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Interference from Broccoli Residue on *Brassica* Germination and Seedling Growth

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Abstract. **Germination bioassays were conducted to assess if water-soluble extracts of broccoli (*Brassica oleracea* L. var. *italica* L.) affect germination of broccoli, cabbage (*Brassica oleracea* L. var. *capitata* L.), and cauliflower (*Brassica oleracea* L. var. *botrytis* L.). Greenhouse experiments also examined the phytotoxic potential of soil previously cropped with broccoli and broccoli plant parts on seedling growth of those species. The first bioassay used nonsterile extracts (NSEs) and filter-sterilized extracts (FSEs) of broccoli leaves. The second bioassay used nonsterile and filter-sterilized leaf extracts (LEs), stem and root extracts (SREs), and whole broccoli plant (leaves, stems, and roots) extracts (WPEs). Broccoli and cabbage germination were not affected by NSEs or FSEs, but the latter reduced cauliflower germination by 22%. LEs and SREs decreased germination speed for broccoli, cabbage and cauliflower. Greenhouse seedlings were grown in soil previously cropped with broccoli or fallow soil at three fertilizer levels. Broccoli soil was phytotoxic to cauliflower but enhanced broccoli and cabbage seedling growth. The differential sensitivity to broccoli plant residue was in the order of cauliflower > broccoli = cabbage, with SR residue having the highest phytotoxic potential.**

Crop sequence can inhibit or stimulate growth of the second crop (Rice, 1984). Allelopathy, or the degree of growth inhibition of one plant on another, is thought to be caused by phytotoxins released by a crop during its growth or by decaying crop or weed residues left in the field (Hegde and Miller, 1990; Putnam and Tang, 1986).

Allelopathic suppression of associated species is widespread in agroecosystems (Wardle et al., 1993). In horticulture, many examples of allelopathy have been reviewed by Putnam (1986). Poor emergence of lettuce (*Lactuca sativa* L.) seedlings was reported when planted immediately after celery (*Apium graveolens* L.) was removed (Shilling et al., 1992). Asparagus (*Asparagus officinalis* L.) root tissues inhibited lettuce emergence and delayed tomato (*Lycopersicon esculentum* Mill.) and asparagus seedling emergence when incorporated to dry soil before seeding (Shafer and Garrison, 1986). Alfalfa (*Medicago sativa* L.) roots inhibited cucumber (*Cucumis sativus* L.) seed germination and were toxic to pregerminated cucumber seeds (Ells and McSay, 1991).

Cruciferous plants possess a wide range of allelopathic potential (Grodzinsky, 1992). They have been found to suppress weeds, leading to a reduction in the use of herbicides (Jimenez-Osornio and Gliessman, 1987). Cabbage soil amendments have been shown to posses fungicidal activity (Keinath, 1996) attributed to the chemical breakdown of glucosinolates (Fenwick et al., 1983). Broccoli amendments reduced *Verticillium dahliae* Kleb. microesclerotia and wilt incidence in cauliflower and was suggested as alternative method to methyl bromide to manage soilborne diseases effectively (Subbarao and Hubbard, 1996). However, decomposing broccoli residue was found to be toxic to lettuce seedlings (Patrick et al., 1963).

Broccoli, cabbage, and cauliflower are three major cruciferous crops that have become important components of cropping systems in southwestern Texas and northern Mexico. After harvest, a significant portion of aboveground broccoli biomass and all roots are left in the field. This investigation was prompted to assess if water-soluble extracts of broccoli plants affect germination of broccoli, cabbage, and cauliflower and to examine the phytotoxic potential of broccoli plants parts on seedling growth of three brassica species.

