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RESEARCH PAPERS

The "stanchezza" (soil sickness) of sweet basil

Andrea Minuto[1], Giovanni Minuto[1], Quirico Migheli[2], Maria Lodovica Gullino[1] and Angelo Garibaldi[1]

[1]Dipartimento di Protezione e Valorizzazione delle Risorse Agroforestali - Patologia vegetale Università, Via Leonardo da Vinci 44, 10095 Grugliasco (Torino), Italy
[2]Dipartimento di Protezione delle Piante, Università, Via Enrico De Nicola 9, 07100 Sassari, Italy

Summary. Seven glasshouse experiments were set up in 1995–1999 to evaluate the efficacy of various treatments in reducing the incidence of "stanchezza" (soil sickness) of sweet basil, an emerging phytopathological problem in the main basil-growing areas of Italy. Complete or partial replacement of the soil was generally effective in increasing plant size and fresh weight of both roots and aerial parts. Steam disinfestation of the soil abolished the symptoms of "stanchezza" during the first growth cycle, but its effect was reduced on the second crop. Leaching the substrate with water and deep tilling had no or only limited effect on plant growth. Amendment with a commercial biostimulating product containing organic phenolic compounds had only partial efficacy. Bench solarization was the most effective means of control of the syndrome and its effect continued for the second crop cycle. No significant reduction of "stanchezza" was achieved with broad-spectrum antibiotic or fungicide mixtures. No significant differences were detected between bacterial and fungal populations in a soil with "stanchezza" and a non-cultivated soil. Further investigation is now required to shed light on the mechanisms underlying this emerging phytopathological problem.

Key words: *Ocimum basilicum*, phytotoxicity, soil sickness, soil microflora, steam disinfestation.

Introduction

Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop in several Mediterranean countries including Italy, France and Israel. In Italy, approximately 80 ha are grown annually with this crop, most production being concentrated in the Riviera Ligure (Garibaldi *et al.*, 1997), where about 50 specialized farms with an average area of 1 ha grow basil year-ound, reaching gross incomes of € 600,000 per ha per year. Basil is grown as a direct-seeded crop by using 10–12 g (1 g = 600 to 800 seeds) of seeds per m^2, resulting in 5,000–6,000 plants per m^2. It is often overseeded after the first two to three harvests of the previous crop and is repeat-harvested after re-growth. This practice results in a total amount of 30–40 g of seeds per m^2 over a crop cycle typically lasting 6–7 months.

Several soilborne pathogens have been described on basil and intensive cultivation favours

Corresponding author: Q. Migheli
Fax: +39 079 229316
E-mail: migheli@uniss.it

some diseases, such as *Fusarium* wilt and crown rot, *Rhizoctonia* and *Sclerotinia* basal rots and *Pythium* damping-off (Garibaldi *et al.*, 1997).

An emerging problem in basil cultivation is the disease known as "stanchezza" (literally "fatigue") to basil growers. In recent years it has been observed that basil cannot be established for more than 1–2 cycles in stands that have been harvested before. The first symptoms, visible in both soil- and bench-grown basil, are: reduced germination, slow penetration of roots into the soil, and poor growth during and after germination. Subsequently, the interval between formation of the cotyledons and emission of the first true leaves is abnormally long, leaf chlorosis and yellowing occur on surviving plants, and the total biomass and yield are significantly reduced.

The symptoms of this soil sickness can be avoided by disinfesting the soil with methyl bromide or steam or by replacing the soil in which the previous crop was grown with non- cultivated soil or substrate. However, the high cost of steaming and the increasing limitations placed on the use of methyl bromide (Ristaino and Thomas, 1997) make it necessary to develop effective alternatives for disease control.

The aim of this work was primarily to test the efficacy of different means of control, permitting more basil crops to be grown on the same soil by limiting the deleterious effects of "stanchezza", and secondly to provide insight into the mechanisms underlying this soil disease.

Materials and methods

Induction of "stanchezza", plant material and growing conditions

Seven experimental trials were carried out between October, 1995 and April, 1999 in a glasshouse at the Centro Regionale di Sperimentazione ed Assistenza Agricola, located at Albenga (Liguria, Italian Riviera). Minimum temperatures in the glasshouse were 12–16°C, maximum temperatures 25-31°C, depending on the season (see Tables 1–6 for timing of each trial).

