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Combined effect of organic amendments, biofumigation and solarization on onion growth and on pink root (*Setophoma terrestris*) severity

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Abstracts
Soilborne diseases are of difficult control in organic horticultural production. We studied the effect of organic amendments combined with solarization to control pink root of onion, caused by Phoma terrestris, as a model pathosystem. Two field trials were carried out on a sandy soil, previously infested experimentally with S. terrestris. The amendments incorporated into the upper 10 cm of the soil were: green residues of Brassicaceae species, fruits of Persian lilac (PLF) (Melia azedarach) and fresh chicken manure. One half of the experimental units were covered with a transparent polyethylene film, during 25 days in late spring (november-december). There were three replicates. Mean soil temperature, measured at a depth of 10 cm, was 4 to 7°C higher in polyethylene-covered than in non-covered soil. Temperature was lowest in unamended soil, intermediate in broccoli- and radish-amended soil and highest in PLF- and manure-amended soil. In the first year trial, soil samples obtained from each treatment were planted with onion seedlings, cv. Valcatorce, and grown in the greenhouse. In the second year trial, onion bulbs were rooted in soil samples. Pink root severity was significantly higher in non-solarized than in solarized soil and was lower in PLF-amended soil than in the other treatments. The beneficial effect of PLF on disease severity remained even in soil samples stored during eight months at 5°C. Root dry weight of onion seedlings and of rooted bulbs was significantly higher in PLF- and manure-amended soil than in the other treatments. A similar tendency was observed for leaf dry weight, in seedlings. It is concluded that PLF incorporated as a soil amendment, alone or in combination with solarization, has the potential to control pink root and to stimulate growth of onion plants.
Keywords*: Persian lilac, Brassicaceae species, chicken manure, leaf dry weight, root dry weight*

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I. Introduction

Soil-borne diseases are difficult to manage in onion and other horticultural crops. Resistant varieties frequently are not available. In conventional production systems, the chemical disinfestation of the seed and of the substrate is a valid means to control some, but not all of such diseases. However, such tools are not available in organic horticulture or similar systems, which depend largely on rotation and other management options, including solarization, biofumigation and organic amendments.

The combination of such approaches might be useful to tackle certain polyetic diseases, such as onion pink root. The causal agent, *Setophoma terrestris* (synonyms *Phoma terrestris*, *Pyrenochaeta terrestris*), is a soil-inhabiting fungus which infects the roots of many plant species, ~~however~~ although economically important diseases occur mainly in onion and other alliaceous crops. In Argentina, pink root is a major yield-limiting factor of the onion crop grown during the warm season (Klingner & Pontis, 1964; Kiehr et al., 1996).

The technique of solarization or solar heating of soil has been developed and introduced by Katan et al. (1976). It consists of covering the soil with a transparent polyethylene sheet during several weeks, mainly during the hot season. The resulting increase of soil temperature kills many of the soil-inhabiting pathogenic fungi, bacteria and nematodes as well as weed seeds and seedlings. However, there are also other factors involved in the elimination of pests and the enhancement of crop growth and yield after solarization (Chen & Katan, 1980; Aloj & Noviello, 1982; Gamliel & Katan, 1992).

The incorporation of green manure or plant residues into the soil is known to reduce the intensity of many soil-borne diseases (Cook & Baker, 1983). According to the early investigations of Sanford in the 1920s (*fide* Garrett, 1965), this effect in part is due to a non-specific mechanism as the added organic matter may increase the overall activity of certain microorganisms which in turn control soil-borne plant pathogens, by mechanisms such as competition or parasitism.

