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Interaction effects of *Allium* spp. residues, concentrations and soil temperature on seed germination of four weedy plant species

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ABSTRACT

Soil in laboratory microcosm experiments was amended with dried and milled crop residues of onion (*Allium cepa* L. cv. Mission) or garlic (*A. sativa* L. cv. California Early). The amendments, along with the additional factors of amendment concentration (0, 1 or 3% (w/w)) and soil temperature (23 or 39 °C), were evaluated with respect to germination of seeds of the weedy annual plants *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass), *Portulaca oleracea* L. (common purslane), *Sisymbrium irio* L. (London rocket) and *Solanum nigrum* L. (black nightshade). Deleterious effects of seed exposure to 39 °C versus 23 °C were demonstrated in ECHCG, POROL, and SSYIR; and to increasing amendment concentration and the [concentration × temperature] interaction in all four plant species tested ($P < 0.05$). The effect of garlic versus onion amendment was significant only in SOLNI, where garlic residues demonstrated more herbistatic and/or herbicidal activity. The results of these experiments showed that the garlic and onion plant residues were capable of causing significant reductions in seed germination during their decomposition in soil, but only at elevated (39 °C versus 23 °C) soil temperature. At the agricultural level, activity of these residues could be exploited as a component of integrated weed management using appropriate crop sequencing, and may extend utility of soil heating treatments (e.g. solarization) for weed control to cooler climates or seasons, or for shorter treatment durations.

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1. Introduction

Plant residues have been used since ancient times as mulches and green manures for subsequently planted crops, in part for their antagonistic effects on soilborne pests. For a period of time beginning in the late 1940s, the pesticidal importance of crop sequencing and residue management was downplayed, due to the ready availability of chemical soil fumigants and disinfestants. Recently, however, restrictions on usage of soil-applied chemicals and interest in sustainable farming practices have spurred renewed interest in incorporating bioactive crop residues into soil to inactivate soilborne pests. Although pest management successes have been reported following soil amendment, especially with cruciferous plants such as cabbage, broccoli, rape, and mustards (Hao et al., 2003), biocidal activity has often been incomplete or unpredictable (Fennimore and Jackson, 2003; Hartz et al., 2005).

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Abbreviations: ECHCG, *Echinochloa crus-galli*; POROL, *Portulaca oleracea*; SSYIR, *Sisymbrium irio*; SOLNI, *Solanum nigrum*.

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Amendments sometimes have been more effective in controlling or weakening populations of certain phytoparasitic nematodes and pathogenic fungi when combined with soil heating methods, particularly solarization (Ramirez-Villapudua and Munnecke, 1988; Gamliel and Stapleton, 1993, 1997; Stapleton and Duncan, 1998; Ploeg and Stapleton, 2001). Solarization by itself has been shown to provide economic weed control in warmer climates (Egley, 1990; Elmore, 1991; Rubin and Benjamin, 1984; Stapleton et al., 2002, 2005), but has limitations which may be improved by combination with organic amendments (Gamliel and Stapleton, 1997). In any case, apart from cruciferous species and cultivars, other plant taxa which can be employed for pest management must be identified and conditions for their optimal effects validated for use in crop sequencing. This is necessary not only to devise management tactics for the broadest range of soilborne pests, but also to provide more economic flexibility for growers as they schedule their crop and/or green manure rotations.

The alliaceous crops onion (*Allium cepa* L.) and garlic (*A. sativum* L.) exhibit multiple bioactive properties and have historically been investigated for both stimulatory and inhibitory effects on a variety of soil microorganisms (Timonin and Thexton, 1950; King and Coley-Smith, 1968; Agrawal, 1978; Bianchi et al., 1997). As with the Brassicaceae, *Allium* spp. produce numerous sulfur-containing chemical products, including volatile compounds arising via cleavage of certain S-alk(en)yl cysteine sulphoxides (Jones et al., 2004) which can act upon a variety of soilborne pests, including fungi, bacteria, and nematodes (Timonin and Thexton, 1950; Nath et al., 1982; Zeidan et al., 1986; Clarkson et al., 2006). The qualitative and quantitative characteristics of the volatile sulfur compounds produced by various members of the Alliaceae differ by cultivar, as well as species (Kallio and Saloninne, 1990; Jones et al., 2004).

