

Reduction of Root-Knot Nematode, *Meloidogyne javanica*, and Ozone Mass Transfer in Soil Treated with Ozone

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Abstract: Ozone gas (O₃) is a reactive oxidizing agent with biocidal properties. Because of the current phasing out of methyl bromide, investigations on the use of ozone gas as a soil-fumigant were conducted. Ozone gas was produced at a concentration of 1% in air by a conventional electrical discharge O₃ generator. Two O₃ dosages and three gas flow rates were tested on a sandy loam soil collected from a tomato field that had a resident population of root knot nematodes, *Meloidogyne javanica*. At dosages equivalent to 50 and 250 kg of O₃/ha, *M. javanica* were reduced by 24% and 68%, and free-living nematodes by 19% and 52%, respectively. The reduction for both *M. javanica* and free-living nematodes was dosage dependent and flow rate independent. The rates of O₃ mass transfer (OMT) through three soils of different texture were greater at low and high moisture levels than at intermediate ones. At any one soil moisture level, the OMT rate varied with soil texture and soil organic matter content. Results suggest that soil texture, moisture, and organic matter content should be considered in determining O₃ dosage needed for effective nematode control.

Key words: free-living nematode, fumigant, management, *Meloidogyne javanica*, methyl bromide, root-knot nematode, ozone, ozone mass transfer.

High value crops, including nursery, strawberry and tomato in California have relied on methyl bromide fumigation for control of weeds, fungi and plant parasitic nematodes. Because of environmental concerns, the use of methyl bromide has been reduced in the United States. We investigated ozone (O₃) as a possible alternative for root-knot nematode (RKN) control.

O₃ is highly reactive with many organic compounds (Razumovskii and Zaikov, 1984) and has been used in soils for environmental remediation of contaminated sites, and degradation of pesticides (Somich et al., 1990; O'Mahony et al., 2006). Because it is an effective disinfecting agent and inhibits or kills bacteria, fungi, viruses, and protozoa (Kim et al., 1999), it has been used for drinking water disinfection and food processing sanitization (Kim et al., 1999; Meunier, 2006). O₃ has also been used to fumigate stored grains and products for control of insects and fungi (Kells et al., 2001). Information on the effects of O₃ on nematodes, however, is limited (Weber et al., 1979; Small & Meek, 1996), and little is known about the direct effect of O₃ application to nematodes in soil.

O₃ is an effective biocide due to oxidation of organic molecules in living organisms. The effectiveness depends on two kinds of reaction of O₃ in soil; one would be the reaction of O₃ free radicals, and the other one would be the slow movement of O₃ through the medium depending on the amount of 'O₃ demanding materials' present (Staehelin and Hoigne, 1985; Kim et al., 1999; Kells et al., 2001). Different soils vary in the amount of O₃ demanding materials that they contain. If the O₃ injected into soil is not decomposed and does

not react with the O₃ demanding materials in the soil, it will pass un-reacted through the soil a greater distance. The O₃ passing through the soil without decomposition or reaction is defined as O₃ mass transfer (OMT). If the amount of O₃ demanding materials in soil is higher, then less O₃ would pass through. We hypothesize that the physical characteristics and conditions of soil will affect OMT through the soil, and thus affect the amount of O₃ oxidization within soil.

Our objectives were to determine the effect of O₃ treatment on reduction of plant parasitic nematodes as well as free-living nematodes in soil, and to test the effects of soil moisture level, texture, and organic matter content on OMT.

MATERIALS AND METHODS

Soil source, physical analysis and moisture level adjustment: Soil was collected from two locations in a field in Irvine, CA (University of California South Coast Research and Extension Center) infested with RKN, from two locations in a pecan orchard in Winton, CA, and from one location in a sugarbeet field in Tracy, CA (Table 1). The soil was mixed, and then sieved through a screen (4 mm opening) to remove rocks and other debris. Soil from each source was analyzed for texture (particle size), organic matter content, and moisture retention by the Division of Agriculture and Natural Resources (DANR) Analytical Laboratory at UC Davis. Soil moisture content (% g of water /g of dried soil x 100) was determined by oven drying. Desired moisture levels lower than in natural soil were made by adding water to oven-dried soil, and higher moisture levels were achieved by adding water to the natural soil.

