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Nematode response to methyl bromide and 1,3-dichloropropene soil fumigation at different temperatures

Shikui Xue,[1] Jianying Gan,[1]* Scott R Yates[1] and J Ole Becker[2]
[1]*US Salinity Laboratory, USDA-ARS, 450 West Big Springs Road, Riverside, CA 92507, USA*
[2]*Department of Nematology, University of California, Riverside, CA 92521, USA*

Abstract: Several heat-based methods, such as soil solarization, are being developed as alternative practices for managing soil-borne pests and pathogens. The effectiveness of these practices is often inconsistent or marginal, thus commanding the need for their integration with other methods. The main objective of this study was to determine synergistic interaction between soil fumigants and temperature. Soil infested with citrus nematode *Tylenchulus semipenetrans* was exposed to methyl bromide or 1,3-dichloropropene at various temperatures. Fumigant degradation was concurrently measured and concentration-time index (*ct*) was calculated and correlated to the recovered nematode population. In untreated soil, nematode survival was not affected by temperatures of 20–30 °C, but was strongly reduced at $\geq$ 40 °C. In fumigated soil, nematode suppression was much greater at 30 °C than at 20 °C, and the *ct* required for nematode elimination at 30 °C was $<$ 50% of that needed at 20 °C for both fumigants. These results suggest that these fumigants became more active with increasing temperature in the sub-lethal temperature range. It also implies that, when integrated with a heat-based practice, reduced rates of fumigants may provide adequate pest control, thus minimizing the environmental input of chemical fumigants.

Keywords: methyl bromide; 1.3-dichloropropene; citrus nematode; concentration-time index; ct; integrated pest management; soil fumigation

1 INTRODUCTION

Over the past five decades, management of soil-borne pests and pathogens has heavily relied on the use of soil fumigants. Many fumigants, however, possess negative attributes, such as acute and chronic toxicity, or mutagenicity. In particular, due to their high vapor pressures, significant fractions of applied fumigants escape into the air during fumigation.[1–5] Detection of high ambient concentrations has led to regulatory actions against several fumigants, including methyl bromide and 1,3-dichloropropene (1,3-D). Thus, there is an urgent need to develop effective and yet environmentally safer soil-borne pest management practices.

Soil solarization is considered as a non-chemical alternative to soil fumigation. In soil solarization, moist soil is covered with transparent or black plastic tarp, which causes thermal and biological suppression of soil-borne pests and pathogens.[6] Temperature increases are typically the greatest near the soil surface, and gradually diminish with depth.[7–9] Consequently, soil solarization provides adequate pest control only near the surface, but is often inadequate below that layer. A number of field studies have shown, however, that when soil solarization is combined with fumigant application at regular or reduced rates, efficacy is significantly improved compared with solarization or fumigation alone.[9–12] The enhanced efficacy was suggested to be caused by synergistic interactions between fumigants and temperature.[10] In containers, the activity of methyl isothiocyanate against *Verticillum dahliae* Kleb and *Fusarium oxysporum* f sp *vasinfectum* (Atk) Sny & Hans increased by four times when the temperature increased from 25 °C to 35 °C.[11] Similar temperature stimulation was also observed for 1,2-dibromoethane and 1,3-D against *V dahliae*.[13]

Synergistic interactions between fumigants and temperature may be useful in that they may be used for designing integrated practices to improve the efficacy of solarization. In particular, if the synergism allows lower rates of fumigants to be used, environmental input of chemical fumigants will also be reduced. So far, however, fumigant–temperature interactions have not been systematically studied, and the relationship between fumigant activity and chemical behavior as a function of temperature is not understood.

The objective of this study was to determine the interaction between soil temperature and activities of

* Correspondence to: Jianying Gan, USDA-ARS, US Salinity Laboratory, 450W Big Springs Road, Riverside, CA 92507, USA
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methyl bromide and 1,3-D against the citrus nematode *Tylenchulus semipenetrans* Cobb. Both fumigants are heavily used worldwide, but have been shown to volatilize excessively into the air during their application.[1–5,14] Methyl bromide is a potent ozone depleting compound, while 1,3-D is a Clean Air Act substance classified by the US Environmental Protection Agency. Minimizing their input into the environment is of great importance.

