Eur J Plant Pathol (2010) 126:387–402
DOI 10.1007/s10658-009-9543-y

Biofumigation with Brassica plants and its effect on the inoculum potential of Fusarium yellows of Brassica crops

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Received: 7 March 2009 / Accepted: 1 October 2009 / Published online: 24 October 2009

Abstract The use of Brassica crops as green manure in the so-called biofumigation treatment has been successfully exploited for the management of soil-borne pathogens and is gaining interest particularly in the case of less intensive agricultural systems. A study was undertaken to investigate possible negative side-effects of biofumigation in order to prevent possible damage caused by wilt pathogens able to attack both plants used for biofumigation as well as agricultural crops. To do so, firstly the response of different Brassicas, including some used in biofumigation, to the formae speciales of *Fusarium oxysporum* known for being pathogenic on Brassica crops was evaluated. Secondly, the effect of green manure treatments on yield, quality of crops, and inoculum densities, infection and survival of *Fusarium oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* was evaluated. In the second part of the work, four Brassica crops, selected for their response (susceptibility or resistance) to *F. oxysporum* f. sp. *conglutinans* and to *F. oxysporum* f. sp. *raphani* were evaluated in order to determine their response to the two pathogens during subsequent crops grown in soil where plants were incorporated as green manure into the soil at the end of each cycle. Moreover, the dynamics of the populations of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* in the soil after several biofumigation cycles was studied. Many of the Brassica crops used for biofumigation tested were susceptible to *F. oxysporum* f. sp. *conglutinans* and or to *F. oxysporum* f. sp. *raphani*. Green manure treatment, carried out by growing nine cycles of biocidal plants, with a short crop cycle of 30–35 days, did not reduce Fusarium wilts on susceptible Brassica hosts. The population of the pathogen was partially increased as a result of the incorporation of tissues of the susceptible plants. When Brassica crops grown were resistant to the two *F. oxysporum* pathogens used for soil infestation, green manure simulation did inhibit both pathogens, thus confirming its biocidal activity. The results obtained under our experimental conditions show that biofumigation treatment is not applicable for soil disinfestation on crops susceptible to the same formae speciales of *F. oxysporum* affecting Brassica species used for biofumigation. Brassica crops resistant to Fusarium yellows should be grown where biofumigation is applied. Moreover, alternation of crops used for biofumigation should be encouraged.

Keywords Soil disinfestation · Soilborne pathogens · *Fusarium oxysporum* f. sp. *raphani* · *Fusarium oxysporum* f. sp. *conglutinans* · Green manure

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Introduction

The Brassicaceae are among the plant families with high content of glucosinolates in their tissues. Brassica plants are also characterised by a high content of other sulphur-containing compounds (Walker et al. 1937; Sang et al. 1984, Mayton et al. 1996; Gimsing and Kirkegaard 2006, 2009). Antifungal volatiles such as allyilsothiocyanate have been found in leaf extracts of various Brassica species (Mayton et al. 1996, Sang et al. 1984; Manici et al. 1997). In the case of *Brassica juncea* it has been shown that the above– and below-ground plant components produce different isothiocyanates, which show different persistence once the plant residues have been incorporated in the soil (Kirkegaard and Sarwar 1998). Motisi et al. (2009) described the non-additive effect of the incorporation of leaves and /or roots of *B. juncea* against *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici* and suggested the existence of a complex interaction between these two type of residues. The toxicity of isothiocyanates or other glucosinolates-related compounds to various microorganisms has been well documented (Gamliel 2000; Bailey and Lazarovits 2003; Mazzola and Cohen 2005, Manici et al. 2000, PiedraBuena et al. 2006, Mattner et al. 2008; Motisi et al. 2009). Chemicals of this group such as methyl-isothiocyanate, the active ingredient of metham sodium and dazomet, are widely used as soil fumigants.

Volatile aldehydes and sulphides are also toxic to soilborne plant pathogens (Lewis and Papavizas 1971). The generation of such compounds in soil amended with Brassica residues have been shown to contribute to pathogen control (Muehlchen et al. 1990). Moreover, numerous bacteria and fungi can decompose cabbage and generate sulphur-containing volatile compounds, mainly sulphide-based (Kadota and Ishida 1972). When introduced in the soil, Brassica residues are decomposed, resulting in generation of various S-containing volatile compounds, such as dimethyl sulphide and dimethyl disulphide (Lewis and Papavizas 1970, 1971, Ramirez-Villapuda and Munnecke 1988). These decomposition products can suppress certain soilborne pathogens, pests and weeds (Lewis and Papavizas 1971, 1974; Pavlica et al. 1978; Matthiessen and Kirkegaard 2006).

Volatile compounds have been reported to inactivate fungal propagules *in vitro* (Smith and Kirkegaard 2002; Manici et al. 1997) or in sterile soil (Manici et al. 2000). Biofumigation can be achieved by incorporating fresh plant material (green manure), seed meals (a by-product of seed crushed for oil), or dried plant material treated to preserve isothiocyanate activity (Gamliel and Stapleton 1993; Brown and Morra 1997: Kirkegaard and Matthiessen 2004; Lazzeri et al. 2004; Matthiessen and Kirkegaard 2006). The incorporation of glucosinolate-containing plant material causes a suppression of diseases and pests related to the release of glucosinolate hydrolysis products, but there may also be a suppression not related to the isothiocyanates, which can be due to the added organic matter which can increase the population of antagonistic organisms in soil, or to the release of toxic compounds which are not of glucosinolate origin (Bailey and Lazarovits 2003; Larkin and Griffin 2007).

The use of Brassica intercrops can also be considered a type of biofumigation, with isothiocyanates being released from the intact roots (Kirkegaard et al. 2000). Thus, Brassica residues can provide varying levels of pathogen control, either alone or when combined with other disinfestation methods. There is considerable interest in many types of agricultural systems (particularly organic and sustainable agriculture) in biofumigation as an alternative to synthetic soil fumigants in horticulture, and for control of soilborne pathogens in extensive agriculture (Gamliel 2000; Gullino et al. 2003; Garibaldi et al. 2008). This is particularly true after the phase-out of methyl bromide, also because Brassica crops used as green manures have the ability to control multiple soil-borne problems (Larkin and Griffin 2007). Biofumigation can be effectively combined with biocontrol agents compatible or resistant to isothiocyanates. The synergistic effect of *Brassica carinata* meals combined with the mycoparasite *Coniothyrium minitans* against *Sclerotinia sclerotiorum* and *S. minor* has been demonstrated under controlled conditions (Marciano et al. 2004). Control of sugar beet damping-off caused by *Pythium ultimum* can be improved with a combined incorporation of *Brassica carinata* seed meal and selected tolerant strains of *Trichoderma* (Galletti et al. 2008).

The field application of this technique is easy and practical (Mattner et al. 2008). Biofumigation shows typical green manure disadvantages, such as only partial or sometimes inconsistent activity in disease suppression, the need for using high amounts of material and consequently the fact that such a technique is not easily applicable in intensive agri-

cultural systems. In order to overcome such constraints, biocidal Brassica plant dried pellets have been produced (Lazzeri et al. 2004).