The basil cv. Genovese Gigante, widely grown in the Liguria region, was used in all the trials. Seeds certified as *Fusarium oxysporum* f. sp. *basilici*-free (S.A.I.S., Cesena, Italy), were sown (4 g m^{-2}) in a soil (18-cm depth, pH 6.2–6.5; Turco, Albenga, Italy) composed of 60:10:20:10 peat-moss:composted pine and poplar bark:manure:clay (v:v), water holding capacity 50% of the vol., which was amended with a $N:P_2O_5:K_2O$ 15:9:15 slow-release fertilizer (25 g m^{-2}) 2 days before sowing.

Plots (5 m^2) were distributed in a complete randomized block design with 3 replicates in Trials 1–3, a single plot per treatment in Trials 4–5, and 4 replicates per treatment in Trials 6–7.

In order to induce the phenomenon of "stanchezza" experimentally, the growing period was extended until plants reached a height of about 25 cm. Plants were then manually harvested, basil seeds were directly re-sown, and a second crop was grown as before. Agricultural practices normally adopted by local growers were followed. After two growth cycles, the substrate was treated as described in the next paragraph. A $N:P_2O_5:K_2O$ 20:10:10 fertilizer (500 mg l^{-1}) was distributed continuously at each watering.

Substrate treatments

In each of the first three trials, soil affected with "stanchezza" was subjected to the following treatments (Tables 1–3): 1) steam disinfestation at 80°C for 30 min; 2) inundation (leaching) with 50 l of water m^{-2}; 3) complete replacement with virgin soil; 4) partial replacement with 30% of virgin soil; 5) deep tilling (18 cm); 6) amendment with Co-Actyl-N (Timac Italia, Milano, Italy), a product claimed as "soil bioregenerating and root development biostimulating agent", which contains organic phenolic compounds (26% w/w organic carbon) and is used by some basil growers in Italy to alleviate the symptoms of "stanchezza" on sweet basil. The first two trials were carried out independently, while in the third, basil was re-sown on the same soil as that used in the second trial, without the soil being treated in any way.

Trials 4 and 5 were carried out to evaluate the effect of bench solarization on basil grown in soil with "stanchezza". In trial 4, a plot with artificially induced "stanchezza" and a plot with previously non-cultivated soil were solarized as described elsewhere (Gullino *et al.*, 1998) before sowing, and compared with two non-solarized controls. In trial 5, the residual effect of solarization was measured by re-sowing basil on the same plots as in trial 4, without treating the soil.

Trial 6 was designed to evaluate if broad-spectrum mixtures of fungicides and antibiotics alleviate the symptoms of "stanchezza". Soil with artificially induced "stanchezza" and non-cultivated soil were treated by incorporating either a mixture of fungicides [(g m^{-2} a.i.): metalaxyl, 0.5; benomyl, 3; prochloraz, 0.5; tolclofos methyl, 2] or a mixture of antibiotics [(g m^{-2} a.i.): tetracycline hydrochloride, 2.5; streptomycin sulphate, 2.5] one week before sowing. In trial 7, the same plots were re-sown with a second basil crop and the same fungicide and antibiotic mixtures were repeated as described, but only in one half of each replicate (0.8 m^2).

Population densities

Relevant microbial populations were assayed in a soil in which "stanchezza" was artificially induced by repeated cropping and in previously non-cultivated soil. Samples (1 g) from each soil were taken in quadruplicate, ground in a mortar and dispersed in 100 ml sterile distilled water. After shaking (250 rpm) for 15 min, appropriate dilutions of each sample were plated on the following substrates: potato dextrose agar (PDA, Merck, Darmstadt, Germany) amended with streptomycin sulphate (100 µg ml^{-1}); *Fusarium* selective medium (Komada, 1975); a selective medium for oomycetes based on P_{10}VP medium (Tsao and Ocaña, 1969) modified by the addition of 15 µg ml^{-1} benomyl (Benlate, 50% a.i., Du Pont, Wilmington, USA), rifampicin (10 µg ml^{-1}) and penicillin (15 µg ml^{-1}) but lacking vancomycin; and a base medium for isolating bacteria consisting of (g l^{-1}) yeast extract, 5; bacto-peptone, 5; glucose, 10; agar, 20 (Merck). Plates were incubated at 25–28°C and developing fungal and bacterial colonies were counted daily.

Evaluation of results and statistical analysis

The effect of the treatments on plant growth was evaluated by measuring plant height (20 groups of 5 plants for each replicate treatment) at monthly intervals until harvest-time. At the end of the trials, the f wt of roots and aerial plant organs (10 groups of 5 plants for each replicate treatment) was determined. Data from experiments 1–3 and 6–7 were subject to an analysis of variance followed by Duncan's multiple comparison test (P=0.05). In experiments 4–5 the mean plant height and the f wt of the aerial plant organs were measured as described from a single replicate treatment and the data were expressed as mean plant height and mean f wt per m^2.

