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Induction of Tolerance to Root-Knot Nematode by Oxycom[1]

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Abstract: Oxycom applications increased plant growth and population levels of *Meloidogyne incognita* on susceptible tomato. A single Oxycom drench at 2,500 ppm applied 7 days prior to inoculation with *M. incognita* provided remediation of plant growth measured 63 days later. This occurred without reducing nematode population levels. Follow-up drenches at 2,500 ppm at 10-day intervals stunted shoots and roots ($P = 0.05$). The same application rates at 20-day intervals did not reduce plant growth. Plants receiving multiple drenches had more galls ($P = 0.05$), females, and second-stage juveniles (J2) per root system compared to plants receiving only the single treatment. Foliar mass and height of plants treated with a single pre-inoculation Oxycom drench were indistinguishable from plants without nematodes. Oxycom treatments activated signaling pathways for plant defense as confirmed by detection of elevated defense gene transcripts in root tissues. The finding of increased reproduction of root-knot nematode without loss of plant growth is consistent with the definition of induced tolerance. Frequency, rate, and timing of applications need further study with other nematodes and various field settings.

Key words: Ethylene, growth stimulation, induced resistance, MAPK activation, nematodes, salicylic acid, tolerance.

Root-knot nematode, *Meloidogyne incognita,* is an economically significant pathogen of many crops that causes extensive damage in temperate regions of the world (Sassar and Freckman, 1988). Production of high-value crops has relied on a variety of tools directly lethal to nematodes, including pre- and post-plant nematicides. Phase-out of methyl bromide (Noling and Becker, 1994), removal of phenamiphos from the U.S. market in 2005 (Bayer Chemical, comm.), and California restrictions of 1,3-dichloropropene and metam sodium (township caps and metam sodium buffer zones, etc.) pose challenges for California's intensive agriculture. Alternative or integrated measures for plant protection against nematodes may involve the chemical activation of the plant's natural defense mechanisms.

Natural plant defense mechanisms can involve preformed physical and chemical barriers or induced mechanisms. In addition to the induced localized hypersensitive response, which requires specific recognition events between the host and its challenging pathogen, plants possess other mechanisms that limit pathogen ingress and reproduction in a systemic manner. For example, salicylic acid and benzothiodiazole (Conrath et al., 1995; Kessmann et al., 1994; Shericca et al., 1998) stimulate increased expression from several defense genes, including some of the protective proteins termed pathogenesis-related proteins, resulting in plant resistance or tolerance to pathogens. Chemical induction of "systemic acquired resistance" is observed with benzothiodiazole in tomato and grapevines against *M. incognita* (Owen et al., 1998) and with hydroxyurea in tomato against *M. javanica* (Glazer and Orion, 1985). Chitosan treatment of tomato roots increased production of defense-related chemicals and enzymes and was correlated with improved resistance to the root-knot nematode (Vasiukova et al., 2001). The lipopolysaccharides of *Rhizobium etli* evoked resistance in potato to a cyst nematode (Reitz et al., 2000). These studies suggest that induced resistance may offer an alternative method for nematode control.

Oxycom is one of the few registered broad-spectrum chemicals available for management of plant pathogens. Studies in bean (Kim et al., 2001) and tobacco (Yang et al., 2002) indicate that Oxycom activates systemic resistance. Preliminary microplot and greenhouse studies, specifically in sandy soil, showed significant growth improvement after Oxycom applications to grapevines (McKenry, unpubl.) and tomato plants (Anwar and McKenry, 2002a). Meanwhile, the associated final population levels of root-knot nematode, *M. incognita,* were highly inconsistent but usually remained high. These findings are typical of a tolerance response rather than a resistance response as defined by most nematologists (Cook and Evans, 1987; Roberts, 1990; Stirling and Cirami, 1998; Trudgill, 1991). To evaluate this hypothesis further we used greenhouse tomatoes to test the effect of an Oxycom drench to roots prior to nematode inoculations with or without follow-up drenches to roots and soil. The impact of Oxycom on known plant defense pathways was assessed by screening for increased transcription of defense genes and the activation of a mitogen-activated protein kinase (MAPK) pathway. Studies in tobacco (Yang et al., 2002) indicate that activation of the MAPK associated with salicylic acid (SIPK) is part of the response that leads to plant defense activation after Oxycom treatments. Whether this activation occurred in tomato was examined in our studies.

Materials and Methods

Oxycom: (Redox Chemicals, Burley, ID) consists of two components. Component A is a 5% stabilized solution of peracetic acid containing 10% to 12% acetic acid and 20% to 22% hydrogen peroxide. Component B contains a mixture of plant nutrients, proprietary stabilizers, and salicylic acid. The two components are

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packed separately and mixed at the time of application. The Oxycom was diluted to 2,500 ppm concentration (V/V) and applied pre-inoculation. In some cases this was followed by repeated post-inoculation drenches.

Nematode inoculum: Eggs of *M. incognita* were extracted from females naturally infecting zucchini (*Cucurbita pepo* L.) roots grown near Selma, California. The galled roots were sealed in Mason glass jars containing 800 ml 2% NaOCl (Hussey and Barker, 1973) and agitated for 4 minutes at 200-cycle min^{-1} on a mechanical shaker (Eberbach, Ann Arbor, MI). Agitation was followed with a thorough rinse in tap water. Eggs were allowed to hatch in a mist chamber to obtain second-stage juveniles (J2) for inoculation of tomato seedlings.