When different Brassica plants were tested for their release of biocidal compounds, mainly isothiocyanates, the highest fungicidal activity and also the highest concentration of isothiocyanates were found in *Brassica juncea* (Smolinska and Horbowicz 1999). Lines of different species of Brassica plants characterised by a higher production of glucosinolates have been obtained (Kirkegaard and Sarwar 1999; Lazzeri et al. 2004).

Some difficulties concerning the use of Brassica crops as soil amendments have been already addressed. Recently, the capability of *Sclerotinia sclerotiorum* to adapt to isothiocyanates and toxic metabolites from Brassica species was reported (Rahmanpour et al. 2009). The possibility that certain pathogens of the plants which are used as green manure can affect amendments, causing more damage to crops has been little investigated. Pattison et al. (2006) reported that some cultivars of *Brassica chinensis*, *B. napus*, *B. juncea* and *Sinapis alba*, used in biofumigation experiments, being susceptible to *Meloidogyne javanica*, can cause an increase of this pathogen in the soil. All studies carried out, did not consider, for example, the potential susceptibility of Brassica crops used in rotation and as green manure to soil-borne pathogens and, consequently, the risk of increasing the inoculum potential of certain pathogens by adopting biofumigation in the practice, when Brassicas are used for both biofumigation and as crops. Particularly, the importance of Fusarium yellows on Brassica crops is very well known and documented. Bosland and Williams (1987), examined an extensive collection of isolates of *Fusarium oxysporum* from Brassica hosts, and characterised on the basis of pathogenicity, isozyme polymorphism, vegetative compatibility and geographic origin, different races of *F. oxysporum* f. sp. *conglutinans*, *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *matthioli* as causal agents of the different yellows.

Fusarium oxysporum f. sp. *conglutinans* and *F. oxysporum raphani* were recently observed in Italy on Brassica crops such as cultivated *(Eruca sativa)* and wild *(Diplotaxis tenuifolia)* rocket (Garibaldi et al. 2006) as well as on a non-Brassica crop such as lamb's lettuce (*Valerianella olitoria*) (Gilardi et al. 2008). Rocket as well as lamb's lettuce are crops potentially subject to biofumigation, due to the difficulty of controlling soil-borne pathogens in such intensive crop systems. However, the use of susceptible crops for biofumigation has the potential for bringing an enrichment and build-up in the population of pathogens in the soil. This becomes a risk to the following crop, if it is susceptible to the same pathogen.

The present study was undertaken in order to investigate this possible negative side-effect of biofumigation in order to prevent possible damage caused by wilt pathogens able to attack both plants used for biofumigation as well as agricultural crops. To do so, firstly the response of several Brassica crops, used for biofumigation, to the formae speciales of *F. oxysporum* known for being pathogenic on Brassica crops was evaluated. Secondly, the effect of green manure treatments on yield, quality of crops, and inoculum densities, infection and survival of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* was evaluated. In this second part of the work, four Brassica crops, selected for their response (susceptibility or resistance) to *F. oxysporum* f. sp. *conglutinans* and to *F. oxysporum* f. sp. *raphani* were evaluated in order to determine their response to the two pathogens during subsequent crops grown in soil for short cycles where plants were incorporated as green manure into the soil at the end of each cycle. Under our experimental conditions we changed the conventional approach of biofumigation to evaluate the long-term effect of this treatment under controlled conditions. Moreover, the dynamics of the populations of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* in the soil after several biofumigation short cycles was studied.

Materials and methods

This study, carried out in growth chambers and glasshouses belonging to the Centre of Competence for Innovation in the Agro-Environmental Sector (AGROINNOVA) of the University of Torino, located at Grugliasco (Torino), is divided into two parts. For clarity, materials and methods are divided into the two parts of the work, while general aspects, such as disease rating and statistical analysis are reported together.

Part 1

The first set of experiments, consisting of five trials, were carried out during the period April 2007-June

Table 1 List of the Brassica species used in the study

Species	Code
Brassica juncea	ISCI[a]61
Brassica juncea	ISCI 99
Brassica juncea	ISCI 20
Eruca sativa	cv Nemat ISCI
Brassica nigra	ISCI 27
Crambe abyssinica	cv Mario ISCI
Rapistrum rugosum	cv Lori ISCI
Brassica carinata	ISCI 7
Lepidium campestre	ISCI 15
Lepidium sativum	ISCI 101
Brassica rapa	cv Silla ISCI
Diplotaxis tenuifolia	Mazzocchi
Sinapis alba	cv Pira ISCI
Barbarea verna	ISCI 100
Rapistrum rugosum	ISCI 03
Brassica juncea	cv Poggiolo ISCI
Sinapis arvensis	cv Iberina ISCI

[a] Centre of Research for Industrial Crops (ISCI)

2008 in order to evaluate the response of different Brassica crops used as biofumigants to inoculation with several *F. oxysporum* isolates, obtained from different hosts.

Plant material and cultivation

Seeds of sixteen Brassica species, listed in Table 1, were obtained from the Centre of Research for Industrial Crops (Bologna). *Diplotaxis tenuifolia* seeds were obtained from the seed company Mazzocchi (Milano, Italy). The material obtained from the Centre of Research for Industrial Crops, coded as ISCI, is under selection because it is characterised by a high content of glucosinolates (Manici et al. 1997). Individual seeds were sown in plug trays (Oktpac160, Arca, Bergamo, Italy) containing a steamed mix substrate Tecno-2: Tiesse-3 (1:1 vol/vol) and maintained in a growth chamber at 28°C with 12 h fluorescent light per day. Tecno-2 (Turco Terricci, Garessio, Italy) is based on peat + clay + NPK + microelements at 2.5 kg m^{-3}, while Tiesse-3 (Turco Terricci, Garessio, Italy) is based on peat + clay + perlite + NPK + microelements at 1 kg m^{-3}. After 20 days, seedlings were removed from the potting mix, and roots were lightly washed, blotted, and artificially inoculated with *F. oxysporum* as described below before being transplanted into 56×40×9 cm trays, filled with 12 l of the same steamed mixture. Each tray, containing 20 plants, represented one replicate. Twenty non-inoculated seedlings/tray for each Brassica crop served as the control. Three replicates were used for each treatment, arranged in a completely randomised block design. Five trials were carried under glasshouse conditions, at temperatures ranging between 25 and 33°C. Each trial lasted 31–35 days.

Isolates of *Fusarium oxysporum*, inoculum production and artificial inoculation

Seven isolates of *F. oxysporum* (Table 2) were tested: three strains belonged to *F. oxysporum* f.sp. *raphani* (Fus Ruc 9A, Fus Ruc 13/03, ATCC 64105), two to *F. oxysporum* f.sp. *conglutinans* (ATCC 16600, Fus Ruc 6), one strain to *F. oxysporum* f.sp. *matthioli* (ATCC 16602) and one strain to *F. oxysporum* f. sp. *conglutinans* from lamb's lettuce (Fus Vale 5/04). The cultures were maintained in tubes on PDA at 8°C.