Ozone treatment system: An O₃ treatment system (G-24, Pacific Ozone Technologies, Brentwood, CA) was set up in a fume hood. It was composed of 1) air feed gas preparation, 2) O₃ generator, 3) O₃ monitor, and 4) a soil treatment column. O₃ was produced from oxygen in the ambient air. A supply of clean and dry air to the

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TABLE 1. Physical characteristics of soils used for OMT rate determination.

Soil type	Crop/Location ^a	Organic matter %	Sand %	Silt %	Clay %	% Water at indicated water potential (-kPa) ^b				
						0	30	100	500	1500
OMT 1										
Loamy sand	Orchard/Winton	0.95	79	17	4	33.3	6.7	4.9	3.5	3.4
Sandy loam	Tomato/Irvine	0.73	62	25	13	35.6	11.2	8.6	7.2	7.0
OMT 2										
Loamy sand	Orchard/Winton	1.01	84	13	3	31.7	6.1	4.3	3.7	3.2
Sandy loam	Tomato/Irvine	0.42	72	18	10	36.9	12.0	8.3	7.1	6.8
Silt loam	Sugarbeet/Tracy	1.62	27	51	22	56.3	27.5	19.7	16.0	15.2

^a All soils were collected from California.

^b Moisture release data were obtained with a tension plate device.

generator is important for generating concentrated O₃. Compressed ambient air was first dried by passage through dual 20 cm x 4 cm silica gel desiccant columns. The dried air (-60°C dew point) was then passed through an oil micron particle filter to remove solids before entering the ozone generator. The O₃ generator provides an electrical discharge that ionizes oxygen, which then recombines as O₃. The O₃ gas used in our experiments was produced constantly at 1% of air (w/w). At standard conditions of 20°C and one atmosphere of pressure, the mass of 1.0 m³ of air is 1.29 kg. In our system, the mass of 1% generated O₃ was 12.9 g per m³ of air. The soil treatment column was made of Schedule 40 PVC pipe 5 cm in diameter and 12 cm high, with a piece of metal screen at the bottom of the column to support soil. A gas inlet was provided in a cap on the bottom of the column and a removable, gas-tight cap with a gas outlet was affixed to the top of the column. The cross-section inner area of the column was 19.625 cm². Because a fixed concentration of O₃ was generated during the experiments, different dosages were obtained by adjusting flow time and flow rate (Table 2). A rotometer was used to measure the O₃ gas flow rate into the inlet of the soil treatment column. An ultraviolet spectrophotometric O₃ analyzer (Hankin Ozone Scarborough, Ontario, Canada) was used to monitor the O₃ concentration before the inlet and after the outlet of the column. The rate of OMT through the soil was calculated by dividing the concentration of O₃ passing through the soil at the outlet by the concentration applied at the inlet.

TABLE 2. Ozone dosage versus gas flow rate and time.

Dosage kg/ha	Gas flow rate ml/min	Gas flow time min
0	0	0
50	8	108
50	45	20
50	145	6
250	8	543
250	45	97
250	145	30

Effect of ozone treatment on soil nematodes: A sandy loam soil (Table 1, OMT 1) naturally infested with RKN, *M. javanica* was used in this experiment. The moisture level of the soil at the time of collection was close to 12% and adjusted to 12% (w/w) before O₃ treatment. Two hundred cm³ of soil from a well mixed sample was loosely packed into the soil treatment column, previously described, to a depth of 10 cm. O₃ was passed through the column from the lower to the upper end and applied at two dosages (50 and 250 kg/ha) with three gas flow rates (8, 45 and 145 ml/min). There were four replicates for each treatment. The experiment was repeated once.

After O₃ treatment, the soil was removed from the column, mixed in a container, set on a lab bench for one hr, and then covered with Parafilm for another 10 hrs. Two 50 cm³ of soil from each replicate was used to extract nematodes by Baermann funnel for 2 days (Qiu et al., 2006). Nematodes were counted with the aid of a dissecting microscope as either RKN second-stage juveniles or free-living nematodes. A composite sample of the free-living nematodes was identified by E. M. Noffsinger. More than 50% were bacterial feeders belonging to genera *Acrobeles*, *Acrobeloides*, and *Eucephalobus* (*Cephalobidae*). Also present were other bacterial feeders, algal feeders, fungal feeders, and insect associates in the genera *Glauxinema*, *Mesodiplogaster* (*Diplogasteridae*), *Mesorhabditis*, *Pelodera* (*Rhabditidae*), *Dorylaimidae*, *Anguinidae*, *Nothotylenchus*, *Aphelenchus* (*Aphelenchidae*), *Aphelenchoides* (*Aphelenchoididae*), *Psilenchus*, and *Tylenchus* (*Tylenchidae*).

