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# Reduction in weed seedling emergence by pathogens following the incorporation of green crop residue

C L MOHLER*, C DYKEMAN*, E B NELSON† & A DITOMMASO*
*Department of Crop and Soil Sciences, Cornell University, Ithaca, NY, USA, and †Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY, USA

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## Summary

Because tillage promotes the germination of many weed species and freshly killed plant material favours the growth of microbial pathogens, we hypothesised that the incorporation of green crop residue should temporarily reduce weed seedling emergence relative to unamended soil. Soil with field-incorporated green crop residue was compared with non-amended soil in glasshouse experiments by sowing several weed species at different times after incorporation. Species included *Abutilon theophrasti*, *Chenopodium album*, *Amaranthus powellii*, *Setaria faberi*, *Echinochloa crus-galli* and, in one year, lettuce and red clover. Soils with green crop residue reduced seedling emergence for 0–4 days after incorporation by an average of 30%. Comparison of emergence in non-sterilised soil with that in sterilised soil, with and without fresh crop residue, indicated that a biological agent caused the depressed emergence. In the third year of the study, the fungi *Fusarium oxysporum* and *F. chlamydosporum* were isolated from seeds exposed to soil amended with green crop residues, and their pathogenicity to seeds and seedlings was confirmed in bioassays. This study indicated that incorporation of fresh crop residue reduces the first flush of weed seedlings following tillage and that this depression in emergence is probably caused by pathogen attack on seeds and seedlings before emergence.

**Keywords:** tillage, seedbank, population dynamics, *Abutilon theophrasti*, *Chenopodium album*, *Setaria faberi*, *Echinochloa crus-galli*, lettuce, red clover, *Fusarium* spp.

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## Introduction

Although the biology of weed seed germination is well studied, the magnitude of weed seedling emergence in an agricultural field is difficult to predict. Weed seedling emergence is affected by multiple factors, including tillage, soil microenvironment, deleterious soil microorganisms and crop residues at the soil surface (Forcella, 1992; Mohler, 1993; Mohler & Teasdale, 1993; Kremer & Li, 2003). Mechanical disturbance of the soil increases seed germination by several mechanisms. Tillage moves previously buried seeds to near the soil surface where germination may be stimulated (Mohler, 1993). By incorporating vegetation and plant residue, tillage allows more light to reach seeds, increases the red: far red ratio of light reaching the soil, raises soil temperatures and increases diurnal soil temperature fluctuations (Cox *et al.*, 1990; Benech-Arnold *et al.*, 2000). All of these stimulate germination of many weed species (Egley, 1986). Tillage also stimulates weed seed germination by venting volatile germination inhibitors (Holm, 1972) and by speeding decay of organic matter, with

*Correspondence*: C L Mohler, Department of Crop and Soil Sciences, 907 Bradfield Hall, Cornell University, Ithaca, NY 14853, USA.
Tel: (+1) 607 255 0199; Fax: (+1) 607 255 2644; E-mail: clm11@cornell.edu

consequent production of nitrate in the absence of plants to remove it from the soil (Pons, 1989; Dou *et al.*, 1995). Although tillage generally promotes germination of weed seeds, the effects of tillage on weed emergence are variable, because of differences among sites in the vertical distribution of seeds in the soil (Mohler, 1993) and soil physical effects on seedling emergence (Mohler & Galford, 1997). Biological processes interact with tillage to determine whether seedlings emerge successfully or die in the soil after germination (Davis & Renner, 2007). Green manure incorporated during tillage can inhibit weed seedling emergence (Dyck & Liebman, 1994; Kruidhof *et al.*, 2011). Suppression of seedling emergence by incorporated green manure has been attributed to three potential mechanisms: (i) the release of allelopathic chemicals, (ii) reduced nitrate because of nitrogen tie-up by soil microbes and (iii) the promotion of seed and seedling pathogens (Wall, 1984; Weston, 1996; Conklin *et al.*, 2002; Kumar *et al.*, 2008). Generally, the work on suppression of weed seedling emergence by crop residues has emphasised allelopathic effects.

Infections caused by fungal and oomycete pathogens, such as *Pythium*, *Fusarium* and *Rhizoctonia* spp., are enhanced or suppressed by crop residue incorporation, depending on the degree of decomposition. Fresh green residues that are cellulose-rich provide a food source for many pathogens (Chung *et al.*, 1988; Manici *et al.*, 2004), whereas more mature composts can provide an environment conducive for growth of beneficial microbes that outcompete or are antagonistic to pathogens (Chung *et al.*, 1988; Noble & Coventry, 2005). Previous studies on the impacts of crop residues on pathogenicity have focused on decomposition during composting; only Conklin *et al.* (2002) have previously investigated how attack by pathogens on a weed species changes through time after incorporation of organic materials.

Tillage thus stimulates weed seed germination, while incorporation of green crop residue by tillage supports a short-term burst of pathogen activity. Consequently, newly germinating weed seeds should experience an increased frequency of pathogen attack when green crop residues are incorporated by the tillage event. Thus, our work presented here was intended to test the following hypotheses: (1) fewer weed seedlings should emerge from soil with incorporated green crop residue than from soil experiencing the same tillage but without the incorporation of green crop residue, (2) this depression in emergence will be ephemeral, with the difference between amended and non-amended soil declining as the residue decomposes and (3) this depression in emergence will be associated with pathogen activity in soil with recently incorporated green residue.