Data from quadruplicate plate counts were subjected to analysis of variance followed by Duncan's multiple range test (P=0.05).

Results

In trial 1, steam disinfestation was most effective in increasing plant height, from 7.7 cm in the untreated control to 18.4 cm (Table 1). Complete

Table 1. Effect of various soil treatments on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height and f. wt. of the roots and aerial plant organs 60 days after sowing on October 30, 1995 (Trial 1).

Treatment[a]	Plant height (cm)[b]	Fresh weight	
		aerial parts (g)[c]	roots (g)[c]
Control	7.7±1.8 a[d]	1.5±0.5 a	0.3±0.1 a
Steam	18.4±1.2 c	4.7±0.6 c	0.4±0.0 b
Inundation	7.0±1.8 a	1.8±0.7 a	0.3±0.1 a
Complete replacement	15.5±1.1 b	3.3±0.3 b	0.3±0.0 a
Partial replacement	15.8±1.2 bc	3.3±0.3 b	0.3±0.0 a
Deep tilling	8.7±2.4 a	1.9±0.2 a	0.3±0.0 a
Co-Actyl-N	6.7±0.4 a	1.1±0.1 a	0.3±0.0 a

[a] See Materials and methods for details.
[b] Mean value measured on 20 groups of 5 plants for each replicate treatment.
[c] Mean value measured on 10 groups of 5 plants for each replicate treatment.
[d] Mean of three replicate values ± S.D. Mean values in each column followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test.

and partial replacement of the soil significantly improved plant size, to 15.5 and 15.8 cm respectively, while inundation, deep tilling and amendment with Co-Actyl-N had no significant effect on plant growth (Table 1). The factors influencing the f wt of aerial parts followed the same pattern, with steam as the most effective means of control, followed by complete or partial replacement, and the other treatments having no significant effect. Fresh weight of the roots was significantly different from the control only after steam disinfestation of the soil.

Similarly, in trial 2, only steam treatment significantly increased plant height, from 15.4 to 20.7 cm (Table 2). Complete and partial soil replacement had a slight but not significant effect, while both washing the soil with water and deep tillage had no effect. Amendment with Co-Actyl-N on the other hand reduced plant height significantly to 11.3 cm. The effect on plant f wt was significant for steam treatment and partial soil replacement only (Table 2).

Trial 3 consisted in sowing a second basil crop in the soil previously used for trial 2, in order to evaluate any residual effect of the soil treatments given in trial 2. Both plant height and the f wt of aerial plant parts were significantly affected only when the soil was completely replaced before the previous crop cycle (Table 3). The f wt of root parts was increased by partial and complete soil replacement and also by Co-Actyl-N- amendment.

Trials 4 and 5 evaluated the effect of bench so-

Table 2. Effect of various soil treatments on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height and f wt of the roots and aerial plant organs 60 days after sowing on January 30, 1996 (Trial 2, first cycle).

Treatment[a]	Plant height (cm)[b]	Fresh weight	
		aerial parts (g)[c]	roots (g)[c]
Control	15.4±1.7 b[d]	3.8±0.8 a	0.5±0.2 a
Steam	20.7±1.7 c	6.9±0.7 b	0.9±0.1 bc
Inundation	14.7±2.1 ab	3.7±0.7 a	0.6±0.3 ab
Complete replacement	17.7±3.2 bc	5.2±2.1 ab	0.9±0.2 bc
Partial replacement	18.3±3.0 bc	6.5±1.6 b	1.1±0.1 c
Deep tilling	14.3±0.3 ab	3.0±0.5 a	0.3±0.1 a
Co-Actyl-N	11.3±1.7 a	3.3±0.5 a	0.9±0.2 bc

[a-d] See Table 1.

Table 3. Effect of various soil treatments on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height and f wt of the roots and aerial plant organs 60 days after sowing on April 4, 1996 (Trial 3, first cycle).