The objectives of this study were to employ microcosms to estimate the effects of decomposing onion and garlic crop residues, amendment concentration and soil temperature with respect to seed germination of four weedy, non-native plant species which are important to agriculture in California (CA), USA. The *Allium* residue concentrations used were similar to those expected to remain in the field after harvest, and the experimental soil temperatures were chosen to simulate either ambient conditions (23 °C) or mild soil solarization (39 °C) in summer weather.

2. Materials and methods

The study consisted of two laboratory experiments in soil microcosms. In order to compare results presented here with those of thermal inactivation of the same weed species, the experimental setup and procedures were similar to those previously described (Dahlquist et al., 2007).

2.1. Experimental plant seeds and soil

All weed seeds used in the first experiment (experiment 1) were collected from plants growing wild in the San Joaquin Valley (SJV) near Fresno, CA (Stapleton et al., 2005). The four species used were the summer annuals *Portulaca oleracea* L. (POROL: common purslane), *Solanum nigrum* L. (SOLNI: black nightshade), and *Echinochloa crus-galli* (L.) Beauv.] (ECHCG: barnyardgrass), and the winter annual *Sisymbrium irio* L. (SSYIR: London rocket). Due to short supply, seeds for barnyardgrass, black nightshade, and London rocket were purchased (Valley Seed Service, Fresno, CA, USA) for use in the second, confirmatory experiment. Hanford fine sandy loam–Typic Xerothents soil was collected from a field at the UC Kearney Agricultural Center which had not received an herbicide application for several seasons. Soil was sifted through a 3 mm mesh sieve to remove roots and other surface debris prior to experimental use.

2.2. *Allium* amendments

The *Allium* soil amendments included leaves, bulbs, and roots of garlic (*A. sativum* 'California Early') and onion (*A. cepa* 'Mission') cull plants and residues collected from commercial fields following harvest in the central SJV. The plant matter was air- and oven-dried, then finely ground in a Thomas-Wiley mill (Arthur H. Thomas, Philadelphia, PA) using a 1mm mesh screen to ensure uniform plant tissue disruption and distribution in soil during the controlled experiments. Biotoxic properties of dried cruciferous plant residues were found to be at least equivalent to those of fresh material in previous studies (Ramirez-Villapudua and Munnecke, 1988; Stapleton and Duncan, 1998). The dried and milled amendments were stored in sealed plastic bags at ambient temperature during the experimental period.

2.3. Preparation of seeds for treatment

Ten seeds of each species were placed in 3.5 cm diameter nylon mesh bags and tied tightly to minimize direct seed contact with soil and amendments in experiment 1. Bags of seeds were then dipped in deionized water and placed between moist paper towels to imbibe water for 24 h prior to treatment. The exception was common purslane, which was dipped only 1 h prior to treatment, as it germinates quickly following imbibition. Seed availability was more plentiful for the second, confirmatory experiment (experiment 2), so 20 seeds of each species were used.

2.4. Preparation of microcosms for treatment

Soil was moistened to approximate field capacity (14–15% H_2O) before amendments were added. Microcosms consisted of 200 g of soil with 0, 1, or 3% (w/w) of milled onion or garlic residues. All samples were thoroughly mixed, then placed in 355 ml plastic cups. Four bags of the target seeds, one of each species, were buried in the center of the soil mass in each cup, then the top of the cup was covered with 0.025 mm (=1 mil) thick, clear polyethylene film secured with a rubber band.

2.5. Treatment

In experiment 1, each amendment concentration (0, 1, or 3%) was evaluated in a separate trial. For each trial, 24 microcosms – 8 each of garlic-amended, onion-amended, and non-amended soil – were incubated at 39 °C and another 24

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microcosms were incubated at 23 °C. Microcosms to be heated were placed in a 385 l, stainless steel water bath, with water temperature maintained at 39 °C using a ‘Techne FTE10A’ (Techne Ltd., Duxford, Cambridge, UK) immersion circulator. Non-heated samples were kept in a water bath maintained at ambient temperature of ca. 23 °C. Temperatures of soil and water were monitored with ‘HOBO Model XT’ (Onset Computer Corp., Bourne, MA, USA) data loggers backed up with periodic thermometer readings. In order to improve sampling and handling logistics in the confirmatory second experiment, the microcosms were blocked by amendment rather than concentration.