Ozone mass transfer through soil: Two experiments, OMT 1 and OMT 2 were performed to determine the effects of soil moisture levels and soil texture on ozone mass transfer. OMT 1 was conducted with a loamy sand soil from Winton, and a sandy loam soil from Irvine, CA (Table 1). The moisture level was adjusted to 0, 3, 5, 8.5, 12, 16 and 20.5% for each type of soil before O₃ treatment. The O₃ system, soil preparation and O₃ treatment were the same as described previously but employed a fixed flow rate of 145 ml/min. Ozone concentration was measured at the bottom of the soil column (inlet) at the beginning of O₃ application and at the top end of the column (outlet) after 10, 20 and 30 min of

continuous O₃ supply, as described previously. The rate of OMT through the soil was calculated by dividing the concentration of O₃ passing through the soil at the outlet by the concentration applied at the inlet. Each treatment was conducted with three replicates, and the experiment was repeated twice.

OMT 2 was conducted with three types of soil (Table 1). Loamy sand and sandy loam soils were collected from the same fields as in OMT 1, but at a different time and from a slightly different location. In addition, a third soil was collected from a sugarbeet field in Tracy, CA. Depending on the soil type, the moisture content was adjusted to 8 to 11 levels ranging from 0 to 18% prior to O₃ treatment. The O₃ system and the flow rate were the same as used for OMT 1. The O₃ concentration at the outlet of the soil column was measured 30 min after the start of the treatment and the OMT rate was calculated as described above. Each treatment was conducted with two replicates and the experiment was repeated once.

Statistical methods: Data were subjected to ANOVA followed by Duncan's New Multiple Range Test or LSD testing at $P = 0.05$ (SuperANOVA, Abacus Concepts, Berkeley, CA, 1989).

RESULTS

Soil characteristics: The moisture release data indicate that the silt loam soil contained more fine pores than the sandy loam and loamy sand soils. Organic matter content was lower in the sandy loam and loamy sand soils, and relatively higher in the silt loam soil (Table 1).

Effect of ozone treatment on soil nematodes: Preliminary statistical analysis on data from the two runs of the experiment showed that the effects of O₃ on nematodes were not significantly different between experiments ($P = 0.5$). The results presented here are pooled data from these two experiments. Treatment with O₃ at both dosages reduced RKN and free-living nematodes ($P = 0.0001$). O₃ treatment at 250 kg/ha reduced RKN 68% (Fig. 1A), free-living nematodes 52% (Fig. 1B), and total nematodes 60% ($P = 0.05$) (Fig. 1C). Treatment with O₃ at 50 kg/ha reduced RKN, free-living, and total nematodes, 24, 19 and 21.5%, respectively. At 250 kg/ha, reductions were 58, 41 and 50% greater for RKN, free-living and total nematodes, respectively, and greater than at 50 kg/ha, ($P = 0.05$). At the same dosage, nematode reductions at flow rates of 8, 45 and 145 ml/min were not significantly different ($P = 0.33$). There was also no interaction of dosage and flow rate on nematode reduction ($P = 0.48$). These analyses indicate that the effect of O₃ treatment on nematodes is dosage dependent and flow rate independent.

Ozone mass transfer through soil: In OMT 1, soil type and moisture level significantly affected the OMT rate ($P = 0.0001$, Fig. 2). Soil type and moisture level also had an interactive effect on the OMT rate ($P = 0.0001$). The