## Materials and methods

### *2005 experiment*

Soil was collected from two adjacent locations in the Cornell Organic Vegetable Cropping System Experiment near Freeville, NY, USA (42°30′N, 76°20′W) (See Chan *et al.*, 2011 or http://www.hort.cornell.edu/extension/organic/ocs/ for a description of that experiment). The soil was a well-drained gravelly loam (loamy-skeletal, mixed, mesic, Glossorboric Hapludalfs). Alley soil corresponded to an area that was kept free of living plants prior to tillage. Plot soil was from an immediately adjacent area in which 2600 kg $ha^{-1}$ dry weight of crop and weed material was tilled into the soil (Table 1). Composted dairy manure solids (2.1% N) were spread on alleys and plots at a rate of 5100 kg $ha^{-1}$ dry weight prior to tillage. Peas (*Pisum sativum* L.) in the green pod stage and weeds, primarily *Amaranthus powellii* S. Wats., were incorporated with a rotary tiller on 15 July 2005. This timing of incorporation corresponds to cover crop incorporation prior to planting vegetables for fall harvest in New York State, and cabbage was transplanted into the plot areas in this and subsequent experiments. Sampling began on the day of tillage when the green matter was incorporated into the soil (i.e. day 0) and continued on days 1, 2, 4, 8, 16 and 33 after incorporation. Samples consisted of the loose surface soil to a depth of about 7 cm and were collected with a shovel.

In this and subsequent experiments, soil was spread in seed trays (flats) and seeded with various annual weeds as described later and then placed in a glasshouse for observation of seedling emergence. Emergence was evaluated in a glasshouse rather than in the field, to maintain more constant environmental conditions, particularly soil moisture, over the observation periods following various soil collection days. Soil was first sieved through a 1.3-cm screen to remove stones. For the plot samples, coarse organic matter that had been screened out was cut into 0.5- to 1-cm pieces and mixed back into the soil. Eight litres of field soil was measured in a graduated cylinder and mixed with 4 L of peat/vermiculite mix without added nutrients. The peat/vermiculite was added to maintain soil structure under glasshouse conditions. Eight 10 × 10 cm (inside dimensions) plastic cell containers were placed in a 28 by 56 cm tray and 500 mL of the soil mixture was placed in each cell and levelled to create a depth of about 4 cm. Four cells were filled with the alley soil mix, and four cells were filled with plot soil mix in each tray. Four replications for each treatment were created for each day of soil collection. Tray positions were re-randomised with the addition of each new soil collection. This

**Table 1** Biomass and C: N ratios for green crop residues incorporated in 2005, 2006 and 2009

| Year | Pea (kg ha$^{-1}$) | Oat (kg ha$^{-1}$) | Weeds (kg ha$^{-1}$) | Total (kg ha$^{-1}$) | C:N* |
|---|---|---|---|---|---|
| 2005 | 1000 | 0 | 1600† | 2600 | 11–14 |
| 2006 | 1300 | 0 | 300‡ | 1600 | 12–15 |
| 2009 | 2600 | 2700 | *c.* 0 | 5300 | 19 |

*Carbon:nitrogen (C:N) ratios were computed based on nitrogen analyses of pea in each year and oat in 2009 and on values for percentage N content of the major weed species in the field as found in Vengris *et al.* (1953) and Alkämper (1976). Carbon content of all materials was assumed to be 41% (LE Drinkwater pers. comm.). The ranges shown for 2005 and 2006 resulted from using the highest and lowest weed N percentages listed in the literature sources.
†Mostly *Amaranthus powellii* with some *Chenopodium album* and other weeds.
‡Mostly *Amaranthus powellii* and *Stellaria media* (L.) Vill.

created a randomised split-plot design, with soil type as the subplot factor.

For each soil source (alley vs. plot), 100 seeds of each of five weed species were sown over the four cells of a half tray. The four cells were subsequently treated as a single unit. The five weed species used were *Abutilon theophrasti* Medicus (velvetleaf), *Chenopodium album* L. (fat-hen, common lambsquarters), *Amaranthus powellii* (Powell amaranth), *Echinochloa crus-galli* (L.) Beauv. (barnyard grass) and *Setaria faberi* Herrm. (giant foxtail). Seeds for this and subsequent experiments were collected from local populations and stored at 4°C in glass jars until used. After sowing, each half tray was evenly covered with a 1 cm layer of the soil mix. Trays were watered daily with a mister. Seedlings were counted and removed as they emerged for 2 weeks after sowing. A few seedlings emerged after this time, but the study was focused on the flush of emergence prompted by tillage, so these late-emerging seedlings were ignored.

Soil samples were collected for seedbank analysis in April prior to the experiment (12 cores of 7 cm diameter by 18 cm deep per plot). Composite samples for each plot were thoroughly mixed, weighed and a 2.5 kg subsample taken. Samples were dried at 40°C and stored at 4°C until the following winter, when the seeds were extracted by elutriation (Gross & Renner, 1989) and counted. All species used in the experiment were absent or had negligible abundance, except for *A. powellii*. For this species, the seeds tested were those added plus those native in the soil.