Table 2 Isolates of *Fusarium oxysporum* used for artificial inoculation

Code	Species and forma specialis	Original host
Fus Ruc 9A/02	*Fusarium oxysporum* f.sp. *raphani*	Wild rocket (*Diplotaxis tenuifolia*)
Fus Ruc 13/03	*Fusarium oxysporum* f.sp.*raphani*	Cultivated rocket (*Eruca sativa*)
ATCC[a] 64105	*Fusarium oxysporum* f.sp.*raphani*	Horseradish (*Cochlearia armoracia)*
ATCC 16602	*Fusarium oxysporum* f.sp. *matthioli*	Stock (*Matthiola incana*)
Fus Vale 5/04	*Fusarium oxysporum* f.sp. *conglutinans*	Lamb's lettuce (*Valerianella olitoria*)
Fus Ruc 6	*Fusarium oxysporum* f.sp. *conglutinans*	Cultivated rocket (*Eruca sativa*)
ATCC 16600	*Fusarium oxysporum* f.sp. *conglutinans*	Cabbage (*Brassica oleracea*)

[a] Source of ATCC strains is available at http://www.atcc.org

Each *F. oxysporum* isolate was grown in 1000-ml Erlenmeyer flasks containing 150 ml of casein hydrolysate. Flasks were incubated on a platform shaker at 200 rpm, at temperatures ranging between 20 and 25°C. After 12 days, fungal liquid cultures were aseptically removed from the flasks and centrifuged at 8,000*g* for 15 min at 10°C. Supernatant was removed and the pellet suspended in 200 ml of sterile distilled water (SDW), followed by homogenisation with a rotary hand blender. The concentration of spores was determined by haemocytometer under the microscope and adjusted to 1×10^6 spores ml^{-1} by adding deionised water. Roots of 20 day-old Brassica seedlings were dipped in the spore suspensions of each isolate for 20 min before being transplanted. Controls for each Brassica species were represented by plants dipped, before being transplanted, in SDW.

Part 2

The second set of trials was carried out during the period April 2008–February 2009 in order to evaluate the long term effect of green manure applied in the short cycle on the survival in the soil of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* under controlled conditions.

Plant material and cultivation

Brassica juncea ISCI 99 (resistant to ATCC 64105 and susceptible to ATCC 16600), *Eruca sativa* Nemat (susceptible to both ATCC 64105 and ATCC 16600), *Barbarea verna* ISCI 100 (resistant to both ATCC 64105 and ATCC 16600), and *Brassica nigra* ISCI 27 (susceptible to both ATCC 64105 and ATCC 16600) were used. Twenty-day old seedlings, corresponding to a density of 101 plants m^{-2}, grown as described for the first set of trials, were transplanted into 48×41×20 cm trays filled with 20 l of steamed Tecno 2: Tiesse 3 (1:1 vol/vol) mix, previously fertilised as described above, artificially infested with *F. oxysporum* f. sp. *raphani* ATCC 64105 and *F. oxysporum* f. sp. *conglutinans* ATCC 16600, as described below. Four replicates were used for each treatment, maintained under glasshouse conditions at temperatures ranging between 25 and 33°C, arranged in a completely randomised block design.

Green manure application

Green manure treatment was applied in the second set of trials by transplanting nine cycles of crops in the same substrate in order to investigate the long-term effect of biofumigation under controlled conditions as described above. Each crop cycle lasted 30–35 days. At the end of each cycle, after completing disease rating, plants (stem and fresh leaves) at 60–80% of the flowering stage were weighed, harvested, cut into 1–3 cm pieces and immediately incorporated and mixed into the soil, simulating a biofumigation treatment under controlled conditions. At the same time, soil moisture was adjusted to field water capacity to favour glucosinolate release. After one day, 20 day-old seedlings were transplanted into the biofumigated soil and a new crop cycle started.

Isolates of *Fusarium oxysporum* and induction of resistance to benomyl

Benomyl-resistant mutants of *F. oxysporum* f. sp. *raphani* ATCC 64105 and of *F. oxysporum* f. sp. *conglutinans* ATCC 16600 were used to distinguish *Fusarium* spp. added to the soil from indigenous isolates of *F. oxysporum*. Ultraviolet light-induced, benomyl-resistant mutant isolates of the two pathogens were produced for use as markers. Microconidia from 10–15 day-old potato dextrose agar (PDA) cultures of *F. oxysporum* f. sp. *raphani* ATCC 64105 and *F. oxysporum* f. sp. *conglutinans* ATCC 16600 were suspended in water and adjusted to 10^6 spores ml^{-1}. A 9 cm Petri dish containing 10 ml of this suspension was exposed to ultraviolet light (Sankyo Denki G30T8 germicidal lamp, Gelaire TC) at a distance of 10 cm for 5, 10, 15, 20, and 25 min in a dark chamber. Approximately 95% of the spores were killed. Survivors were plated on a Komada medium added with 10 and 30 mg l^{-1} of benomyl (Benlate, 50 % w.g., DuPont, USA). Mutants able to grow on benomyl, were transferred again onto plates with Komada containing 10 and 30 mg l^{-1} of benomyl. The colonies growing under these conditions were considered benomyl-resistant. Fifteen resistant mutants for each of the two isolates of *F. oxysporum* were selected and maintained in tubes on Komada with 10 mg l^{-1} of benomyl at 8°C. Pathogenicity of the benomyl-resistant mutants was tested and compared with that of the wild-type parent isolates by

inoculating 20 day-old seedlings of *Eruca sativa* by root dipping as described below. The isolates showing the highest virulence were reisolated. Two resistant mutants of *F. oxysporum* f. sp. *raphani* ATCC 64105, coded as RB 1 and RB 2, and one of *F. oxysporum* f. sp. *conglutinans*, ATCC 16600, coded as RB 1, selected on the basis of the pathogenicity test, were used in the second part of the work.

Survival and population dynamics of *F. oxysporum* in biofumigated soils

The inoculum of *F. oxysporum* f. sp. *raphani* ATCC 64105 RB and *F. oxysporum* f. sp. *conglutinans* ATCC 16600 RB, used for infesting the substrate after steaming, consisted of chlamydospores prepared in talc, as described by Locke and Colhoun (1974). The concentration of chlamydospores in talc was assessed by plate dilution on Komada medium with 10 mg l^{-1} of benomyl. Moisture regime was kept at 65–75% field capacity. Fifteen days after steaming, soil was infested with the chlamydospores prepared in talc at two dosage levels, corresponding respectively to a final concentration of 10^3 and 10^4cfu ml^{-1} of soil. Controls were represented by infested soil without plants and by plants transplanted into non-infested soil.