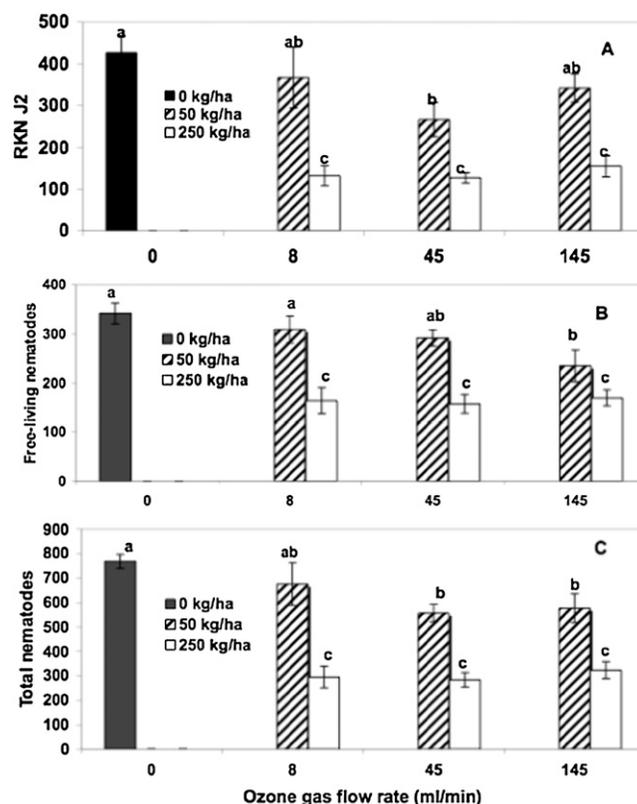


FIG. 1. Reduction of nematodes by O₃ treatment. Nematodes from soil after O₃ treatment were recovered by Baermann funnel method and counted as RKN second stage juveniles (A) and free-living nematodes (B) and the sum of RKN and free-living nematodes (C). Bars are means of 4 replicates with standard errors. Bars with same letters indicate that they are not significantly different ($P = 0.05$) according to LSD. Solid bar is control without O₃ application, hatched bars 50 kg O₃/ha, and opened bars 250 kg /ha. Dosages of O₃ (kg/ha) were determined by the horizontal area of the soil treatment column and application time calculated based on O₃ concentration and flow rates.

OMT rates after 10, 20 and 30 min of treatment were zero or near zero at soil moisture levels of 3%, 5% and 8.5% for the loamy sand soil (Fig. 2A), and at moisture levels of 5%, 8.5% and 12% for the sandy loam soil (Fig. 2B). At 0%, 16% and 20.5% soil moisture level, the OMT rates after 10 min were significantly higher for loamy sand than for sandy loam soil (Fig. 2). At most moisture levels, OMT rates were similar after 10, 20 or 30 min for both soil types. After 30 min of treatment, soil with 0% moisture had the highest OMT. For both types of soil, the OMT rates were highest in very dry (0%) and very wet (20.5%) soil, and lowest at intermediate (5% and 8.5%) soil moisture levels. The curves of the OMT rates versus the soil moisture levels from dry to wet were U-shaped. Consistent results were obtained from both trials.

Results for OMT 2 were similar to those for OMT 1. Soil type, moisture level, and their interaction significantly affected the OMT rates ($P = 0.0001$). The highest OMT rates were at very dry and very wet soil conditions, and the lowest OMT rates were at the intermediate moisture levels for each of three types of soil (Fig. 3).

