

## Field Application of Brassicaceous Amendments for Control of Soilborne Pests and Pathogens

**I. A. Zasada**, Former Graduate Research Assistant, and **H. Ferris**, Professor, Department of Nematology, University of California, Davis 95616; **C. L. Elmore**, Professor and **J. A. Roncoroni**, Research Associate, Department of Vegetable Crops, University of California, Davis 95616; **J. D. MacDonald**, Professor, **L. R. Bolkan**, Research Associate, and **L. E. Yakabe**, Graduate Research Assistant, Department of Plant Pathology, University of California, Davis 95616

Corresponding author: Inga Zasada. [zasadai@ba.ars.usda.gov](mailto:zasadai@ba.ars.usda.gov)

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### Abstract

Field studies were conducted to evaluate brassicaceous amendments in combination with solarization against diverse soilborne organisms in cut-flower production systems. Across a diversity of California climates, the application of broccoli (*Brassica oleracea* var. *botrytis*) did not consistently reduce populations of *Fusarium* spp., citrus nematode (*Tylenchulus semipenetrans*), or weeds. Solarization in combination with broccoli amendments did not consistently improve pest suppression in cool coastal regions, whereas solarization was an important component of suppression in the hot central valley. When the biomass of broccoli was increased from 4 to 8.4 dry tons/ha, there was a decrease in survival of weeds and citrus nematode but the effect on *Fusarium* spp. survival was not consistent. A horseradish (*Armoracia lapathifolia*) amendment reduced nematode populations compared to broccoli, but increased field bindweed (*Convolvulus arvensis* L.). Our research demonstrates that soilborne organisms vary greatly in their susceptibility to brassicaceous amendments. The citrus nematode was consistently suppressed by brassicaceous amendments, while the effect on *Fusarium* spp. and weeds was variable. To achieve consistent and reliable pest suppression in amendment-based management systems, it is essential to determine and understand the component mechanisms active against specific soilborne organisms.

### Introduction

The coastal regions of California represent a component of California's highly valued ornamental industry. The ornamental industry has relied heavily upon the use of methyl bromide/chloropicrin as a tool for the control of soilborne organisms and is seriously threatened by the pending loss of methyl bromide. Because of the diversity of the crops represented in this commodity group, the small-sized operations, and the small acreage involved, this industry has received comparatively little attention with respect to methyl bromide alternatives research relative to higher value crops.

Biofumigation refers to the suppression of soilborne organisms by biocidal compounds released into the soil during the decomposition of plant material or animal by-products (1). Plants in the brassicaceae that contain the secondary metabolites glucosinolates have been used in this pest management approach (4). Glucosinolates have low biological activity but their derived products (isothiocyanates) constitute an important group of bioactive molecules and have pesticidal activity. There are many reports of isothiocyanate-producing plant residues having efficacy against weeds (3), nematodes (5) and fungi (10), and a diversity of brassicaceae have been tested for soilborne pest management (9,11).

Growers of ornamental crops along California's coast are challenged in the search for alternatives to methyl bromide. The climate is often cool, with an intruding marine fog layer that renders solarization generally ineffective. Additionally, the small size of fields and their proximity to housing and commercial developments makes impractical the use of pesticidal materials that require large buffer zones. Thus, alternative approaches for the control of soilborne organisms, such as biofumigation, would be highly desirable if efficacious. The objectives of this project were: (i) to evaluate biofumigation in the coastal and central regions of California, (ii) to investigate factors that have potential to influence efficacy of brassicaceous amendments, (iii) to compare broccoli amendments with other brassicaceous amendments for soilborne pest suppression, and (iv) to determine the effect of brassicaceous amendments on the survival of a range of soilborne pests.

### Buried Inoculum Protocol

Five grams of soil containing mixed stages of the citrus nematode (*Tylenchulus semipenetrans*) or chlamydospores of *F. oxysporum* f. sp. *dianthi* were placed in 3.8- $\times$ -7.6-cm, 40-mesh nylon sachets. The sachets were sealed with hot glue and had a length of colored string attached to denote the intended depth of placement in the plot (Fig. 1). When sachets were removed from soil at the end of experiments, those containing nematodes were assayed for survivors within 48 hours of removal. Sachets that originally contained *F. oxysporum* f. sp. *dianthi* were air dried, ground, and then serial dilutions were prepared, spread onto petri dishes, and *Fusarium* spp. survival determined.