Nine crop cycles were grown in the same soil and pathogen population dynamics were evaluated every three cycles. Crops were watered regularly and soil was maintained at a moisture regime corresponding to 65–75% soil capacity. Five soil cores, randomly collected from each tray with a stainless cork borer previously disinfected, were combined to make one composite soil sample (80–100 g) per tray. Three sub-samples of 5 g of soil were taken from each sample and used to determine the soil population of the *F. oxysporum* RB mutants by soil dilution plating, using 10^{-2}, 10^{-3} and 10^{-4} dilutions on Komada medium with 10 mg l^{-1} of benomyl. Three plates/dilution were used. Populations were monitored three times, after three, six and nine crop cycles and determined as cfu g^{-1} of air-dried soil.

Disease evaluation and statistical analysis

Symptoms of yellows were evaluated visually, starting 10 days after transplanting. Disease development was evaluated every week by recording the number of plants without symptoms and with typical symptoms. Dead plants were counted and removed. At the end of each trial, re-isolation from infected plants was carried out on Komada medium (Komada 1975) to confirm the presence of *F. oxysporum* as the causal agent of the symptoms observed. Biomass production was evaluated at the end of each trial by measuring fresh weight of the above-ground parts of the plants. In the first set of trials, plant weight is reported as percent of the weight of control plants. In the second set of trials, disease severity and biomass were evaluated at the end of each crop cycle, while pathogen population dynamics was evaluated three times, every three cycles.

The scale used for disease evaluation ranged from 0 to 4 (0 = no symptom; 1 = slight discolouration of root, no vascular discolouration, first symptoms of leaf chlorosis; 2 = severe leaf chlorosis and initial symptoms of wilting; 3 = severe wilting symptoms; 4 = dead plant). Data are expressed as disease index (DI) at the end of the trials, from 0 to 100, calculated using the formula: $DI = [\sum (i \times x_i)]/(4 \times \text{total of plant}) \times 100$ with i=0–4 (x_i is the number of plant with rating i).

The data sets from the five trials of the first set of trials were combined. The data were arcsine-transformed and analysed together by univariate ANOVA with Tukey's test using SPSS software 13.0.

In the second set of trials, disease severity and biomass were evaluated at the end of every crop cycle, while pathogen population dynamics were determined every three cycles.

The influence of disease severity on biomass production at the end of the first set of trials and for each biofumigation cycle was analysed by calculating Pearson's correlation coefficient.

Results

Part 1

Response of cruciferous crops to different formae speciales of *Fusarium oxysporum*.

The five trials carried out in order to check the response of 17 Brassicas to *F. oxysporum* f. sp. *matthioli*, *F. oxysporum* f. sp. *raphani*, *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* from lamb's lettuce provided consistent and similar results. The tested crops showed a similar and consistent response

to the tested strains throughout all the trials. All tested crops were resistant or partially resistant to *F. oxysporum* f. sp. *matthioli* ATCC 16002 (Table 3). *Eruca sativa* and *Crambe abyssinica* were susceptible to the three tested strains of *F. oxysporum* f. sp. *raphani*. They showed the same reaction also to *F. oxysporum* f. sp. *conglutinans* Fus Vale 5/04 and ATCC 16600, while they were both partially resistant to *F. oxysporum* f. sp. *conglutinans* Fus Ruc 6 (Table 3). Most of the tested Brassicas (*E. sativa*, *B. nigra*, *C. abyssinica*, *R. rugosum*, *L. campestre*, *L. sativum*, *B. rapa*, *D. tenuifolia*, *S. arvensis*) were susceptible to *F. oxysporum* f. sp. *raphani* ATCC 64105 (Table 3). Again, several Brassica crops (*E. sativa*, *B. nigra*, *C. abyssinica*, *L. sativum*, *D. tenuifolia*, *B. verna*, *R. rugosum*) were susceptible to *F. oxysporum* f. sp. *raphani* Fus Ruc 13/03 (Table 3). The response of the tested crops to the three strains of *F. oxysporum* f. sp. *conglutinans* tested was quite different: they were all resistant or partially resistant to the strain Fus Ruc 6, most of them, with the exclusion of *B. verna*, were susceptible or highly susceptible to strain ATCC 16600, and three of them (*B. nigra*, *C. abyssinica* and *L. sativum*) were susceptible to the isolate Fus Vale 5/04 (Table 3). The three tested selections of *Brassica juncea* were all resistant to all tested strains, with the exception of *F. oxysporum* f. sp. *conglutinans* ATCC 16600 (Table 3).

The values of the biomass measured reflected the response to the different pathogens: in the presence of susceptibility or high susceptibility, biomass was low or very low (Table 4). In some cases, such as for *C. abyssinica* in the presence of *F. oxysporum* f. sp. *raphani*, it was equal or close to 0 g (Table 4). A significant negative correlation between disease index and biomass was observed in the case of most tested species, except *B. carinata* and *B. rapa* (Table 5). When correlation was evaluated with single strains of the pathogens, correlation between disease incidence and biomass was always significant, although it showed lower values in the case of the isolates of *F. conglutinans* Ruc 6 and *F. raphani* Ruc 9A/02 (Table 5).

Part 2

Production and characterisation of benomyl-resistant mutants of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani*.

Mutation rates for the recovery of benomyl-resistant mutants were in the range of 10^{-2}–10^{-3}. Fifteen resistant mutants for each of the two isolates of *F. oxysporum* were successfully recovered from the parent isolates after repeated screenings with ultraviolet light. The RB isolates (ATCC 64105 coded RB 3–15 and ATCC 16600 coded RB 2–15) varied regarding the characteristics measured, with some isolates revealing significantly impaired capabilities relative to the wild-type parents in growth, and pathogenicity. Only isolates ATCC 16600 RB1, ATCC 64105 RB1 and RB2 equalled or surpassed the parent wild-types for all characteristics measured in initial tests and were found to be similar to the wild-types in further evaluations for pathogenicity (Table 6). On the basis of these tests, it was concluded that these isolates could be used to adequately represent *F. oxysporum* f. sp. *conglutinans* ATCC 16600 and *F. oxysporum* f. sp. *raphani* ATCC 64105 in soil tests. The benomyl-resistance was found to be a stable and reliable marker throughout all phases of this research. Isolates ATCC 16600 RB1 and a mixture of ATCC 64105 RB1 and RB2 were used for the study of the benomyl-resistant strains of *F. oxysporum* population dynamics.

Response of selected Brassica crops to benomyl-resistant strains of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani*

When the susceptibility of selected Brassicas to *F. oxysporum* f. sp. *conglutinans* ATCC 16600 RB was tested, *B. verna* (ISCI 100) confirmed its resistance, with no symptoms at both tested inoculum dosages of the pathogen. *Eruca sativa* confirmed its high susceptibility, which persisted at similar values throughout all cycles at both inoculum dosages. Also *B. juncea* was susceptible throughout all cycles, with a higher disease index at the first cycle at the higher inoculum dosage (Table 7). The biomass evaluated at the end of each cycle confirmed the results of the pathogenicity test: in the presence of high disease severity, biomass was vey low if compared to the non-inoculated control (Table 8). A significant negative correlation between disease index and biomass was observed at all cycles (Table 5).