Tomato seedlings: Fifteen-day-old seedlings of cherry tomato (*Lycopersicon esculentum* Mill.) cv. Sweetie grown in transplant trays were planted singly in 15-cm-diam. pots filled with autoclaved soil (80% sand, 10% silt, 10% clay). Beginning 7 days after transplant, Oxycom was drenched at 2,500 ppm to a selected number of plants. Half of the plants were treated only once and designated as a single application regime. The other half received additional soil drenches at 10-day intervals throughout their growth period, and these were designated as a multiple application regime. Two days after the first Oxycom application, plants were inoculated with freshly hatched J2 of *M. incognita*. Fifteen hundred J2 suspended in 10 ml of water were pipeted into three equidistant 3-cm-deep holes surrounding the root zone of each plant. Inoculation holes were refilled with steam-sterilized soil, and pots were watered immediately to moisten the soil. The control plants that were not inoculated with nematodes were similarly treated with sterilized water. Plants were fertilized every 2 weeks with Hoagland's solution (Hoagland and Arnon, 1950).

Experiment 1: The effects of the two Oxycom applications on susceptible cherry tomato cv. Sweetie to *M. incognita* were compared in a greenhouse at 30 ± 4 °C. Treated pots were placed on a greenhouse bench in a completely randomized design with 10 replications of each. A non-inoculated control was included to compare plant growth and nematode reproduction with and without Oxycom applications.

Treatment effects on plant-growth parameters were assessed 35 and 63 days after inoculation (DAI), with nematode population levels being recorded at final harvest only. Foliage was clipped twice to record the photosynthetic leaf area and shoot weight. Leaf area was measured with an Li-3100 area meter (LI-COR, Inc., Lincoln, NE). At the final harvest, plants were removed from pots and their roots carefully washed free of soil. Roots were visibly rated for severity of galls using a 1-to-5 scale, where 0 = no galls, 1 = 1 or 2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 > 100 galls per root system (Taylor and Sassar, 1978).

The root system of each plant was divided into two equal halves—one for J2 extraction and the other for determining female population within roots. Roots were stained with acid fuchsin (Byrd et al., 1983) and spread in a film of glycerin between two glass plates. The glycerin improves optical qualities of the system, prevents drying, and helps to hold the plates together. Number of females within the roots was determined under a dissecting microscope. The other half of each root system was incubated in a mist chamber for 5 days to hatch eggs. Females and J2 per gram of root were calculated. Nematode reproduction was assessed by calculating nematode reproduction rate $R = P_f/P_i$, where P_i = initial inoculum level and P_f = final population at harvest (Sassar et al., 1984).

Experiment 2: The effect of repeated Oxycom drenches on reproduction of *M. incognita* and on plant growth of susceptible tomato cv. Blitz F1 (De Ruiter Seeds, Columbus, OH) was studied in a greenhouse at 30 ± 4 °C. This cultivar was chosen to broaden the scope of our experience with Oxycom. Tomato seedlings at 15 days old were transplanted into individual 15-cm plastic pots filled with autoclaved soil (80% sand, 10% silt, 10% clay). Plants were allowed 7 days to heal any transplant injuries. Three treatments were tested: *M. incognita*-infected tomato plants, *M. incognita*-infected tomato plants drenched with Oxycom, and a water control. The first Oxycom application was drenched at 2,500 ppm 2 days before inoculation. Two additional drenches of Oxycom were made 20 and 40 DAI. Soil surrounding the roots of each plant was infested with approximately 5,000 *M. incognita* eggs in 5 ml tap water 2 days after the first Oxycom application. The control plants received 5 ml tap water only. Five pots of each treatment were placed on a greenhouse bench in a completely randomized design. Sixty-three days later, the plants were removed from the pots. Shoot weight, leaf area, and yield were recorded and then roots were washed free of soil and weighed. Eggs were extracted from galled roots by placing them in an 800-ml sealed glass jar with 2% NaOCl (Hussey and Barker, 1973) and shaken for 4 minutes at 200 rpm on a rotary shaker (Eberbach, Ann Arbor, MI). Extracted eggs were rinsed thoroughly in tap water and counted using a stereomicroscope. Nematode reproduction was assessed as in experiment 1.

Defense gene transcript accumulation: Roots from mature plants at 63 DAI were immersed directly into liquid nitrogen and ground to powder. The powdered material was treated with Tri-Reagent (MRC, Cincinnati, OH) to extract RNA following the manufacturer's protocol. Samples of 10 µg were separated on 1.5% formaldehyde agarose gels and transferred to nylon membranes. Membranes were hybridized at 55 °C overnight with digoxigenin-labeled tobacco RNA probes (Roche, Indianapolis, IN). Probes used were for the defense genes, *PR-1a, PR-1g, PR-5,* and phenylalanine ammonia lyase (*PAL*) (Cutt et al., 1988; Eyal et al., 1992; Nelson et al., 1992; Pellegrini et al., 1994). After high stringent

TABLE 1. Effect of Oxycom on reproduction of *Meloidogyne incognita* (Mi) on roots of cherry tomato cv. Sweetie.