### *2006 experiment*

The methods of soil collection in 2006 were similar to those of 2005, except that plot and adjacent alley soil was collected from four replications of one treatment of the cropping systems experiment. Plot areas only were spread with composted dairy manure solids (2.03% N) at a rate of 4100 kg ha$^{-1}$ (DW), and peas and weeds (Table 1) were rotary tilled to incorporate the green biomass on 19 July 2006. Alley areas had again been kept free of plants prior to tillage. Soil was collected from each plot and adjacent alley area on days 0, 1, 2, 4, 8 and 16 after tillage. The identity of replicates from the field was retained through subsequent soil processing and experimentation.

The soil was processed as in 2005. Soil samples from both plot and alley areas were divided in half, and one-half of each was autoclaved at 120°C for 1 h in autoclave bags to kill any microorganisms present. Autoclaving had minimal effect on soil structure. The other half was brought to the glasshouse and used directly. Treatments for 2006 thus consisted of sterilised plot soil, unsterilised plot soil, sterilised alley soil and unsterilised alley soil. Two L of soil was spread in 25 × 25 cm trays, one per treatment unit, and 100 seeds each of *A. theophrasti*, *C. album*, *A. powellii*, *E. crus-galli* and *S. faberi* were sown in each tray. Seeds were covered with 1 cm of the soil mixture, and the trays were wet by capillary action. Thereafter, trays were watered daily with a mister. Trays were arranged in a fully randomised design with trays re-randomised with each additional collection. Seedlings were counted and removed as they emerged for 2 weeks after sowing. The glasshouse was maintained near 29/21°C day/night temperature. This temperature regimen is typical of temperatures in late July to early August in Ithaca, NY, USA. The glasshouse provided more consistent temperatures over collection dates than would have occurred if emergence observations had been made in the field.

Seedbank samples were collected and analysed as for 2005. All weed species used in the study were absent or had negligible abundance except *A. powellii*. This species was subsequently dropped from the analysis, because the soil sterilisation would have killed the seeds already in the soil and thereby confounded the results.

### *2009 experiment, set-up and seedling emergence*

Soil sampling in 2009 was similar to that in 2006. Alleys were kept free of plants as in previous years. A cover crop of peas and oats (*Avena sativa* L.) (Table 1) was flail mowed, plots were spread with composted chicken manure (5.73% N) at a rate of 720 kg ha$^{-1}$ (DW) and

both plots and adjacent alleys were rotary tilled on 13 July 2009. As in 2006, samples were collected from four replicate pairs of plot and alley locations. Samples were collected on days 0, 1, 2, 4, 8 and 16 after tillage. Soil was processed as in 2005 and 2006, divided into equal subsamples, one of which was autoclaved at 120°C for 1.5 h and one of which was not.

Each type of soil was spread in 28 × 56 cm trays at a rate of 4540 mL tray$^{-1}$ to create a depth of about 3 cm of soil. Seeds of red clover (*Trifolium pratense* L.), lettuce (*Lactuca sativa* L.), *A. theophrasti* and *C. album* were sown over the entirety of each tray. *Echinochloa crus-galli* and *S. faberi* were each sown in only half of each tray to ease seedling identification later. The number of seeds sown for each species was determined from preliminary Petri dish germination tests (data not shown), with the number set to produce *c.* 100 seedlings per tray. The inclusion of lettuce and red clover was to assess whether depressed emergence would be observed in crop species as well as weeds. *Amaranthus powellii* was left out of the experiment, because of high background levels of this species in the soil seedbank. After sowing, trays were covered with 1 cm of the soil mixture and wet by capillary action.

Trays were arranged in the glasshouse in four blocks, with replicates corresponding to those in the cropping systems experiment from which the soil was collected. The four soil treatments were arranged randomly, and spaces left for insertion of later collection dates. Seedlings were counted and removed for 2 weeks after sowing. The glasshouse was maintained near 29/21°C day/night temperature, which is typical of late July to early August temperatures in Ithaca, NY, USA.

Seedbank samples were collected and analysed as for 2005 and 2006. All species used in the experiment were absent or had negligible abundance except *C. album*. This species was subsequently dropped from the analysis because the soil sterilisation would have killed the seeds already present in the soil and thereby confounded the results.

### *Analysis of seed pathogens in the 2009 experiment*

Twenty seeds of each species were separately placed in 2.5 × 5 cm nylon mesh packets to facilitate the subsequent recovery of seeds from soil to assess pathogen infection. Packets were placed at 1 cm depth in each of the trays containing unsterilised plot soil collected on day 2. Packets were retrieved from the soil after 5 days and brought to the laboratory for analysis. The seeds essentially acted as bait to attract soil pathogens that attack the species under investigation.

Seeds were removed from the packets, and two subsamples of five seeds each were placed on water agar plates amended with rifampicin and penicillin (WARP) (Nelson & Hsu, 1994). The WARP plates were sealed with Parafilm and incubated at 18°C for 4 days to allow any fungi and oomycetes colonising the seeds to grow out onto the agar. Mycelia growing from seeds were hyphal-tipped and transferred to V8 medium (200 mL Campbell's V8 juice + 3 g $CaCO_3$ + 8.5 g Bacto Agar + 800 mL $dH_2O$) and incubated at 31°C. Seeds of some species were colonised by several morphologically distinguishable fungi. All fungi or oomycetes emerging from seeds were tentatively identified, based on gross morphology. Four isolates from *C. album*, three isolates each from *E. crus-galli* and *A. theophrasti*, two isolates from lettuce and one isolate each from *S. faberi* and red clover were evaluated for pathogenicity in preliminary bioassays.