2.6. *Determining seed germination after treatment*

After periods of 2, 4, and 7 days of incubation, cohorts of amended and non-amended microcosms were removed from the waterbath. Bags containing target seeds were exhumed and separated from soil, opened and seeds were visually checked for germination. Seeds were counted as germinated if the radicle had emerged and the plumule emerged to a length of 3 mm. Germinated seeds were counted and removed. Remaining, non-germinated seeds from each bag were placed in 100 mm × 15 mm Petri dishes on 70 mm diameter disks of Whatman #1 filter paper moistened with 1.4 ml of deionized water. Petri dishes were placed in transparent plastic food storage boxes and incubated in a ‘Revco Model BOD50A14’ incubator (Revco Scientific Inc., Asheville, NC, USA) incubator on a cycle of 8 h at 20 °C and 16 h at 30 °C, and exposed to a fluorescent grow lamp during the 30 °C cycle only. Water was added to Petri dishes as needed during the incubation period to prevent dehydration. Seeds were checked for germination at regular intervals of 3–5 days for a period of 30 days. Germinated seeds were counted and discarded. To estimate viability of non-germinated seeds at the end of the 30-day period, seeds of POROL, ECHCG, and SSYIR were gently squeeze-tested (Taylorson, 1970), while those of SOLNI were subjected to triphenyl tetrazolium chloride (TTC) viability testing (International Seed Testing Association, 1985). The taxa subjected to squeeze testing had seeds that were judged too small to accurately use TTC staining.

2.7. *Experimental design and data analysis*

The two experiments were set up as completely randomized blocks, with three blocks designed to facilitate the intensive sampling and handling procedures, each replicated four times. Treatments consisted of the amendment types, amendment concentrations and incubation temperatures described above, and the response variables were cumulative germination for each species. Data were subjected to analysis of variance using the general linear model (GLM) procedure of SAS software (SAS Institute, Cary, NC, USA). Assumptions for use of GLM were tested using univariate tests for normality and homogeneity of variance. Separation of means was accomplished within the GLM procedure using a Tukey’s Honestly Significant Difference (HSD) test. In cases where there were no significant amendment or experiment effects, data were pooled accordingly.

3. Results

3.1. *General effects of treatment components*

Experimental waterbaths maintained target temperatures within 1 °C during experiments. Seed germination responses to treatments were inconsistent after the 2- and 4-day exposure periods, but variability decreased after 7 days of treatment. Apart from germination data, non-germinated seeds of all species were tested for viability at the end of the 30-day incubation period. There was greater inherent variability when classifying a seed as viable using the squeeze test, and so the response variable used was germination. Most non-germinated seeds were clearly non-viable when assessed via the squeeze technique, and only a small percentage was considered potentially viable: 0–8% of ECHCG, 1–3% of POROL, and 4–11% of SISYR. Apparent viability of non-germinated SOLNI seeds was 10–23%, according to TTC assay.

The weedy plant species used in the experiments responded consistently with respect to decreased germination at the higher temperature and with increasing amendment concentration (Figs. 1–4). For three of the four species tested, no significant differences in germination were apparent between seeds buried in onion-amended versus garlic-amended soil. Differences between the two amendments were found only for SOLNI ($P < 0.0104$), with constituents from garlic residues more deleterious to seed germination than from onion (Fig. 2a and b).

