

Dose response of weed seeds and soilborne pathogens to 1,3-D and chloropicrin

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Abstract

InLine (Dow AgroSciences LLC, Indianapolis, IN, USA), a commercial formulation of 1,3-dichloropropene (1,3-D; 61%) plus chloropicrin (33%), is one of the potential replacements to methyl bromide for soil fumigation. The efficacy of 12 InLine concentrations (80–19,520 $\mu\text{mol kg}^{-1}$) to control five species of weed seeds and four soilborne pathogens was evaluated in a laboratory dose–response study after 24 h of exposure to fumigants in a sandy loam soil at 20 °C in microcosms. Logistic dose–response models were used to estimate the effective concentration to reduce weed seed and pathogen viability by 50% (LC₅₀) or 90% (LC₉₀). Among the weeds, the seed of *Portulaca oleracea* was the most sensitive to soil fumigation with InLine (LC₅₀ = 352 $\mu\text{mol kg}^{-1}$, LC₉₀ = 583 $\mu\text{mol kg}^{-1}$), followed by *Stellaria media* and *Polygonum arenastrum* with LC₉₀ values of 780 and 1636 $\mu\text{mol kg}^{-1}$ soil, respectively. The seeds of *Malva parviflora* and *Erodium cicutarium* were not sensitive to fumigation up to the highest InLine dose of 19,520 $\mu\text{mol kg}^{-1}$ soil. Among the pathogens, *Pythium ultimum* (LC₅₀ = 30 $\mu\text{mol kg}^{-1}$ soil, LC₉₀ = 46 $\mu\text{mol kg}^{-1}$ soil) was the most sensitive and *Verticillium dahliae* (LC₅₀ = 625 $\mu\text{mol kg}^{-1}$ soil, LC₉₀ = 2735 $\mu\text{mol kg}^{-1}$ soil) was the least sensitive to InLine fumigation. *Phytophthora cactorum* and *Fusarium oxysporum* exhibited intermediate susceptibility to this soil treatment (LC₅₀ ≤ 397 $\mu\text{mol kg}^{-1}$ soil, LC₉₀ ≤ 1113 $\mu\text{mol kg}^{-1}$ soil). In this sandy loam soil, InLine at a concentration of 1636 $\mu\text{mol kg}^{-1}$ reduced the viability of *Portulaca oleracea*, *S. media*, *Polygonum arenastrum* seeds and all fungi pathogens tested (except for *V. dahliae*) by 90% at 20 °C after 24 h exposure.

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1. Introduction

Many crops are susceptible to soilborne pathogens such as *Pythium ultimum*, *Phytophthora cactorum*, and *Verticillium dahliae* as well as to competition from weeds. Soilborne pathogens and weeds can significantly reduce yield and quality of field crops. Additionally, weeds may serve as hosts for insects and pathogenic nematodes and microorganisms (Chandler, 1990; Himelrick and Dozier, 1991; Vallad et al., 2005) and they can increase production costs (Goodhue et al., 2004). The standard treatment for management of soilborne pathogens, nematodes, and

weeds in many high-value crop production systems for more than 40 years has been preplant soil fumigation with a 2:1 mixture of methyl bromide (CH₃Br; MeBr) and chloropicrin (CCl₃NO₂; CP). The Montreal Protocol led to stringent regulations on MeBr production and consumption and stimulated the research on all areas of integrated pest management including alternative biocides (USEPA, 1993; UNEP, 1997). The loss of MeBr will greatly affect agricultural, silvicultural, and horticultural production unless safe and efficacious alternatives are found. Several alternative fumigants have been suggested as MeBr replacements for high-value fruit, nut and vegetable crops and tested in field experiments to evaluate their efficacy to control pathogens and weeds while maintaining high yields (Ajwa and Trout, 2004; Fennimore et al., 2003; Gilreath

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et al., 2004a, b, c, 2006, Haar et al., 2003). Currently registered alternatives to MeBr are CP, 1,3-dichloropropene ($C_3H_4Cl_2$, 1,3-D), 1,3-D plus CP (Telone C35 for broadcast and InLine for drip applications; Dow AgroSciences LLC, Indianapolis, IN, USA), and methyl isothiocyanate (MITC) generators such as Dazomet or Basamid (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione; CERTIS USA LLC, Columbia, Maryland), sodium methyldithiocarbamate ($CH_3NHCSS Na$, trade name Vapam; AMVAC Chemical Corporation, Los Angeles, California, or Sectagon; Tessenderlo Kerley, Inc., Phoenix, Arizona) and potassium methyldithiocarbamate ($CH_3NHCSS K$, trade name Kpam; AMVAC Chemical Corporation) (Ajwa et al., 2003). An experimental chemical alternative to MeBr is iodomethane (CH_3I , also referred to as methyl iodide, trade name Midas; Arysta Lifesciences, Cary, NC) (Ajwa et al., 2003).