For these preliminary bioassays, three V8 plugs of one fungal isolate and 50 seeds of the species from which the isolate was originally obtained were placed together in plates containing 15 mL of 0.25–0.50 mm autoclaved sand and covered with an additional 15 mL of sand. Control plates had seeds but no plugs. Plates were watered with sterile water until thoroughly moistened. Plates were placed in a growth chamber set to 12 h days at 30°C and 12 h nights at 10°C and incubated for 7 days, at which time plates were removed and seedlings were assessed for disease. The percentage of seedlings emerged was calculated, and any additional symptomology, such as seed rotting, radicle discoloration and seedling damping-off, were recorded. In most cases where isolates were pathogenic, seeds were rotted prior to emergence. From observations on these plates, four isolates were determined to be potentially pathogenic (data not shown). To identify these isolates to species, they were then cultured on V8 agar in 90-mm Petri dishes on top of a layer of cellophane for subsequent DNA extraction and internal transcribed spacer (ITS) sequencing. Cultures were scraped off of the surface of the cellophane, lyophilised overnight and then kept at −20°C under argon in 2.2-mL microcentrifuge tubes. DNA was extracted from 0.5 g mycelium with the Qiagen DNeasy Plant Mini kit (Qiagen Scientific, Inc., Germantown, MD, USA) with one modification. Mycelium was ground in its dry lyophilised form, so the addition of liquid nitrogen was not necessary. All subsequent steps were conducted according to the manufacturers' instructions.

PCRs for DNA extracted from live cultures were carried out using the ITS1 and ITS4 primer pair (White *et al.*, 1990). All PCR reactions contained 10 mM Trizma HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 μM of each primer, 200 μM of each dNTP, 1 unit of Sigma REDTaq genomic polymerase and 0.5 μL of template DNA per 25 μL reaction volume. DNA was amplified with a Bio-Rad MyCycler™ (Bio-Rad, Inc., Hercules, CA, USA)

thermal cycler using the following PCR conditions: initial denaturation at 94°C for 5 min then 30 cycles, denaturation at 94°C for 30 s, annealing at 47°C for 90 s, extension at 72°C for 60 s and a final extension for 10 min. Sequencing was performed at the Cornell University Life Sciences Core Laboratories Center on an Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase. Sequences were compiled and edited in Sequencher 4.8 (Gene Codes) to remove vector sequences and eliminate poorly resolved regions. Sequence affinities to known taxa were determined based on BLAST searches of the NCBI GenBank database.

Owing to the variable germination of weed seeds, larger bioassays were required to better determine the pathogenicity of the four fungal isolates identified as potentially pathogenic in the preliminary bioassays. All four isolates were tested against seeds of all six weed and crop species. Plates were set up as for the preliminary bioassay but with three replicate plates of 50 seeds for each seed species by isolate combination. These plates were monitored for 3 weeks after sowing, with emergence counts at 1 and 3 weeks. Emerged seedlings were removed with forceps.

### *Data analysis*

Differences among the interactions of treatments with collection dates were tested using repeated measures analysis of variance (ANOVA) implemented with SAS Proc GLM (SAS Institute, 2008). We report the results of the univariate tests with *P*-values corrected using the Lecoutre enhancement of the Huynh–Feldt method. Univariate tests have more power than multivariate tests when the number of subjects (in this case, treatments × replications) is small (ATSSCG, 1997), which was particularly the case in 2005. For the purposes of analysis, species were treated as separate experiments. Only within-subject effects are reported and discussed, because the effect of treatments averaged over all soil collection days is of little interest. Differences among treatments at individual collection days were also evaluated using separate ANOVAS. A two-way chi-square test of independence was conducted on the totals of emerged seedlings in inoculated versus uninoculated plates for each seed species by fungal isolate combination.

## Results

### *2005 experiment*

ANOVA indicated significant interactions between soil and collection day for *A. theophrasti* ($P < 0.001$) and *C. album* ($P < 0.05$). Significant differences in seedling emergence from the two types of soil were observed for days 0, 1, 2 or 4 for some but not all weed species (Fig. 1). All five weed species showed the greatest depression in emergence, on day 1 or on day 2. After reaching a minimum for soil collected 1–2 days after tillage, seedling emergence increased with collection day for all species except *C. album*. Seedling emergence patterns for alley samples were similar to those for plot samples but with less depression in emergence for samples collected soon after tillage.

