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Running title: Management of lettuce Fusarium wilt

EFFECT OF DIFFERENT ORGANIC AMENDMENTS ON LETTUCE FUSARIUM WILT AND ON SELECTED SOIL-BORNE MICROORGANISMS

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Abstract: In this study we have evaluated the effect of different organic amendments on lettuce Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae* in pots under controlled conditions. We have also evaluated their effects on the density of the pathogen, on the total fungi and on fluorescent *Pseudomonas* spp. after two subsequent lettuce crops. We found a significant reduction in the severity of the symptoms of *F. oxysporum* f. sp. *lactucae* after the use of *Brassica carinata* pellets (reduction of between 52% and 79%) and compost (49% to 67% reduction), while *Brassica* green manure and cattle and chicken manure only provided partial control of Fusarium wilt. However, we also observed variations in effectiveness for the same treatment in repeated trials. In general, we observed an increase in *Pseudomonas* and a decrease in fungal populations in the growing medium, which was obtained by mixing a blonde sphagnum peat and a sandy loam soil with *B. carinata* pellet and compost after two consecutive cropping cycles. The prolongation of the *Brassica* and compost treatments, from 30 to 60 days, did not significantly affect disease severity, plant growth and the microbial population of the total fungi or *Pseudomonas*. We obtained the largest lettuce biomass in the non–inoculated growing medium amended with Brassica flour, chicken manure, *Brassica carinata* pellets and compost, as a consequence of fertilization. The treatment with *B. juncea* green manure, *B. carinata* (pellets and flour) and compost applied 30 days before planting led to promising results and merits further investigation for use under field conditions. Giovanna – both the past and present perfect could be used, but for various reasons, I think we should use the past throughout (also to change something!).

Introduction

The management of Fusarium wilt pathogens, which are responsible for severe losses on a number of economically important crops, is currently being investigated, with special attention being paid to the use of measures with a low environmental impact (Blok *et al*., 2000; Reuveni *et al*., 2002; Borrero *et al*., 2006; Klein *et al*., 2011; Gullino *et al*., 2012; Borrego-Benjumea 2014 a,b; Garibaldi *et al*., 2014a; Gilardi *et al.*, 2014a,b).

The use of organic amendments (compost, animal manure, vegetable residues, organic waste) on several soil-borne pathogens has been exploited intensively for many years and has received renewed interest after the phase-out of methyl bromide (Bailey & Lazarovits 2003; Bonanomi *et al.*, 2007; Klein *et al.*, 2011). Studies carried out under different environmental conditions have shown the capability of different members of the *Brassica* species, such as *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. carinata*, *B. campestris* and *B. oleracea*, to control several soil-borne pathogens (Matthiessen & Kierkegaard, 2006; Mazzola *et al.*, 2007; Motisi *et al.*, 2009; Hansen & Keinath, 2013). The biofumigation potential of the various species and cultivars of brassicas can vary: for instance, *Brassica juncea* cv. Pacific gold has been reported to have a higher biomass and glucosinolate content than seven other *B. juncea* cultivars (Antonious *et al.*, 2009). A similar specific cultivar suppression effect of *Rhizoctonia* root rot, due to *B. rapa* amendment, was described by Kasuya *et al*. (2006). Brassica plants have been applied as fresh plant material (green manure), a composite of *Brassica* plants, seed meal and dried plant material, either alone or combined with other disinfestation methods, against soil-borne pathogens in several pathosystems (Gamliel 2000; Matthiessen & Kirkegaard, 2006; Kasuya *et al*., 2006; Mazzola *et al*., 2007; Ascencion *et al*., 2015). Among the various organic amendments that are available, composts have been exploited intensively because of their efficacy in the management of soil-borne pathogens, and many examples of their role in disease suppression are well-known (Bonanomi *et al.*, 2007). Some examples of the positive effect of chicken compost, alone or combined with soil solarisation, have been reported against *Meloidogyne incognita* and *Pythium ultimum* on lettuce (Gamliel & Stapleton, 1993) and against tomato Fusarium wilt (Borrego-Benjumea *et al*., 2014a).