Materials and Methods

Leaf extract bioassay. Dry broccoli leaves ground to pass through a 1-mm^2 sieve (50 g) were combined with 500 mL of deionized distilled water (DW). This extraction ratio was similar to the allelopathic studies reported by Nielsen et al. (1960) and Martin et al. (1990). The mixture was stirred for 24 h at 24 °C. No attempt was made to extract under low temperature since we were trying to mimic field temperatures. One-half of the supernatant liquid was passed through a double layer of Whatman no. 1 filter paper, while the remaining supernatant liquid was run through a sterile filter with a pore size of 0.2 µm (Nalge Co., Rochester, N.Y.). Filtration excluded microorganisms capable of causing decomposition that may have produced phytotoxins (Harper and Lynch, 1982; Shilling et al., 1986). The nonsterile leaf extracts (NSLEs) and the filter-sterile leaf extracts (FSLEs) were refrigerated at 5 °C until use.

Seeds of 'Galaxy' broccoli, 'Green Cup' cabbage, and 'Snow Crown' cauliflower were surface-sterilized with 1% sodium hypochlorite solution for 10 min and rinsed three times with DW. Fifty seeds of each brassica species were placed in 100 × 15-mm petri dishes lined with two sheets of Whatman no. 1 filter paper. NSLEs, FSLEs, or DW, previously equilibrated at room temperature for 1 h, were added to each petri dish at 3.5 mL and then placed on a thermogradient table at 18 °C. Extracts or water were subsequently added at ≈1 mL every other day. Germinated seeds were counted daily for 17 d. Seeds were considered germinated when radicle reached 2 mm in length.

Leaf, root, and stem extract bioassay. A second germination bioassay with NS and FS L, stem and root (SR), and whole broccoli plant (leaves, stems, and roots) (WP) extracts were prepared as described before. The pH and electrical conductivity (EC) of the NS and FS LEs, SREs, and WPEs were measured with

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an ion–pH analyzer (model 250; Corning Science Products, Corning, N.Y.) and conductivity meter (model 19000-00; Cole-Parmer Instrument Co., Chicago). The pH and EC ($dS \cdot m^{-1}$) values were 6.0 and 15.5 for FSLEs, 5.8 and 15.4 for NSLEs, 5.5 and 11.5 for FSSREs, 5.4 and 11.6 for NSSREs, 6.4 and 11.7 for FSWPEs, and 6.0 and 12.9 for NSWPEs. The pH and EC values were regressed against total percentage germination and days to 50% germination (T_{50}). Germination tests were also performed to determine whether the small numerical differences in pH and EC would affect germination by adjusting the pH and EC of all NS and FS extracts to that of the extract treatment showing the lowest pH and lowest EC. The pH and EC were adjusted using 5 M hydrochloric acid and calcium chloride, respectively.

Broccoli soil residue greenhouse experiment. Four cubic meters of soil was collected from the top 30 cm of soil (Uvalde silty clay loam soil: fine, mixed, hyperthermic Aridic Calciustoll) from two adjacent plots: one had soil planted for 3 consecutive years with broccoli crops with residue incorporated 30 cm deep (broccoli soil) and another had soil that had been fallowed for 3 years (fallow soil) and had been previously planted to rye (*Secale cereale* L.) and corn (*Zea mays* L.) during the last 10 years. Broccoli soils did not have visible decomposed plant parts. Elemental soil analysis (N, P, K, Ca, Mg, Zn, Fe, Mn, Cu, Na, and S) and pH were not different between the broccoli and fallow soil samples (data not shown). The EC of the saturation extract of the soils were 1.3 for broccoli soil and 0.8 $dS \cdot m^{-1}$ for fallow soil. Field corn was planted to assess growth uniformity on areas from which broccoli and fallow soil samples were taken. Corn growth on broccoli soil was severely stunted and chlorotic. Conversely, corn on fallow soil had normal growth with no visual phytotoxic effects. Similarly, corn grown in areas where plant-free alleys were in previous broccoli crops had normal growth. Broccoli or fallow soil samples were divided into three subplots that received no fertilizer (0), the recommended fertilizer rate (RR) at 67N–22P ($kg \cdot ha^{-1}$), or double the recommended rate (2RR) at 134N–44P. The fertilizer was ground to pass a 1-mm^2 sieve and then mixed well with the soil. Three seeds of broccoli, cabbage, or cauliflower were planted in 15 × 15 cm (diameter × height; 1900-cm^3) pots containing either fertilized or unfertilized fallow or broccoli soil. Pots were then placed on greenhouse tables lined with polyethylene sheets. Pots were subirrigated to field capacity. One week after sowing, emergence was counted and seedlings were thinned to one plant per pot. Four plants per treatment were sampled destructively 30 d after seeding (DAS) and every 14 d thereafter for 8 weeks (44, 58, 72, and 86 DAS). The seedlings were removed from pots and shoots were cut off at the crown surface. Roots were washed in water, blotted, placed in polyethylene bags, and stored at 7 °C for 1 or 2 d. Stem diameter, shoot length, and leaf area were measured immediately after cutting, and the dry mass of shoots (stems and leaves) was determined after oven drying at 65 °C for 5 d. Fresh roots were partitioned into basals (arising from the basal region of the hypocotyl) and laterals (arising from the taproot) and dry mass were determined. Leaf area was measured using a digital image analysis system (Decagon Devices Inc., Pullman, Wash.) with a spatial resolution of 512 × 488 pixels using a MicroSoft DOS 3.3 AgImage Plus software.