### *2006 experiment*

In 2006, the interaction of collection day with source (plot versus alley) was significant for *A. theophrasti*, and the interaction of day with source and sterilisation was significant for *E. crus-galli* (Table 2). As in 2005, differences in emergence among treatments were greatest on about day 2 of the experiment (Fig. 2). Emergence of *C. album* in unsterilised plot soil never differed from that in unsterilised alley soil, but on day 2, seedling emergence in sterilised soil from the alley was greater than that in unsterilised soil from either location (Fig. 2). *Abutilon theophrasti* and *E. crus-galli* showed significantly less emergence on day 2 for the unsterilised plot soil treatment compared with the other three treatments, and *S. faberi* had less emergence in the unsterilised plot treatment on day 2 than in either of the sterilised treatments (Fig. 2). For *A. theophrasti* and *E. crus-galli*, emergence in unsterilised plot soil tended to increase with later collection days, as predicted. Like the 2005 data, graphs of the various treatments resembled one another in general form, despite significant differences during early collection days.

### *2009 experiment: seedling emergence*

The interaction of collection day by sterilisation significantly affected emergence of all species except *E. crus-galli*, for which the interaction was nearly significant (Table 3). The collection day by source interaction was significant for red clover and approached significance for *S. faberi*. The interaction of day by source by sterilisation approached significance for *A. theophrasti*, lettuce and red clover.

Seedling emergence was significantly depressed in the unsterilised plot soil relative to at least two of the three controls at one or more collection days for four species: *S. faberi*, *E. crus-galli*, lettuce and red clover (Fig. 3). The greatest depression in seedling emergence was observed at day 2 for these species. The unsterilised alley soil showed lower emergence than the sterilised treatments for most species, but the depression was generally less substantial than for the unsterilised plot soil treat-

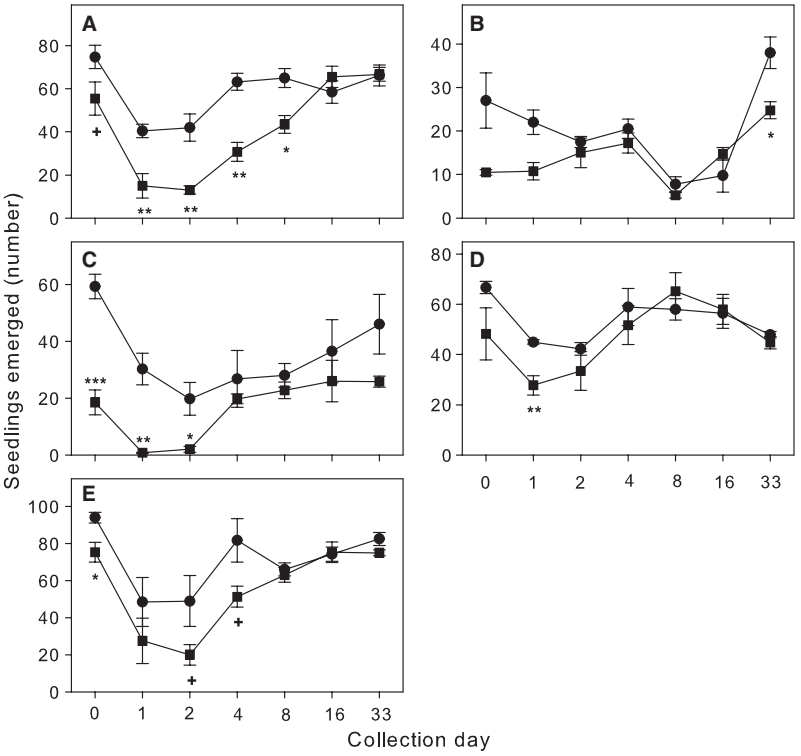


**Fig. 1** Seedling emergence in response to soil collection day for (A) *Abutilon theophrasti*, (B) *Chenopodium album*, (C) *Amaranthus powellii*, (D) *Setaria faberi* and (E) *Echinochloa crus-galli* in plot versus alley soil samples in 2005. Bars show SEM. Collection days in which a significant difference between treatments occurred are marked for each weed species. Significance levels: $+P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Symbols: —•— Alley, —■— Plot.

ment. Collection day had little effect on emergence of *E. crus-galli*, *S. faberi* or red clover in the two sterilised treatments.

### *2009 experiment: seed pathogens*

ITS sequencing indicated that Isolate 1 was closely related to *Phanerochaete sordida*. Isolate 2 was identified as *Fusarium chlamydosporum*, and isolates 3 and 4 were identified as *F. oxysporum*. The four potential pathogen isolates varied in their effects on the six plant species tested (Table 4). *Fusarium oxysporum* isolate 3 did not significantly reduce the emergence of any of the species. *Fusarium chlamydosporum* isolate 2 and *F. oxysporum* isolate 4 both significantly reduced the emergence in *E. crus-galli*, lettuce and red clover.

## Discussion

In accord with hypothesis 1, the unsterilised plot soil treatment generally had less seedling emergence than other treatments for plantings made the first few days after green residue incorporation. In accord with hypothesis 2, this response was transient, because plantings made after day 4 or day 8 resulted in emergence that did not differ between the unsterilised plot soil and the other treatments. Because the sterilised plot soil did not show depressed emergence in the early days, the suppression of emergence by incorporated green residues was probably due to a biological agent rather than allelopathic chemical effects. This observation supports hypothesis 3.