However, some of the incorporated plant remains also may exert other, more specific effects, such as the build-up of toxic compounds. This is the case with members of the Brassicaceae and some related families the tissues of which are rich in glucosinolates. These sulfur compounds, when degraded, release volatile isothiocyanates and related substances, which are toxic to pathogenic fungi and other microorganisms, nematodes and germinating seeds (Lewis & Papavizas, 1971). This sanitation effect is enhanced by covering the soil with a plastic sheet which retains the volatiles for more time in the soil environment (Ramirez-Villapudua & Munnecke, 1987; Zavaleta-Mejía & Rojas, 1990; Deadman et al., 2006; Guerrero et al., 2010). Kirkegaard et al. (1993) have coined the term biofumigation for this approach. A similar effect as with brassica residues may be achieved with other organic amendments which eventually release toxic volatiles such as ammonia and other nitrous compounds (Lazarovits et al., 2001). Thus, the term biofumigation should not be limited to the application of plant materials obtained from brassicaceous plants only.

Persian lilac or chinaberry (*Melia azedarach* L., Meliaceae), a close relative of the neem-tree (*Azedarach indica* A. Juss.), is an exotic tree of Asian origin much cultivated as a street tree in Argentina and many other countries. It produces a large number of fruits which remain on the tree throughout the winter season and can be easily collected. The fruits and leaves of Persian lilac, incorporated into the soil, enhance the growth of onion seedlings and reduce the severity of pink root caused by *Setophoma terrestris* (Kiehr et al., 1995), while the extracts of different plant organs inhibit diverse fungi (Carpinella *et al*., 1999; 2003; Ashraf & Javaid, 2007; Shafique et al., 2007).

We studied the effect of Persian lilac fruits and other organic amendments combined with a transparent polyethylene covering of the soil to control pink root of onion as a model pathosystem.

II. Material and Methods

Two field trials were carried out on a sandy soil (Table 1) at Bahía Blanca, Argentine (38º 44' S; 62º 16' W).

Table 1. Chemical characterisation of soil used onion plants trials.

MO	CE	pH	Nt	Pe	Kdisp
%	dS m^{-1}		%	ppm	
1.93	0.58	7.7	0.094	19.1	414.25

Abbreviations: MO, materia orgánica; CE, conductividad eléctrica; Nt, Nitrógeno total; Pe, fósforo extractable; K, potasio .

The experimental plots were weeded, drip-irrigated and inoculated with *S. terrestris*. Combined inocula were used, made up of seven highly aggressive isolates of *S. terrestris* in the first trial and ten isolates in the second trial. The inoculum was produced by mixing two times autoclaved wheat grains with PDA (Potato Dextrose Agar) cultures of the fungus isolates. This mixture was incubated in sterile conditions in the laboratory during 20 days. The inoculum was incorporated in the upper 10 cm of the soil, at a rate of 1 kg m^{-2} in the first and of 0.8 kg m^{-2} in the second trial. After an incubation period of 10 days in the field, the organic amendments were incorporated into the upper soil layer.

In the first trial, the following amendments were used: T_1: fruits of Persian lilac (PLF) (*Melia azedarach*; 2 kg m^{-2}); T_2: green residues of broccoli (Bro) (*Brassica oleracea* var. *italica*; 5 kg m^{-2}); T_3: green residues of radish (Rad) (*Raphanus sativus*; 5 kg m^{-2}); T_4: fresh chicken manure (Man) (7 kg m^{-2}). Once incorporated they were irrigated and covered with a transparent polyethylene sheet (100 µm). There was an un-amended control, with (T_5) and without polyethylene cover (T_6). The plots were drip-irrigated and remained covered during 25 days in late spring (november). There were three replicates with the plots distributed totally at random.

The second trial was carried out in a similar manner, but during summer, maintaining the polyethylene cover during 24 days. The amendments included: T_1, T_6: fruits of Persian lilac (PLF2) (2 kg m^{-2}); T_2, T_7: fruits of Persian lilac (PLF5) (5 kg m^{-2}), T_3, T_8: green residues of cauliflower (Cau) (*Brassica oleracea* var. *botrytis*; 5 kg m^{-2}) and T_4, T_9: fresh chicken manure (Man) (5 kg m^{-2}) as well as an un-amended control (T_5, T_{10}). Half of the plots were covered with polyethylene (T_1-T_5) and the other half were not (T_6-T_{10}), resulting in a two-factorial design with three replicates.