Fig. 1. Example of buried inoculum sachets used in all brassicaceous field and microplot experiments.

### Field Trials

Field and pot trials were conducted at three diverse climatic regions in California. In all experiments, soil temperatures were recorded by Onset Stowaway microloggers (Onset Computer Corporation, Pocasset, MA). The soil treatments and methods differed from site to site. All brassicaceous amendments were incorporated as fresh material, but are expressed on a dry weight basis.

Watsonville, CA is near the Pacific coast, but far enough inland to escape the strongest effects of the coastal fogs. Soil beds were prepared and the area sprinkler-irrigated to field capacity. Treatments were replicated four times in a randomized complete block design as follows: broccoli residue at 5.6 tons/ha, tarped; metam sodium (Amvac, Los Angeles, CA) at 240 kg/ha, tarped and untarped; and tarped and untarped controls. Machine-chopped broccoli residue was applied directly to the plot surface at the appropriate rates and hoe-incorporated to a depth of 15 cm. Designated plots were covered with a clear 1.1-mm-thick polyethylene tarp (Leco Industries, St. Laurent, Quebec) and anchored along the edges of the bed with soil. Prior to treatment, sachets of *F. oxysporum* f. sp. *dianthi* were installed to depths of 5, 15, and 30 cm in the center of each plot. Four weeks after treatment, the sachets were retrieved and viable propagules quantified. In addition, weed species and number from within 0.25 m<sup>2</sup> quadrats were determined.



Fig. 2. Field experiment evaluating different rates of broccoli amendments in a calla lily production system, Monterey Bay Academy, CA, 2000.

Field trials were established in a cool, coastal region near the Pacific shoreline at Monterey Bay Academy (MBA), CA. The experimental design and application of amendment material were similar to those described for Watsonville, with the following exceptions. In 1999 the treatments were: broccoli residue at 5.6 tons/ha, tarped and untarped; and metam sodium at 478 kg/ha, tarped. In 2000 the treatments were: broccoli residue at 4 tons/ha, tarped and untarped; broccoli residue at 5.6 and 8.4 tons/ha, tarped; and metam sodium at 478 kg/ha, tarped. Tarped and untarped

controls were included during both years. At the time of treatment, sachets of *F. oxysporum* f. sp. *dianthi* (1999 and 2000) and citrus nematode (2000 only) were placed at depths of 5, 15, and 30 cm. Designated plots were covered with a clear polyethylene tarp (Fig. 2). The treatment duration was 6 weeks, after which the tarps were removed and sachets were recovered immediately to assay for survival. During both years, weed species and number from 0.25 m<sup>2</sup> quadrats were determined.

An experiment was conducted in the hot central valley of California at Davis in 2000 using a system of microplots wherein large plastic pots (75 liter) were set into 46-cm-deep trenches (Fig. 1). After the pots were placed into the trenches, soil was backfilled into the trenches around the pots. Soil was added into the bottoms of the pots to form a layer 30 cm below grade. At this point sachets containing the citrus nematode and *F. oxysporum* f. sp. *dianthi* were laid, and seeds of common purslane (*Portulaca oleracea* L.), redroot pigweed (*Amaranthus retroflexus* L.), annual bluegrass (*Poa annua* L.), little mallow (*Malva parviflora* L.) and field bindweed (*Convolvulus arvensis* L.) were sprinkled on the surface. These were then covered with soil that was added and packed until the level in pots was 15 cm below grade, at which point another series of test organisms was placed. The same procedure was repeated at 5 cm. Broccoli-treated pots received machine-chipped broccoli mixed with soil at pre-determined rates and applied to specific depths. After placement of the test organisms and treatment set up, all pots were irrigated to approximately field capacity. The following treatments were replicated four times in a randomized complete block design: broccoli at 2.6 and 5.2 tons/ha, tarped; broccoli at 3.6 tons/ha, tarped and untarped, metam sodium at 358 kg/ha, tarped; and a tarped and untarped control. Tarped treatments were covered with clear polyethylene. Pots were destructively sampled 6 weeks after treatment. Sachets were removed to assay for nematode and fungal survival. Soil from each depth containing weed seeds was placed in a container and the number of viable seed was determined by germination.



Fig. 3. Placement of broccoli, Brussels sprouts, and horseradish amendments on the soil surface prior to incorporation, Davis, CA, 2001.