Although allelopathy may have played a role in the suppression of emergence by crop residues in these experiments, the effect of allelopathy was likely secondary to that of pathogens. Peas have not been shown to be particularly allelopathic. For example, the study by Akemo *et al.* (2000) showed pure pea cover crops had the highest percentage weed ground cover, whereas mixed rye (*Secale cereale* L.)-pea cover crops had the lowest. Water extracts of *Amaranthus retroflexus*, a close relative of *A. powellii*, inhibited germination and early

**Table 2** ANOVA summary (*P* levels) for seedling emergence in the 2006 experiment

| | *Abutilon theophrasti* | *Chenopodium album* | *Setaria faberi* | *Echinochloa crus-galli* |
|---|---|---|---|---|
| Collection day | 0.074 | NS | <0.001 | 0.068 |
| Day*Source | 0.016 | NS | NS | NS |
| Day*Sterilisation | NS | NS | NS | 0.028 |
| Day*Source*Sterilisation | NS | NS | NS | 0.010 |

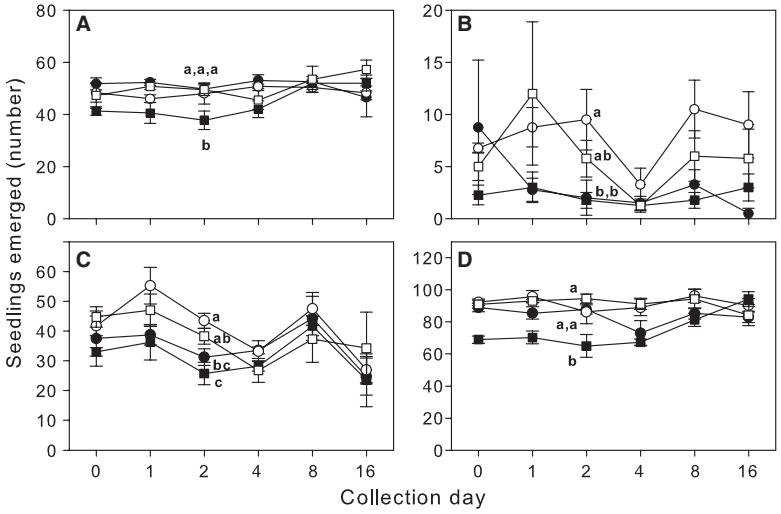


**Fig. 2** Seedling emergence in response to soil collection day for (A) *Abutilon theophrasti*, (B) *Chenopodium album*, (C) *Setaria faberi* and (D) *Echinochloa crus-galli* in 2006. Bars show SEM. Significant differences among treatments are shown for day 2; data points with the same letter are not significantly different at $P < 0.05$. Symbols: —•— Alley unsterilised, —○— Alley sterilised, —■— Plot unsterilised, —□— Plot sterilised.

growth of cotton (*Gossypium hirsutum* L.) and sorghum (*Sorghum bicolor* (L.) Moench) in laboratory bioassays (Munger *et al*., 1984), but water extracts of most plant species have such an effect. Mature residue of *A. retroflexus* inhibited the growth of maize (*Zea mays* L.) and soyabean (*Glycine max* (L.) Merr.) in sand culture (Bhowmik & Doll, 1983), but whether vegetative residue of the sort we used would affect seeds and seedlings in soil seems doubtful. Young residue of *A. retroflexus* was less inhibitory than mature residue in laboratory bioassays (Munger *et al*., 1984) and young plants of *A. powellii* have a low concentration of secondary plant compounds as evidenced by their mild flavour and high palatability as salad greens. Allelopathy of oat residue is well documented (Fay & Duke, 1977; De Bertoldi *et al*., 2009). Elimination of the suppression by sterilisation and the isolation of pathogens from germinating seeds in the 2009 experiment indicate that the observed suppression in soil with incorporated oat-pea residue may have been due to pathogens, rather than allelopathy in this study.

Autoclaving treatments could have affected nutrient and organic matter dynamics of the soil (Williams-Linera & Ewel, 1984). Although all soil sterilisation treatments are known to influence some chemical and physical soil properties, autoclaving has become the standard for eliminating microbial activity from soils (Trevors, 1996), and it is superior to fungicide applications for this purpose. Soil applications of fungicides are known to be problematic because of their selectivity to certain taxa of fungi, soil adsorption, bacterial degradation and other non-target impacts on plants (Munnecke, 1972). Autoclaving may have increased N release by thermal decomposition of residue, release from microbial biomass, or by inhibiting microbial sequestration of N (Powlson & Jenkinson, 1976). However, because of the low C:N ratios of the residue, particularly in 2005 and 2006, no sequestration of N would be expected. Even if nitrate concentration differed between the sterilised and unsterilised treatments, this could not explain the lower emergence of *A. theophrasti* and lettuce in the plot unsterilised treatment, because germination of *A. theophrasti* is not affected by N (Fawcett & Slife, 1978) and the lettuce seeds used lacked dormancy.