A number of interactions among the tested treatment components of temperature, amendment and concentration gave significant differences in seed germination over both experiments. The relationship of [higher temperature × increasing amendment concentration] had the most consistently deleterious effects on seed germination, resulting in

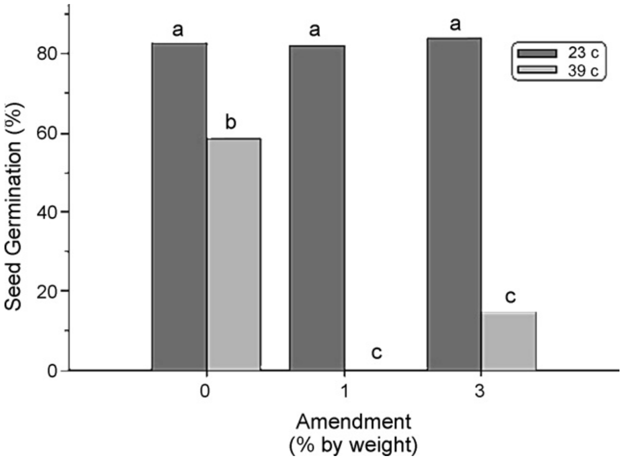

Fig. 1 – Interaction effects of temperature and *Allium* spp. soil amendment concentration on the 30-day, cumulative germination of *Echinochloa crus-galli* seeds following exposure to treatments for 7 days. As no significant differences were found between *A. cepa* and *A. sativum* amendments, or between two experiments, those data were pooled appropriately. Different letters over columns indicate differences according to Tukey’s HSD test ($P < 0.05$).

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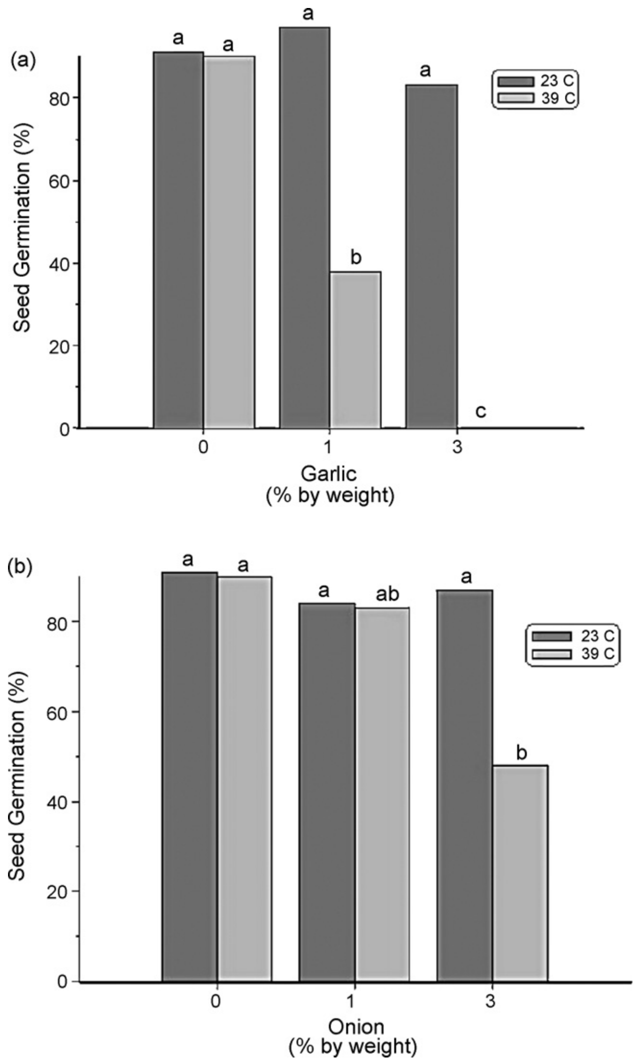

Fig. 2 – Interaction effects of temperature and (a) onion (*Allium cepa*) or (b) garlic (*A. sativum*) soil amendment concentration on the 30-day, cumulative germination of *Solanum nigrum* seeds following exposure to treatments for 7 days. As no significant differences were found between two experiments, those data were pooled accordingly. Different letters over columns indicate differences according to Tukey's HSD test ($P < 0.05$).