	Number of galls, females, and J2 per root system			Reproduction factor	Number of females and J2 per gram of root	
	Galls	Females	J2		Females	J2
Meloidogyne incognita	406b	425c	217b	0.15b	29b	15b
Oxycom + Mi Single application	426b	465b	551b	0.38b	29b	33b
Oxycom + Mi Multiple applications	483a	515a	3,783a	2.52a	43a	303a

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

washing at 55 °C, immunological detection of bound probes was performed according to the standard protocol for CDP-Star (Roche, Indianapolis, IN).

In-gel kinase assay: To detect MAPK activity, in-gel kinase assays were performed using myelin basic protein (MBP) (Invitrogen, Carlsbad, CA) as a substrate. Tomato leaves from 4 week-old plants raised in the greenhouse in commercial potting soil were sprayed with 5,000 ppm Oxycom, 1 mM salicylic acid, or water to run off. Leaves were removed at 0, 30 minutes, 1 hour, and 3 hours as designated and immediately frozen in liquid nitrogen. Leaves were ground in liquid nitrogen to a fine powder using a mortar and pestle, and the material was suspended in extraction buffer (100 mM HEPES pH 7.5, 5 mM EDTA, 5 mM EGTA, 10 mM DTT, 10 mM Na_3VO_4, 10 mM NaF, 50 mM beta-glycerolphosphate, 1 mM phenylmethylsulfonyl fluoride, 5 mg/ml aprotinin, 5 mg/ml leupeptin, 10% glycerol [Zhang and Klessig, 1997]). The suspension was centrifuged at 10,000 g for 40 min, and supernatants were transferred into clean tubes, quickly frozen in liquid nitrogen, and stored at 80 °C. The protein concentration in the extracts was determined using the Bio-Rad protein assay kit (Hercules, CA) with bovine serum albumin as the standard.

Proteins in the tomato leaf extracts (15 µg) were separated by electrophoreses in a 10% SDS-polyacrylamide gel containing 0.25 mg/ml MBP. After electrophoresis, SDS was removed and renaturation was permitted according to the procedures of Zhang and Klessig (1997). The gel was incubated with gamma-^{32}P-ATP (3000 Ci/mmol) for 90 minutes. The unincorporated gamma-^{32}P-ATP was removed by washing. The gel was then dried and exposed to X-ray film (Intermountain X-ray Corp., Salt Lake City, UT). Prestained markers (Bio-Rad, Hercules, CA) were used to check the size of proteins with MBP kinase activity.

Data analysis: All data were subjected to analysis of variance using SAS procedures, and differences among treatment means were separated with Duncan's multiple-range test at $P \leq 0.05$ (SAS Institute, Cary, NC). Orthogonal partitioning of treatments was performed for both experiments.

RESULTS

Experiment 1: A pre-inoculation drench of Oxycom to 15-day-old tomato plants enhanced reproduction of *M. incognita* without altering plant parameters ($P = 0.05$). Multiple drenches of Oxycom at 10-day intervals increased the reproduction factor (Pi to Pf) by 16-fold ($P = 0.05$) (Table 1). *Meloidogyne incognita*-infected tomato plants receiving multiple applications also exhibited significantly greater galling ($P = 0.05$) and greater numbers of adult females ($P = 0.05$) compared to plants receiving only the pre-inoculation treatment. When population development was calculated by extracting the Pf per gram of root, the multiple drench treatments still resulted in higher incidence of females, a greater

TABLE 2. Effects of two Oxycom treatments on growth of cherry tomato cv. Sweetie inoculated with *Meloidogyne incognita* (Mi).

					Plant weight (g)			
	Leaf area (cm^2)		Height (cm)		Top		Root	
Treatments	35 DAI*	63 DAI	35 DAI	63 DAI	35 DAI	63 DAI	35 DAI	63 DAI
Control	707.0a	2,067.4a	48.7ab	112.3a	30.3a	102.6a	7.3a	17.1a
Meloidogyne incognita	609.4bc	1,678.8c	60.1a	122.0a	27.9ab	89.5b	5.8ab	14.6a
Oxycom Single application	655.1ab	1,957.3ab	49.2ab	110.6a	28.0ab	98.6a	7.2a	16.7a
Oxycom Multiple application	396.2d	1,305.6d	35.7c	81.1b	16.0c	59.8c	4.1c	12.2b
Oxycom + Mi Single application	692.9ab	1,781.1bc	58.9a	121.0a	30.7a	94.2a	5.8ab	16.2a
Oxycom + Mi Multiple applications	541.6c	1,057.1e	45.6bc	115.1	23.5b	64.3c	4.7c	11.4b

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.
* = Days after inoculation.

TABLE 3. Effect of pre-inoculation and follow-up applications of Oxycom at 20-day intervals on *Meloidogyne incognita* (Mi) reproduction and tomato growth.