The combination of 1,3-D plus CP can provide significant control of many plant pathogens in soil, but it has had limited efficacy for control of weeds or volunteer plants, such as *Zantedeschia aethiopica* and *Ranunculus* species (Duniway, 2002). The combination of 1,3-D and CP enhanced the control of soilborne pathogenic fungi and increased strawberry yield relative to fumigation with 1,3-D similarly to mixtures of MeBr and CP or methyl iodide and CP (Wilhelm et al., 1961, Hutchinson et al., 2000). Recent field studies showed that InLine provides good control of soilborne pathogens and many weeds (Fennimore et al., 2003). However, relatively high application rates are required for results equivalent to the standard, MeBr plus CP, fumigation. For example, in strawberry, a minimum rate of 336 kg ha^{-1} of InLine per treated bed area is required to achieve yield equivalent to 390 kg ha^{-1} of MeBr plus CP broadcast shank application (Ajwa et al., 2003). Stringent regulations to mitigate risks of human exposure limit the utility of 1,3-D products. These include extensive buffer zones (90 m away from occupied structures), personal protective equipment to limit worker exposure, and in California, a cap on the annual use per township (93 km^2) (Duniway, 2002).

Limited information is available on the minimum concentration of InLine required to control major weeds and soilborne pathogens in fumigated soils. The objectives of our study were to develop logistic dose–response models for InLine in a sandy loam soil with five weed species and four soilborne plant pathogens. The research, conducted in a laboratory, was designed to determine InLine concentrations required in a sandy loam soil to reduce weed seed and pathogen viability by 50% (lethal concentration, LC_{50}) and 90% (LC_{90}).

2. Materials and methods

2.1. InLine concentrations

The commercial formulation of InLine (61% 1,3-D plus 33% CP, 94.1% active ingredients, a.i.) (Dow Agro-

Sciences LLC, Indianapolis, IN, USA) was applied to the soil to achieve final concentrations in the soil of 0, 80, 200, 400, 600, 800, 1000, 2000, 4000, 6000, 8000, 9760 and $19,520 \mu\text{mol kg}^{-1}$ [dry wt.]. These rates are equivalent to InLine concentrations in the gaseous phase of the microcosm of 0, 50, 125, 250, 375, 500, 625, 1250, 2500, 3750, 5000, 6100, and $12,200 \mu\text{M}$, or field application rates ranging between 0 and 4600 kg ha^{-1} . InLine application rates in water were based on label application directions for this fumigant in the field (500 to 1500 mg l^{-1}) (CDMS, 2006), represented in our study by the intermediate concentration rates.

2.2. Soil type and microcosm study

Soil samples were collected from the top 15 cm at the California Strawberry Commission Research facilities near Watsonville ($121^\circ 50'W$, $36^\circ 54'N$), CA, USA, on a plot without a known history of fumigation. The soil was classified as an Elder sandy loam (coarse-loamy, mixed, thermic, Cumulic Haploxeroll), with a mean particle size distribution of 62% sand, 26% silt, and 12% clay. The pH was 7.75 (H_2O) and 7.08 (0.01 M $CaCl_2$), and the organic C content was 6 g kg^{-1} soil. This sandy loam soil was selected for this study because it is a typical soil used for strawberry and cut flower production in California. Also, this soil was naturally infested with *Pythium ultimum* (50–100 propagules g^{-1} soil), *Verticillium dahliae* (30–45 microsclerotia g^{-1} soil) and *Fusarium oxysporum* (850–1200 propagules g^{-1} soil). Moist field soil was passed through a 2-mm sieve prior to use.

To ensure that population densities of *Pythium ultimum* were sufficient to evaluate fumigant efficacy, experimental soils were amended with pathogen inoculum produced in the laboratory. The procedure that was used took advantage of the saprophytic phase of the pathogens life cycle to produce inoculum similar to what would be present in field soils (Martin, 1992). Briefly, air dried soil was amended with dried, crushed sugar beet leaves ($0.25 \text{ g } 100 \text{ g}^{-1}$ soil), placed in a small plastic autoclave bag, stoppered with a foam plug, and autoclaved for 50 min. Sterile water was added to bring the soil to -10 kPa and an agar culture of two strains of the pathogen recovered from the same soil used in this experiment was added. After 7 d incubation at room temperature with occasional mixing, the soil was removed and dried under a laminar flow hood. Dried soil was mixed and propagule densities determined by dilution plating as described below.

Additional samples of experimental soil were infested with inoculum of *Phytophthora cactorum*. Inoculum of the pathogen was produced as described previously in jars of V8 juice-oat-vermiculite substrate (Bhat et al., 2006). A pathogen was allowed to permeate the substrate for 1 month, during which time it formed mycelium and oospores. The infested substrate was poured into a wrap of cheesecloth and washed in tap water to leach away unassimilated nutrients. The washed inoculum was

thoroughly mixed with the experimental soil (1 part substrate/5 parts soil, v/v). The soil mixture was divided into 30 ml samples, each wrapped snugly in a synthetic fabric pouch (i.e., a “sample bag”) that was tied shut with a nylon cord. The sample bags were placed in the microcosms as described below.

