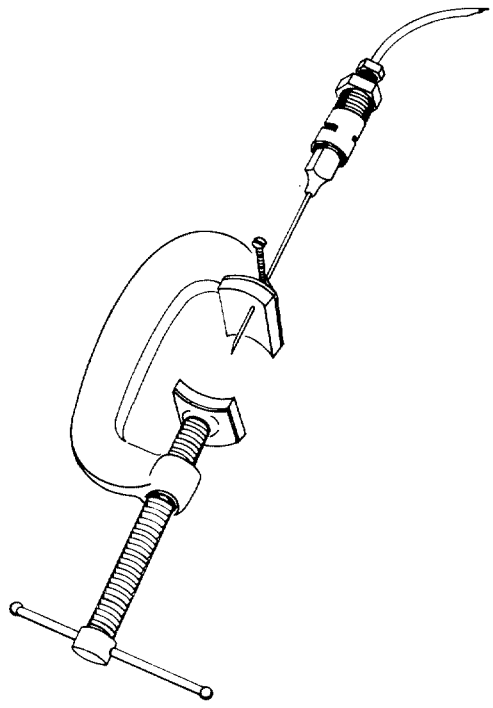

FIG. 1. Modified "C" clamp for pressure injection; illustration of hyprodermic needle and Luer-Lok assembly.

The procedure for injection was the same in greenhouse and field studies. In order to expose the greatest cross-sectional area of wood to the injected solution while minimizing injury, a 2.34-mm hole was drilled completely through the seedling or vine trunk. Holes were drilled as close to the soil level as was practical. The injection hole was drilled with a Skil cordless 9.5-mm drill (300 rpm) and high-speed, twist bits. The hypodermic needle, in the modified "C" clamp, was inserted into the hole and the clamp closed around the trunk. Solutions were injected at 12.3 kg/cm^2. After application, injection holes were sealed with a grafting compound.

A 0.1% solution of Eosin was used as a marker to determine characteristics of transport (2). For volumes, times, and distances of transport, see Table 1. In greenhouse studies, for the testing of phytoxicity and methods of application, a single volume of 5 ml of oxamyl was used. The plants and concentrations of oxamyl tested were: sour orange, *Citrus aurantium* L.; 'Thompson seedless' grape, *Vitis vinefera* L.; and black walnut, 1- and 2-year-old *Juglans hindsii* Jeps. at 0.25 mM, 1.25 mM, 6.25 mM; peach 'Lovell', *Prunus persica* Batsch. at 0.25 mM, 1.25 mM, 6.25 mM, 31.25 mM, and 156.25 mM.

Field phytotoxicity studies were conducted in the spring and fall on 2.5-year-old St. George rootstocks, *Vitis rupestris* Scheele, 25 to 35 mm in trunk diameter. Each treatment was replicated 6 times. Each treatment was injected at 8.8 kg/cm^2 with a volume of 26.2 ml of material. The following chemicals and concentrations were tested in the spring and fall: carbofuran, 0.25 mM, 1.25 mM, 6.25 mM; oxamyl, 0.25 mM, 1.25 mM, 6.25 mM, 31.25 mM; sulfocarb, 0.25 mM, 1.25 mM, 6.25 mM, 31.25 mM; phenamiphos, 0.25 mM, 1.25mM, DBCP, 0.01 mM, 0.05 mM, 0.25 mM; acetone solution, 5% and 10%. In the spring, an additional replicated series of phenamiphos, 1.25 mM, and DBCP, 1.25 mM, received three times the volume (78.6 ml) of the regular treatments.

Nematicidal treatments were conducted on trees and vines that had a trunk diameter, at the point of injection, of at least 12.5 mm. Various volumes and concentrations (Tables 2, 3) of the following were tested: carbofuran, oxamyl, phenamiphos,

TABLE 1. Solute transport of pressure injected 0.1% eosin in selected plant species.

Plant	Trunk Diameter (mm)	Injection time (min)	Pressure (kg/cm^2)	Volume injected (ml)	Stained tissue above injection (cm)	Smallest roots stained
Olive	7	60	7.0	6.8	62	+
Apple	7	16	10.5	2.5	58	+
Orange	8	20	10.5	8.2	50	+
Peach	6	50	14.1	3.4	58	+
Grape	20	9	10.5	4.6	100	+
Walnut	6	38	17.6	1.5	26	+

TABLE 2. Nematode control one month after trunk injection with 5 ml of nematicidal solutions at 12.3 kg/cm² (spring application).