Treatments	Leaf area (cm^2)	Plant weight (g)			Eggs per gram of root	Reproduction factor
		Top	Root	Fruit		
Control	1,424.3a	101.1a	22.6a	122.1a	—	—
Meloidogyne incognita	948.5b	79.9b	19.1a	93.5b	10,404a	45a
Oxycom + Mi	1,592.5a	115.9a	28.2a	128.2a	10,608a	50a

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

reproduction level, and an earlier egg hatch. Oxycom increased levels of *M. incognita* by 17.5% and 9.7% for multiple or single treatments, respectively.

Plant growth: The two Oxycom application regimes showed differential influence on plant growth. The pre-inoculation drench was more effective in promoting growth than multiple follow-up applications. Plants receiving multiple applications were stunted ($P = 0.05$), attained less leaf area, and generally accumulated less biomass than all other treatments ($P = 0.05$) (Table 2). Plants receiving multiple treatments also demonstrated foliage phytotoxicity symptoms, including intraveinal bleaching of older leaves leading to their eventual desiccation.

During the first 35 DAI, *M. incognita* reduced leaf area ($P = 0.05$) compared to that of the non-treated control. A single Oxycom treatment reduced early detrimental impacts of nematodes; however, follow-up applications of Oxycom reduced leaf area of nematode-infected plants even further ($P = 0.05$).

Upon termination of the experiment (63 DAI) *M. incognita* was responsible for a 19% reduction in leaf area ($P = 0.05$) and 13% reduction in top weight ($P = 0.05$) but no reduction in plant height or total root weight. Pre-inoculation treatments with Oxycom provided significant benefits in top weight of nematode-infected plants, rendering growth of treated plants indistinguishable from the non-inoculated check. Multiple applications of Oxycom reduced leaf area, top weight, and root weight without reducing plant height ($P = 0.05$).

Experiment 2: Meloidogyne incognita reproduced abundantly with similar RF (final egg numbers $[P_f]/P_i$) and eggs per gram of root, whether treated or not (Table 3). Growth differences on treated or non-treated plants were not significant except for shoot weight. Vegetative growth of *M. incognita*-infected tomato plants benefited from the Oxycom drenches ($P = 0.05$). Increases of 40%, 31%, and 27% in leaf area, shoot weight, and yield, respectively, were attributable to Oxycom treatments to nematode-infected plants. Whether treated or not, the root growth of nematode-infected plants was not different from those uninfected.

Orthogonal comparison: Growth of *M. incognita*-infected tomato plants was improved by pre-inoculation and follow-up multiple treatments of Oxycom ($P = 0.05$) (Table 4). A single pre-inoculation application improved shoot weight of *M. incognita*-infected tomato plants. Follow-up applications at 20-day intervals provided growth ($P = 0.05$) benefits to *M. incognita*-infected tomato plants over control as well as *M. incognita* alone, which is strong evidence for tolerance.

Defense gene transcript accumulation: The roots showed enhanced accumulation of transcripts for three PR proteins—PR-1a, PR-1g, and PR-5—as well as PAL. There was an effect of dosage for the PR genes: the transcript levels being higher with the multiple applications of Oxycom. Infection of the tomato roots by the nematodes did not cause strong expression of *PR-1a* or *PR-1g*. Slight induction of *PR-5* and *PAL* over the levels seen in the control roots was observed (Fig. 1).

MAPK-activation: The Oxycom treatment rapidly induced the activity of a 48 kDa protein kinase that phosphorylated myelin basic protein. A kinase of the same

TABLE 4. Orthogonal comparison of *Meloidogyne incognita* (Mi)-infected tomato plant growth parameters treated with single pre-inoculation treatment and multiple 20-day-interval treatments of Oxycom.

Comparison	Single pre-inoculation Oxycom treatment[1]		Multiple 20-day-interval Oxycom treatments[2]		
	Leaf area (cm^2)	Shoot weight (g)	Leaf area (cm^2)	Shoot weight (g)	Yield (g)
Oxycom + Mi	1,781.1	94.1	1,592.6*	115.9*	128.4*
vs.	vs.	vs.	vs.	vs.	vs.
Mi alone	1,678.8	89.5	948.5	79.7	93.5
Oxycom + Mi	1,781.0	94.0	1,592.6	115.9*	128.4
vs.	vs.	vs.	vs.	vs.	vs.
Control (No Oxycom, no Mi)	2,067.5*	102.4	1,424.3	101.1	122.1

* indicates significant difference at $P = 0.05$ according to Planned F test.
1 = derived from experiment 1; 2 = derived from experiment 2.