Soil temperature was measured daily with a digital multifunction thermometer, at 8:00 and and 15:00 hs, at a depth of 1, 5 and 10 cm (1. trial) and of 3 and 10 cm (2. trial).

After removing the polyethylene sheets the soil of each plot was handhoed repeatedly during the next days. Soil samples were taken from the upper 10 cm of each plot, mixed thoroughly, sieved through a 5 mm-mesh and stored in plastic bags until use. Onion seedlings, cv. Valcatorce, were grown from seeds superficially sterilized with NaOH, in moistened filter paper. Sixteen uniform healthy-looking plantlets were pricked out in plastic pots filled with the soil samples, three for each plot, and held in a growth room, at about 27°C. The pots were distributed totally at random. Two similar tests were carried out; the first one immediately after field sampling and the second one after eight months storage of soil aliquots in a cold chamber held at 5°C.

Survival of plants was recorded 21, 37 and 50 days after transplanting, in the first test, and after 8, 22, 36 and 44 days, in the second test. Plants were harvested after 50 (1. test) or 47 days (2. test) and washed carefully. The roots were evaluated for the intensity of reddish discoloration (pink root symptom) and root destruction, using an intensity scale reaching from 0 to 3 (0 = not visibly affected, 1 slightly, 2 moderately, 3 severely affected). Severity of both variables was calculated according to the formula: Severity = $(0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3)/(n_0 + n_1 + n_2 + n_3) \times 3$, where n_x is the number of seedlings in each category. Roots and leaves were severed, dried separately at 60°C for one day and weighed.

In the second trial, medium-sized onion bulbs, cv. Valcatorce, were used instead of seedlings. The loose dry bulb scales and roots of healthy-looking bulbs were removed and the remaining outer dry scale was rinsed with ethanol and flamed superficially in the region of the disk. Such bulbs were planted in plastic pots filled with the sampled soil. Each experimental unit consisted in 36 planted bulbs. The pots were distributed at random in an artificially illuminated growth cabinet, at 28+/-3°C.

The rooted bulbs were harvested after 35 days determining fresh weight of leaves, and degree of root coloration and destruction as well as root dry weight.

The data were transformed, when necessary, and submitted to one-way ANOVA, in the first trial, and two-way ANOVA, in the second trial. In the case of significant differences, the mean values of the treatments were compared using the Fisher`s LSD test at 5% (Di Renzo, et al., 2017). Root discoloration and destruction, as categorical variables, were not submitted to statistical analysis.

III. Results

Soil temperature

In both trials, soil temperatures were consistently higher at 15:00 than at 8:00 hs of daytime. At 15:00 the upper soil layers had clearly higher temperatures than the deeper ones. In trial 1 (November), there was a difference of about 10 ºC between 1 cm and 10 cm soil depth in the plastic-covered plots. In trial 2 (February), the differences between 3 and 10 cm were about 6 ºC in the non-covered and about 11 ºC in the covered treatments. At 8:00 the differences due to soil depth were less pronounced (Tables 2 and 3).

In the first trial, the general mean temperature (all days, hours, soil depths and replicates) of the non-covered and non-amended treatment was 26.4 ºC, that of the polyethylene-covered non-amended one was 32.0 ºC. In the second trial, the corresponding values were 26.3 and 33.8 ºC, respectively. That means a net increase of 5.6 ºC in the first and 7.5 ºC in the second trial, due to solarization only.

Table 2. Mean soil temperature (°C) at different depths and daytimes. Trial 1.

Treatment	Amendment	Plastic cover	8:00			15:00			General mean
			1 cm	5 cm	10 cm	1 cm	5 cm	10 cm	
1	PLF	yes	27.7	25.3	25.5	50.1	45.4	39.8	35.6
2	Bro	yes	24.8	23.6	24.3	48.6	44.4	39.3	34.2
3	Rad	yes	26.0	24.3	24.6	47.8	43.2	38.3	34.1
4	Man	yes	27.9	26.0	26.8	50.3	45.9	40.6	36.3
5	None	yes	25.4	23.6	23.9	43.8	39.7	35.5	32.0
6	None	no	22.4	19.6	19.8	35.0	32.2	29.5	26.4

Abbreviations: PLF, fruits of Persian lilac 2 kg m^{-2}; Bro, green residues of broccoli, 5 kg m^{-2};Rad, green residues of radish, 5 kg m^{-2}; Man, fresh chicken manure, 7 kg m^{-2}.