Among the tested crops, *E. sativa* and *B. nigra* were, respectively, highly susceptible and susceptible to *F. oxysporum* f. sp. *raphani* ATCC 64105 RB, while *B. juncea* was resistant (Table 9). Again,

Table 3 Average disease index and reaction of different Brassica crops to *Fusarium oxysporum*

N	Species and code	Disease index (0–100) and reaction													
		Fusarium oxysporum f.sp. *matthioli*		*Fusarium oxysporum* f.sp. *raphani*						*Fusarium oxysporum* f.sp. *conglutinans*					
		ATCC 16602		Fus Ruc 13/03		Fus Ruc 9A/02		ATCC 64105		Fus Vale 5/04		Fus Ruc 6		ATCC 16600	
		D.I. [a]	Reaction [b]	D.I.	Reaction	D.I.	Reaction	D.I.	Reaction	D.I.	Reaction	D.I.	Reaction	D.I.	Reaction
1	*Brassica juncea* (ISCI 61)	3.3 ab[c]	R	3.5 a	R	3.1 ab	R	17.5 abc	PR	2.8 a	R	0.8 a	R	45.6 abcde	S
2	*Brassica juncea* (ISCI 99)	14.9 ab	PR	0.0 a	R	2.5 ab	R	0.8 a	R	0.0 a	R	0.0 a	R	97.9g	HS
3	*Brassica juncea* (ISCI 20)	21.5 ab	PR	4.2 a	R	2.8 ab	R	9.0 ab	R	0.8 a	R	4.4 abc	R	84.8 defg	HS
4	*Eruca sativa* (Nemat)	17.2 ab	PR	38.6bc	S	44.7 e	S	32.8 abcde	S	40.8cd	S	26.2 d	PR	78.4 cdefg	HS
5	*Brassica nigra* (ISCI 27)	12.4 ab	PR	76.5de	HS	10.7 abcd	PR	69.4 ef	HS	17.9 abcd	PR	10.0 abcd	R	60.2 bcdefg	S
6	*Crambe abyssinica* (Mario)	1.5 ab	R	100.0f	HS	40.6 e	S	98.3 f	HS	44.8 d	S	21.8cd	PR	31.4 ab	S
7	*Rapistrum rugosum* (Lori)	15.9 ab	PR	29.0b	PR	11.4 abcd	PR	62.5 def	HS	19.5 abcd	PR	13.8 abcd	PR	73.8 bcdefg	HS
8	*Brassica carinata* (ISCI 7)	3.8 ab	R	1.7 a	R	2.6 ab	R	4.4 a	R	1.4 a	R	7.2 abc	R	53.3 bcdef	S
9	*Lepidium campestre* (ISCI 15)	0.0 a	R	2.8 a	R	11.7 abcd	PR	69.0 ef	HS	0.8 a	R	9.7 abcd	R	56.9 bcdefg	S
10	*Lepidium sativum* (ISCI 101)	9.7 ab	R	54.8cd	S	16.2 abcd	PR	50.5 cde	S	38.4 bcd	S	21.7cd	PR	37.9 abc	S
11	*Brassica rapa* (Silla)	21.9 b	PR	19.5 ab	PR	17.0 bcd	PR	42.2 bcde	S	11.1 abc	PR	19.0 bcd	PR	88.5 efg	HS
12	*Diplotaxis tenuifolia*	8.4 ab	R	35.6 bc	S	19.7cd	PR	47.9 cde	S	23.2 abcd	PR	15.6 abcd	PR	86.7 defg	HS
13	*Sinapis alba* (Pira)	11.8 ab	PR	3.8 a	R	6.0 abc	R	17.5 abc	PR	6.9 a	R	3.1 ab	R	97.1 fg	HS
14	*Barbarea verna* (ISCI 100)	0.0 a	R	91.0 ef	HS	0.7 a	R	29.0 abcd	PR	8.5 ab	R	10.4 abcd	R	5.1 a	R
15	*Rapistrum rugosum* (ISCI 03)	9.2 ab	R	33.1 bc	S	22.6 d	PR	59.8 de	S	19.7 abcd	PR	5.6 abc	R	64.2 bcdefg	HS
16	*Brassica juncea* (Poggiolo)	13.7 ab	PR	17.9 ab	PR	4.6 abc	R	4.0 a	R	4.0 a	R	2.4 ab	R	42.6 abcd	S
17	*Sinapis arvensis* (Iberina)	6.9 ab	R	17.9 ab	PR	10.1 abcd	R	48.5 cde	S	9.5 ab	R	8.8 abcd	R	74.0 bcdefg	HS

The values are the average of five trials

[a] D.I. Disease index (0–100)

[b] Reaction: R, resistant D.I. 0–10; PR, partially resistant, D.I. 11–30; S: susceptible, D.I. 31–60; HS: highly susceptible, D.I. 61–100

[c] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

Table 4 Average relative biomass of different Brassica crops inoculated with different formae speciales of *Fusarium oxysporum*

N	Species and code	Biomass[a]							Healthy control (Fresh epigeal biomass m^{-2})
		Fusarium oxysporum f.sp. *matthioli* ATCC 16602	*Fusarium oxysporum* f.sp. *raphani*			*Fusarium oxysporum* f.sp. *conglutinans*			
			Fus Ruc 13/03	Fus Ruc 9A/02	ATCC 64105	Fus Vale 5/04	Fus Ruc 6	ATCC 16600	
1	*Brassica juncea* (ISCI 61)	97.3 a[b]	89.8 abcd	96.7 a	85.9 ab	89.1 ab	91.2 a	77.4 a	1121.4
2	*Brassica juncea* (ISCI 99)	75.0 ab	101.7 a	80.7 abcde	96.1 ab	97.3 a	74.7 a	1.6 i	1046.9
3	*Brassica juncea* (ISCI 20)	67.9 b	92.1 abc	90.9 ab	101.5 a	87.3 ab	63.8 a	20.8 fghi	986.2
4	*Eruca sativa* (Nemat)	60.2 b	68.1 de	57.8 de	80.5 abcd	56.2 d	63.8 a	25.2 fghi	1070.5
5	*Brassica nigra* (ISCI 27)	69.6 ab	42.4 f	91.1 ab	42.9 ef	76.7 bcd	72.1 a	37.4 defg	814.3
6	*Crambe abyssinica* (Mario)	67.5 b	0.0 g	29.4 f	4.4 g	36.1 e	48.6 a	70.9 ab	1284.4
7	*Rapistrum rugosum* (Lori)	66.5 b	77.4 cde	55.8 e	42.2 ef	55.1 d	70.0 a	29.8 efgh	1015.6
8	*Brassica carinata* (ISCI 7)	79.7 ab	100.3 ab	63.4 cde	88.3 ab	79.3 abc	74.3 a	64.9 abc	1200.9
9	*Lepidium campestre* (ISCI 15)	74.6 ab	88.7 abcd	59.9 cde	27.1 f	75.7 bcd	43.9 a	22.9 fghi	612.1
10	*Lepidium sativum* (ISCI 101)	84.8 ab	40.9 f	70.7 bcde	48.9 ef	61.6 cd	71.1 a	55.7 abcde	472.3
11	*Brassica rapa* (Silla)	67.8 b	83.1 abcde	80.9 abcde	75.8 bcd	68.4 bcd	78.5 a	45.1 bcdef	1049.6
12	*Diplotaxis tenuifolia*	87.8 ab	62.3 e	75.2 abcde	62.7 cde	70.6 bcd	76.3 a	13.2 ghi	553.1
13	*Sinapis alba* (Pira)	79.1 ab	81.2 abcde	80.8 abcde	82.4 abc	61.6 cd	83.8 a	3.8 hi	1121.4
14	*Barbarea verna* (ISCI 100)	65.4 b	5.3 g	100.7 a	50.5 e	69.4 bcd	46.3 a	67.4 abc	742.0
15	*Rapistrum rugosum* (ISCI 03)	70.3 ab	78.5 bcde	77.9 abcde	51.8 e	82.3 abc	87.6 a	34.7 efg	808.0
16	*Brassica juncea* (Poggiolo)	61.4 b	85.4 abcd	85.1 abc	103.1 a	72.6 bcd	77.7 a	63.6 abcd	1056.7
17	*Sinapis arvensis* (Iberina)	70.5 ab	90.3 abcd	81.7 abcd	59.8 de	76.2 bcd	91.6 a	42.0 cdef	1203.6