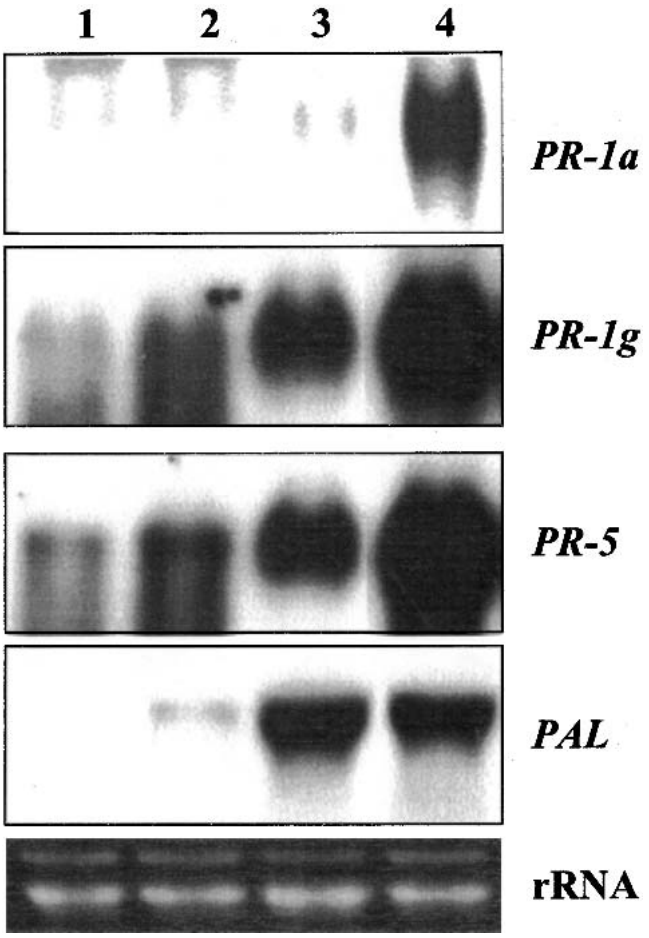

FIG. 1. Comparison of transcript levels for PR genes and PAL in tomato roots after challenge by nematodes and treatment with Oxycom. Tomato plants were without treatment (lane 1) as a control, colonized with nematodes (lane 2), or exposed to a single drench of 2,500 ppm Oxycom without nematodes (lane 3) or multiple drenches of 2,500 ppm Oxycom (lane 4) without nematodes. Total RNA was isolated from the roots, and hybridization analysis was performed as described in Materials and Methods. The pattern of ethidium bromide-stained gel of rRNA bands is provided to showed equal loading of the gel. The data are for one of two experiments that show the same results.

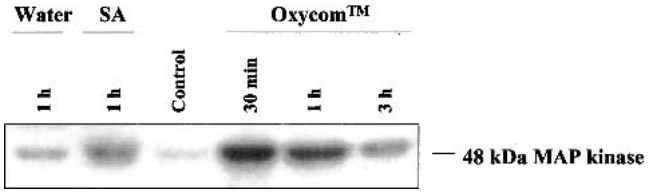

FIG. 2. Activation of a 48 kDa MBP kinase by treatment of tomato leaves with Oxycom or SA. Tomato leaves were treated with water as a control, 1 mM SA, or 5,000 ppm Oxycom. At the designated times after treatment, proteins were isolated and in-gel kinase assays were performed using MBP as a substrate as described in Materials and Methods. Prestained markers were used to determine the size of proteins with MBP kinase activity. The data are from one of two experiments where the same results were observed.

size was also activated by treatment with 1 mM salicylic acid. Activation of the kinase by Oxycom was observed at 30 minutes and was still strong at the 1-hour time point but was reduced by 3 hours (Fig. 2).

DISCUSSION

Root-knot nematodes, *Meloidogyne* spp., pose particular difficulties for pest management because of their wide host range, short generation time, and high reproduction rate. Their habitat is limited to root systems and surrounding soil, with their point of entry at the root tips. Tactics such as nematicides and plant resistance generally reduce nematode population levels and nullify nematode damage long enough to produce healthy crops. In some cases, cultural practices that improve plant health can also lessen nematode impact (McSorley, 1998).

We document here a different tactic to reduce plant damage due to nematodes. Oxycom is a product having broad-spectrum effects, including benefits to plant growth and enhancement of plant resistance to above- and below-ground pest population growth (Kim et al., 2001; Yang et al., 2002). This study shows that Oxycom enhanced plant tolerance to root-knot nematode but not its resistance. This tolerance was exhibited with a single pre-inoculation treatment and (or) with follow-up drenches to the soil. Dosage rates are critical because multiple soil applications under the conditions of this study reduced plant health while stimulating nematode reproduction. In field applications, an Oxycom drench is usually followed by additional water so that the product does not remain for extended periods around only a portion of the root (McKenry, obs.). This dilution effect on the Oxycom application was not studied here.

At 30 °C about 700 and 1,200 degree-days will have been accumulated at 35 and 63 days or 1.5 to 2.5 nematode generations, respectively (Melakeberhan et al., 1989). It appears that Oxycom treatments can increase the rate of nematode development. The enhanced reproduction by *M. incognita* following applications of Oxycom was coupled with improved vegetative growth and enhanced plant reproduction. Earlier flowering and fruiting were observed in Oxycom-treated tomato plants, an effect correlated with salicylic acid (Raskin, 1992), which is a component of the Oxycom formulation.

Plant resistance may slow nematode penetration (Anwar and McKenry, 2002b, 2002c), slow nematode development (Anwar et al., 2000), or prevent reproduction (Anwar et al., 2000) by activating a variety of biochemical and molecular defense mechanisms (Bowles, 1990; Potenza et al., 1996). Mechanisms operating to render infected plants tolerant are generally related to plant characteristics and environmental conditions (Seinhorst, 1970; Trudgill, 1991). Increased nutrition (Trivedi and Barker, 1986), water (McSorley and Duncan, 1995; Trivedi and Barker, 1986; Wilcox-Lee and Loria, 1987), synthesis of secondary metabolites includ-

ing pathogenesis-related proteins (Stintzi et al., 1993), and differential allocation of resources (Kessler and Baldwin, 2002) have been associated with a tolerance response to nematodes and other plant pathogens.