In the first trial, all organic amendments increased the general mean temperature over that of the non-amended control. The difference was greatest with 7 kg m^{-2} of chicken manure, followed by 2 kg m^{-2} of PLF, and smallest with 5 kg of the two brassicacea each (Table 2). In the second trial, the difference was greatest with PLF5, followed by Man and PLF2, while 5 kg of cauliflower did not elevate the temperature in comparison with the non-amended control (Table 3).

Table 3. Mean soil temperature (°C) at different depths and daytimes. Trial 2.

Treatment	Amendment	Plastic cover	8:00		15:00		General mean
			3 cm	10 cm	3 cm	10 cm	
1	PLF2	no	19.9	22.6	35.4	29.0	26.7
2	PLF5	no	20.5	24.0	37.1	31.2	28.0
3	Cau	no	19.6	22.1	34.7	28.3	26.2
4	Man	no	20.1	22.7	36.5	30.0	27,3
5	None	no	19.5	21.9	35.2	28.6	26.3
6	PLF2	yes	24.6	28.4	48.7	37.4	34.8
7	PLF5	yes	25.9	29.8	50.6	38.4	36.2
8	Cau	yes	24.3	27.2	47.2	36.7	33.9

9	Man	yes	25.1	28.4	48.5	38.1	35.0
10	None	yes	24.1	27.0	47.3	36.8	33.8

Abbreviations: PLF2, fruits of Persian lilac 2 kg m^{-2}; PLF5, fruits of Persian lilac, 5 kg m^{-2} ; Cau, green residues of cauliflower, 5 kg m^{-2};Man, fresh chicken manure, 5 kg m^{-2}.

Seedling tests (Trial 1)

Two onion seedling tests were carried out with soil samples taken from the field plots of the first trial, the first one shortly after soil sampling and the second one after eight months storage at 5º C.

Table 4. Survival rate (%) (SUR), root destruction (RDES) and root discolouration (RDIS) of onion seedlings at 50 days (1. test) and 44 days (2. test) after transplanting.

Treatment	Amendment	Plastic cover	1. test			2. test		
			SUR	RDES	RDIS	SUR	RDES	RDIS
1	PLF	yes	65	1.1	0.6	91* b	0.2	0.6
2	Bro	yes	51	1.2	0.6	81 b	0.9	0.9
3	Rad	yes	56	1.1	0.6	79 b	1.1	1.0
4	Man	yes	58	1.0	0.4	43 a	0.9	1.0
5	None	yes	69	1.1	0.8	87 b	1.0	1.0
6	None	no	50	1.8	0.9	85 b	0.8	1.0

* Values in the columns followed by the same letter do not differ for Fisher's DMS at 5%.
Abbreviations: PLF, fruits of Persian lilac 2 kg m^{-2}; Bro, green residues of broccoli, 5 kg m^{-2};Rad, green residues of radish, 5 kg m^{-2}; Man, fresh chicken manure, 7 kg m^{-2}.

As can be seen in Table 4, survival rate was not significantly affected in the first test. However, in the second one, chicken manure had a much lower percentage of surviving plants than the other treatments.

Root destruction and root discolouration, as categorical variables, were not submitted to statistical analysis. In the first test, root destruction was higher in the unamended nonsolarized soil than in any of the solarized treatments. There was a similar tendency with respect to root discoloration (Table 4). In the second test, carried out after eight months storage, only the Persian lilac-amended soil remained active in protecting the roots from destruction and pink root discoloration (Table 5).