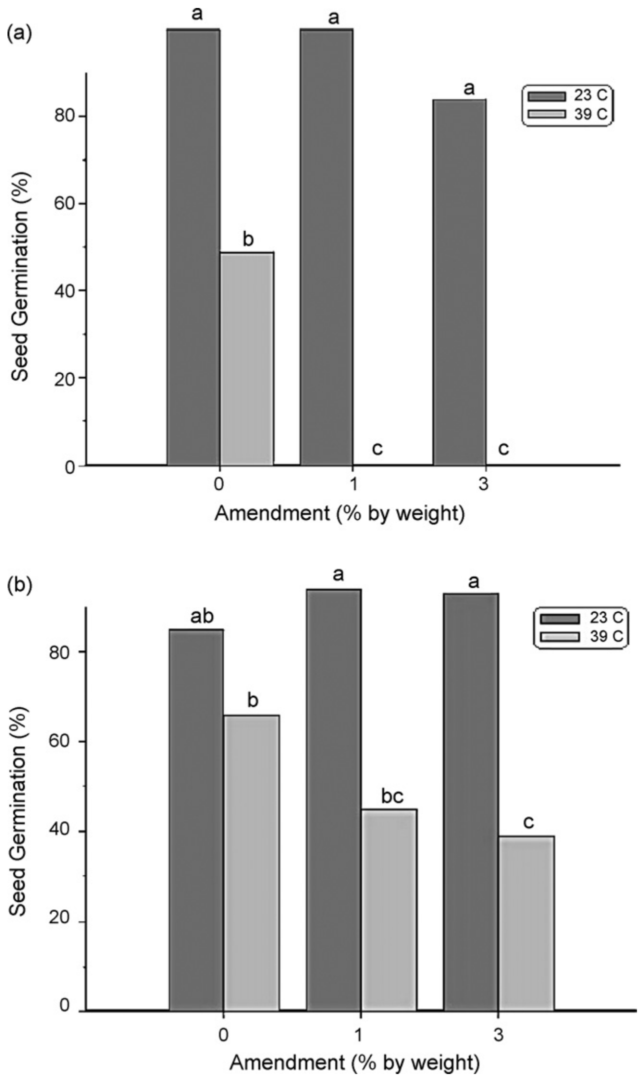

Fig. 3 – Interaction effects of temperature and *Allium* spp. soil amendment concentration on the 30-day, cumulative germination of *Sisymbrium irio* seeds in (a) experiment 1 and (b) experiment 2 following exposure to treatments for 7 days. As no significant difference was found between *A. cepa* and *A. sativum* amendments, those data were pooled accordingly. Different letters over columns indicate differences according to Tukey's HSD test ($P < 0.05$).

significant reductions in seed viability of all species, except POROL, in both experiments. On the other hand, the interactions of [amendment × rate] and [temperature × amendment] were inconsistent in affecting seed germination.

3.2. *Effects of treatment factors on individual weedy plant species*

ECHCG (*E. crus-galli*). The interaction of [temperature (23 or 39 °C) × amendment concentration (0, 1, or 3%)] was highly significant ($F = 16.45$; $P < 0.0001$) in reducing seed germination of ECHCG in both experiments (Fig. 1), but no significant difference was detected between exposure to onion versus garlic amendment. Data pooled from the two experiments showed that, after 7 days incubation at 23 °C, there was no significant, deleterious effect of either onion or garlic amendment on seed germination, regardless of residue concentration. However, when incubated at 39 °C without amendments, there was a 29% decrease in germination; and at 39 °C with amendments, viability decreased by 82–100% over the non-amended, non-heated control (Fig. 1).

SOLNI (*S. nigrum*). None of the main effects alone were considered for seeds of SOLNI, as all treatment components were involved in statistically significant interactions. Interactions of [temperature (23 or 39 °C) × rate (0, 1, or 3%)] and [temperature × amendment (garlic, onion, non-amended)] were significant in both experiments. Unlike the other weedy plant species tested, differences between onion and garlic