Improved health of nematode-infected plants correlates with enhanced root growth. An increase of 19% root growth of *M. incognita*-infected tomato plants that received Oxycom over *M. incognita*-infected tomato plants indicates the effectiveness of Oxycom in improving either root functions or total biomass to exploit more soil. Anwar and Van Gundy (1989) reported that root length has an impact on physiological processes and increases the total root absorbing area, thus leading to enhanced absorption of nutrients and water and, consequently, better foliar growth.

The molecular assays suggest the possibility that tolerance in the tomato toward the nematode may be related to activation of certain plant defense genes. The single and multiple applications of Oxycom resulted in strong activation of the defense genes *PR-1g, PR-5,* and *PAL.* Infection by the nematodes alone did not result in high expression of any of these genes. PAL is the first enzyme of the phenylpropanoid pathway, and gene expression is induced during a resistant hypersensitive response to TMV or to a fungal elicitor (Pellegrini et al., 1994). Induction of *PAL* in the tomato roots was consistent with activation of the SA-pathway of defense (Yang et al., 2001). The pathogenesis related protein PR-1g is believed to be a basic protein with expression conditioned by ethylene (Eyal et al., 1992). High expression from the *PR-5* gene, encoding an osmotin, is correlated to regulation by SA (Uknes et al., 1992) as well as ABA and ethylene (Grillo et al., 1995). The possibility that ethylene production is part of the response of the plant to the Oxycom treatment is likely because other ethylene-related genes are increased in transcription when *Arabidopsis* is treated with this product (Miller et al., unpubl.). An ethylene effect may have increased nematode populations in the Oxycom-treated roots. Wubben et al. (2001) found that ethylene enhanced the susceptibility of *Arabidopsis* to the sugar beet cyst nematode.

Only the multiple treatments with Oxycom induced accumulations of the *PR-1a* mRNA (Cutt et al., 1988). Although PR-1a is a marker for the SA pathway, systemic expression does not occur in tobacco following SA applications alone (Chamnongpol et al., 1998). The junior authors have demonstrated that, in tobacco, a combination of ROS and SA enhanced expression of this promoter and permitted a systemic response (Blee et al., unpubl.). These data confirm the interaction suggested between SA and ROS (Chamnongpol et al., 1998), where an increase in the level of hydrogen peroxide in transgenic tobacco tissues was proposed because of a deficit in catalase activity. These workers also suggested that the ROS might be acting by increasing the production of ethylene. This interaction between ROS and SA in enhancing the expression of certain genes is consistent with the presence of the ROS, peracetic acid, and SA in the Oxycom product. Other interactions between SA and ethylene are documented and discussed in a review on ethylene and signal transduction by Wang et al. (2002).

The enhanced expression of *PR-1a, PR-5,* and *PAL* suggested that tomato responded to Oxycom treatment by activation of the SA-associated defense pathway. We confirmed this possibility by demonstrating that Oxycom treatment of leaf tissues activated a protein kinase of the same size as that which was activated by SA (Zhang and Klessig, 1997). The transient activation of this MAPK activity agreed with the response of tobacco to Oxycom (Yang et al., 2002). Activation in tomato of a 48-kDa MBP protein kinase was also reported to be a consequence of wounding or exposure to the oligosaccharide elicitors, polygalacturonic acid and chitosan (Stratmann and Ryan, 1997). In this context it is interesting that chitosan treatments enhanced nematode resistance (Vasiukova et al., 2001).

Our findings suggest that an array of defense proteins associated with the SA- and ethylene-pathways were being induced by the Oxycom treatments. Additional studies are needed to reveal whether any of the defense genes were active in limiting the symptomatic effects of the nematode, such as reduced growth, while permitting their populations to remain high. Effective activation of defense also was consistent with the deleterious effect of multiple treatments of Oxycom. Energy loss in building defenses and restriction of cell wall elongation by their modifications as part of the defense strategy (Kim et al., 2001) could account for the stunting effect of the multiple Oxycom treatments. Stunted growth by chemical applications that induced systemic resistance was observed under field conditions (Romero et al., 2001). In several transformed plants, over-expression of defense genes has resulted in reduced plant performance (Heil and Baldwin, 2002).

Our findings also suggest that improvement of plant tolerance to root-knot nematode is an alternative approach to minimizing crop damage. Our data with the Oxycom product are consistent with chemical induction of plant tolerance and growth. Although it is well established that plant damage is related to nematode density (Barker and Noe, 1987; McSorley and Duncan, 1995; Seinhorst, 1967), some plant cultivars may be more tolerant of the same nematode population density than others (Trudgill, 1991). Effectiveness of this material may depend on soil texture. Rate and timing of application of the chemicals inducing tolerance must be investigated to maximize efficacy. Use of Oxycom as the solo treatment may be adequate in some field settings, but in others it could be used in combination with other products that specifically reduce nematode population densities.

Literature Cited

Anwar, S. A., M. V. McKenry, and J. Faddoul. 2000. Reproductive variability of field populations of *Meloidogyne* spp. on grape rootstocks. Journal of Nematology 32:265–270.