Infested soil was thoroughly mixed with field soil, and placed in microcosms consisting of 230 ml jars containing 150 g soil [dry wt.] and preconditioned at 20 °C for 24 h before being used in the study. On the day of the fumigation, sample bags containing soil infested with *Phytophthora cactorum* and mesh bags containing 30 viable seeds each of *Polygonum arenastrum* (Common Knotweed), *Stellaria media* (Common Chickweed), *Portulaca oleracea* (Common Purslane), *Malva parviflora* (Little Mallow), and *Erodium cicutarium* (Redstem Filaree) were buried in jars at 2.5 and 4.0 cm soil depth, respectively. Weed seed bags were imbibed in moist field soil at 20 °C for 24 h prior to the study. Imbibition is completed in less than 1 d at room temperature (Baskin and Baskin, 2001). Previous studies indicated that dry seeds of *S. media* and *Portulaca oleracea* were not sensitive to fumigation with CP, but seeds moistened with water were sensitive (Haar et al., 2003). Seeds of *M. parviflora* and *E. cicutarium* were not sensitive to CP fumigation dry or wet.

The experimental design consisted of 12 fumigant concentrations and a nonfumigated control in four replicated microcosms. InLine was applied by a syringe as freshly prepared aqueous solution to bring soil moisture content from initially 4% to an average of 15% (w/w). The control microcosms received water instead of InLine solution to adjust to the same soil moisture content. Microcosms were sealed with an air-tight lid and exposed to the fumigant for 24 h at 20 °C. Exposure time was selected based on InLine field studies indicating that the maximum concentration of CP and 1,3-D in the soil gaseous phase was reached within 18 to 24 h (Ajwa and Trout, 2004; Ajwa et al., 2003).

2.3. Parameters

2.3.1. Soil sampling and retrieval of mesh bags and sample bags

After 24 h of fumigation, microcosms were vented under a laminar flow hood for 5 min. Then soils were mixed, and vented again for another 30 min to release any remaining volatile fumigant. Preliminary studies showed that >95% of InLine in microcosms dissipates within 30 min after opening the jars. The sample bags containing *Phytophthora cactorum* and weed seeds were retrieved, and soil samples were taken for analysis of survival of *V. dahliae*, *F. oxysporum* and *Pythium* sp. within 2 h after venting the microcosms. The microcosm study was repeated once in time for data validation.

2.3.2. Weed seed viability

Seeds were removed from bags, sorted by species, and tested for viability colorimetrically with tetrazolium

chloride (TTC) (Grabe, 1970). The seeds were allowed to imbibe on filter paper (Whatman no. 1) moistened with 1 ml of sterile deionized water in plastic Petri dishes (100 mm diam., 15 mm high). Petri dishes were sealed with parafilm and placed in a germination chamber at 21 °C in darkness for 20–24 h. Imbibed seeds were cut with a scalpel and placed in another Petri dish, cut side down, on filter paper moistened with 1 ml of a 0.1% (w/v) 2,3,5-tetrazolium chloride solution. Petri dishes were sealed with parafilm and returned to the germination chamber for another 20–24 h, and then evaluated under a microscope for staining of the embryo. Seed viability was calculated according to

$$\text{Seed viability (\%)} = (\text{no. stained seeds/no. total seeds})100. \quad (1)$$

2.3.3. Survival of fungi and oomycetes

2.3.3.1. Survival of *V. dahliae*. Soils were air-dried in the laboratory (23 °C ± 2 °C), mixed thoroughly, and pulverized using mortar and pestle. From each sample, 10 g of soil was placed in snap cap vials and mixed with 2.5 ml of a dl-methionine solution (7.5 mg ml⁻¹) (Kapulnik et al., 1985). Vials were capped and incubated in the dark at 30 °C for 1 week. The vials were then opened and allowed to air-dry for 1 week at 22–24 °C. Samples were repulverized and dispensed onto Petri dishes containing modified NP-10 selective medium (Kabir et al., 2004) using the modified Anderson sampler (Butterfield and DeVay, 1977). With the Anderson sampler, 0.5 g of pulverized soil from each sample was distributed over two replicates of six Petri dishes. Plates were incubated in the dark at 22–24 °C for 3 weeks. After incubation, the surfaces of the agar media were gently washed under running tap water to dislodge and remove soil particles. Washed Petri dishes were examined for *V. dahliae* microsclerotia clusters using a dissecting microscope with transmitted light. Counts from the two replications were combined for mean values and expressed as microsclerotia g⁻¹ dry soil.

2.3.3.2. Survival of *F. oxysporum*. Soil samples were air-dried in the laboratory (23 °C ± 2 °C), mixed thoroughly, and pulverized using mortar and pestle. The number of *F. oxysporum* propagules in each sample was determined by dilution plating on modified Komada's medium (Komada, 1975) on four replicated plates. Counts from the four replicates were combined for mean values and expressed as colony forming units (cfu) g⁻¹ dry soil.