A field trial established at Davis, CA in 2001 included: broccoli leaves and stalks at 4.0 tons/ha, tarped and untarped; Brussels sprouts (*B. oleracea* L. var. *gemmifera*) at 5 tons/ha, tarped and untarped; horseradish mature leaves at 4 tons/ha, tarped and untarped; and tarped and untarped controls. Treatments were replicated four times in a split-plot design. The plant material was placed on top of the beds (Fig. 3) and incorporated to approximately 10 cm. After incorporation, sachets of the citrus nematode and *F. oxysporum* f. sp. *dianthi* were inset to various depths as

described above. A clear polyethylene tarp was applied by machine over all plots and the tarp was cut away to expose the soil in plots meant to be untarped.

Tarped plots were sealed on all sides with soil. Treatment duration was 6 weeks, after which the tarp was removed and sachets were retrieved. Weed species number within 0.25 m<sup>2</sup> quadrats were counted immediately after tarp removal.

**Statistical Analysis.** Differences in soil organism survival among amendment treatments were determined by ranked analysis of variance or analysis of variance and means separated by Tukey's adjustment for multiple comparisons. Data were log transformed, when appropriate, to meet the assumptions of normality and homogeneity of variance (SAS, Cary, NC). Percent reduction of test organisms is expressed as a percentage of the untarped control.

### Effect of Brassicaceous Amendment Application Rates on Soilborne Organism Survival

Broccoli residues were tested for effects on target organisms across all experiments using different application rates. Increasing the amount of broccoli had differential effects on organisms. When broccoli incorporation was increased from 4.0 to 8.4 tons/ha at MBA in 2000, citrus nematode reduction increased from 78 to 100% at 15 cm (Fig. 4). Although not significant, there was also a reduction in total weed survival (comprised of redroot pigweed, annual bluegrass, and little mallow) as the rate of broccoli increased. Over this same range of treatments, there was no negative effect on the survival of *Fusarium* spp. (*data not shown*). We could not detect any effect of broccoli residues at 2.6 to 5.2 tons/ha at Davis in 2000.

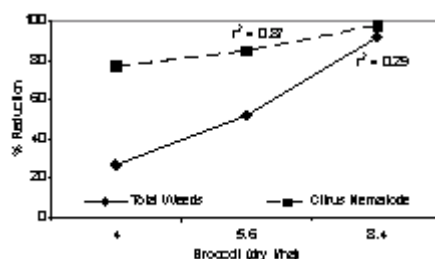


Fig. 4. Effect of increasing rates of tarped broccoli amendments on percent reduction of citrus nematode and total weed populations during 2000, MBA, CA. Percent reduction is expressed as a percentage of the untarped control. Each point is the mean of four replications. Increasing rates of broccoli were significantly different for citrus nematode ( $P < 0.001$ ) but not for total weed populations.

Inconsistent pest and pathogen suppression across broccoli amendment rates may have resulted from inadequate glucosinolate composition of the incorporated material. The toxicity of brassicaceous amendments, and of their associated isothiocyanates, is dependent upon the structure of the compound and the pest species being targeted (3,10,20). Based upon the nature and concentrations of specific isothiocyanates produced, broccoli may not be the best choice for an amendment (12). The glucosinolate profiles of *B. oleraceae* vary greatly with variety (12), age of plant (15), and plant part (14). The broccoli used in these experiments was collected after the harvest of broccoli florets. While this may be the logical time for incorporation from a production perspective, it may not coincide with maximum concentrations of glucosinolates in the plant.

### Effect of Broccoli Amendments on Survival of Target Organisms

Our research demonstrates that soilborne organisms vary in their susceptibility to brassicaceous amendments. The citrus nematode was affected the most by brassicaceous amendments and the numbers recovered from soil were significantly reduced with higher rates of amendments (Fig. 4). However, the citrus nematode is more sensitive to isothiocyanates than other plant-parasitic nematodes (20) and direct application of data from trials with this

nematode to other plant-parasitic nematodes demands caution. The control of *Fusarium* spp. with soil amendments was variable across all sites. In our experiments at coastal locations in California, broccoli amendments did not significantly reduce the number of viable chlamydo spores. Finally, weed control was the most variable across treatments and sites compared to the other organisms.