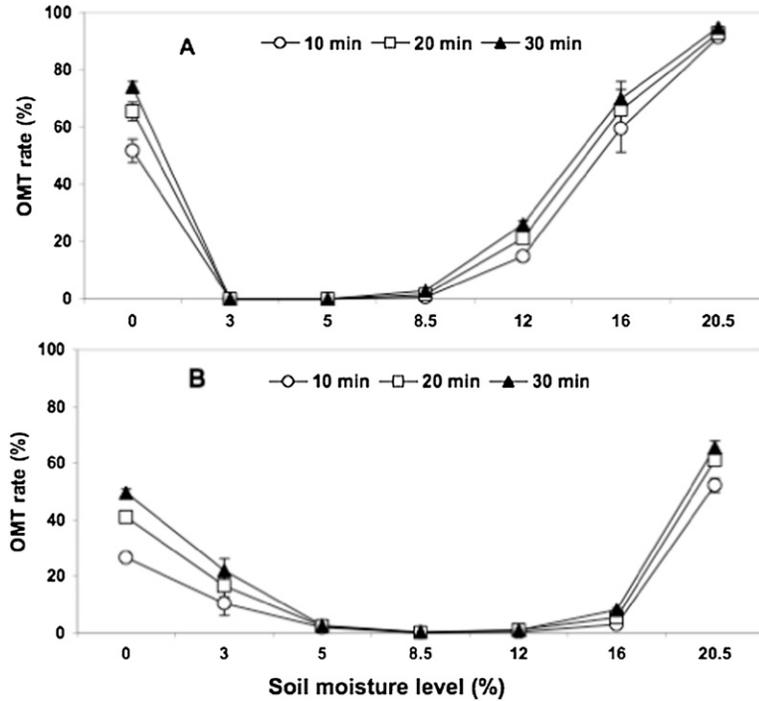


FIG. 2. Ozone mass transfer (OMT) through soil (Experiment 1). Rates of OMT were determined on two types of soil, loamy sand (A) and sandy loam soil (B) with moisture levels from 0 to 20.5% after 10 (opened circle), 20 (opened square) or 30-min (solid triangle) application of 1% O₃ with 145 ml/min flow rate. The rate of OMT was calculated by dividing the concentration of O₃ passing through the soil at the outlet by the concentration applied at the inlet. Data are means of 3 replicates. Bars on curves were standard errors.

The curves of the OMT rates versus the soil moisture levels were also U-shaped. The U-shape for soils with a high content of sand, low content of clay or low level of soil organic matter (SOM) was less open than the curves for soils with less sand, higher clay or SOM content. Simple linear regression analysis for 0% soil moisture showed positive correlation for the OMT rate (*Y*) versus sand content (*X*) ($Y = 0.44X + 45, P = 0.0001, r^2 = 0.91$), negative correlations for the OMT rate versus clay con-

tent ($Y = -1.4X + 87, P = 0.0001, r^2 = 0.89$), and for the OMT rate and SOM content ($Y = -16.3X + 88, P = 0.03, r^2 = 0.5$). There was a similar trend at high moisture levels.

DISCUSSION

We have found that O₃ injected into soil significantly reduced plant parasitic nematodes and the reduction

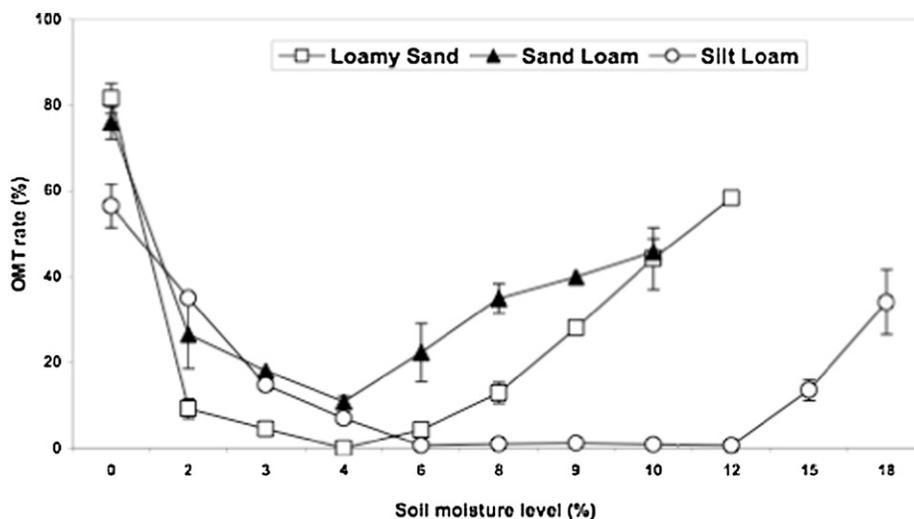


FIG. 3. Ozone mass transfer (OMT) through soil (Experiment 2). Rates of OMT were determined on three types of soil with moisture levels from 0 to 18% after 30-min application of 1% O₃ with 145 ml/min flow rate. The types of soil were loamy sand (opened squares), sandy loam (solid triangles) and silt loam (opened circles). The OMT rate was calculated by dividing the concentration of O₃ passing through the soil at the outlet by the concentration applied at the inlet. Bars on curves are standard errors with means of two replicates.

was dosage dependent and flow rate independent. We also demonstrated that soil types and conditions affected OMT rate. The OMT rate was affected by soil characteristics and conditions.

There are two modes of O₃ degradation in a medium, instantaneous decomposition and direct reaction to O₃-demanding materials (Stahelin & Hoigne, 1985; Hunt and Marinas, 1997; Kim et al., 1999; Kells et al., 2001; Lim et al., 2002). Though the rates of OMT through the soil in our studies can not be used to distinguish these two modes, they can be used to estimate the differences in amount of O₃-demanding materials for different soil types or different soil conditions. The major O₃-demanding materials in soil are organic matter and metal oxides on the soil surface (Lim et al., 2002). It has been proposed that O₃ is decomposed not only in the presence of organic matter, but also in the presence of moisture or on rough surfaces (Alder and Hill, 1950; Turner et al., 1973).