Broccoli–plant residue greenhouse experiment. Three seeds of broccoli, cabbage, and cauliflower were planted in 15 × 15 cm (diameter × height; 1900-cm^3) pots containing broccoli residue and fertilizer rates depending on the treatment assignment. Broccoli residue treatments were no residue or control (C), L, SR, and WP. Fertilizer rates (in $kg \cdot ha^{-1}$) were 0 (no fertilizer), RR (67N–22P), and 2RR (134N–44P). All residues were separately dried and ground to pass a 1-mm^2 sieve. Pots were subirrigated as described before and fertilized with a soluble fertilizer at 116, 25, and 96 mg N, P, and K/L every 2 weeks. Seedlings were thinned to one plant per pot 1 week after emergence. Shoot and root components were measured 4 weeks after thinning following the procedures described before. Leaf color was determined using a ColorMate/45 color analyzer (Milton Roy, Rochester, N.Y.) following the spectrophotometric technique of Santos (1992), where leaf color is indexed based on the chromaticity $(a/b)^2$ values. Higher $(a/b)^2$ values indicate less leaf chlorosis.

Statistical procedures. Germination bioassays were analyzed as a split plot in a randomized complete-block design using four replications, with brassica species as main plots and extracts as subplots. The greenhouse studies for the broccoli–soil (broccoli and fallow) and broccoli–plant residue (C, L, SR, and WP) were split plots with four replications. In each experiment, broccoli residue was the main plot and fertilizer rate (0, RR, or 2RR) was the subplot. Data for each group were analyzed using ANOVA and GLM procedures of SAS (SAS Institute, Cary, N.C.), with means separated by Fisher's Protected LSD (0.05).

Table 1. Effect of broccoli extracts on germination and days to 50% germination (T_{50}) of broccoli, cabbage, and cauliflower seeds. Germination bioassays were performed in petri dishes at 18 °C in darkness.

	Broccoli		Cabbage		Cauliflower	
Extract[z]	Germination (%)	T_{50}	Germination (%)	T_{50}	Germination (%)	T_{50}
			First bioassay			
DW	87	4.0	97	3.0	97	5.0
NSE	85	6.8	95	6.0	91	8.5
FSE	91	6.5	91	5.7	75	12.5
LSD(0.05)	NS	1.1	NS	1.4	7	3.5
			Second bioassay			
DW	100	2.3	98	2.1	97	3.0
NSLE	84	7.1	80	5.5	77	7.3
FSLE	66	6.8	82	6.1	57	7.9
NSSRE	96	5.1	97	2.7	87	5.5
FSSRE	93	4.2	96	3.6	80	6.7
LSD(0.05)	8	0.6	NS	0.6	16	0.8

[z]DW = distilled water; NSE = nonsterilized extract; FSE = filtered-sterilized extract; NSLE = nonsterilized leaf extract; FSLE = filtered-sterilized leaf extract; NSSRE = nonsterilized stem and root extract; FSSRE = filtered-sterilized stem and root extract.
[NS]Nonsignificant.