Others have found that brassicaceous amendments can reduce populations of *V. dahliae* in the same geographic locations (18,19). In California, the incidence of Verticillium wilt was reduced with broccoli at approximately 7 dry tons/ha, and almost complete control of *F. oxysporum* f. sp. *conglutinans* was achieved by the use of solarization plus dried cabbage amendments (13). However, in a different study, a cabbage amendment did not improve the efficacy of solarization in reducing populations of *Phytophthora* sp. (6). Other researchers found that the suppression of fungal pathogens including *Fusarium oxysporum* f. sp. *asparagi*, *Rhizoctonia solani*, and *V. dahliae* was no greater in plots amended with broccoli at 40 wet tons/ha than in plots amended with grass (2). The reported inconsistencies associated with brassicaceous research might be explained by the array of plant sources, physical environments, and target organisms used for these studies.

### Benefits of Combining Brassicaceous Amendments with Tarping

Combination of solarization with other pest management techniques is frequently suggested for marginal environmental conditions or to decrease treatment duration (17).

In most cases, reductions in target organism populations in the tarped versus untarped broccoli amendments were not different in our coastal research sites. This is similar to previous research in a California coastal area (18). There was an exception at Watsonville in 1999 (Fig. 5), where all weeds except common purslane were controlled under tarps when temperatures reached 35°C for 97 hours. The winter annual weed, common groundsel (*Senecio vulgaris*), germinates under cool temperatures and has been effectively controlled with solarization (7); however under the same conditions common purslane, a summer annual, germinated in all tarped treatments except those that included a broccoli amendment. It is likely the combination of tarping and broccoli reduced the germination of common purslane, due to the production and retention of isothiocyanates. The differences between tarped and untarped treatments were significant in experiments conducted at Davis due to an overriding solarization effect in this inland valley location where ambient temperatures and solar radiation were much greater (see "Effect of Tarping Only").

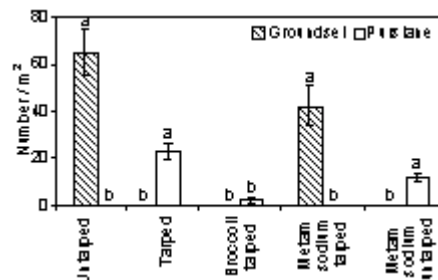


Fig. 5. Effect of amendments, metam sodium, and 4 weeks of tarping on common groundsel and common purslane control immediately after tarp removal during 1999, Watsonville, CA. Each bar is the mean of four replications and the same letter above the bars indicates lack of statistical difference according to Tukey's adjustment for multiple comparison test ( $P < 0.001$ ). Error bars represent standard errors.

## Comparison of a Broccoli Amendment with Other Brassicaceous Amendments

We attempted to apply higher concentrations of specific glucosinolates by using other brassicaceous amendments. When target organisms were exposed to other brassicaceous amendments, differences in efficacy were observed. For the citrus nematode at 30 cm, Brussels sprout and horseradish amendments reduced citrus nematode survival 39 and 59%, respectively, compared to the untarped control, while broccoli was ineffective (Fig. 6). This same effect was not observed at 15 cm. The reason for the inconsistency in citrus nematode suppression between depths is unclear. Broccoli was the only brassicaceous amendment which reduced bindweed populations compared to the untarped control (Fig. 6). Weeds other than bindweed were not affected by the type of brassicaceous amendment. There was a non-significant reduction in *Fusarium* spp. with brassicaceous amendments compared to the uncovered control at 30 cm. There was no difference in *Fusarium* spp. reduction between amendments. Although Brussels sprouts and broccoli have similar glucosinolate profiles, Brussels sprouts contain larger concentrations of sinigrin (12). Horseradish contains mainly sinigrin (8) but at higher concentrations than the other two amendments. This higher concentration of sinigrin in horseradish may have contributed to the increased reduction in citrus nematode in our field studies.



Fig. 6. Effect of brassicaceous amendment on percent reduction of *Fusarium* spp., bindweed and citrus nematode at 30 cm during 2001, Davis, CA. Percent reduction is expressed as a percentage of the untarped control. Each bar is the mean of four replications. Reduction in pest survival between treatments was significantly different for bindweed ( $P < 0.05$ ) and the citrus nematode ( $P < 0.01$ ), but not for *Fusarium* spp. ( $P < 0.10$ ). A star above a bar indicates lack of difference with the untarped control ( $P < 0.10$ ) according to Tukey's adjustment for multiple comparison test.