2.3.3.3. Survival of *Pythium ultimum*. One gram of air-dried soil was added to 10 ml sterilized water blanks (triplicate tubes for each sample), vortexed to mix the soil and 500 µl distributed evenly over a Petri dish containing a semi-selective *Pythium* medium (5 plates per tube). The medium consisted of corn meal agar (17 g l⁻¹, BBL, Kansas City, MO) amended immediately after autoclaving with 0.1% Tween 20 followed by pimaricin

(10 mg l⁻¹), penicillin (100 mg l⁻¹), ampicillin (250 mg l⁻¹), rifampicin (10 mg l⁻¹), rose bengal (50 mg l⁻¹) and Benomyl 50WP (20 mg l⁻¹, DuPont, Wilmington, DE) after the medium had cooled to 50 °C (Martin, 1992). After 24 h incubation at 25 °C the surface of the plates was washed free of soil under a gentle stream of water and the number of colonies counted. Plates were placed back into the incubator and subsequent counts made again after 24 h. The total colony counts were expressed as cfu g⁻¹ dry soil and the presented data reflect the mean of the three replicates.

2.3.3.4. Survival of *Phytophthora cactorum*. Soil samples infested with *Phytophthora cactorum* were assayed for pathogen survival by dilution plating. A subsample from each soil sample was air dried in an open Petri dish placed in a fume hood overnight. For each dried subsample, 2 g of the soil were added to 10 ml of sterile distilled water in a 20-ml test tube and vortexed for 1 min. Immediately afterwards, 0.1 ml of the soil suspension was withdrawn and spread evenly on a semi-selective medium on each of two Petri dishes filled with a semi-selective medium consisting of corn meal agar (17 g l⁻¹, BBL, Kansas City, MO), pimaricin (10 mg l⁻¹), ampicillin (250 mg l⁻¹), rifampicin (10 mg l⁻¹), and pentachloronitrobenzene (25 mg l⁻¹) in a 100-mm-diameter Petri dish. The plates were incubated for 7 d at 18 °C. After incubation, the debris on the surface of each plate was gently cleaned under running tap water with a soft cheesecloth sponge. Colonies with the characteristic of *Phytophthora cactorum* were counted and then observed microscopically to confirm their identity. The colony counts were expressed as cfu g⁻¹ dry soil.

2.4. Data analysis

The experiment was designed as a randomized complete block with four replicates. The relationship between the percentage of weed seed and pathogen mortality and the logarithm of the InLine concentration was described according to the sigmoidal logistic probability model (2),

$$Y = \frac{a}{1 + e^{-x-x_0/b}}, \quad (2)$$

where Y is the weed seed or pathogen mortality (percent mortality relative to an untreated control) as a function of the logarithm of the InLine concentration (x). Sigmoidal logistic probability models were computed using SigmaPlot 2001 (Systat Software Inc., Point Richmond, CA, USA). Logistic dose–response curves were used to estimate the InLine concentration required to achieve a weed seed and pathogen mortality of 50% (LC₅₀) or 90% (LC₉₀) using SAS probit procedure (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was used to assess treatment effects, and when the F -test revealed an error probability of $\leq 5\%$ ($P \leq 0.05$), the means were separated using Duncan's Multiple Range test with SPSS (version 12.07 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Dose-response of weed seeds to soil fumigation with InLine

Sensitivity of weed seeds towards soil fumigation with InLine varied considerably within the weed species studied. Sigmoidal dose–response curves of weed seeds exposed to different concentrations of InLine indicated a strong relationship between increases in fumigant concentrations and mortality rates of *Polygonum arenastrum*, *S. media*, and *Portulaca oleracea* seeds with R^2 values ≥ 0.97 (Figs. 1 and 2), while *M. parviflora* and *E. cicutarium* were the weeds least sensitive to this fumigant. *S. media* and *Portulaca oleracea* were more sensitive to fumigation with InLine than *Polygonum arenastrum*. More than 50% seeds of the former two weed species were killed during a 24 h exposure to 800 $\mu\text{mol InLine kg}^{-1}$ soil, while a concentration of 1000 $\mu\text{mol kg}^{-1}$ was required for an equivalent efficacy on *Polygonum arenastrum*. Seeds of *M. parviflora* and *E. cicutarium* were relatively unresponsive to fumigation with InLine. Only 8% and 34% of seeds of *E. cicutarium* and *M. parviflora*, respectively, were killed