Results and Discussion

Germination bioassays. In the first germination bioassay, crop × extract interaction was significant for germination and T_{50}. Therefore, main effects were partitioned for each crop (Table 1). Broccoli and cabbage seed germination were not affected by incubating seeds with NSEs or FSEs. In contrast, FSEs significantly reduced cauliflower germination by 22% compared to DW, while NSEs did not affect germination (Table 1). These results agree with those suggesting that soil microorganisms have a role in dissipating phytotoxicity. Extracts with soil particles have been shown to be less effective in causing allelopathy than those without soil particles (Patrick et al., 1964). Corn pollen extract inhibited growth of several crops: the weakest inhibition (28%) was measured in nonsterilized soil and the strongest inhibition (54%) was measured in sterilized soil (Jimenez et al., 1983).

Percent germination has been used to assess the adverse effect of allelopathic material on potential crop stand (Hegde and Miller, 1990; Hicks et al., 1989; Martin et al., 1990). However, percent germination may lose its utility as an index when seed vigor is taken into account. For this bioassay, T_{50} served better as an index of speed of germination. Pooled percent germination of cabbage and cauliflower grown on broccoli extract were inversely related ($r^2 = -0.93$) to their respective T_{50}. Extracts increased T_{50} relative to DW control on all brassica species, but the delay in germination was in the order of cauliflower > broccoli = cabbage (Table 1). Cauliflower seeds incubated in FSEs had a higher T_{50} than NSEs and DW. However, cabbage and broccoli had similar T_{50} in either FSEs or NSEs, but a lower T_{50} when incubated in DW. This bioassay was incapable to determine whether other confounding factors in FSEs or NSEs (e.g., salts) affected T_{50} relative to DW in cabbage and broccoli.

In the second bioassay, broccoli LEs decreased final percentage germination of broccoli and cauliflower seeds incubated at 18 °C (Table 1). The reduction in germination was greater for the FSLEs than for the NSLEs. As described for the first bioassay, cauliflower was the most sensitive brassica species to broccoli extracts. SREs did not affect final germination, except for cauliflower, which exhibited a 17% reduction imposed by the FS portion compared with the control DW. Broccoli plant extracts did not affect total germination of cabbage (Table 1).

Broccoli plant extracts increased T_{50} relative to DW on broccoli, cabbage, and cauliflower (Table 1, second bioassay), and the delay in germination was greater for LEs compared with the SREs (Table 1) . FSSREs delayed T_{50} relative to the NS fraction in cauliflower and cabbage. It is unclear why FSSREs decreased T_{50} compared with NSSREs in broccoli.

The single linear regression equation of T_{50} as affected by EC (combined NS and FS extracts) was $T_{50} = 0.98 + (0.00647 \times EC)$; $r^2 = 0.94$, while the correlation between EC and percent germination was low ($r^2 = 0.131$). Patrick et al. (1963) noted that increased salinity could enhance phytotoxicity of plant extracts, with the response being concentration dependent. No direct relationship between pH of the pooled NSEs and FSEs and percent germination of the three brassica species was observed. Shafer and Garrison (1986) noted that pH and EC of amended soil with asparagus root tissue did not affect tomato, lettuce, and asparagus seedling growth.

Broccoli soil residue. For broccoli seedlings, the main effects of soil, fertilizer rate, and sampling date differed significantly for root and shoot components (Table 2). Basal and lateral root dry mass, shoot dry mass, and stem diameter were significantly enhanced when broccoli seedlings were grown on broccoli than fallow soil. These growth components increased independently of fertilization. Leaf area was 52% and 111% greater for broccoli seedlings grown on broccoli soil compared to fallow soil at RR or 2RR, respectively (data not shown). The partitioning of the signifi-

Table 2. Effects of fallow or broccoli soil residue on root and shoot components of broccoli, cabbage, and cauliflower seedlings. Means were pooled across all sampling dates and fertilizer rate.