By monitoring the population densities of soil microorganisms and pathogens in soil, it is possible to obtain a better understanding of the complex effect of organic amendments or crop residues on soil microorganisms, including pathogens (Njoroge *et al*., 2008; Lu *et al*., 2010; Ferrocino *et al*., 2014; Ascencion *et al*., 2015). Several parameters have been explored in attempts to describe the suppressiveness proprieties of soil (Janvier *et al.*, 2007). The build-up of disease suppression could be related to chemical, physical and biological characteristics, or to specific microorganisms as well as to metabolic activity produced by several groups of microorganisms (Hoitink & Boehm, 1999;

Weller *et al*., 2002; Kasuya *et al*., 2006; Janvier *et al.*, 2007). Among these, *Pseudomonas* spp. has long been studied to evaluate its contribution to disease suppression in several cropping systems (Bonamomi *et al*., 2007; Hiddink *et al*.,2005; Weller *et al*., 2002). The density of *Pseudomonas* spp. has been associated with soils of low receptivity to Fusarium wilt and rot diseases in several crops (Alabouvette, 1990; Gamliel & Katan, 1993; Hiddink *et al*., 2005). Gamliel & Katan, (1993) observed disease suppression by Pseudomonads in solarised and non-solarised soil against *Fusarium oxysporum* f. sp. *vasinfectum*, *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici*, but not against *F. oxysporum* f. sp. *melonis*. Despite the many approaches that have been developed to evaluate the effect of organic amendments on soil-borne pathogens and to study their effect on soil microorganisms, further research is still needed in order to foster their practical implementation.

This study was carried out, in pot trials, in order to evaluate the effect of different organic amendments on lettuce Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae.* This pathogen is seed-transmitted and occurs in most countries in which lettuce is grown and causes serious economic losses (Garibaldi & Gullino, 2010, Matheron & Gullino, 2012). The effect of incorporating different organic amendments on the density of *F. oxysporum* f. sp. *lactucae*, on the total fungi and on fluorescent *Pseudomonas* spp. after two subsequent lettuce crops has also been evaluated.

Materials and methods

Layout of trials and plant material

Four trials were carried out in 20-L plastic pots (56.5 x 42 x 20 cm) filled with a mixture (30:70 v/v) of a blonde sphagnum peat (pH 6.1, C-total 175 g/kg, N-total 7 g/kg, organic matter 32.4%, Turco, Albenga) with a sandy loam soil, which had the following characteristics: sand, 68.8% ± 5;

silt, 6.8% ± 5; clay, 26% ± 5; pH, 7.1; organic matter content, 2.4%; cation exchange capacity, 5.7 meq100 g^{-1} soil. The growing medium was not steamed before use.

Lettuce plants of cv. Crispilla, which is highly susceptible to Fusarium wilt (Gilardi *et al*., 2014 b), were transplanted 20-25 days after sowing into treated and untreated growing medium (20 plants/pot). Two subsequent crop cycles were carried out in the same growing medium. At the end of each cropping cycle, lettuce plants were removed from the soil, at the full maturity stage, for Fusarium wilt and yield evaluation. In all the trials, soil treatment with organic amendments was carried out for 30 days before the transplanting. A 60 day treatment with *Brassica*-derived products (flour, pellets and green manure) and compost was also tested in trials 3 and 4 (Table 1). The pots were maintained in a greenhouse at temperatures ranging from 25 to 28°C and watered daily.

Artificial inoculation with the pathogen and organic amendment application

One isolate of *Fusarium oxysporum* f. sp. *lactucae*, resistant to 10 mg L^{-1} of benomyl (FusLat10RB), was used. Resistance to benzimidazoles permits an easier re-isolation of the pathogen from an infested growing medium on a Komada semi-selective medium to which a benzimidazole fungicide has been added. A talc formulation of the *F. oxysporum* f. sp. *lactucae* strain was mixed with the soil at 1-6x10^4 Colony Forming Units (CFU) ml^{-1} 24 h before starting the treatments.

Immediately before starting the trials, the growing medium was irrigated with 3.5-L/pot, which corresponded to 75% of the soil moisture capacity. The treated growing medium was covered with transparent polyethylene (PE) sheets (50 μm thick) and sealed immediately after () application of the soil amendments, which had previously been incorporated into the soil to the full depth of the pots. The soil mulch was removed 30-60 days later. The treated and untreated growing medium were kept in a growth chamber at an air temperature that ranged from 28 to 30°C and a soil temperature that ranged from 26.5 to 28.7°C at a depth of 10 cm (Digital Data Logger EM50, Decagon Devices, USA).