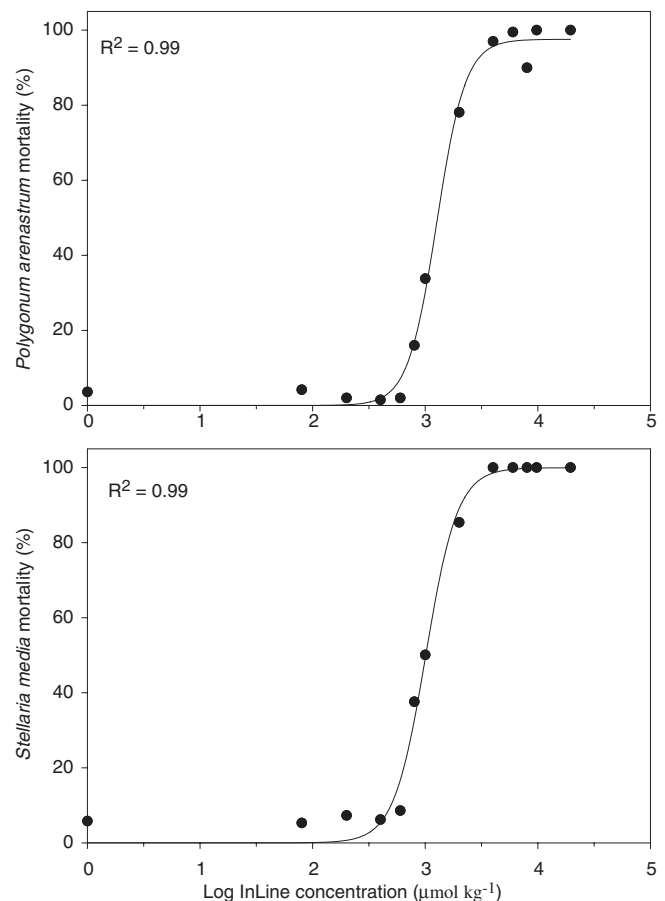


Fig. 1. Response of seeds of *Polygonum arenastrum* and *Stellaria media* to different InLine concentrations after fumigation of a sandy loam soil for 24 h at 20 °C.

during a 24 h exposure to the highest InLine concentration of $19,520 \mu\text{mol kg}^{-1}$ tested in this experiment (Data not shown). The slope b of the logistic curve of the nonlinear regression models indicated that the sensitivity of weed seeds towards soil fumigation with InLine decreased in the order *Portulaca oleracea* > *S. media* > *Polygonum arenastrum* > *M. parviflora* > *E. cicutarium* (Table 1).

Probit analysis on logistic dose curves revealed that the concentration required to control 50% (LC_{50}) of the seeds of *Portulaca oleracea*, *S. media* and *Polygonum arenastrum* varied between 352 and $854 \mu\text{mol InLine kg}^{-1}$ soil, while concentrations of 583 to $1636 \mu\text{mol InLine kg}^{-1}$ soil were required to kill 90% (LC_{90}) of the seeds of these weeds during a 24 h exposure at 20°C (Table 1). InLine concentrations of $\leq 19,520 \mu\text{mol kg}^{-1}$ soil did not control seeds of *M. parviflora* and *E. cicutarium* during a 24 h exposure at 20°C .

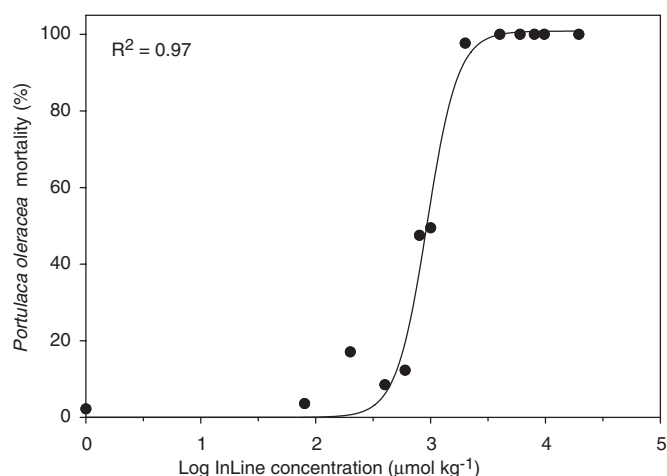


Fig. 2. Response of seeds of *Portulaca oleracea* to different InLine concentrations after fumigation of a sandy loam soil for 24 h at 20°C .

Table 1

Estimated parameters for nonlinear regression of weed seed and soil-borne pathogen mortality percentages on the concentration of InLine ($\mu\text{mol kg}^{-1}$ soil) required to control 50% (LC_{50}) and 90% (LC_{90}) growth at 20°C for 24 h exposure time

Species	b^a	R^2	LC_{50}		LC_{90}			
			Lower limit ^b	Upper limit ^b	Lower limit ^b	Upper limit ^b		
Weeds								
<i>Polygonum arenastrum</i>	97.5	0.99	800	854	915	1529	1636	1763
<i>Stellaria media</i>	99.9	0.99	428	454	484	728	780	844
<i>Portulaca oleracea</i>	100.8	0.97	371	352	392	546	583	630
<i>Malva parviflora</i>	15.5	0.85	—	> 19520	—	—	> 19520	—
<i>Erodium cicutarium</i>	6.8	0.65	—	> 19520	—	—	> 19520	—
Pathogens								
<i>Verticillium dahliae</i>	91.3	0.96	488	625	757	2455	2735	3098
<i>Fusarium oxysporum</i>	105.6	0.92	280	306	332	1048	1113	1188
<i>Pythium ultimum</i>	100.0	0.99	28	30	32	42	46	52
<i>Phytophthora cactorum</i>	104.2	0.98	397	412	426	957	993	1033

^aSlope at the inflection point of the logistic curve indicating the susceptibility of the species to the fumigant ($P \leq 0.01$).