In the first test, leaf dry weight and total dry weight were significantly higher in the solarized treatments than in the nonsolarized control; Persian lilac was the most effective amendment. In the second test, only Persian lilac had significantly higher root, leaf and total dry weights than the control (Table 5). The proportion of root dry weight to total dry weight was not significantly affected by the different treatments (Table 5).

Table 5. Root (RDW), leaf (LDW) and total dry weight (TDW) (mg per plant), as well as proportion RDW/ TDW. Trial 1; first and second seedling test.

Treatment	Amendment	Plastic cover	1. test				2. test			
			RDW	LDW	TDW	RDW/TDW	RDW	LDW	TDW	RDW/TDW
1	PLF	yes	0.76	5.10*c	5.86* b	0,13	0.69*b	2.96*c	3.65*b	0.18
2	Bro	yes	0.90	3.79 ab	4.69 ab	0,19	0.47 ab	2.05abc	2.52 ab	0.17
3	Rad	yes	0.68	4.65 bc	5.33 b	0,13	0.36 a	1.68 ab	2.04 a	0.16
4	Man	yes	0.95	4.85 bc	5.80 b	0,16	0.54 ab	2.75 bc	3.29 ab	0.16
5	None	yes	1.07	4.44 bc	5.50 b	0,19	0.34 a	1.57 a	1.93 a	0.17
6	None	no	0.56	2.73 a	3.30 a	0,17	0.40 ab	1.57 a	1.98 a	0.20

* Values in the columns followed by the same letter do not differ for Fisher's DMS at 5%.
Abbreviations: PLF, fruits of Persian lilac 2 kg m^{-2}; Bro, green residues of broccoli, 5 kg m^{-2};Rad, green residues of radish, 5 kg m^{-2}; Man, fresh chicken manure, 7 kg m^{-2}.

Onion bulb test (Trial 2)

In the second trial, onion bulbs were rooted in the soil samples collected from the field plots.

Table 6. Root destruction (RDES), root dry weight (RDW) and leaf fresh weight (LFW) of rooted onion bulbs. Second trial.

Treatment	Amendment	Plastic cover	RDES	RDW	LFW
1	PLF2	no	1.3	0.49*ab	55.62
2	PLF5	no	0.9	0.53 abc	55.77
3	Cau	no	2.0	0.55 abc	51.67
4	Man	no	1.7	0.52 abc	53.86
5	None	no	1.9	0.52 abc	44.09

6	PLF2	yes	0.8	0.47 ab	50.66
7	PLF5	yes	0.5	0.63 bc	58.32
8	Cau	yes	1.0	0.59 abc	53.66
9	Man	yes	0.8	0.82 c	60.84
10	None	yes	0.9	0.41 a	48.60

* Values in the columns followed by the same letter do not differ for Fisher's DMS at 5%.
Abbreviations: PLF2, fruits of Persian lilac 2 kg m^{-2}; PLF5, fruits of Persian lilac, 5 kg m^{-2} ; Cau, green residues of cauliflower, 5 kg m^{-2};Man, fresh chicken manure, 5 kg m^{-2}.

Root destruction was not submitted to statistical analysis (see above). However, from the figures (Table 6), it can be seen, that the degree of root desintegration is markedly higher in the non-solarized treatments than in the solarized ones. Similarly, soils amended with Persian lilac fruits showed the lowest values of root destruction. Only very few and scattered plants exhibited faint pink root symptoms, thus data of root discoloration are not shown.

The values of root dry weight were log-transformed before being submitted to ANOVA. There was no significant effect when comparing solarized with non-solarized treatments (p=0.21). However, there were significant differences between amendments (p=0.005). There was also a significant interaction between the two factors (p=0.017). The comparison of means shows that, in the covered plots, the amendments chicken manure and the high dosis of Persian lilac have a significantly higher root dry weight than the un-amended control. In the non-covered plots there were no differences between the different treatments (Table 6).

No significant differences were found with respect to leaf fresh weight neither concerning plastic-covering (p=0.76) nor amendments (p=0.40). The lowest values were found in the unamended control plots (Table 6) suggesting that the organic amendments had a beneficial effect on leaf growth.