Values are the average of five trials

[a] The biomass was calculated as follows: biomass = $Ai/A \times 100$ (Ai represents the weight of treatment m^{-2} and A represents the weight of healthy control m^{-2})

[b] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

biomass measured at the end of each crop cycle, reflected the response of the different Brassica crops to the pathogen: a high disease incidence corresponded to a strong yield reduction (Table 10). When the correlation between disease index and biomass was evaluated for the two pathogens in the different crop cycles, a significant negative correlation was always observed, with the only exception of the isolate of *F. raphani* in the first crop cycle (Table 5).

Green manure application

The application of green manure treatment was not able to reduce the incidence of *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani*, in any cycle on all the crops susceptible to one or both pathogens (Tables 7 and 9). In the last crop cycle, disease index was similar or higher than in the first cycle.

Pathogen survival

The usage of benomyl-resistant mutants of both pathogens permitted easy and reliable reisolation of the strains used for soil infestation. *Fusarium oxysporum* f. sp. *conglutinans* after three crop cycles persisted in the soil, at significantly lower levels in the case of *B. verna*, a crop resistant to the pathogen. In the case of *B. juncea* and *E. sativa*, both susceptible to the pathogen, after three crop cycles, the pathogen was present at values higher than those introduced with soil infestation (Table 11). In the sixth crop cycle, the pathogen concentration in the soil generally increased slightly, with the exception of *B. juncea* in the presence of the highest initial inoculum. In the ninth crop cycle

Table 5 Correlation between disease index and corresponding biomass in Part 1(referred to Tables 3 and 4) and Part 2 (referred to Tables 8 and 10)

Part 1[a]						Part 2 [b]				
Species and codes	Pearsons coefficients	Significance of correlation [c]	Isolates of *Fusarium oxysporum* f. sp.	Pearsons coefficient	Significance of correlation	Cycle of green manures	*F. conglutinans* ATCC 16600 RB		*F. raphani* ATCC RB 1 + RB 264105	
							Pearsons coefficient	Significance of correlation	Pearsons coefficient	Significance of correlation
Brassica juncea (ISCI 61)	−0.268	*	*matthioli* ATCC 16602	−0.449	*	I cycle	−0.821	*	−0.283	NS
Brassica juncea (ISCI 99)	−0.860	*	*raphani* Fus Ruc 13/03	−0.859	*	II cycle	−0.449	*	−0.711	*
Brassica juncea (ISCI 20)	−0.781	*	*raphani* Fus Ruc 9A/02	−0.391	*	III cycle	−0.352	*	−0.608	*
Eruca sativa (Nemat)	−0.669	*	*raphani* ATCC 64105	−0.884	*	IV cycle	−0.664	*	−0.699	*
Brassica nigra (ISCI 27)	−0.650	*	*conglutinans* Fus Vale 5/04	−0.644	*	V cycle	−0.546	*	−0.769	*
Cramby abissinica (Mario)	−0.841	*	*conglutinans* Fus Ruc 6	−0.188	*	VI cycle	−0.577	*	−0.723	*
Rapistrum rugosum (Lori)	−0.444	*	*conglutinans* ATCC 16600	−0.775	*	VII cycle	−0.711	*	−0.730	*
Brassica carinata (ISCI 7)	−0.220	NS				VIII cycle	−0.892	*	−0.785	*
Lepidium campestre (ISCI 15)	−0.614	*				IX cycle	−0.792	*	−0.773	*
Lepidium sativum (ISCI 101)	−0.842	*								
Brassica rapa (Silla)	−0.164	NS								
Diplotaxis tenuifolia (Mazzocchi)	−0.806	*								
Sinapis alba (Pira)	−0.723	*								
Barbarea verna (ISCI 100)	−0.750	*								
Rapistrum rugosum (ISCI 03)	−0.707	*								
Brassica juncea (Poggiolo)	−0.604	*								
Sinapis arvensis (Iberina)	−0.525	*								

[a] susceptibility of different brassica crops to different isolates of *Fusarium oxysporum*.

[b] green manure simulation

[c] * significant correlation ($P<0.05$) ; NS = not significant correlation ($P>0.05$).

Table 6 Pathogencity of the mutants (RB) of *Fusarium oxysporum* f.sp *raphani* ATCC 64105 and *Fusarium oxysporum* f.sp *conglutinans* (ATCC16600) resistant to benomyl on rocket (*Eruca sativa*), compared to that of the wild types (WT)

Original strain	Benomyl ppm	UV treatment (min)	Code	Disease index (0–100)	
				First trial	Second trial
ATCC 64105	30	10	RB1[a]	50	50
	10	15	RB2[a]	60	35
	30	5	RB10	40	–[b]
	30	5	RB11	60	20
	30	15	RB12	75	15
	30	15	RB13	30	–
	30	20	RB14	25	–
	30	20	RB15	60	10
	10	10	RB7	40	–
	10	10	RB3	40	–
	10	5	RB4	80	20
	10	25	RB5	60	–
	10	15	RB6	60	25
	30	10	RB9	65	20
	10	25	RB8	10	–
	–	–	WT	63	47
ATCC 16600	10	5	RB 1[a]	100	100
	30	5	RB12	15	5
	–	–	WT	100	67

[a] Mutants selected for further work

[b] Not tested

the population decreased slightly, still remaining at values higher than the initial ones. The lower population of the pathogen was observed with the resistant Brassica crop (*B. verna*) (Table 11).