^bConfidence interval estimates ($P < 0.05$).

3.2. Dose–response of fungal and oomycete pathogens to soil fumigation with InLine

Increasing concentrations of InLine significantly increased mortality rates of *V. dahliae*, *F. oxysporum*, *Pythium ultimum* and *Phytophthora cactorum* with R^2 values ≥ 0.91 ($P \leq 0.01$) (Figs. 3 and 4). Among the pathogens evaluated, *Pythium ultimum* was the most sensitive to InLine fumigation, followed by *F. oxysporum* and *Phytophthora cactorum* with intermediate sensitivities, and *V. dahliae* with the least sensitivity. An InLine concentration of $200 \mu\text{mol kg}^{-1}$ controlled almost 100% of the colony forming units of *Pythium ultimum*, while a fumigant concentration of $6000 \mu\text{mol kg}^{-1}$ InLine was required for an equivalent control of *V. dahliae*. According to the Duncan's Multiple Range test increases in the fumigant rate beyond $4000 \mu\text{mol kg}^{-1}$ did not result in significantly further reductions in *V. dahliae* microsclerotia, or propagules of *F. oxysporum* and *Phytophthora cactorum* viability (data not shown). At $4000 \mu\text{mol InLine kg}^{-1}$ soil, mortality rates for these pathogens varied between 82% and 100%.

Probit analysis on logistic dose curves revealed that the minimum concentration required to kill 50% (LC_{50}) of the pathogen samples ranged between 30 and $625 \mu\text{mol InLine kg}^{-1}$ soil for *Pythium ultimum* and *V. dahliae*, respectively (Table 1). Concentrations of 46 to $2735 \mu\text{mol InLine kg}^{-1}$ soil were required to kill 90% (LC_{90}) of these pathogens during 24 h exposure at 20°C . InLine concentrations of $\leq 1113 \mu\text{mol kg}^{-1}$ soil were sufficient to control 90% propagules of *F. oxysporum* and *Phytophthora cactorum*.

4. Discussion

A logistic dose–response model was used to evaluate the sensitivity of important species of weed seeds and soilborne

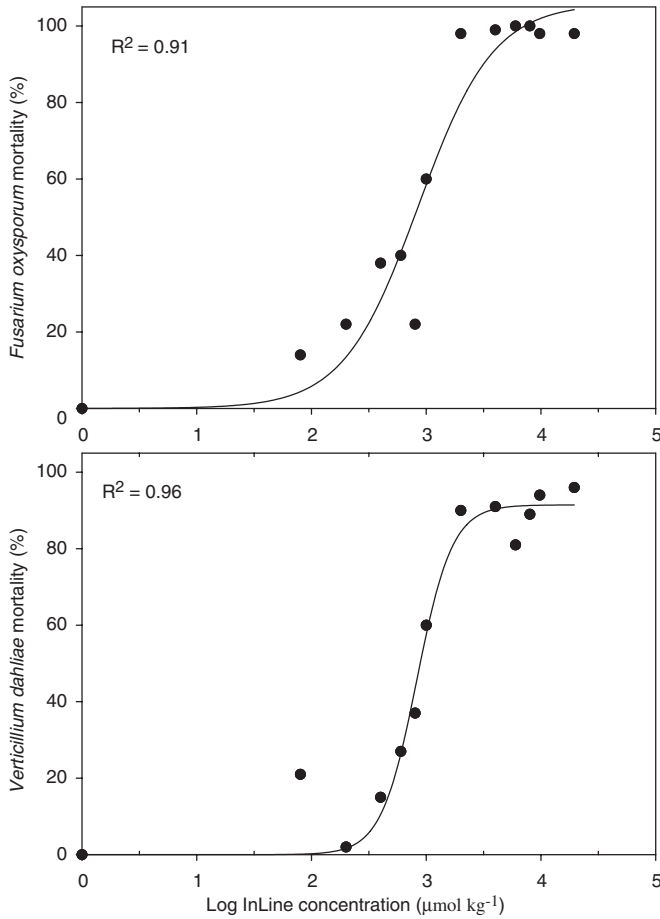


Fig. 3. Response of *Verticillium dahliae* and *Fusarium oxysporum* to different InLine concentrations after fumigation of a sandy loam soil for 24 h at 20 °C.