Anwar, S. A., and M. V. McKenry. 2002a. Effect of Oxycom on growth of tomato and reproduction of *Meloidogyne incognita*. Nematology: International Journal of Fundamental and Applied Nematological Research 4:141.

Anwar, S. A., and M. V. McKenry. 2002b. Penetration and development of *Meloidogyne arenaria* on two grape rootstocks. Journal of Nematology 34:143–145.

Anwar, S. A., and M. V. McKenry. 2002c. Penetration, development, and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* spp. Nematropica 30:9–17.

Anwar, S. A., and S. D. Van Gundy. 1989. Influence of four nematodes on root and shoot growth parameters in grape. Journal of Nematology 21:276–283.

Barker, K. R., and J. P. Noe. 1987. Establishing and using threshold population levels. Pp. 75–81 *in* J A. Veech and D. W. Dickson, eds. Vista on nematology. Hyattsville, MD: Society of Nematologists.

Bowles, D. J. 1990. Defense-related proteins in higher plants. Annual Review of Biochemistry 59:873–907.

Byrd, D. P., T. Kirkpatrick, Jr., and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142–143.

Chamnongpol, S., H. Willekens, W. Moeder, C. Langebartels, H. Sandermann, Jr., M. V. Montagu, D. Inze, and W. V. Camp. 1998. Defense activation and enhanced pathogen tolerance by H_2O_2 in transgenic tobacco. Proceedings of National Academy of Sciences 95:5818–5823.

Conrath, U., Z. Chen, J. R. Ricigliano, and D. F. Klessig. 1995. Two inducers of plant resistance, 2,6-dichloroisonicitinic acid and salicylic acid, inhibit catalase activity in tobacco. Proceedings of the National Academy of Sciences 92:7143–7147.

Cook, R., and K. Evans. 1987. Resistance and tolerance. Pp. 179–231 *in* R. H. Brown and B. R. Kerry, eds. Principles and practice of nematode control in crops. Orlando, FL: Academic Press.

Cutt, J. R., D. C. Dixon, J. P. Carr, and D. F. Klessig. 1988. Isolation and nucleotide sequence of cDNA clones for the pathogenesis-related protein PR1a, PR1b, and PR1c of *Nicotiana tabacum* cv. *Xanthi* nc induced by TMV infection. Nucleic Acids Research 16:9861.

Eyal, Y., 0. Sagee, and R. Fluhr. 1992. Dark-induced accumulation of a basic pathogenesis-related (PR-1) transcript and a light requirement for its induction by ethylene. Plant Molecular Biology 19:589–599.

Glazer, C., and D. Orion. 1985. An induced resistance effect of hydroxyura on plants infected by *Meloidogyne javanica*. Journal of Nematology 17:21–24.

Grillo, S., A. Leone, Y. Xu, M. Tucci, R. Francione, P. M. Hasegawa, L. Monti, and R. A. Bressan. 1995. Control of osmotin gene expression by ABA and osmotic stress in vegetative tissues of wild-type and ABA-deficient mutants of tomato. Physiologia Plantarum 93:498–504.

Heil, M., and I. T. Baldwin. 2002. Fitness costs of induced resistance: Emerging experimental support for a slippery concept. Trends in Plant Science 2:61–67.

Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Circular 347. University of California, Agricultural Experimental Station, Berkeley, CA.

Hussey, R. S., and K. R. Barker. 1973. Comparison of methods for collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

Kessler, A., and I. T. Baldwin. 2002. Plant responses to insect herbivory: The emerging molecular analysis. Annual Reviews of Plant Biology 53:299–328.

Kessmann, H., T. Staub, J. Ligon, M. Oostendrop, and J. Ryals. 1994. Activation of systemic acquired resistance in plants. European Journal of Plant Pathology 100:359–369.

Kim, Y. C., K. A. Blee, J. Robins, and A. J. Anderson. 2001. Oxycom under field and laboratory conditions increases resistance response in plants. European Journal of Plant Pathology 107:129–136.

McSorley, R. 1998. Alternative practices for managing plant-parasitic nematodes. American Journal of Alternative Agriculture 13: 98–104.

McSorley, R., and L. W. Duncan. 1995. Economic thresholds and nematode management. Advances in Plant Pathology 11:147–170.

Melakeberhan, H., H. Ferris, M. V. McKenry, and J. T. Gaspard. 1989. Overwintering stages of *Meloidogyne incognita* in *vitis vinifera*. Journal of Nematology 21:92–98.

Nelson, D. E., K. G. Raghothama, N. K. Singh, P. M. Hasegawa, and R. A. Bressan. 1992. Analysis of structure and transcriptional activation of an osmotin gene. Plant Molecular Biology 19:577–588.

Noling, J. W., and J. O. Becker. 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. Journal of Nematology 26:642–646.

Owen, K. J., C. D. Green, and B. J. Devrall. 1998. Systemic acquired resistance against root-knot nematodes in grapevines. 7th International Congress of Plant Pathology 2,1.4.38 (Abstr.).