Treatment	Rate mM	Average number *Pratylenchus*/gram of root[a]			
		Grape *P. vulnus*	Apple *P. penetrans*	1-year walnut *P. vulnus*	2-year walnut *P. vulnus*
Check	—	106	517	239	13
Sulfocarb	0.25	16**	—	71***	—
	1.25	37*	379	160	—
	6.25	40*	180***	286	—
	31.25	3**	264	75***	—
Oxamyl	0.25	28*	—	120	—
	1.25	8**	520	134	—
	6.25	28*	428	69***	—
	31.25	11**	160***	30*	—
Carbofuran	0.25	71	—	314	—
	1.25	19**	206***	159	—
Phenamiphos	0.25	20**	447	—	3
	1.25	27*	196***	—	11
DBCP	0.05	85	—	—	4
	0.25	5**	—	—	12
	1.25	18**	337	—	10

[a]Asterisks (*, **, ***) indicate significant control at $P = 0.05$, 0.01, and 0.10, respectively.

TABLE 3. Control of *Pratylenchus vulnus* on Thompson seedless grape one month after trunk injection with nematicidal carbamate and phosphate solutions at 12.3 kg/cm².

Treatment	Rate mM	ml injected	Nematodes[a] gram of root
Check	—	—	8.3
Sulfocarb	0.05	5	2.2**
Sulfocarb	0.05	25	0.3**
Sulfocarb	0.25	25	1.2**
Oxamyl	0.05	5	1.9**
Oxamyl	0.05	25	0.5**
Oxamyl	0.25	25	1.7**
Carbofuran	1.25	25	4.2*
Phenamiphos	0.05	5	8.2

[a]Asterisks (*, **) indicate significant control at $P = 0.05$ and 0.01, respectively.

sulfocarb (aldicarb sulfoxide), and 1-3 dibromo-3-chloropropane (DBCP). Two times of application were selected: late spring, when trees and vines were actively growing, and midsummer, when fruit was maturing. The spring application was performed on 6-month-old Thompson seedless grapes and 1- and 2-year-old seedlings of *J. hindsii* growing in 20-cm diameter pots. Plants were inoculated with 5,000 *Pratylenchus vulnus* 3 months prior to nematicidal injections. Also included were 2-year-old, bare-root apple seedlings, *Malus sylvestris* ('Golden delicious' on 'Malling 106') field-infected with *P. penetrans*, which were planted in 20-cm pots and maintained 6 months prior to injection. Treatments were replicated four times. Chemicals, rates, and concentrations are listed in Table 2.

The midsummer application involved only Thompson seedless grape cuttings grown 6 months prior to injection. Potting and inoculation with *P. vulnus* was carried out as previously described. However, one set of pots was inoculated 1 month prior to injection (Table 3) and a second set of pots was inoculated 3 months prior to injection. Both experiments were replicated 4 times. The vines in the pots inoculated 3 months prior to application were treated with 25 ml of DBCP pressure injected (12.3 kg/cm²) at concentrations of 0.05 and 0.25 mM.

At harvest (one month after injection for all experiments), the total root system of each plant was individually weighed and placed in a mist chamber extractor for 1 week; then nematodes were collected and counted. The soil from each pot was wet-screened and placed on a Baermann funnel for 3 days, and then the soil population of nematodes was counted.

RESULTS

Our transport studies with Eosin injections included seedlings or cuttings of

Lovell peach, Thompson seedless grape, Golden delicious apple on Malling 106, olive, orange and black walnut. When leaves began to guttate, the dye was immediately evident. The distances that a 0.1% solution of Eosin moved from the point of injection, as well as the time for the solute transport, are in Table 1. The volume of chemical to be injected was determined by the amount of solution the plant would take either before guttation began or the solution ceased to flow, regardless of pressures (up to 17.6 kg/cm^2) used. The volume of solution that could be injected varied from 2% of the plant volume (roots, stems, and leaves) to 30%. Among the plant species tested, the various volume percentages were: almond, 2; apple, 5; grape, 8; orange, 9; olive, 9; peach, 11; and walnut, 30%. Of these plants, walnut was generally the most difficult to inject; volumes of injected solution in walnut varied from 3 to 30% of the plant volume. This resistance to injection was unexpected because severed walnut branches, 1 m long with tip removed, proved to be no hindrance to solution movement at a pressure of 3.5 kg/cm^2.

The only phytotoxic effects observed in greenhouse experiments were on Lovell peach injected with 5 ml oxamyl at 31.25 mM (6,881.3 mg/ml) and 156.25 mM (34,406.3 mg/ml) and on apples injected with 5 ml oxamyl at 31.25 mM and 5 ml of sulfocarb at 31.25 mM (6,946.9 mg/ml).