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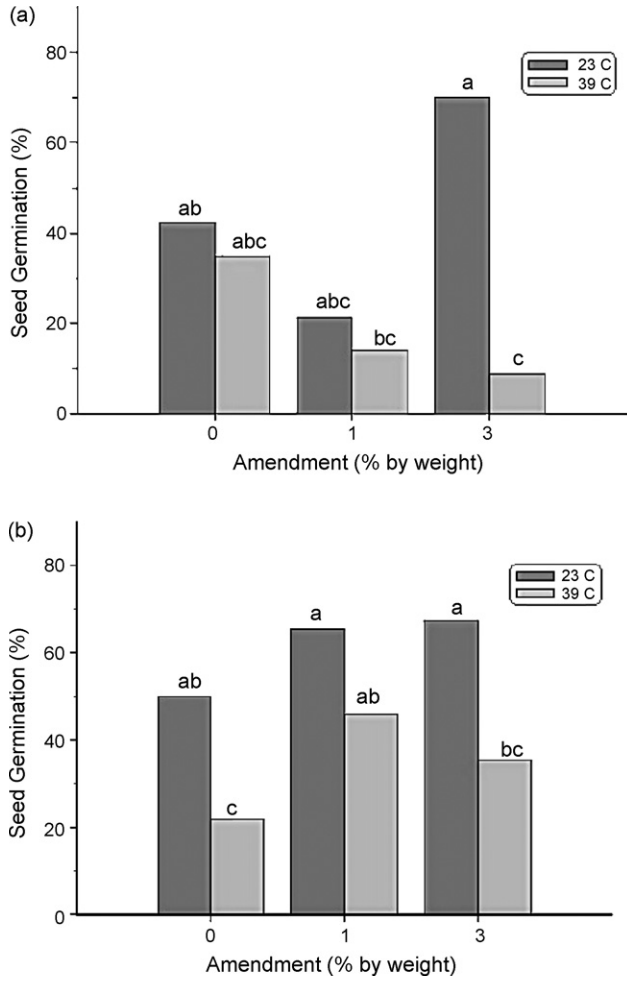

Fig. 4 – Interaction effects of temperature and *Allium* spp. soil amendment concentration on the 30-day, cumulative germination of *Portulaca oleracea* seeds in (a) experiment 1 and (b) experiment 2 following exposure to treatments for 7 days. As no significant difference was found between *A. cepa* and *A. sativum* amendments, those data were pooled accordingly. Different letters over columns indicate differences according to Tukey's HSD test ($P < 0.05$).

amendment were found in SOLNI (Fig. 2; $F = 6.75$; $P < 0.0105$), with garlic amendment more deleterious to seed germination than onion. However, this effect was detected only at 39 °C and not at 23 °C (Fig. 2a and b). Seeds of SOLNI were not affected by either amendment at 23 °C, but seed germination declined by at least 75% of the non-amended, 23 °C treatments when the amended microcosms were incubated at 39 °C. In both experiments, 3% garlic-amended soil at 39 °C significantly reduced seed germination to a greater extent than onion-amended soil by completely inhibiting seed germination (Fig. 2a and b).

SSYIR (*S. irio*). A significant experiment effect was found for SSYIR ($F = 5.83$; $P < 0.018$), but it was due to the magnitude of the treatment effects, rather than to inconsistency of response (Fig. 3a and b). The interaction of [temperature (23 or 39 °C) × amendment concentration (0, 1, or 3%)] was significant in both experiments. Similarly to SOLNI, seeds of SSYIR were not affected by either amendment at 23 °C, but seed germination declined significantly when exposed to either amendment at 3% concentration at 39 °C in each of the two experiments (Fig. 3a and b).

POROL (*P. oleracea*). Data for POROL were analyzed by individual experiment, since there were different responses for each (Fig. 4). Of the four weed species tested, seeds of POROL were the most tolerant of treatment factors. Soil amended with either onion or garlic and incubated at 23 °C had little effect on POROL seed germination in either experiment (Fig. 4a and b). When incubated at 39 °C in experiment 1, a similar response to the other species occurred, where seed germination declined with increasing amendment concentration (Fig. 4a). At 3% concentration, seed germination was reduced by 79%, as compared to the non-amended, 23 °C treatment. In experiment 2 at 39 °C, however, a significant response did not occur (Fig. 4b). In experiment 1, the temperature effect was insignificant, but the [temperature × rate] interaction was significant; while in experiment 2, the opposite relationship was found.