Treatment[a]	Plant height (cm)[b]	Fresh weight	
		aerial parts (g)[c]	roots (g)[c]
Control	19.1±6.7 a[d]	2.7±2.0 ab	0.5±0.3 a
Steam	29.2±2.5 a	3.9±1.0 ab	0.7±0.3 a
Inundation	17.8±11.9 a	1.5±1.1 a	0.3±0.2 a
Complete replacement	42.2±0.9 b	10.4±2.8 c	1.6±0.2 c
Partial replacement	25.2±7.9 a	5.1±1.4 b	1.1±0.2 b
Deep tilling	26.7±0.9 a	3.0±0.3 ab	0.4±0.1 a
Co-Actyl-N	17.8±1.0 a	4.7±0.9 b	1.2±0.1 bc

[a-d] See Table 1.

larization on "stanchezza" of sweet basil. An increase in plant height and f wt as a consequence of solarization was evident in both soils, although the lack of replicated plots made it impossible to perform the statistical analysis (Table 4). Moreover, the effect of solarization continued into the second crop cycle, doubling the average plant height on soil with "stanchezza" (from 5.3 cm in the untreated control to 10.7 cm) and increasing the f wt from 152 to 945 g m^{-2} (Table 4).

No significant reduction in "stanchezza" was achieved with broad-spectrum antibiotic or fungicide mixtures. Plant height and the f wt of aerial parts and roots were not affected by the mixtures, while a significant reduction of growth was observed when basil was established on soil with "stanchezza" (Tables 5 and 6). A slight, although not significant, phytotoxic effect was recorded after the second antibiotic treatment (Table 6).

Bacterial and fungal populations in soil samples with "stanchezza" did not differ from corresponding populations isolated from non-cultivated soil. Total fungi isolated on PDA amended with oxytetracycline and streptomycin ranged from 1.5 to 1.8×10^5 colony forming units (cfu) g^{-1}. On media selective for *Fusarium* and for oomycetes, $2.1–2.8\times10^3$ and $5.6\text{-}6.0\times10^2$ cfu g^{-1} respectively were recovered. Total bacterial population ranged from 2.6 to 3.2×10^7 cfu g^{-1}. It is worth noting that in the plots with soil "stanchezza", plants were often attacked by the soil-borne basil pathogens *R. solani* and *F. oxysporum* f. sp. *basilici*, although the number of *F. oxysporum*-like colonies in virgin and sick soil samples was similar.

Table 4. Effect of bench solarization on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height (cm per plant) and f wt (g m^{-2}) of the aerial plant organs 30 days after sowing on August 20, 1996 (Trial 4, first cycle) and on October 1, 1996 (Trial 5, second cycle).

Soil affected by "stanchezza"	Solarization	First cycle		Second cycle	
		Plant height (cm)	Fresh weight (g m^{-2})	Plant height (cm)	Fresh weight (g m^{-2})
Yes	No	14.9	502	5.3	152
Yes	Yes	19.0	1,834	10.7	945
No	No	19.0	1,210	6.7	321
No	Yes	22.3	1,738	8.5	622

Table 5. Effect of soil treatment with two fungicide and antibiotic mixtures on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height and f. wt. of the roots and aerial plant organs 50 days after sowing on September 30, 1998 (Trial 6).

Treatment[a]	Soil with "stanchezza"	Plant height (cm)[b]	Fresh weight	
			aerial parts (g)[c]	roots (g)[c]
—	No	20.1±1.9 b[d]	2.8±0.1 b	0.6±0.0 b
Antibiotic mix[e]	No	19.8±1.5 b	2.5±0.2 b	0.7±0.1 b
Fungicide mix[f]	No	19.8±0.4 b	2.8±0.2 b	0.7±0.2 b
—	Yes	11.6±2.6 a	2.8±0.7 a	0.5±0.1 a
Antibiotic mix	Yes	14.0±2.8 a	2.4±0.4 a	0.4±0.1 a
Fungicide mix	Yes	11.5±2.8 a	2.7±0.7 a	0.5±0.1 a

[a-d] See Table 1.
[e] Antibiotic mixture consisted in (g m^{-2} a.i.): tetracycline hydrochloride, 2.5; streptomycin sulphate, 2.5.
[f] Fungicide mixture consisted in (g m^{-2} a.i.): metalaxyl, 0.5; benomyl, 3; prochloraz, 0.5; tolclofos methyl, 2.

Table 6. Effect of soil treatment with a fungicide and an antibiotic mixture on "stanchezza" (soil sickness) of sweet basil. Results are expressed as plant height and f wt of the roots and aerial plant organs 45 days after sowing on March 1, 1999 (Trial 7).