Brassica carinata, used as defatted seed meal (Biofence, N organic 6%, P 2.2%, K 2%, organic C 52%, Triumph, Italy), and formulated as flour (Biofence 10, N organic 6%, P 2.2%, K 2%, organic C 45%, Triumph, Italy), was mixed in the soil at 2.5 g L^{-1}.

Brassica juncea (ISCI99) was sown at 1-1.2 gm^{-2} in plastic pots filled with sterile blonde Sphagnum peat. The pots were kept in a greenhouse at 24-26°C for 40-50 days until use, that is, the stage that corresponded to 80% of flowering. The plants were weighed, harvested, cut into 1 to 3 cm pieces, and incorporated into the soil, thus simulating a biofumigation treatment.

A compost (Ant's Compost, AgriNewTech, Torino, Italy), prepared from biodegradable municipal solid waste, derived from food and green waste, in a dynamic industrial system, and which had been allowed to compost for three months (pH = 7.5, moisture= 48%, Organic C = 20% d. m., Organic N = 1.2% d.m., P_2O_5 = 0.8% d.m., K_2O = 1.1% d.m.), was used at 10 kg m^{-2}.

The chicken manure was formulated in pellets (Organic N 3% P_2O_5 3%, FA.CO from Fantino G & M, Cuneo, Italy), while the cattle manure was obtained from local organic farms and used after 9 months of storage.

Quantification of the pathogen and of the selected microbial population in the soil

The soil dilution plating method was used to quantify the survived resistant pathogen and the selected microbial population in the soil (Ferrocino *et al*., 2014). Soil samples of 100 g were taken from each plastic pot at the end of the second cultivation cycle in trials 1 and 2 (Table 1). Soil sub-samples (5 g) were mixed for 30 min with 45 ml of Ringer's solution amended with 5 µl of Tween 20 (Sigma, Milan, Italy). Within 24 h after the sampling, 10-fold dilutions were prepared in Ringer's solution (Merck Millipore, Germany), and aliquots of 1 ml of the selected dilutions were spread in triplicate onto selected agar media. In order to evaluate the pathogen density after the soil treatment, the FusLat10RB strain of *Fusarium oxysporum* f. sp. *lactucae* was used and spread onto a semi-selective medium (Komada, 1975) to which 10 mg $L^{-1}$of benomyl had been added; the medium was then incubated at 20°C for 4 days. The total fungal population was evaluated using

Potato Dextrose Agar (PDA, Merck), to which 25 mg L^{-1} of streptomycin sulfate had been added. The total *Pseudomonas* spp. population was also evaluated using *Pseudomonas* agar with a cetrimide-fucidin-cephalosporin (CFC) supplement (Oxoid, Milan, Italy). The CFUs were counted 48h to 72 h after incubation at 23°C. The mean value of three independent plates is reported in Tables 2 and 3.

Fusarium wilt evaluation and analysis

The effectiveness of different treatments on the severity of *F. oxysporum* f. sp. *lactucae* on lettuce was evaluated weekly during the trials, and the wilted plants were counted and removed. In the final evaluation, 4 weeks after transplanting, the severity of symptoms was assessed using a disease severity (DS) scale, ranging from 0 to 100, where 0 = healthy plant, 25 = initial leaf chlorosis, 50 = severe leaf chlorosis and initial wilting symptoms during the hottest hours of the day, 75 = severe wilting and severe leaf chlorosis symptoms; 100 = plant totally wilted and leaves completely necrotic. In order to evaluate the effect of each treatment on plant growth, the total fresh plant biomass was weighed at the end of each crop cycle. Data were analysed to explore the correlations between the nominal reduction in fresh weigh of the plants grown in the inoculated soil (fresh weight of plants grown in treated and untreated non-inoculated soil– fresh weight of plants grown in treated and untreated inoculated soil) and the disease severity of the plants grown in the inoculated soil by calculating the Pearson's correlation coefficient (R).

The adopted experimental scheme was a completely randomized block design with three (trials 1 and 2) and four (trials 3 and 4) replicates per treatment.

The data were subjected to analysis of variance (ANOVA) and statistically analysed using Tukey's HSD test (P=0.05). Before the analysis, the population densities (CFUs/g of dry soil) were logarithmically transformed, while the disease severity data were arcsine transformed in order to normalize the distribution of the variance.