2 EXPERIMENTAL METHODS

2.1 Soil, chemicals and nematodes

Arlington fine sandy loam (coarse-loamy, mixed, thermic Haplic Durixeralf), sampled at the University of California, Riverside Agricultural Experimental Station, was used in this study. The soil consisted of 64% sand, 29% silt, 7% clay and 0.92% organic matter (OM), and had a pH of 7.2. Soil was sieved through a 2-mm mesh without air-drying, and stored at room temperature before use.

Methyl bromide (>99.5%, Matheson Gas Products Inc, East Rutherford, NJ, USA) was introduced into a Teflon sampling bag (Fisher Scientific Inc, Pittsburgh, PA, USA) in a fume hood and used as the stock gas (vapor density 3.7 mg ml^{-1}) for all the treatments. A 1,3-D 800 g litre^{-1} EC, (Telone EC, Dow AgroSciences LLC, Indianapolis, IN, USA), was used for 1,3-D treatment. Standard 1,3-D (98%) was purchased from Chem Service (West Chester, PA, USA).

The citrus nematodes were obtained from an infested orchard at the University of California, Riverside Citrus Research Center. Infested citrus roots were cut into 1-cm pieces and placed on Baermann funnels for approximately 24 h at 24 °C to collect second-stage juveniles of *T semipenetrans*.

2.2 Treatment and incubation

Fifty g of soil was weighed into 175-ml glass serum bottles and inoculated with 1.0 ml of a solution containing approximately 600 citrus nematodes. Four different rates were used for methyl bromide (0, 3.1, 5.6, and 7.3 mg kg^{-1}) and 1,3-D (0, 1.1, 2.2, and 6.4 mg kg^{-1}). For methyl bromide treatments, a gas-tight micro-syringe was used to inject a known amount of the compound into the sample bottles, and the treated bottles were immediately crimp-sealed with aluminum caps and Teflon-faced butyl rubber septa. For 1,3-D treatments, Telone EC was mixed with water, and 0.5 ml of the fumigant solution was added to the samples with a pipette. The initial soil moisture was adjusted to 10% (w/w) for all treatments. The closed sample bottles were immediately transferred to water baths and incubators with preset temperatures. Four incubation temperatures, of 20, 30, 40, and 45 °C, were used, and the variation in each temperature was $< \pm 1$ °C.

2.3 Bioassay and residue analysis

After 6, 12, 24, 48 and 96 h of incubation, six replicated samples from each treatment were removed. Four of the replicates were used for nematode extraction on Baermann funnels, followed by nematode enumeration. Nematode recovery was normalized over nematode population enumerated at time zero from untreated soil samples. The other two replicates were used for analysis of residual fumigant concentration in soil. Sample vials were chilled in a freezer at −21 °C and then decapped while the soil was frozen. Ethyl acetate (40 ml) and anhydrous sodium sulfate (40 g) were added, and the vials were then immediately recapped. The samples were thawed at room temperature, mechanically shaken for 30 min, and an aliquot of the solvent supernatant was transferred into GC vials for analysis. Preliminary experiments showed that the recovery of fumigant was >95% for the above procedure. Analysis of fumigants was done on a HP 6890 gas chromatograph (GC) equipped with an electron capture detector (ECD). The GC conditions were 30 m × 0.25 mm × 1.4 μm RTX-624 capillary column (Restek Co, Bellefonte, PA, USA), 1.1 ml min^{-1} column flow, 240 °C inlet temperature, and 300 °C detector temperature. The oven temperature for methyl bromide was initially held at 40 °C and then ramped to 150 °C at 20 °C min^{-1}. An isothermal 110 °C oven temperature was used for the elution of 1,3-D isomers. Under these conditions, methyl bromide, *Z*- and *E*-1,3-D were eluted 2.3, 3.6 and 3.8 min, respectively, after injection.