	Root component		Shoot component				
Soil residue (S)	Basal root dry mass (g)	Lateral root dry mass (g)	Shoot dry mass (g)	Shoot length (cm)	Stem diam (mm)	No. of leaves	Leaf area (cm^2)
				Broccoli			
Fallow	0.09	0.07	0.40	11.0	2.2	4.4	44.8
Broccoli	0.13	0.12	0.51	12.6	2.4	5.0	76.2
Significance	**	**	**	**	**	**	**
Interaction							
S × F[z]	NS	NS	NS	**	NS	*	**
S × SD[y]	*	**	**	**	NS	**	**
				Cabbage			
Fallow	0.06	0.06	0.32	8.6	1.9	4.8	42.4
Broccoli	0.08	0.09	0.38	9.2	2.1	5.2	63.8
Significance	NS	*	NS	*	NS	NS	*
Interaction							
S × F	NS	NS	**	NS	NS	*	**
				Cauliflower			
Fallow	0.05	0.05	0.31	9.2	2.0	4.8	38.2
Broccoli	0.03	0.03	0.18	7.8	1.8	4.8	28.0
Significance	*	NS	*	**	NS	NS	NS
Interaction							
S × SD	NS	NS	NS	*	*	NS	NS

[z]F = fertilizer rate.
[y]SD = sampling date.
NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.

cant soil × sampling date interaction for shoot and root components indicated that growth enhancement of broccoli seedlings occurred only 58 d after seeding (data not shown). We hypothesize that this promotion in leaf growth could have been triggered by stimulatory factors produced after that time. Patrick et al. (1964) pointed out that it takes 10 to 25 d from the start of decomposition to the peak of allelochemical production. Stimulatory effects are usually obtained following a decline in toxicity of the allelochemicals (Patrick et al., 1963).

Broccoli soil had a slight effect on the growth of root and shoot components of cabbage seedlings (Table 2). Seedlings grown on broccoli soil had 50% more lateral root dry mass and were slightly taller with larger leaf area than when grown on fallow soil, while basal root dry mass, shoot dry mass, and stem diameter were not affected by broccoli soil. Considering soil × fertilizer interaction, seedlings on broccoli soil at RR had increased shoot dry mass, leaf number, and leaf area compared to 0 fertilizer level (data not shown), while doubling the fertilization to 2RR did not translate to a further increase in shoot dry mass, leaf number, and leaf area compared to RR (data not shown).

Cauliflower seedling growth was significantly suppressed by broccoli soil compared to seedlings grown on fallow soil. They had 40% and 42% less basal root dry mass and shoot dry mass, respectively (Table 2). No effect of broccoli soil was measured on lateral root dry mass, stem diameter, leaf number, or leaf area. Increasing fertilizer rate from 0 to 2RR did not alleviate the suppression of growth in cauliflower (not shown). Patrick et al. (1963) found that, when extracts of decomposing field residues of broccoli were bioassayed, they inhibited root growth of lettuce. Over time, cauliflower seedlings showed severe stunting, weak petioles, and leaf discolorations such as yellowing, bronzing, and purpling usually associated with N and secondary nutrient deficiencies. Similar symptoms were observed on lettuce and spinach plants when residues of broccoli and broadbean (*Phaseolus lunatus* L.) were left on soils in Salinas Valley, Calif. (Rice, 1984).

Broccoli–plant residue. For broccoli seedlings, the main effects of residue differed significantly for root and shoot components (Table 3). Over time, L, SR, and WP residues had a slight inhibitory effect on stem length (9%) and shoot dry mass (18%), with some chlorosis when grown on SR residue. The partitioning of the residue (R) × fertilizer (F) interaction for leaf area indicated that there was a leaf growth promotion produced by L and WP residue with no fertilizer in broccoli (Fig. 1). Further increases in fertilizer rate from RR to 2RR increased leaf area for C, L, and SR but not for WP residue. Similar trends were observed for root dry mass, shoot dry mass and stem length (not shown). It appears that the degree of inhibition may not only be related to the fertilization level and residue type, but probably with residue concentration and degree of decomposition associated with fertilizer levels.