## Effect of Tarping Only

On the coast of California at MBA, during both years, tarping with clear plastic sheets resulted in moderate increases in maximum soil temperatures; 7 and 6°C at 5 and 30 cm, respectively (Table 1). Further inland, at Watsonville, tarping increased maximum soil temperatures by 12 and 5°C at 5 and 15 cm, respectively. At Davis, in the central valley of California, the maximum soil temperature recorded at 5 cm in tarped, broccoli-amended treatments was 63°C. Tarping the soil increased maximum soil temperatures 12, 10, and 4 degrees at 5, 15, and 30 cm, respectively (Table 1). The addition of broccoli residues to tarped treatments had no significant effect on maximum soil temperatures or cumulative exposure at MBA or Davis.

Table 1. Maximum soil temperatures during biofumigation experiments in California.

Location	Treatment	Soil Depth (cm)		
		5	15	30
		Temperature (°C)		
Watsonville 1999	Untarped	31	27	-
	Tarped <sup>a</sup>	43	32	-
MBA 2000	Untarped	36	-	24
	Tarped <sup>b</sup>	43	34	30
	Tarped + broccoli <sup>bc</sup>	41	36	32
Davis 2000	Untarped	49	38	37
	Tarped <sup>a</sup>	61	48	42
	Tarped + broccoli <sup>bd</sup>	63	47	41

<sup>a</sup> Four weeks solarization.

<sup>b</sup> Six weeks solarization.

<sup>c</sup> Broccoli at 5.6 dry tons/ha.

<sup>d</sup> Broccoli at 3.6 dry tons/ha.

At Davis, in the central valley of California there was an effect of increased soil temperatures on survival of target organisms (*data not shown*). At 5 cm (where soil temperatures were most elevated) all target organisms were reduced > 94%. At 15 cm, *Fusarium* spp. and citrus nematode survival in the tarped control was reduced 87 and 100%, respectively, compared to the untarped control. At 30 cm a maximum temperature of 42°C (Table 1) was not high enough to reduce *Fusarium* spp. survival, although nematode survival was reduced by 21%. Temperatures of 48 and 42°C achieved at 15 and 30 cm, respectively, were not sufficient to reduce weed seed viability.

### Conclusions and Future Research Needs

The amount of biomass left in a field after the harvest of broccoli on the coast of California is approximately 5 dry tons/ha (16). In our experiments, biomass ranged from 2.6 to 8.4 dry tons/ha. At these levels, weed, nematode, and fungal populations were not consistently reduced across or within trials. Based upon the average concentration of sinigrin in broccoli (12), and knowing the citrus nematode specific toxicity of its allyl isothiocyanate glucosinolate-degradation product (20), broccoli at approximately 115 dry tons/ha would be necessary to reduce citrus nematode survival by 90%. One could also predict that horseradish material at 20 dry tons/ha would be required for citrus nematode suppression. Clearly, the amount of plant material needed to achieve toxic levels of isothiocyanates for consistent nematode management would be difficult to obtain. The use of broccoli varieties containing higher concentrations of glucosinolates would reduce the biomass needed to generate toxic concentrations of specific isothiocyanates. However, in these studies we have reported 100 and 60% citrus nematode reduction with broccoli at 8.4 dry tons/ha and horseradish at 4 dry tons/ha, respectively. The production of other glucosinolate-degradation products, in addition to isothiocyanate, or changes in the soil environment after amendment appear to have played a role in citrus nematode suppression.

These studies and others illustrate the considerable complexity of optimizing an amendment-based pest management system. Suppression of soilborne organisms by brassicaceous amendments was variable and inconsistent. *Fusarium* spp. and total weed survival were never consistently reduced regardless of application amount, while citrus nematode populations were reduced with increasing levels of broccoli. Consistent and reliable soilborne pest management with brassicaceous amendments will not be achieved in fields without a better understanding of the biological and chemical components involved. Future research should focus on determining the nature and amounts of glucosinolates in different plant parts and their change with time, the

decomposition and fate of isothiocyanates in soil, quantifying lethal concentration levels for isothiocyanates and other glucosinolate-degradation products to a range of soilborne pests and, ultimately, designing pest management systems for specific target organisms based on an integration of all this information.



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