2.4 Calculation of concentration–time index (*ct*)

The exposure of nematodes to soil fumigants was calculated as a concentration–time index, which was integral of methyl bromide or 1,3-D concentration over time:

$$ct(t) = \int_0^t c(t)dt \tag{1}$$

where $ct(t)$ is the concentration–time index up to time t (h), $c(t)$ is the fumigant concentration at time t in soil (mg kg^{-1}), and t is measured in hours: (h). Assuming that fumigant degradation obeys the first-order kinetics, the rate constant (k_T, h^{-1}) for temperature T (K) can be estimated from residual concentrations of fumigants at different times:

$$c(t) = c_0 e^{-k_T t} \tag{2}$$

Thus, eqn (1) can be written as:

$$ct(t) = \int_0^t c_0 e^{-k_T t} dt \tag{3}$$

Degradation rate of a pesticide is known to closely depend on temperature, and the effect can be described by the Arrhenius equation:

$$k_T = A e^{-E/RT} \tag{4}$$

where A is Arrhenius coefficient (h^{-1}), E is activation energy (J mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is temperature in K. Thus,

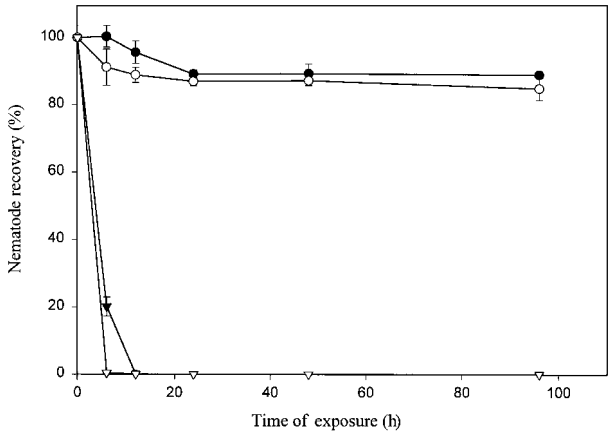

Figure 1. Responses of the citrus nematode *Tylenchulus semipenetrans* to temperature in untreated Arlington sandy loam: (●) at 20°C, (○) at 30°C, (▼) at 40°C, (▽) at 45°C.

eqn (3) can be rearranged as eqn (5) for calculation of *ct*:

$$ct(t) = \frac{-c_0}{Ae^{-E/RT}} e^{-Ae^{-E/RT_t}}\Big|_0^t \qquad (5)$$

3 RESULTS AND DISCUSSION

3.1 Nematode response to temperature in untreated soil

Nematode survival was not significantly affected by temperatures in the range of 20–30 °C in untreated soil (Fig 1). The nematode recovery, after normalization with the recovery at zero time, remained essentially unchanged throughout the incubation period. When the temperature was further increased to 40 °C, however, nematode recovery rapidly decreased, and no nematode was recovered after 12 h of exposure. Since solarization rarely causes temperature increases in deep soil layers (>25 cm) to exceed 35 °C, soil solarization alone may result in poor nematode control in subsoil, as observed in many field studies.[8]