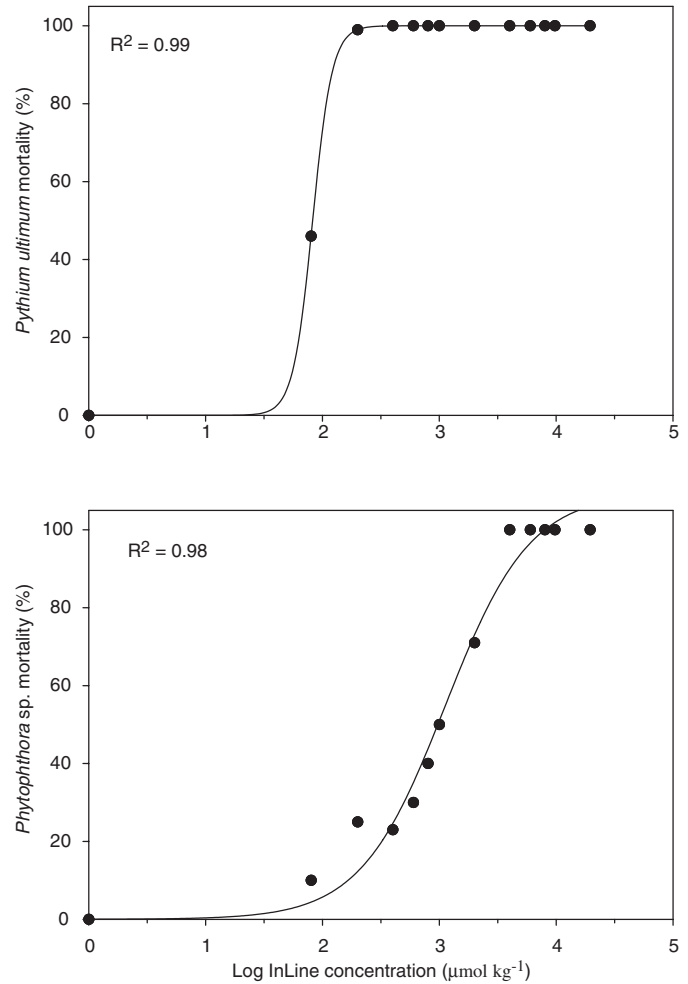


Fig. 4. Response of *Pythium ultimum* and *Phytophthora* spp. to different InLine concentrations after fumigation of a sandy loam soil for 24 h at 20 °C.

pathogens to 12 concentrations of InLine ranging from 80 to 19,520 μmol InLine kg⁻¹ soil (equivalent to air concentrations of 50–12,200 μM). This model was significant at $P \leq 0.01$ for all fumigant doses over a 24-h exposure time, and therefore provides a tool to describe the efficacy of different InLine doses to control weeds and pathogens in fumigated Elder sandy loam soil with water potential of -10 kPa. The interpretation of our results is limited by the facts that data were obtained in laboratory experiments under artificial conditions, and that the technique of inoculum preparation for some pathogens (i.e., *Phytophthora cactorum*) may influence the sensitivity of these pathogens to fumigation relative to native populations in the soil. Moreover, soil type and soil moisture content during fumigation will affect the efficacy of the fumigant. Previous studies showed that the fumigant efficacy in sandy loam soils was highest at a moisture content of 14–15% (Zhang et al., 1998; Haar et al., 2003).

Our results indicated that InLine can provide satisfactory control of selected weeds and soilborne pathogens in addition to pests listed on the InLine label such as nematodes, symphylans and wireworms (CDMS, 2006). However, the horticultural acceptability of different rates

of InLine in a given field will depend to a large extent upon the spectrum and distribution of pathogens in the field. For example, among the weed species, *Portulaca oleracea*, *S. media* and *Polygonum arenastrum* were sensitive to InLine, while this fumigant did not provide adequate control of *M. parviflora* and *E. cicutarium* at the concentration range, exposure time and temperature conditions tested in this study.

A laboratory dose–response experiment with the fumigant CP showed that the lowest concentration to kill 50% of the seed during 72 h exposure was 100 μM for *Portulaca oleracea*, 150 μM for *S. media* and 1000 μM *Polygonum aviculare* (Haar et al., 2003). *M. parviflora* and *E. cicutarium* have been shown to be resistant to soil fumigation in field evaluations of CP, metam sodium, and methyl bromide (Haar et al., 2003; Fennimore et al., 2003). Virtually impermeable film (VIF) trapped the fumigant for a longer period near the soil surface, and thereby increased the dose (fumigant concentration multiplied by time) and prolonged the exposure of weed seeds to the fumigant (Fennimore et al., 2005). Furthermore, factors that play a

role in controlling seed sensitivity towards fumigants are seed coat hardness and impermeability (i.e., physical dormancy) to moisture and fumigants. *M. parviflora* and *E. cicutarium* belong to plant families with physical dormancy and hard seed coats (Baskin and Baskin, 2001). Penetrating the hard seed coats with fumigants is difficult to overcome under field conditions. Therefore, fumigant combinations, herbicides and mulches are more reliable methods to control hard-coated weed seeds (Hutchinson et al., 2003; Johnson and Fennimore, 2005). Hutchinson et al. (2003) reported effective fumigant concentrations to kill 50% of *Cyperus esculentus* of 53.2, 52.4, 20.2, 18.5, 6.5, and 3.7 μM for MeBr, 1,3-D, methyl iodide, CP, metam Na, and propargyl bromide, respectively. Combining each fumigant with 17% CP resulted in an increased *C. esculentus* control for all fumigants with exception of MeBr/CP and methyl iodide/CP combinations.