4. Discussion

Under the controlled and optimized conditions of this microcosm study, the inhibitory influence of finely milled and re-hydrated garlic or onion residues on germination of seeds of ECHCG, SOLNI, POROL, and SSYIR, when incubated for 7 days at ambient soil temperature (23 °C), was clearly shown to be modest or insignificant. On the other hand, similar incubation of seeds at the mildly elevated soil temperature of 39 °C produced consistently deleterious effects. In certain field studies, combinations of solarization with organic residues have given results no better than solarization alone, indicating that soil heating at 40–70 °C produced a comparatively more drastic effect on targeted pests than organic amendments (Stapleton, 1998). The 39 °C treatment employed in the present study simulated a mild solarization treatment under field conditions, with the plastic covering the microcosms retaining moisture and heat, as well as volatile compounds (JJS, unpublished) generated by the onion and garlic residues.

In this study, the treatment interactions of [(higher) temperature × (increasing amendment) rate] and [(higher) temperature × (either) amendment] were significant and more deleterious to seed germination of the summer annuals ECHCG and SOLNI, and the winter annual, SSYIR, than to the summer annual POROL (Figs. 1–4). It is well known that POROL often exhibits tolerance to solarization under field conditions (Elmore, 1991; Verdu and Mas, 2004). The seed germination responses of all these species during treatments in the field would be dependent on a far greater complexity of interacting factors, including genetic variation, age of seeds, depth of seeds in soil, concentration of organic amendment, soil moisture and temperature, length of exposure, presence and activity of antagonistic and/or stimulatory microorganisms, and others. Also, it should be emphasized that the extent of amendment tissue disruption and homogeneous distribution throughout the soil profile in the field would not be as optimal as provided for in these microcosm studies.

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In an isothermal inactivation study conducted in microcosms containing silica sand rather than field soil (Dahlquist et al., 2007), 39 °C was found to be sublethal to seeds of ECHCG and POROL, and marginally lethal to SOLNI seeds. However, using similar experimental methodology in field soil-filled microcosms, the *Allium* spp. amendments used in this study usually provided significantly deleterious effects on weed seeds when incubated at 39 °C, even at a concentration of 1% (w/w). Several seeds of all species tested and in all treatments were found to be colonized by fungi upon removal from the soil microcosms. In previous studies, *Allium* spp. amendments have stimulated growth of certain bacterial and fungal groups in rhizosphere soil (Timonin and Thexton, 1950) and have triggered germination of the phytopathogenic fungus *Sclerotium cepivorum* (Coley-Smith and King (1969). On the other hand, the same authors and others (Tansey and Appleton, 1975; Agrawal, 1978; Singh and Singh, 1980; Nath et al., 1982; Zeidan et al., 1986; Bianchi et al., 1997; Clarkson et al., 2006) found that the onion and/or garlic extracts or amendments were inhibitory to many soil and root organisms.

As a laboratory feasibility study, the experimental results reported herein suggested limited potential for the residues of onion and garlic crops to influence weed seed germination at ambient soil temperature. However, when combined with even mild soil heating at 39 °C, the incorporation of the *Allium* crop residues contributed to a consistent, inhibitory effect on germination of the weed seeds tested. If components of the seed banks in agricultural or horticultural soils can be prevented from germinating for even a single season, weeds will be less competitive with desired plants, and fewer mature weeds will be present to contribute progeny toward maintaining or increasing the seed bank population. However, as exemplified by POROL in this study, considerable variability among responses of the various species in the weed community will be expected, especially under marginal conditions for soil heating produced by weather, climate, soil depth, and other considerations. Under suitable conditions for solarization, either in the field or nursery, soil temperatures may exceed 70 °C near the soil surface (Stapleton et al., 2002, 2005) and 39 °C would be found at levels deep enough in the soil so as to preclude successful seedling emergence of many weedy plant species. As solarization is limited by climatic and local weather conditions and by declining temperatures with increasing soil depth, the incorporation of crop amendments such as onion and garlic prior to solarization may enhance its effectiveness by reaching seeds at greater depths, requiring less time to achieve desired results, and/or providing weed control at lower temperatures. In addition, the integrated use of *Allium* amendments with solarization has the potential to extend economic weed management into geographic areas of marginal suitability and/or shorten the treatment period required without the need for supplemental herbicide application or cultivation.

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