Results

Effect on disease severity

During trials 1 and 2, Fusarium wilt severity in the inoculated untreated control pots ranged from 62.1 to 63.3 in the first lettuce cycle and from 50 to 83.3 in the second cycle (Tables 2 and 3). Disease severity (DS) was lower in trial 3, where it ranged from 32 to 39.7 in the first cycle and from 33.4 to 37.5 in the second cycle, than in trial 4, where the values ranged from 44.4 to 59.6 in the first cycle and from 40.9 to 60.9 in the second cycle (Tables 5 and 6).

In trial 1, both of the tested formulations of *Brassica* (flour and pellets) led to a significant reduction in the Fusarium wilt of lettuce, that is, from 63.3 to 24.6 and 22.9, respectively. When the *Brassica* treatment was applied as green manure, disease severity was observed to be more severe than in the untreated control (Table 2). Chicken manure and compost significantly reduced disease severity to values of 36.2 and 32.5, respectively, while cattle manure did not reduce Fusarium wilt significantly. The positive effect of the *Brassica* formulated amendments and chicken manure on disease reduction was again observed in the second lettuce cycle, but the compost was no longer effective (Tables 2 and 7).

In trial 2, all the tested organic amendments significantly reduced Fusarium wilt severity, compared to the inoculated and untreated control at the end of the first lettuce cycle, with the exception of chicken manure. In general, this significant efficacy was not observed in the second cycle (Tables 3 and 7).

In trial 3, in the presence of lower disease severity in the inoculated untreated control of 32.2-39.7, all the tested organic amendments, with the exception of chicken manure, significantly reduced lettuce Fusarium wilt severity, with values ranging from 8.4 to 23.1 at the end of the first cycle. This positive effect was still evident during the second crop cycle (Tables 5 and 7). The efficacy of chicken manure appeared to be improved at the end of the second cycle, with a significant Fusarium wilt reduction of 43.1%. No significant effect was found by extending the Brassica and compost soil treatment period from 30 to 60 days (Table 5).

In trial 4, in the presence of higher Fusarium wilt severity (59.6-60.9) for the inoculated untreated control, the best efficacy was provided by *B. carinata* pellets, applied for 30 and 60 days, with DS values of 12.2 and 15.0, respectively. For the first cycle, *Brassica* formulated as flour or used as green manure and compost also significantly reduced Fusarium wilt severity, to 33.8, 38.3 and 36.9, respectively, compared to the inoculated and untreated control. The lettuce plants grown in the growing medium treated with chicken manure were affected more by *F. oxysporum* f. sp. *lactucae* than those grown in the inoculated and untreated control growing medium (Table 6).

Effect on lettuce biomass

In trial 1, *Brassica* flour, chicken manure and compost provided the best effect on the fresh weight of lettuce grown in a non-inoculated growing medium. A significant negative correlation between the Fusarium wilt severity of the inoculated plants grown in the growing medium treated with the different amendments and the fresh weight reduction was observed at the end of the first cycle (R= -0.91**) and second crop cycle (R = -089**). At the end of the first cycle in the inoculated plots, the largest biomass was observed for the treatments that provided the best disease control. At the end of the second cycle, no differences in fresh weight were observed, compared to the inoculated and untreated control, the only exception being the plants grown in pots amended with the *Brassica* flour (Table 4).

In trial 2, in the same way as in trial 1, chicken manure and compost significantly increased the lettuce fresh weight in the non-inoculated plots, compared to the untreated control. This effect was also observed in the second cycle. Similar results, in terms of lettuce yield, were observed in the first and second cycles in the infested soil treated with compost and *B. carinata* pellets (Table 4). The lettuce biomass production was not significantly affected by the disease, and no linear correlation was in fact observed between these parameters.

In trial 3, the lettuce yield was slightly more variable between treatments in the second cycle than in the first cycle. The best fresh weight results were again provided by chicken manure and compost in both cycles. This positive effect was maintained, even in the presence of the pathogen, when *Brassica carinata* was used either as pellets or as a flour formulation (Table 5). A negative correlation between the severity of Fusarium wilt in the inoculated pots and the fresh weight reduction of plants grown in the growing medium treated with different amendments was only detected at the end of the first cycle (R = - 0.89**).

In trial 4, no significant differences were observed in plant fresh weight between treatments or between the inoculated and untreated control plants, with the exception of the results obtained with *B. carinata* flour and pellets, which caused a significant increase of 457.9 and 395.7 g/pot, respectively (Table 6). The fresh weigh reduction of the lettuce grown in the inoculated and treated soil at the end of both cycles was not significantly influenced by disease severity.