IV. Discussion

In the present experiments, two principles of soil-borne disease control have been applied: 1. incorporation of different organic amendments into the soil and 2. covering the soil with a transparent plastic sheet (solarization). The combination of both gives place to, 3. a biofumigation effect with some of the amendments. Possible effects on both, plant and pathogen, as well as on different components of the environment, biotic and abiotic ones, may be expected. Therefore, physical (soil temperature) and biological traits (plant survival, root symptoms, plant growth and partition) were measured.

Covering the soil with a polyethylene sheet increased soil temperature. The differences recorded lie within the range reported in other places (Porter and Merriman, 1983; Katan, 1985; Ghini et al., 1994). The time of tarping the soil in our two experiments, 25 and 24 days, is at the lower limit of the recommended time period. In addition, in the first trial the treatment was carried out in November where solar intensity might not be very effective. Thus, extending the period of solar heating up to six or eight weeks, preferentially in the months of January-February, is expected to improve the beneficial effects of the treatment. Deadman et al. (2006) concluded that the effects on pathogen population levels, disease incidence and plant growth in cucumber (*Cucumis sativus* L.) were greater during summer growing seasons than during the winter. When biosolarization with *Brassica carinata* residues was initiated in autumn, disinfectant efficacy decreased and the survival of *Phytphthora capsici* inoculum increased using either pellets alone or mied with fresh sheep manure (Guerrero et al., 2010).

The incorporation of organic amendments, with and without plastic cover, also raised soil temperature. This effect might be due to the increase of exothermic metabolic activity due to the organic matter added. At an equal dose, fruits of Persian lilac is clearly the most effective of the amendments tested in raising soil temperature, thus, it is concluded, that the latter amendment has an additional specific effect, possibly due to the release of certain substances. The incorporation of three different brassicaceous residues is much less effective than Persian lilac or chicken manure. The increase of temperature in solarized soil is known to inactivate soilborne pathogens (Katan et al., 1976), However, there are also other factors involved in the elimination of pests and the enhancement of crop growth and yield after solarization, for example an increase in organic matter and mineral nutrients in the treated soil (Chen & Katan, 1980). In addition, the exudates of roots and seeds of tomato have been shown to change their composition in solarized compared to nonsolarized soil, facilitating the colonization with fluorescent pseudomonads and reducing that of fungi in the rhizosphere (Gamliel & Katan, 1992).

Soil solarization, alone or in combination with other treatments, has been demonstrated to effectively control pink root disease and to enhance plant growth and yield of onion in different places and situations (Katan et al., 1980; Rabinowitch et al., 1981; Hartz et al., 1989; Cho et al., 2000; Lee et al., 2007). In the present experiments, solar heating alone, without taking into account the effect of amendments, improved the survival rate, increased the leaf and total dry weight of seedlings and reduced the root destruction rate in seedlings and rooted bulbs.

The organic amendments tested in the present investigation only slightly affected survival rate, root destruction and dry weight in the seedlings test of the first trial. In the rooted bulbs, Persian lilac fruits and chicken manure increased root dry weight significantly. There was no remarkable effect of the brassicaceous amendments at all.

It has been shown that different extracts from fruits, seeds and leaves of Persian lilac exhibit fungistatic and fungicidal effects in vitro (Carpinella et al., 1999; 2003; Ashraf & Javaid, 2007; Javaid & Rehman, 2011; Neycee et al., 2012). However, investigations in vivo so far are extremely rare. Leaf extracts have been shown to affect the survival of *Alternaria alternata*, *Fusarium solani* and *Cladosporium* sp. on wheat seeds (Shafique et al., 2007). In experiments carried out under greenhouse conditions, soil amendments of Persian lilac fruit have been shown to stimulate the growth of young onion plants and to reduce the severity of pink root in this crop (Kiehr et al., 1995).

V. Conclusion

This background, together with the results reported here, do merit further investigations on the usefulness of Persian lilac, in combination with other approaches, in the control of onion pink root and other plant diseases.

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