3.2 Nematode response to temperature in fumigated soil

In fumigated soil, nematode population suppression was a result of combined fumigant activity and temperature effect (Figs 2 and 3). For both methyl bromide and 1,3-D, nematode recovery was strongly affected by the initial fumigant rate and soil temperature, as well as by the length of exposure. For instance, at 20 °C and after 24 h of incubation, nematode recovery was 45, 22 and 0% in soil that was treated with methyl bromide at 3.1, 5.6 and 7.3 mg kg^{-1}, respectively (Fig 2a). Under the same conditions, nematode recovery was 52, 27 and 0% in soil treated with 1,3-D at 1.1, 2.2 and 6.4 mg kg^{-1}, respectively (Fig 3a). In the soil that received the highest rate of either methyl bromide or 1,3-D, nematodes were essentially eliminated within the first 24 h at all temperatures. It must be noted that fumigant application rates used in this study were very low compared with field application rates. The application rates used were only 3.9–9% of the field rate for methyl bromide, and 5–30% of those for 1,3-D. Control of nematodes at such low fumigant rates was apparently due to the good containment of fumigants by the closed containers. Fumigation in containers also eliminates invasion and recolonization by nematodes and fungi from infested but sub-lethally treated soil areas that would occur in the field.[15] This was in agreement with Baines *et al*[16] who reported that between 9 and 15 times higher rates of the fumigants were required to eliminate plant parasitic nematodes and pathogenic fungi under field conditions than in containers trials. In practice, containment of fumigants in soil may be improved by the use of a surface cover, especially less permeable films.[17,18] When an impermeable film was used as surface tarp, lower dosages of methyl bromide were shown to be as effective as full dosages for pest control.[17] The use of lower fumigant rates and better

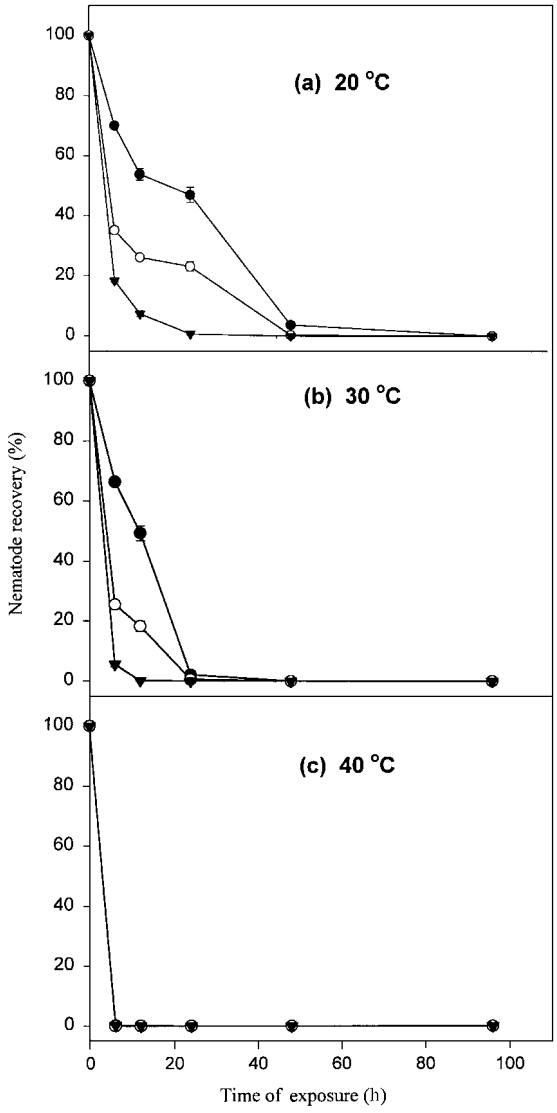

Figure 2. Responses of the citrus nematode *Tylenchulus semipenetrans* to temperature in Arlington sandy loam after treatment with methyl bromide at (●) 3.1 mg kg^{-1}; (○) 5.6 mg kg^{-1} and (▼) 7.3 mg kg^{-1}.