In cabbage, broccoli residue had minimum effect on seedling growth. Although there was a significant R × F interaction for all shoot components (Table 3), differences were negligible (data not

Table 3. Effects of broccoli plant residue on root and shoot components of broccoli, cabbage and cauliflower seedlings.

	Root	Shoot		Leaf		
Residue (R)	dry mass (mg)	Dry mass (mg)	Length (cm)	No.	Area (cm^2)	Color[z]
			Broccoli			
Control	34	203	12.0	4.0	28.4	0.70
Leaves	30	166	11.6	4.0	25.1	0.66
Stem and root	31	168	11.3	3.9	24.5	0.62
Whole plant	31	165	11.2	3.8	24.8	0.66
LSD(0.05)	NS	26	0.5	NS	2.4	0.04
Interaction						
R × F[y]	**	**	**	NS	**	NS
R × SD[x]	NS	NS	NS	*	NS	NS
			Cabbage			
Control	24	162	8.7	4.3	27.2	0.65
Leaves	27	155	8.9	4.2	26.5	0.63
Stem and root	25	150	8.4	4.2	24.2	0.58
Whole plant	25	155	8.6	4.3	26.5	0.60
LSD(0.05)	NS	NS	0.2	NS	NS	0.04
Interaction						
R × F	NS	**	**	**	**	**
R × SD	*	NS	NS	NS	NS	NS
			Cauliflower			
Control	26	182	10.2	5.2	27.1	0.60
Leaves	27	193	10.5	5.1	28.6	0.56
Stem and root	23	178	10.0	5.1	25.7	0.51
Whole plant	29	206	10.4	5.2	31.2	0.55
LSD(0.05)	NS	23	0.2	NS	2.2	NS
Interaction						
R × F	NS	**	**	NS	**	NS
R × SD	NS	NS	NS	NS	**	NS

[z]Leaf chlorosis indexed by $(a/b)^2$ as described in the text (higher values = less, lower = more).
[y]F = fertilizer rate.
[x]SD = sampling date.
[NS,*,**]Nonsignificant or significant at $P = 0.05$ or 0.01, respectively. Means separated by Fisher's Protected LSD.

shown). The most detectable difference occurred for leaf number, which was reduced when grown on SR residue at RR and 2RR (Fig. 1), but a slight increase was obtained with L residue at 0 and WP residue at RR and 2RR.