Although there was no external phytoxicity noted in grapes, there was discoloration of the pith and some necrosis of stem tissue immediately surrounding the injection site at the rates of 6.25 mM or greater of oxamyl. Notable injection site tissue damage of this type was eliminated in subsequent experiments by the application of grafting compound to the injection holes.

In the field, no phytotoxic effects were noted with St. George grape rootstock injected in the fall when vines were dormant and examined in the spring when vines showed active, new growth. There were no detectable differences in shoot length, leaf size, or fruit set when rootstock injected in the fall was compared the following spring with nontreated controls. Acetone (5% and 10% solutions) was included in these field tests because it was used to effect solution of the nematicides in water at the higher concentrations. The same results were obtained with the spring-treated vines, *i.e.* no detectable toxicity.

The control by the various nematicidal treatments in grape, apple, and black walnut is shown in Table 2. Little control of *P. vulnus* and *P. penetrans* was obtained with low rates of carbofuran or DBCP on any plant tested. The higher rates on grape provided significant control. The lower rates of sulfocarb, oxamyl, and phenamiphos suppressed nematode populations ($P = 0.01$). The highest concentration of sulfocarb and oxamyl gave similar results. Only slight control ($P = 0.10$) of *P. penetrans* was effected on apple. With walnut, the best result ($P = 0.05$) was obtained with oxamyl at 31.25 mM; there were indications of control ($P = 0.10$) with sulfocarb at 0.25 mM and 31.25 mM and oxamyl at 6.25 mM. No differences were obtained with 2-year-old walnuts treated with phenamiphos or DBCP.

In the final two experiments with Thompson seedless grape (Table 3), both concentration and volume of injected nematicides were varied. Emphasis was on the earlier, successful lower concentrations with increased volumes. All injection treatments (Table 3), of oxamyl and sulfocarb gave significant control of *P. vulnus* ($P = 0.01$), but carbofuran (1.25 mM, 25 ml injected) was less effective ($P = 0.05$). Phenamiphos (0.05 mM, 5 ml injected) provided no control of this nematode. DBCP injected at 25 ml of 0.05 mM into grape reduced *P. vulnus* in the roots ($P = 0.05$). The higher concentration (0.25 mM) did not effect control.

Nematicidal injections in all these experiments had no measurable effect upon the soil population of *P. vulnus* or *P. pentrans*, irrespective of the time of application, material injected, or volume.

DISCUSSION

Our study of solute transport was designed to determine whether an injected solution could, under pressure, be distributed to all portions of the plant. Sachs (3) previously conducted experiments on the amount of solute redistributed to various

parts of the plant. He measured an increase of injected solute in the root system and a decrease in the shoot system between 1 and 14 days after injection. Nyland and Sachs (2) reported clear xylem sap exuded from cut surfaces almost immediately after injections were commenced. In our trials, the dye was evident in the first guttated droplets.

The lack of external phytoxicity noted in these experiments was unexpected. There was no evidence in grapes that the seasonal timing of treatments played any role in phytoxicity. Some of the vines bore berries at the time of injection; yet the treatments had no visible effect on fruit maturity or vine condition. Efficacy of nematicidal activity, however, may well be coordinated with season. Our data show that the nematicidal activity of sulfocarb and oxamyl was lower in the spring during active growth, whereas carbofuran, phenamiphos, and DBCP showed the greatest nematicidal activity during the same period. During midsummer, sulfocarb and oxamyl showed their highest activity, whereas carbofuran, phenamiphos, and DBCP declined in activity.

It may be that the slight tissue necrosis noted in early experimentation influenced nematicidal activity and movement. The application of grafting compound to the wound area, lowering concentrations, or increasing volumes injected avoided the problem and may have added to the increased control levels recorded. Also the lower concentrations and increased volume injected were more likely to maintain lethal concentrations for a longer period of time.

Our experiments illustrated the feasibility of pressurized trunk injection of nematicides for the control of parasitic nematodes of perennial trees and vines. Pressurized injection of nematicidal solutions offers a potentially effective method of overcoming many of the hindrances to the success of conventional application methods, as steep hillsides, rocky ground, inclement weather, heavy clay soils, high organic soils, or too much or too little soil moisture. By being directed to the target organism from within the tree or vine, environmental pollution of the soil, water and air is kept to a minimum as compared to the level of pollution in standard methods of soil fumigation or repeated spray programs.

The amount of chemicals retained within the plant and/or fruit when this method of application is used is unknown.

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