On the basis of the above-stated reasoning, microbial pathogens appear to be the most likely cause of suppressed emergence following incorporation of green residue in this study. In addition, however, pathogenic fungi were cultured from germinating weed seeds exposed to soil with incorporated green residue. Incorporation of green crop residue has been shown to increase pathogens in other studies (Grünwald *et al*., 2000; Manici *et al*., 2004). Seedling emergence in the experiments reported here generally increased for progressively later plantings, after a minimum for the

**Table 3** ANOVA summary ($P$ levels) for seedling emergence in the 2009 experiment

| | *Abutilon theophrasti* | *Setaria faberi* | *Echinochloa crus-galli* | Lettuce | Red clover |
|---|---|---|---|---|---|
| Collection day | <0.001 | <0.001 | NS | <0.001 | <0.001 |
| Day*Source | NS | 0.061 | NS | NS | 0.022 |
| Day*Sterilisation | 0.006 | <0.001 | 0.055 | 0.001 | 0.033 |
| Day*Source*Sterilisation | 0.098 | NS | NS | 0.085 | 0.073 |

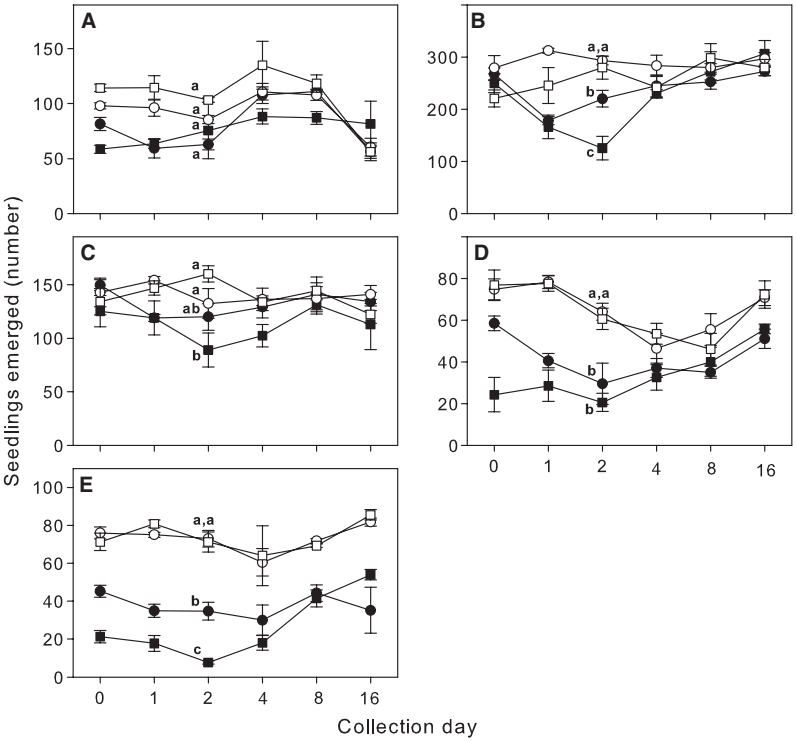


**Fig. 3** Seedling emergence in response to soil collection day for (A) *Abutilon theophrasti*, (B) *Setaria faberi*, (C) *Echinochloa crus-galli*, (D) lettuce and (E) red clover in 2009. Bars show SEM. Significant differences among treatments are shown for day 2; data points with the same letter are not significantly different at the $P < 0.05$ level. Symbols: —•— Alley unsterilised, —○— Alley sterilised, —■— Plot unsterilised, —□— Plot sterilised.

planting made on about day 2. This increase was probably related to the degree of decomposition of the residues (Chung *et al.*, 1988; Grünwald *et al.*, 2000).

The pattern of seedling emergence from unsterilised alley soil reflected that from unsterilised plot soil, but with significantly less suppression in emergence for plantings made soon after tillage and residue incorporation. Although no green residue was incorporated in the alley soils, tillage may have stimulated a lesser increase in pathogen activity. This is supported by the frequent, although only occasionally significant, difference in emergence between sterilised and unsterilised alley soil soon after tillage.

Some of the parallels in the emergence patterns of all treatments over time may be attributed to natural variation in soil conditions among collection days. Although soil was watered daily after spreading in trays in the glasshouse, soil moisture in the field varied substantially across collection days, and this affected soil structure in the trays and may have affected chemical and biological conditions as well. Additionally, glasshouse conditions, particularly the amount of sunlight,

**Table 4** Number of seedlings (of the 150 tested) emerging from sand plates uninoculated (U) or inoculated (I) with the indicated pathogen isolate and corresponding chi-square test results (1 degree of freedom test). Significance levels: NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. N/A indicates inoculated seeds had a qualitatively greater seedling emergence

| | *Abutilon theophrasti* | | *Echinochloa crus-galli* | | *Setaria faberi* | | Lettuce | | Red clover | |
|---|---|---|---|---|---|---|---|---|---|---|
| Isolate* | I | U | I | U | I | U | I | U | I | U |
| 1 | 57 | 34 | 86 | 96 | 26 | 45 | 100 | 58 | 47 | 50 |
| 2 | 48 | 28 | 70 | 98 | 37 | 27 | 23 | 56 | 32 | 64 |
| 3 | 36 | 38 | 95 | 104 | 43 | 30 | 44 | 61 | 43 | 46 |
| 4 | 56 | 63 | 83 | 106 | 36 | 26 | 36 | 85 | 16 | 61 |
| Significance | | | | | | | | | | |
| 1 | N/A | | NS | | * | | N/A | | NS | |
| 2 | N/A | | ** | | N/A | | *** | | *** | |
| 3 | NS | | NS | | N/A | | NS | | NS | |
| 4 | NS | | * | | N/A | | ** | | *** | |

*Isolate 1, close to *Phanerochaete sordida*; isolate 2, *Fusarium chlamydosporum*; isolates 3 and 4, *F. oxysporum*.

varied through time and may have promoted or delayed germination and emergence. These uncontrolled variables would be expected to affect all treatments similarly. In 2005, temperatures in the glasshouse greatly exceeded the set temperature on warm, sunny days; high temperatures recorded at a nearby weather station during the first 6 days following incorporation may account for the large parallel dips in emergence from both plot and alley soils for early collection days. In contrast with 2005, glasshouse temperature was well regulated in the 2006 and 2009 experiments.