Fusarium oxysporum f. sp. *raphani* in the third crop cycle survived in the soil at significantly similar values in the case of the three tested crops. In the sixth cycle, significant increases in pathogen concen-

Table 7 Incidence of Fusarium wilt on selected Brassica crops inoculated with *Fusarium oxysporum* f.sp. *conglutinans* ATCC 16600 RB resistant to benomyl in different cycles of biofumigation

Cycle	Fusarium wilt expressed as disease index (0–100)								
	Healthy control			Soil infested with cfu ml^{-1} of soil 10^3			Soil infested with cfu ml^{-1} of soil 10^4		
	B. juncea ISCI 99 (HS)[a]	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)	*B. juncea* ISCI 99 (HS)	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)	*B. juncea* ISCI 99 (HS)	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)
1	0.0 a[b]	0.0 a	0.0 a	15.6 a	69.4 a	0.0 a	56.9 a	87.2 a	0.9 a
2	0.0 a	0.0 a	0.0 a	41.6 ab	85.9 ab	0.0 a	55.3 a	82.8 a	0.9 a
3	0.0 a	0.0 a	0.0 a	52.5 ab	86.9 ab	0.0 a	48.8 a	75.6 a	0.0 a
4	0.0 a	0.0 a	0.0 a	52.5 ab	86.9 ab	0.0 a	55.0 a	75.6 a	0.0 a
5	0.0 a	0.0 a	0.0 a	40.6 ab	88.8 ab	0.0 a	38.8 a	90.9 a	0.0 a
6	0.0 a	0.0 a	0.0 a	38.8 ab	83.4 ab	0.0 a	45.9 a	75.9 a	0.0 a
7	0.0 a	0.0 a	0.0 a	65.6 b	88.1 ab	4.4 a	30.3 a	84.4 a	0.0 a
8	0.0 a	0.0 a	0.0 a	79.4 b	95.6 b	4.7 a	59.4 a	80.3 a	0.0 a
9	0.0 a	0.0 a	0.0 a	46.9 ab	92.8 b	0.0 a	34.7 a	71.6 a	0.0 a

[a] Reaction: HS highly susceptible, disease index 61–100; S: susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

Table 8 Fresh epigeal biomass of selected Brassica crops inoculated with *Fusarium oxysporum* f.sp. *conglutinans* ATCC 16600 RB resistant to benomyl in different cycles of biofumigation

Cycle	Fresh epigeal biomass m^{-2} (g)								
	Healthy control			Soil infested with cfu ml^{-1} of soil 10^3			Soil infested with cfu ml^{-1} of soil 10^4		
	B. juncea ISCI 99 (HS)[a]	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)	*B. juncea* ISCI 99 (HS)	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)	*B. juncea* ISCI 99 (HS)	*Eruca sativa* Nemat (HS)	*B. verna* ISCI 100 (R)
1	2162.1 a[b]	2189.5 abcd	1623.5 a	1783.5 a	1077.7 a	1622.5 a	1008.6 ab	309.5 bc	1251.5 a
2	2100.1 a	2229.7 abc	1132.1 bc	893.8 bc	879.1 ab	936.0 bc	1212.9 a	822.2 abc	826.7 abc
3	1382.6 bcd	2729.7 a	1085.4 bcd	812.5 cde	1008.6 a	803.4 cde	718.5 abc	1425.3 a	856.7 abc
4	1682.4 abc	2639.7 ab	1423.8 ab	516.8 b	483.2 bc	1032.5 b	428.9 bc	983.2 ab	925.8 ab
5	2178.4 a	1822.7 cde	595.5 f	202.2 e	178.4 c	531.0 e	640.8 abc	178.4 c	420.7 c
6	1963.9 ab	1869.4 bcd	732.2 def	266.8 cde	269.3 c	712.4 cde	499.5 abc	526.9 bc	492.9 bc
7	1331.3 cd	1388.7 de	693.1 ef	80.8 de	127.0 c	610.3 de	257.6 bc	455.3 ac	878.0 abc
8	805.4 d	1110.3 e	711.4 ef	88.9 e	44.7 c	578.3 e	179.4 c	287.6 bc	754.1 bc
9	1033.0 d	1232.7 e	1033.0 cde	210.4 de	47.3 c	681.4 de	245.4 bc	283.5 bc	937.0 ab

[a] Reaction: HS highly susceptible, disease index 61–100; S: susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

tration were observed in the case of *E. sativa* and *B. nigra*, the two crops susceptible to the pathogen. In the ninth crop cycle a slight decrease was observed. However, population density remained always higher than at the beginning of the trial. The lower density was observed in the case of *B. juncea*, which is resistant to Fusarium yellows (Table 12).

Discussion

This study showed that many of the Brassica crops of interest for biofumigation are susceptible to *F. oxysporum* f. sp. *conglutinans* and to *F. oxysporum* f. sp. *raphani*. Particularly, *E. sativa* (cv. Nemat), used as catch crops for nematodes, is susceptible to both

Table 9 Incidence of Fusarium wilt on selected Brassica crops inoculated with *Fusarium oxysporum* f.sp. *raphani* ATCC 64105 RB resistant to benomyl in different cycles of biofumigation

Cycle	Fusarium wilt expressed as disease index (0–100)								
	Healthy control			Soil infested with cfu ml^{-1} of soil 10^3			Soil infested with cfu ml^{-1} of soil 10^4		
	B. juncea ISCI 99 (R)[a]	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)	*B. juncea* ISCI 99 (R)	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)	*B. juncea* ISCI 99 (R)	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)
1	0.0 a[b]	0.0 a	0.0 a	0.0 a	14.4 a	12.2 a	0.0 a	27.2 a	17.5 a
2	0.0 a	0.0 a	0.0 a	0.0 a	52.8 b	25.9 ab	0.3 a	70.3 b	35.6 bc
3	0.0 a	0.0 a	0.0 a	0.9 a	70.3 bc	24.1 ab	0.3 a	74.7 bc	37.5 bc
4	0.0 a	0.0 a	0.0 a	0.0 a	83.8 c	36.6 ab	0.0 a	88.1 bc	44.4 bc
5	0.0 a	0.0 a	0.0 a	0.9 a	89.1 c	45.6 b	0.0 a	93.1 bc	44.7 bc
6	0.0 a	0.0 a	0.0 a	0.0 a	89.1 c	37.5 ab	0.0 a	95.3 c	35.3 bc
7	0.0 a	0.0 a	0.0 a	0.0 a	93.8 c	35.6 ab	0.0 a	90.6 bc	35.0 bc
8	0.0 a	0.0 a	0.0 a	0.0 a	93.8 c	22.8 ab	0.0 a	93.1 bc	30.9 ab
9	0.0 a	0.0 a	0.0 a	0.0 a	96.3 c	28.8 ab	0.0 a	94.7 bc	51.6 c

[a] Reaction: HS highly susceptible, disease index 61–100; S: susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

Table 10 Fresh epigeal biomass of selected Brassica crops inoculated with *Fusarium oxysporum* f.sp. *raphani* ATCC 64105 RB resistant to benomyl in different cycles of biofumigation