Effect on microbial populations

In trial 1, the number of CFU/g of benomyl-resistant mutants of *F. oxysporum* f. sp. *lactucae* was significantly lower in the growing medium treated with *B .carinata* flour than the untreated control, with no differences being observed between the other treatments, with the exception of the compost and *B. juncea* green manure, which resulted in a similar number for the inoculated and untreated control. The total fungal population was also reduced significantly by the *B. carinata* flour, compared to the untreated control. The *Pseudomonas* spp. population at the end of the second crop cycle was significantly higher in all of the amended growing medium. compared to the non-amended control. The highest *Pseudomonas* spp. values were recorded after the use of *Brassica* (flour and pellets) and compost amendments (Table 2). In trial 2, all the tested amendments significantly reduced the pathogen population: the lowest value was observed in the growing medium amended with *B. carinata* pellets, which, at the same time, significantly reduced the total fungal population and improved the number of *Pseudomonas* spp. at the end of the second crop cycle (Table 3).

Discussion

Managing Fusarium wilt with non-chemical measures (varietal resistance, organic amendments, biological control agents, cultural practices) has received increasing attention, because of the increasing restrictions on the use of chemical fungicides. Some of these measures are also exploited to reduce the inoculum density of the pathogen in the soil, a factor that is involved directly in the efficacy of disease suppression. The role of organic amendments in the suppression of soil-borne diseases, which involves inducing specific microbial, biological and chemical changes, has been reviewed in several pathosystems (Gamliel & Stapleton, 1993; Bailey & Lazarovits, 2003; Bonamomi *et al*., 2007; Borrego-Benjumea *et al*., 2014 a, b; Termorshuizen 2012; Ascencion *et al*., 2015). In the present study, the impact of amendments based on *Brassica,* either as plants or dry matter (as pellets and flour), cattle and chicken manure and compost has been evaluated on lettuce Fusarium wilt, considering the impact on yield, as well as through an evaluation of the soil bacterial and fungal population dynamics. Organic amendments are often used to improve soil quality as they can change the general suppressiveness of soil, because of the enriched microbial activity. Many *Pseudomonas* spp. have been identified from different soils, considering their suppression roles in various crop species, with both antagonistic and plant growth promoting activities (Alabouvette *et al*., 1990). The suppression mechanism of *Pseudomonas* against Fusarium wilt is not completely clear, as it includes induced resistance in the plants, competition and antibiotic production, and some examples of specificity have been established (Gamliel and Katan, 1993; Weller *et al*., 2002; Borrero *et al*., 2006). The results of the present study show that the *Brassica* amendments used in this study can induce a reduction in Fusarium wilt of up to 50%, compared to an untreated control. In general, an increase in the population density of the *Pseudomonas* species as well as a significant reduction in the total fungal population and pathogen density were observed in the growing medium after two consecutive lettuce cropping cycles.

When compost and Brassica seed meal were used alone or combined in a simulated biosolarisation treatment of soil, artificially infested with F. *oxysporum* f. sp. *basilici* and *F. oxysporum* f. sp. *conglutinans* (Fusarium wilt agents of basil and rocket, respectively), the aerobic mesophilic bacteria and *Pseudomonas* spp. populations were generally improved, compared to the untreated control (Ferrocino *et al*., 2014). An increase in *Pseudomonas* populations was observed after amending a sandy soil with *Brassica napus* seed meal at rates below 1% v/v (Mazzola *et al*., 2001). In the present pot trials, which were carried out under controlled conditions at a moderate incubation temperature of the soil and with artificial inoculation, a significant reduction in the severity of *F. oxysporum* f. sp. *lactucae* was observed when pellets of *B. carinata* (between 52% and 79%) and compost (between 49% and 67%) were used, while cattle and chicken manure only provided partial control (Table 7). The results of Borrego-Benjumea *et al*. (2014a) point out the positive effect of a soil application of poultry manure at 1%, incubated at 23±2°C and 35°C, in reducing tomato Fusarium wilt by 40% and 95%, respectively. Gamliel and Stapleton (1993) also indicated the ability of composted chicken manure, at 10 t/ha, to suppress *Pythium ultimum* on lettuce. In these experiments, chicken manure showed inconsistent results in the reduction of lettuce Fusarium wilt, and the lower incubation temperature during our trials may have prejudiced prejudiced the effectiveness of the treatment. Inconsistencies in Fusarium wilt management when *Brassica* green manure has been used have also been reported by several authors (Blok *et al*., 2000; Bonanomi *et al*., 2007; Njoroge *et al*., 2008; Borrego-Benjumea *et al*., 2014 a).