The varied results and frequent lack of significance across all weed and crop species in the chi-square analysis of the bioassay data are probably due to the high dormancy rate in some seed lots. The scale of the bioassays had to be increased several times before finding a sample size that showed significant differences in emergence between inoculated and uninoculated seeds of some species; for other species, even larger sample sizes might have provided a better test.

The identification of fungal isolates 2, 3 and 4 were consistent with hypothesis 3, and the significant reduction in seedling emergence seen in three of the species (*E. crus-galli*, lettuce and red clover) following inoculation with *Fusarium* species isolated from seeds exposed in the experiment is not surprising. Both *Fusarium chlamydosporum* and *F. oxysporum* are known as weed pathogens. Boari and Vurro (2004) demonstrated a 50–60% reduction in seedling establishment for the broomrape species *Orobanche ramosa* L. by both *F. oxysporum* and *F. chlamydosporum* during 4 weeks of monitoring following *Fusarium* inoculations. *Fusarium oxysporum* has also been shown to reduce establishment of *Orobanche cumana* Wallr. by 80% and *Striga hermonthica* (Delile) Benth. by 90% after soil inoculation (Sauerborn *et al*., 2007). Although the exact *forma speciales* of *F. oxysporum* isolate 4 recovered in these studies could not be determined, it could possibly be synonymous with *F. oxysporum* f. sp. *lactucae* [the causal agent of fusarium wilt and root rot of lettuce (Matheron & Koike, 2003)] and would also likely cause symptoms on seedlings. Alternatively, *F. oxysporum* Isolate 4 may be a generalist pathogen that also attacks lettuce.

Interpreting the response of *S. faberi* to inoculation with *Phanerochaete sordida* is difficult. *Phanerochaete sordida* is a known wood-rotting fungus and is not typically associated with seed decay (Tuomela *et al*., 2002). *Phanerochaete sordida* significantly reduced emergence of only *S. faberi*, and the effect may be attributed to some peculiarity in the interaction of these particular species.

When seeds were collected for pathogen isolations 5 days after planting, few showed emerged radicles. Because individuals are subject to attack by additional suites of pathogens after they germinate (Kirpatrick & Bazzaz, 1979), additional pathogen species may have attacked seedlings between radicle emergence and seedling emergence in the experiments. The presence of known pathogens isolated from seeds in the 2009 experiment and the substantial effect of autoclaving on seedling emergence both point to a role for pathogens in the suppression of seedling emergence following green residue incorporation, but further study is clearly needed to fully understand the phenomenon.

Seedling emergence of both weeds and crops was consistently depressed for a few days following the incorporation of green residue. An interesting result of this study was the brevity of the suppression: the effect generally peaked for plantings carried out on day 2, and the suppression of emergence by residue was rarely significant for plantings made later than day 4. The study reported here is one of the few that assayed soil by planting at successive times after residue incorporation. Conklin *et al*. (2002) assayed unamended soil and soil amended with red clover green residue plus compost at successive dates by observing growth and disease symptoms on *Brassica kaber* (DC.) L.C.Wheeler and maize seedlings. The suppressive effect of the amendments on *B. kaber*, attributed largely to *Pythium* spp., lasted several weeks in their study, but maize was unaffected. Dabney *et al*. (1996) found that a crimson clover (*Trifolium incarnatum* L.) cover crop reduced the stand of sorghum (*Sorghum bicolor* (L.) Moench.) when killed 1 or 7 days before planting, but not when killed 14 days or more before planting. The crimson clover produced substantially less biomass when killed earlier than 7 days before planting, however, and this could at least partly explain the lack of a suppressive effect for the early clover termination dates.

The experiments presented here indicate that delaying planting of crop seeds for *c*. 1 week following green residue incorporation may be sufficient to eliminate effects on crops of additional pathogen activity associated with the residue. If crop planting is delayed after residue incorporation, most weed seedlings will usually be killed by final seedbed preparation; so, any effect of pathogens on weed density when planting is delayed would probably be slight. In contrast with direct seeded crops, crops can often be transplanted into freshly incorporated residue without apparent harm. In 2006 and 2009, cabbage was transplanted into the plots used as soil sources within 30 h of residue incorporation; this cabbage showed no signs of disease relative to the cabbage in plots that did not have residue, and we have similarly transplanted other vegetable crops into freshly incorporated green residue without ill effects (CL Mohler & BA Caldwell, unpubl. obs.). Minimal attack

on transplants is reasonable, because transplants typically require a few days to recover before many roots grow out of the plug, and by then, pathogen effects of residue incorporation are waning. The first flush of weed seedling emergence, however, may be partially suppressed, and this will ease weed management.

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