Similar to other fumigants, O₃ degradation (or O₃ demand) and SOM content are positively correlated (Choi et al., 2002; Gan and Yates, 1996). Incorporation of fumigants into soil organic matter and their transformation were demonstrated by radioactive labeling (Xu et al., 2003). Incorporation causes the fumigant to degrade and become inactive against soil organisms. Generally, the SOM content is higher in soils with higher clay and lower sand contents (Bosatta and Agren, 1997). Efficiency of contaminant removal by ozonation is affected by soil physical properties. Soil with higher clay content decreases the level of contaminant removal (Goi et al., 2006; O'Mahony et al., 2006) because clay soil has high ozone demand. A similar interpretation is possible from our results. At the same moisture level, the OMT rates decreased as the soil clay content increased or as the sand content decreased from loamy sand to silt loam soil. In comparison with clay soil, sandy soil has larger pore spaces thus facilitating easier transport of gaseous ozone through the soil.

The OMT rates and the amount of O₃ demanding materials were inversely correlated. Though O₃ reacted with SOM, only the SOM on the soil particle surface was accessible to O₃ (Giese and Christensen, 1954). The total soil surface area depended on soil particle size. Soil with smaller particles provided a larger surface area, possessed a lower OMT rate, and was presumed to have a higher O₃ demand. Generally, the finer the soil texture, the higher dosage of fumigant needed (McKenry & Roberts, 1985). Soil with intermediate moisture levels increased O₃ demand, or increased the number of O₃ activity sites.

Soil condition also affected ozonation efficiency. Dry soil has more air space between soil particles and no solid-liquid interface. Ozone is more stable in air than in water, and could be readily transported through dry soil. As soil moisture increases, water first accumulates as a film on the surface of soil particles and then oc-

cupies the air space between soil particles. As soil moisture increases, O₃ becomes less stable, dissolves in pore water, and subsequently decomposes. Water can also lead to an increase in dissolved organic carbon that can react with the ozone dissolved in the aqueous phase. Thus, moisture in soil increases O₃ demand (Masten and Davies, 1997). In our study, soil with intermediate moisture levels had the lowest OMT rate regardless of the type of soil. Air-dried soil treated with O₃ showed greater removal of chemical contaminants compared to soil with higher water content (Goi and Trapido, 2004; O'Mahony et al., 2006). This could be due to the degradation of O₃ in the moist soil. Increased soil moisture content also increases the degradation of other fumigants, such as 1, 3-dichloropropene (1, 3-D) (Gan et al., 1999). However, it is possible that increased moisture in the soil prevented intimate contact of O₃ with soil surfaces. Although soil with comparatively high moisture has more solid-liquid interface, O₃ cannot access these sites readily if the water barrier around soil particles is high. The overall area of water surface in wetter soil is decreased compared with soil with intermediate moisture levels because interstitial spaces between soil particles become filled with water thus preventing interaction of ozone with organic materials on the soil particle surface. This could account for soil in our study with near saturation tending to have a high OMT rate.

Although the levels of reduction varied among crops and fields, when O₃ was injected into bedded soil using buried drip tubes at rates similar to those tested in our laboratory trials, significant reductions in population levels of RKN and increases in crop yields were demonstrated in carrot and tomato fields (Pryor, 1999). If the reduction of RKN achieved in the laboratory (68%) were achieved in the field, it might be great enough to achieve a statistically significant reduction in damage, but not enough to produce a marketable crop of carrots. This same level of reduction would be more likely to produce a marketable crop in less sensitive crops such as tomatoes, peppers, or cotton (Ferris and Roberts, 2010). At the present time, O₃ applications to soil would be more expensive than currently available soil fumigants. The use of O₃ for soil fumigation would become more feasible if the trend for limitations on the use of other soil fumigants continues, or if the costs of perceived environmental and health effects were factored into the equation.

Fumigant distribution in soil affects pathogen control efficiency. Effective O₃ treatment may also be limited by other factors. For example, fumigants degrade faster at higher temperatures (Gan et al., 1999). When field fumigation is designed, the effects of temperature, soil texture and moisture, and depth of injection should be considered in determining adequate O₃ dosage. The effectiveness of O₃ fumigation on other pathogens, weeds or insects within soil should also be evaluated.

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