Field trials have shown that applications of 1,3-D and CP in combination with pebulate herbicide or fosthiazate can increase the control of problem weeds, such as *Cyperus rotundus* L. and *C. esculentus* (Noling and Gilreath, 2001; Gilreath et al., 2004b; Santos et al., 2006). Two theories have been proposed to describe the synergism of fumigant or fumigant/herbicide combinations. The first theory suggests that one fumigant may cause changes in cell wall permeability that, in turn, may increase the uptake or decrease the efflux of a second fumigant from the cell (Cohen and Levy, 1990). Another hypothesis suggests that exposure of an organism to one fumigant may alter cell processes to an extent that exposure to a second compound is more toxic than under single exposure to the latter compound (Samoucha and Gisi, 1987).

Among the pathogens evaluated, *Pythium ultimum* was the most sensitive and *V. dahliae* the least sensitive pathogen to fumigation with InLine. InLine showed an intermediate efficacy for controlling propagules of *F. oxysporum* and *Phytophthora cactorum* in soils. The greater sensitivity of *Pythium ultimum* to the fumigant compared to the closely related *Phytophthora cactorum* could in part be due to the resting structures each pathogen produces. Whereas *Phytophthora cactorum* survives in the soil as a thick-walled oospore, *Pythium ultimum* survives as a thick-walled oospore and a thin-walled hyphal swelling. The oospore in this species is constitutively dormant and does not readily germinate whereas the hyphal swelling does (Martin and Loper, 1999) and is likely to be more sensitive to fumigants due to its thin wall. The isolates of *Pythium ultimum* used in this study produces large numbers of hyphal swellings in culture and are likely the primary propagules detected when quantifying the pathogen in the infested soil used to amend the test soils. *Pythium ultimum* also showed a relatively high sensitivity to soil fumigation with MeBr relative to *F. oxysporum* (Minuto et al., 1999).

A dose–response study on the efficacy of soil fumigation with MeBr and methyl iodide against a range of soilborne pathogens including *F. oxysporum*, *Phytophthora* spp.,

Pythium ultimum, and *V. dahliae* revealed that *Pythium ultimum* and *Phytophthora* spp. were the most sensitive pathogens and *V. dahliae* was among the least sensitive species (Munnecke et al., 1978, Hutchinson et al., 2000). Concentrations of methyl iodide required to reduce viability of *F. oxysporum*, *V. dahliae*, and *Pythium ultimum* with pathogen inoculum produced on colonized seeds for 7 d by 50 (EC₅₀) were 101, 95 and 8.6 μM . Corresponding values for the fumigant MeBr were 148, 176, and 16 μM , respectively (Hutchinson et al., 2000). Some of the factors responsible for the resilience of *V. dahliae* propagules include the aggregated cell mass that comprise microsclerotia and the melanized nature of microsclerotia. Melanin is known to protect fungi and other microorganisms from various abiotic and biotic stresses (Butler and Day, 1998), and it can be hypothesized that it renders the microsclerotia resistant to certain thresholds of soil fumigants.

The results of this study revealed that an InLine concentration of 1636 $\mu\text{mol kg}^{-1}$ soil (equivalent to 382 kg ha^{-1} , less than maximum label rate of 480 kg ha^{-1}) over 24 h was sufficient to kill $\geq 90\%$ of major weed seeds and propagules of soilborne pathogens, with exception of *V. dahliae*. Our study found that 2735 $\mu\text{mol InLine kg}^{-1}$ soil is needed to kill 90% of *V. dahliae* in this soil. Thresholds for weed and pathogen pressure in soil can vary significantly depending on the crop and cultivar. For example, 2 microsclerotia g^{-1} soil of *V. dahliae* can kill 50% of strawberry plants (Harris and Yang, 1996), whereas no disease symptoms appear on lettuce plants at 100 microsclerotia g^{-1} soil (Subbarao, unpublished data). Therefore, in some cases, it may be advantageous to adjust rates of InLine application to accommodate the prevailing combination of weeds, pathogens, crops and cultivars expected at a given site. For example, currently available strawberry cultivars showed no resistance to Verticillium wilt (Martin and Bull, 2002), and varying sensitivity to different species *Pythium* and *Rhizoctonia* (Martin, 2003). Therefore, controlling *V. dahliae* might require simultaneous or sequential application of another fumigant such as chloropicrin or metam sodium (Ajwa and Trout, 2004). Further research on fumigant efficacy as a function of soil type, sensitivity of various crops and their cultivars to varying pathogen and weed densities, and on methods to control fumigant-resistant weeds is needed.

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