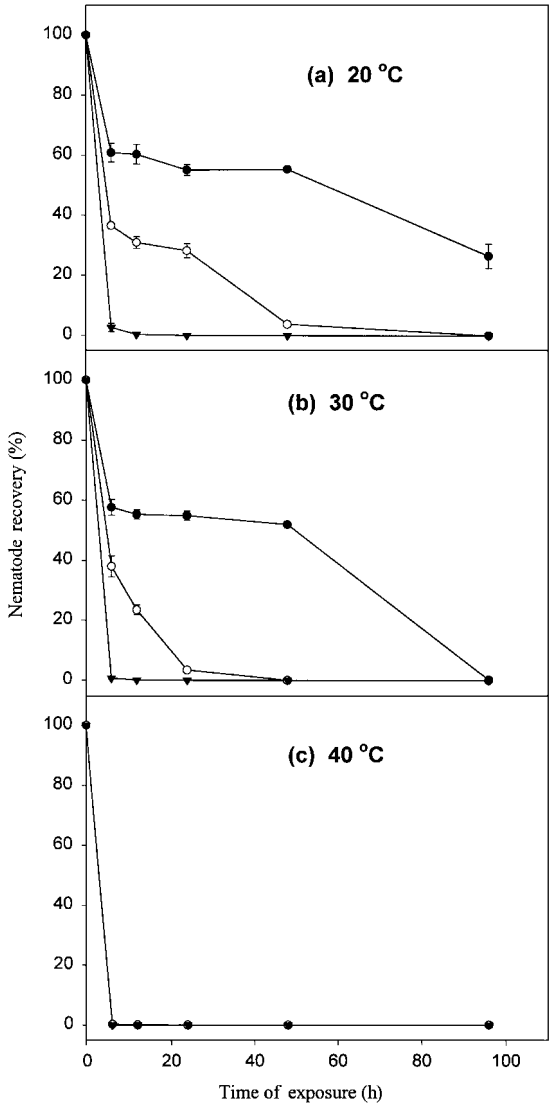

Figure 3. Responses of the citrus nematode *Tylenchulus semipenetrans* to temperature in Arlington sandy loam after treatment with 1,3-dichloropropene at (●) 1.1 mg kg^{-1}, (○) 2.2 mg kg^{-1} and (▼) 6.4 mg kg^{-1}.

containment will result in substantially reduced fumigant emissions.[18,19]

For the same application rate, nematode suppression was closely related to the temperature of incubation. When methyl bromide was used at 3.1 mg kg^{-1}, the time required for nematode elimination decreased sequentially from 96 h at 20 °C, to 48 h at 30 °C and 6 h at 40 °C. At a rate of 5.6 mg kg^{-1}, nematode elimination occurred within 48 h at 20 °C, 24 h at 30 °C and 6 h at 40 °C. A similar trend was also observed for 1,3-D treatments. When soil was treated with 1,3-D at 1.1 mg mg^{-1}, complete nematode elimination was not achieved at 20 °C even at the end of a 96-h incubation period, when approximately 30% of nematodes were recovered. However, complete nematode eradication occurred within 96 h at 30 °C and 6 h at 40 °C. At the rate of 2.2 mg kg^{-1}, nematode elimination was achieved within 96 h at 20 °C, 48 h at 30 °C and 6 h at 40 °C.

Nematode suppression at 40 °C in fumigated soils was similar to that in untreated soil, indicating that temperature alone was sufficient to provide the nematicidal activity. However, nematode responses to temperature were significantly different between 20 °C and 30 °C. In general, much less time was required for the same rate to reduce nematode recovery to zero at 30 °C than at 20 °C (Figs 2 and 3). Likewise, at all fumigant rates, after exposure for the same time, greater suppression of nematodes occurred at 30 °C than at 20 °C, and the effect was especially significant after an initial period of exposure. For instance, at 24 h after treatment, in soil treated with methyl bromide at 3.1 mg kg^{-1}, nematode recovery at 20 °C was 45%, compared with only 2% at 30 °C. At 5.6 mg kg^{-1}, nematode recovery at 20 °C was 22%, but zero at 30 °C. Similarly, in soil treated with 1,3-D at 2.2 mg kg^{-1}, nematode recovery was 27% at 20 °C after 24 h of incubation, while only 3% was detected at 30 °C. Reduced nematode recovery at 30 °C for the same fumigant rate implies that nematodes became more susceptible to fumigant exposure at 30 °C than at 20 °C. This was in contrast with nematode responses in untreated soil, where nematode survival was found to be similar when temperature increased from 20 °C to 30 °C. These results indicate that synergistic reaction occurred between temperature and fumigant at 30 °C, which resulted in a higher fumigant activity at 30 °C than at 20 °C.