Pellegrini, L., O. Rohfritsch, B. Fritig, and M. Legrand. 1994. Phenylalanine ammonia-lyase in tobacco. Molecular cloning and gene expression during the hypersensitive reaction to tobacco mosaic virus and the response to a fungal elicitor. Plant Physiology 106:877–886.

Potenza, C. L., S. H. Thomas, E. A. Higgins, and C. Sengupta-Gopalan. 1996. Early root response to *Meloidogyne incognita* in resistant and susceptible alfalfa cultivars. Journal of Nematology 28:475–484.

Raskin, I. 1992. Role of salicylic acid in plants. Annual Review of Plant Physiology and Plant Molecular Biology 43:439–463.

Reitz, M., K. Rudolph, I. Schroder, S. Hoffmann-Hergarten, J. Hallmann, and R. A. Sikora. 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. Applied Environmental Microbiology 66:3515–3518.

Roberts, P. A. 1990. Resistance in nematodes: Definitions, concepts, and consequences. Pp. 1–15 *in* J. L. Starr, ed. Methods for evaluating plant species for resistance to plant-parasitic nematodes. Hyattsville, MD: Society of Nematologists.

Romero, A. M., C. S. Kousik, and D. F. Ritchi. 2001. Resistance to bacterial spot in bell pepper induced by acibenzolar-S-methyl. Plant Disease 85:189–194.

Sassar, J. N., C. C. Carter, and K. R. Hartman. 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. Crop Nematode Research and Control Project. Cooperative Publications, Department of Plant Pathology, North Carolina State University.

Sassar, J. N., and D. W. Freckman. 1988. A world perspective on nematology: The role of the society. Pp. 7–14 *in* J A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists.

Seinhorst, J. W. 1967. The relationship between population increase and population density in plant-parasitic nematodes. III. Definition of terms, host status, and resistance. IV. The influence of external conditions on the regulation of population density. Nematologica 13:429–442.

Seinhorst, J. W. 1970. Dynamics of populations of plant-parasitic nematodes. Annual Review of Phytopathology 8:131–156.

Shericca, W. M., B. Vernooij, S. Titatarn, M. Starrett, S. Thomas, C. C. Wiltse, R. A. Frederiksen, A. Bhandhufalck, S. Hulbert, and S. Uknes. 1998. Induced resistance response in maize. Molecular Plant-Microbe Interactions 11:463–658.

Stintzi, A., T. Heitz, V. Prasad, S. Weidemann-Merdinoglu, and S. Kauffmann. 1993. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. Biochimie 73:687–706.

Stirling, G. R., and R. M. Cirami. 1998. Resistance and tolerance of grape rootstocks to South Australian populations of root-knot nematode. Australian Journal of Experimental Agriculture and Husbandry 24:277–282.

Stratmann, J. W., and C. A. Ryan. 1997. Myelin basic protein kinase activity in tomato leaves is induced systemically by wounding and

increases in response to systemin and oligosaccharide elicitors. Proceedings of the National Academy of Sciences 94:11085–11089.

Taylor, A. L., and J. N. Sassar. 1978. Identification of *Meloidogyne* species. Pp. 101–105 *in* A. L. Taylor and J. N. Sassar, eds. Biology, identification, and control of root-knot nematodes (*Meloidogyne* Species). Raleigh, NC: North Carolina State University Graphics.

Trivedi, P. C., and K. R. Barker. 1986. Management of nematodes by cultural practices. Nematropica 16:213–236.

Trudgill, D. L. 1991. Resistance to and tolerance of plant-parasitic nematodes in plants. Annual Review of Phytopathology 29:167–192.

Uknes, S., B. Mauch-Mani, M. Moyer, S. Potter, S. Williams, S. Dincher, D. Chandler, A. Slusarenko, E. Ward, and J. Ryals. 1992. Acquired resistance in *Arabidopsis*. The Plant Cell 4:645–656.

Vasiukova, N. I., S. V. Zinov'eva, L. I. Il'inskaia, E. A. Perekhod, G. I. Chalenko, N. G. Gerasimova, A. V. Il'ina, V. P. Varlamov, and O. L. Ozeretskovskaia. 2001. Modulation of plant resistance to diseases by water-soluble chitosan. Prikl Biokhim Mikrobiol 37:115–122.

Wang, K. L. C., H. Li, and J. R. Ecker. 2002. Ethylene biosynthesis and signaling networks. The Plant Cell S131–151.

Wilcox-Lee, D., and R. Loria. 1987. Effects of nematode parasitism on plant-water relations. Pp. 260–266 *in* J A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists.

Wubben, M. J., II, H. Su, S. R. Rodermel, and T. J. Baum. 2001. Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in *Arabidopsis thaliana*. Molecular Plant-Microbe Interactions 14:1206–1212.

Yang, K. Y., Y. Liu, and S. Zhang. 2001. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. Proceedings of the National Academy of Sciences 98:741–746.

Yang, K. Y., K. A. Blee, S. Zhang, and A. J. Anderson. 2002. Oxycom treatment suppresses *Pseudomonas syringae* infection and activates a mitogen-activated protein kinase pathway in tobacco. Physiological and Molecular Plant Pathology 61:249–256.

Zhang, S., and D. F. Klessig. 1997. Salicylic acid activates a 48-kD MAP kinase in tobacco. The Plant Cell 9:809–824.