The here observed variable efficacy of organic amendments on disease and pathogen control could be due to several factors, and among these, their microbial activity is of particular importance. The effect of amendments on soil microorganisms is in general influenced by several factors, such as the type of soil, the type of amendment and dosage used as well as the temperature (Mazzola *et al*., 2001; 2007; Ochiai *et al*., 2007; Borrego-Benjumea *et al*., 2014a). For instance, soil suppressiveness on banana Fusarium wilt has also been associated with lower fungal population densities (Peng *et al*., 1999). The lack of Fusarium wilt control observed in trial two in the chicken manure amended growing medium could be the result of an increase in the total fungi and a lower density of the *Pseudomonas* population. Moreover, the high disease severity observed in trials 1, 2 and 4 may have influenced the effectiveness of the different amendments tested on Fusarium wilt control. In general, the tested amendments have shown a positive effect on disease suppression after one lettuce cultivation cycle, with the exception of the *B. carinata* pellet amendment, which was able to provide a significant Fusarium wilt reduction in three out of four trials in the second cropping cycle (Table 7). The long-term impact on disease suppression of such amendments could be due to a shift in the soil microbial population, and it depends to a great extent on the pathosystem (Motisi *et al.*, 2009; Bonamomi *et al*., 2007; Mazzola *et al*., 2007; Ferrocino *et al.*, 2014; Ascencion *et al*., 2015).The effect of *Brassica juncea*, *B. napus*, or *Sinapis alba* seed meal in reducing the replant disease of apple caused by *Rhizoctonia* spp. seems to be independent of the biofumigation potential and to be mainly affected by the stimulation of the resident beneficial microbial community, e.g. *Streptomyces* spp. (Mazzola *et al*., 2007). However, the role of soil biology as a Fusarium wilt controlling mechanism, following the incorporation of organic amendments, requires further investigation in lettuce systems.

In this study, which was carried out under controlled conditions, the prolongation of the *Brassica* and compost treatments, from 30 to 60 days, did not significantly affect disease severity or plant growth. The largest lettuce biomass was observed in the non-inoculated growing medium amended with Brassica flour, chicken manure, *Brassica carinata* pellets and compost in the first cycle. This positive effect was also evident in the second cycle for chicken manure and compost amendments. In the present study, in general, the lettuce fresh weight was not associated with a reduction in wilt severity, and a possible nutritional effect has been involved. In a previous study, *Brassica carinata* pellets and compost applied as a mixture, or combined with a short period of soil solarisation, did not improve the level of disease management of Fusarium wilt in basil or rocket, compared with soil solarisation alone. However, such treatments significantly increased the biomass of both crops, thus positively affecting yield (Gilardi *et al.*, 2014 a).

Organic amendments could affect disease control by improving the nutrition of the host, thus increasing its resistance, or by reducing the inoculum potential of soil-borne pathogens in the soil. An increase in the ammonia concentration and in the soil pH, factors that are involved in the suppression of Fusarium wilt, has been reported for soil amended with organic amendments rich in nitrogen (Borrego-Benjumea *et al*., 2014a,b).

In conclusion, the potential exists for the practical use of soil amendments in the management of Fusarium wilt of monoculture lettuce grown in short rotations: *Brassica carinata* (pellets and flour) and compost represent a good opportunity to integrate pest management programmes, with a positive impact on reducing the inoculum potential of the pathogen after two consecutive crop cycles. Some encouraging results pertaining to disease management through amendment applications in field experiments, after preliminary studies in laboratory conditions, have been reported by several authors for different pathosystems (Blok *et al*., 2000; Mazzola *et al*., 2007; Larkin & Griffin, 2007; Klein *et al*., 2011; Hansen & Keinath, 2013; Borrego-Benjumea *et al*., 2014a,b).The variability of treatment efficacy in the wilt disease severity of lettuce that has so far been seen means that further work is required to improve these treatments before they are suitable for use in commercial lettuce crop production.

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