3.3 Synergistic interaction between temperature and fumigation

Cumulative exposure of nematodes to fumigants was calculated as concentration-time index (*ct*) in order to describe quantitatively the synergism between temperature and fumigant activity. In this study, analysis of fumigant residual concentration at different times showed that with every 10 °C increase in temperature, fumigant degradation increased by more than twice. Fumigant degradation in soil fitted well to first-order kinetics as in eqn (2). Correlation between nematode survival and *ct* for 20 °C and 30 °C treatments is shown in Fig 4 for methyl bromide and Fig 5 for 1,3-D. Data for the highest rate and temperature (40 °C) are not shown because of the lack of comparison due to immediate nematode elimination. Temperature had a profound effect on the relationship between nematode suppression and *ct*, and similar trends were observed for both fumigants at both rates. For the same initial rate and after the same exposure to the fumigant, greater nematode suppression consistently occurred at 30 °C than at 20 °C (Figs 4 and 5). For instance, in soil treated with methyl bromide at 3.1 mg kg^{-1}, a cumulative exposure for 66 mg kg^{-1} h at 30 °C reduced nematode recovery to 2%, while exposure for 69 mg kg^{-1} h at 20 °C only reduced the recovery to 45% (Fig 4a). In soil treated with 1,3-D at 2.2 mg kg^{-1}, exposure to the fumigant for 45 mg kg^{-1} h at 30 °C resulted in an elimination of nematodes, but exposure for 47 mg kg^{-1} h at 20 °C only decreased nematode recovery to 23% (Fig 5b). Using the lowest

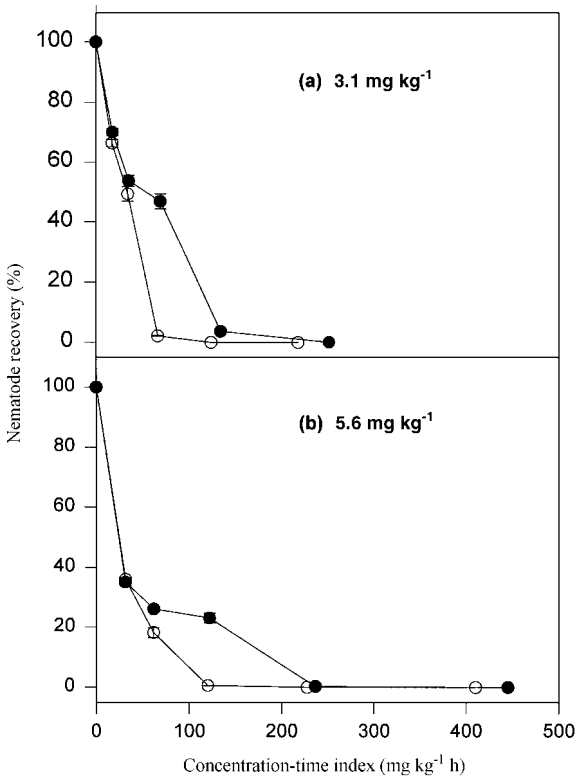

Figure 4. Concentration-time indexes for methyl bromide against the citrus nematode *Tylenchulus semipenetrans* at (●) 20 °C and (○) 30 °C. (a) 3.1 mg kg^{-1}; and (b) 5.6 mg kg^{-1}.