Treatment[a]	First treatment	Second treatment	Soil with "stanchezza"	Plant height (cm)[b]	Fresh weight	
					aerial parts (g)[c]	roots (g)[c]
—	—	—	No	34.8±0.8 b[d]	6.3±1.0 b	2.3±0.5 c
Antibiotic mix[e]	Yes	No	No	35.6±1.3 b	6.2±1.0 b	2.5±0.6 c
	Yes	Yes	No	32.6±1.9 b	6.1±1.1 b	2.2±0.7 bc
Fungicide mix[f]	Yes	No	No	37.8±1.0 b	6.2±1.2 b	2.4±0.3 c
	Yes	Yes	No	39.1±13.8 b	6.1±0.6 b	2.8±1.0 c
—	—	—	Yes	19.4±4.7 a	3.8±0.9 a	1.3±0.2 ab
Antibiotic mix	Yes	No	Yes	16.1±5.5 a	3.2±1.3 a	1.4±0.4 ab
	Yes	Yes	Yes	15.4±3.4 a	3.0±1.1 a	1.2±0.3 a
Fungicide mix	Yes	No	Yes	16.7±6.3 a	3.2±1.1 a	1.2±0.3 a
	Yes	Yes	Yes	17.7±5.9 a	3.7±1.0 a	1.4±0.2 ab

[a-d] See Table 1.
[e] Antibiotic mixture consisted in (g m^{-2} a.i.): tetracycline hydrochloride, 2.5; streptomycin sulphate, 2.5.
[f] Fungicide mixture consisted in (g m^{-2} a.i.): metalaxyl, 0.5; benomyl, 3; prochloraz, 0.5; tolclofos methyl, 2.

Discussion

"Stanchezza" is an emerging disease in the Liguria region, the main basil production area in Italy. Low germination, poor growth, leaf chlorosis and yellowing occur when basil is re-sown for more than 1–2 cycles into an existing basil stand. Several plant species, such as forage grasses, cereals and vegetables, have shown similar autotoxicity responses when re-establishment is attempted (Singh *et al*., 1999). In the case of alfalfa, soil-sickness was correlated with the release of phytotoxic compounds from three-day-old seedlings, with consequent inhibition of alfalfa seed germination and of seedling elongation (Guenzi *et al*., 1964; Miller, 1983, 1992; Chung and Miller, 1995; Chung *et al*., 2000). Similarly, autointoxication of rice plants in poorly drained soils in Taiwan is caused by several compounds released by decomposing rice straw (Chou and Chiou, 1979; Chou *et al*., 1981). Plant species which have recently been shown to suffer from autotoxicity problems include *Tagetes erecta* (Kaul, 2000) and cucumber (Yu and Matsui, 1997; Yu *et al*., 2000).

Although "stanchezza" of basil can be eliminated by treating the soil with methyl bromide, constraints in the use of this fumigant make it advisable to look for a reliable, cost-effective and ecologically sustainable alternative means of control.

In the present study, complete soil replacement was effective in controlling the disease, but this treatment in most cases is impossible for obvious economic and ecological reasons. The symptoms of "stanchezza" can however be reduced and even completely eliminated by soil steaming or solarization.

Bench solarization (Gullino *et al*., 1998) appears particularly attractive because of its low cost and high efficacy. It is effective also against *Fusarium* wilt and *Rhizoctonia* basal rot and can be easily applied by basil growers, as it enables the same soil to be reused and reduces the need for expensive soil-steaming devices. Moreover, the carry-over effect of bench solarization on the subsequent basil cropping cycle was not achieved by steam disinfestation. Unfortunately, the fact that only a single replicate per treatment was used in the two solarization experiments made proper statistical analysis of the data impossible. Future trials will have to examine more closely how solarization improves the health of intensively grown basil. Partial soil replacement and soil steaming can be regarded as alternatives to soil solarization for subsequent cropping cycles.

The effectiveness of both steaming and solarization in reducing "stanchezza" suggests that the disease is probably due to a complex of biotic and abiotic factors, such as phytotoxic compounds released into the soil by decomposing basil plants. At least some of these compounds are temperature-sensitive, and their presence does not significantly affect the soil microflora. A thorough evaluation of these compounds is needed to understand the role of their autotoxicity and the manner of their biodegradation. The longer “stanchezza”-protection of basil plants after soil solarization compared with soil steaming may be explained by the partial and selective effect of solarization on the soil microbial components (Katan and DeVay, 1991). Some of the surviving micro-organisms may have a role in the breakdown of phytotoxic metabolites accumulated during the previous cropping cycle.

Nonetheless, the hypothesis that phytotoxic compounds cause “stanchezza” does not square with the efficacy of methyl bromide in reducing symptoms on subsequent basil crops. Further investigation is required to shed light on the mechanisms underlying this emerging phytopathological problem.

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