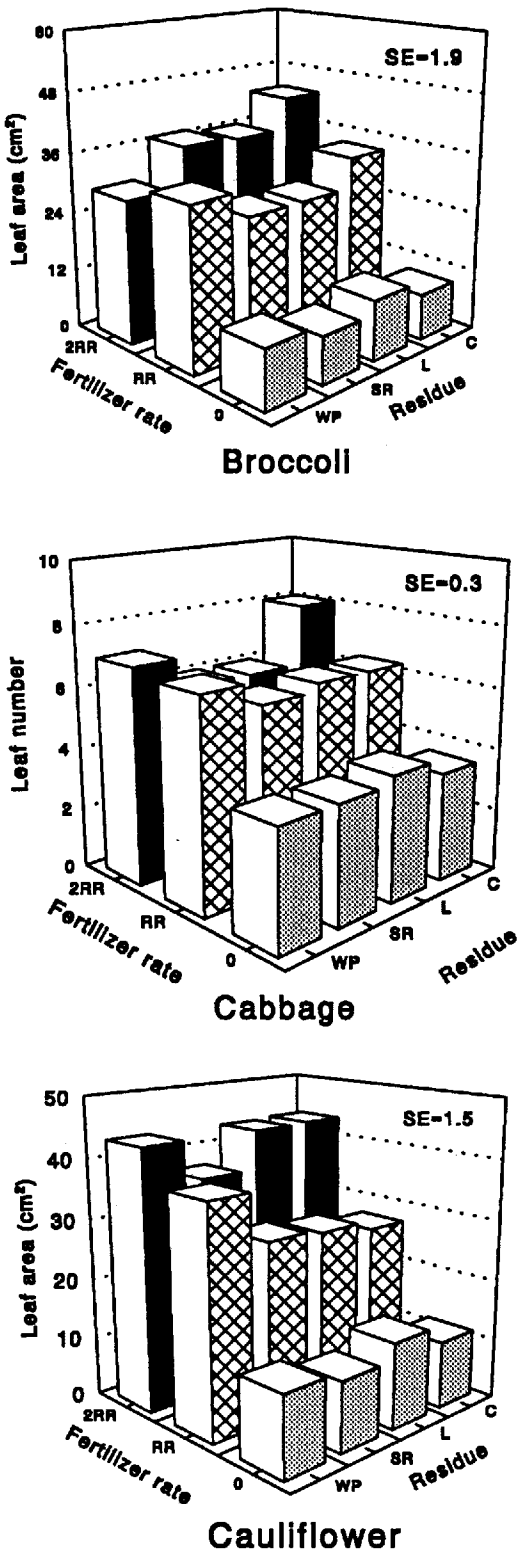

Fig. 1. Leaf area on broccoli, leaf number on cabbage, and leaf area on cauliflower seedlings as affected by broccoli residue: whole plants (WP), stems and roots (SR), leaves (L), and control (C) at three fertilization levels: no fertilizer (0), recommended fertilizer rate (RR) at 67N–22P ($kg \cdot ha^{-1}$), or double the recommended rate (2RR) at 134N–44P.

In cauliflower, broccoli residue did not adversely affect root dry mass (Table 3). The partitioning of the R × F interaction for leaf area shows that leaf area was not affected in the absence of fertilization (Fig. 1). At RR there was a growth enhancement for WP residue, while further increase in fertilization did not enhance growth. Compared to the control, leaf area gain was slightly suppressed when seedlings were grown with SR residue at 2RR. Similar trends were measured for shoot dry mass and stem length (not shown).

Stand establishment and early growth can be adversely affected by the influence of the preceding crop. The bioassays used here showed that cauliflower seed germination was more sensitive to broccoli soil and broccoli extracts than broccoli or cabbage. The greenhouse studies showed similar trends on broccoli and cabbage, while a slight suppression of shoot growth was measured for cauliflower seedlings. In the absence of fertilizer, broccoli leaf residue may promote growth of broccoli seedlings, while some growth suppression may be considered in cauliflower amended with SR tissues. Stems from grain sorghum [*Sorghum bicolor* (L.) Moench] were found to be the most phytotoxic plant part inhibiting wheat (*Triticum aestivum* L.) seedling growth (Ben-Hammouda et al., 1995).

Competition for essential resources and the use of allelochemicals are two main mechanisms that plants use to ensure their dominance and eventual survival. In this study, no attempt was made to isolate phytotoxins or allelochemicals and other compounds such as sugars, glucosinolates, amino acids or fatty acids that might be present in broccoli soil, broccoli plant extracts, or both. It is also unknown how easily those factors leach out of the tissues on extraction or even during decomposition. Complex processes that include allelochemical retention via adsorption to the soil surfaces, inactivation through chemical transformation with or without microbial mediation, or via losses due to leaching, dilution, or both should be considered. Regardless of a single process or combination of such processes, they can nullify any extra potency that a residue has on exposure to the soil, resulting in a modification of the inhibitory potential, which may or may not be effective in a particular crop.

In this study, broccoli residue inhibited speed of germination, total germination, and seedling growth of cauliflower. The speed of germination was also reduced (higher T_{50}) for broccoli and cabbage, but both reached same germination levels as the control. Broccoli soil was phytotoxic to cauliflower but enhanced broccoli and cabbage seedling growth. Higher phytotoxic potential occurred for seedlings grown on SR residues. Further studies on residue concentration, age, and composition over time would be beneficial in the elucidation of the differential growth interference of broccoli residues. This information is valuable for the formulation of rotational sequences for brassica species used in intensive cropping systems.

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