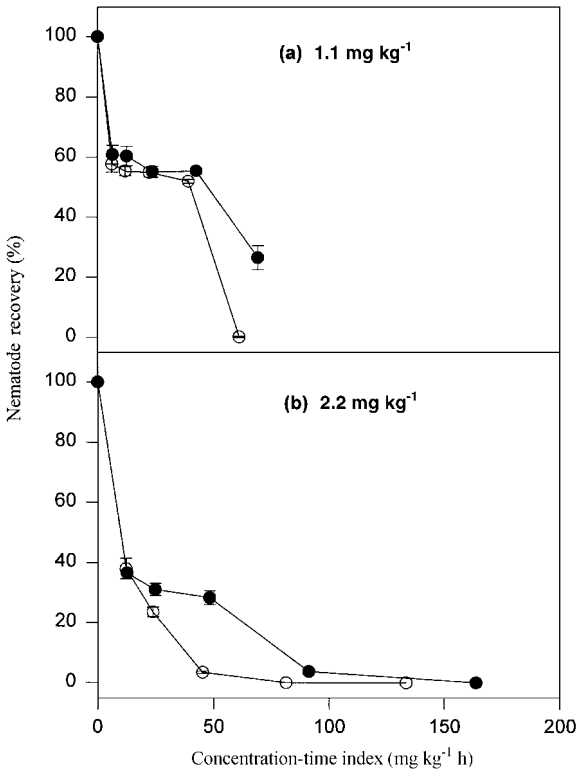

Figure 5. Concentration-time indexes for 1,3-dichloropropene against citrus nematode *Tylenchulus semipenetrans* at (●) 20 °C and (○) 30 °C. (a) 1.1 mg kg^{-1}; and (b) 2.2 mg kg^{-1}.

treatment rate, lethal *ct* values, ie the *ct* at which nematode recovery was reduced to zero, were selected and correlated with temperature (Fig 6). Clearly, as soil temperature increased, lethal *ct* rapidly decreased. At 40 °C, little or no fumigant was needed to kill the nematode, as temperature alone was sufficient to provide the suppression. At 30 °C, the lethal *ct* values were only about 50% or less of that at 20 °C for both methyl bromide and 1,3-D (Fig 6).

The positive correlation of fumigant activity with soil temperature has implications for practical application. For instance, application of fumigants during soil solarization may overcome inadequate pest suppression in deep soil layers, thus improving the overall pest control throughout the soil profile, as observed in several field studies.[9–12] Use of reduced rates of fumigant is possible because temperature in the subsoil layers is elevated, although not to a lethal level, by solarization. Such integration will also reduce the use and hence the environmental input of chemical fumigants, compared with fumigation alone. As pathogens residing near the surface are controlled by soil solarization, the volume of soil that needs fumigant exposure is smaller. Integrated solarization-fumigation may allow solarization to be conducted in regions that have less suitable climate or soil conditions for solarization, or the duration of soil solarization to be shortened. However, the feasibility of such integration should be evaluated with consideration of cost and applicability. In solarized soil, the high soil moisture content will retard, while the high temperature will accelerate, the movement of fumigants.[20,21] In addition, different pest species have different sensitivity to high temperature.[12] Synergism of temperature and fumigants should be further evaluated using other pests and pathogens as well as representative field conditions.

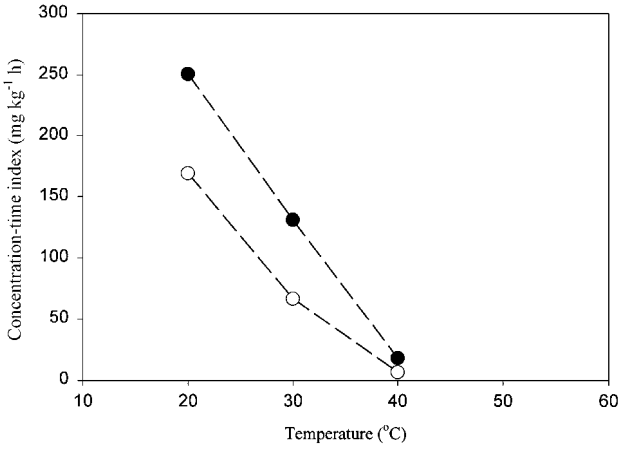

Figure 6. Lethal concentration-time indexes for (●) methyl bromide and (○) 1,3-dichloropropene against *Tylenchulus semipenetrans* as a function of temperature.

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