Cycle	Fresh epigeal biomass m^{-2} (g)								
	Healthy control			Soil infested with cfu ml^{-1} of soil 10^3			Soil infested with cfu ml^{-1} of soil 10^4		
	B. juncea ISCI 99 (R)[a]	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)	*B. juncea* ISCI 99 (R)	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)	*B. juncea* ISCI 99 (R)	*Eruca sativa* Nemat (S)	*B. nigra* ISCI 27 (HS)
1	2210.4 a[b]	2206.8 a	2134.7 a	2160.6 a	2149.9 a	2078.8 a	2066.1 a	2066.6 a	2075.2 a
2	2283.0 a	2187.0 a	1469.5 bc	1769.8 ab	1632.1 ab	1679.9 a	2109.8 a	1058.4 b	1248.0 b
3	1968.5 ab	2352.1 a	691.6 d	1301.8 bc	1285.1 bc	1006.6 b	1453.8 b	785.6 bc	672.3 c
4	1490.9 b	2498.5 a	1843.5 ab	947.2 cd	504.6 cd	928.9 b	954.3 c	428.4 bcd	571.1 cd
5	2036.6 ab	2448.2 a	1787.1 ab	1031.0 cd	250.5 d	735.3 bc	794.7 cd	108.7 d	468.0 cd
6	2057.4 ab	1788.6 ab	1657.5 b	842.5 cd	201.2 d	703.8 bc	697.7 cd	167.2 cd	240.3 d
7	1728.7 ab	1142.8 b	1221.5 c	692.1 d	79.3 d	372.0 cd	526.4 d	157.0 cd	414.1 cd
8	777.4 c	1103.2 b	628.6 d	599.6 d	89.9 d	220.0 d	550.8 d	92.0 d	411.1 cd
9	689.5 c	1099.1 b	769.8 d	571.1 d	99.6 d	171.2 d	544.2 d	50.8 d	302.8 d

[a] Reaction: HS highly susceptible, disease index 61–100; S: susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

pathogens, *B. nigra* is susceptible to *F. oxysporum* f. sp. *raphani* and *B. juncea*, which is considered one of the most effective biocidal plants, is highly susceptible to *F. oxysporum* f. sp. *conglutinans*. A good inverse correlation between disease index and biomass was observed. As a consequence, green manure treatment, carried out by growing different cycles of biocidal plants, with a short crop cycle of 30–35 days,

Table 11 Population dynamics of *Fusarium oxysporum* f.sp. *conglutinans* ATCC 16600 RB1 in soils biofumigated with different Brassica species in the third, sixth and ninth crop cycles

Host (reaction) [a]	Soil infested with cfu ml^{-1} of soil	Pathogen concentration (cfu g^{-1} soil)					
		In the third cycle		In the sixth cycle		In the ninth cycle	
		Mean value	Log10[b]	Mean value	Log10	Mean value	Log10
B, juncea ISCI 99 (HS)	–	0	–	0	–	0	–
Eruca sativa Nemat (HS)	–	0	–	0	–	0	–
B. verna ISCI 100 (R)	–	0	–	0	–	0	–
–[c]	10^3	–	–	–	–	5.5×10^2	2.6 a
–	10^4	–	–	–	–	9.3×10^2	2.9 b
B, juncea ISCI 99 (HS)	10^3	6.5×10^4	4.8 c[d]	1.0×10^5	5.0 b	3.2×10^4	4.4 cd
Eruca sativa Nemat (HS)	10^3	6.0×10^4	4.8 abc	7.0×10^4	4.9 ab	3.6×10^4	4.5 d
B. verna ISCI 100 (R)	10^3	1.2×10^4	4.0 ab	4.0×10^4	4.5 a	2.3×10^4	4.2 c
B, juncea ISCI 99 (HS)	10^4	7.0×10^4	4.9 c	5.0×10^4	4.7 ab	3.1×10^4	4.4 cd
Eruca sativa Nemat (HS)	10^4	7.0×10^4	4.8 bc	6.0×10^4	4.8 ab	3.5×10^4	4.6 d
B. verna ISCI 100 (R)	10^4	1.3×10^4	4.0 a	3.0×10^4	4.4 a	1.9×10^4	4.2 c

[a] Reaction: HS: highly susceptible, disease index 61–100; S susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Data were analysed using log10 values

[c] control without the plants

[d] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

Table 12 Population dynamics of *Fusarium oxysporum* f.sp. *raphani* resistant to benomyl ATCC 64105 RB 1 and 2 in soils biofumigated with different Brassica species in the third, sixth and ninth crop cycles

Host (reaction) [a]	Soil infested with cfu ml^{-1} of soil	Pathogen concentration (cfu g^{-1} soil)					
		In the third cycle		In the sixth cycle		In the ninth cycle	
		Mean value	Log10[b]	Mean value	Log10	Mean value	Log10
B. juncea ISCI 99 (R)	–	0	–	0	–	0	–
Eruca sativa Nemat (S)	–	0	–	0	–	0	–
B. nigra ISCI 27 (HS)	–	0	–	0	–	0	–
–[c]	10^3	–	–	–	–	1.6×10^3	3.0 a
–	10^4	–	–	–	–	4.1×10^3	3.5 b
B. juncea ISCI 99 (R)	10^3	2.0×10^4	4.5 a[d]	4.0×10^4	4.6 a	2.4×10^4	4.3 c
Eruca sativa Nemat (S)	10^3	5.0×10^4	4.8 a	7.0×10^4	4.9 b	2.5×10^4	4.3 c
B. nigra ISCI 27 (HS)	10^3	5.0×10^4	4.8 a	7.0×10^4	4.9 b	5.7×10^4	4.6 d
B. juncea ISCI 99 (R)	10^4	3.5×10^4	4.3 a	3.0×10^4	4.5 a	2.2×10^4	4.3 c
Eruca sativa Nemat (S)	10^4	1.1×10^5	4.7 a	1.5×10^5	5.2 c	4.0×10^4	4.6 d
B. nigra ISCI 27 (HS)	10^4	5.9×10^4	4.7 a	1.6×10^5	5.2 c	6.0×10^4	4.7 d

[a] Reaction: HS: highly susceptible, disease index 61–100; S susceptible, disease index 30–60; R: resistant, disease index 0–10

[b] Data were analysed using log10 values

[c] Control without the plants

[d] Values in the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$)

did not reduce Fusarium wilts on susceptible Brassica hosts. This is due to the fact that the population of the pathogen increased as a result of the incorporation of tissues of the susceptible plants and therefore became too difficult to control. In our study the initial baseline was steamed substrate in order to exclude any effect from microbial (including pathogenic) populations already present Marschner & Rumberger (2004). No wilt decline was observed after repeated growing of the same crop.

When Brassica crops were resistant to the two *F. oxysporum* isolates used in our study for soil infestation, green manure simulation did inhibit both pathogens, thus confirming its biocidal activity. The growth inhibition of *F.oxysporum* f. sp. *conglutinan*s caused by gases emitted from decomposing cabbage residues was already reported by Ramirez-Villapudua and Munnecke (1988). A study carried out on *F. oxysporum* from forest nurseries showed that conidial formation was little affected, mycelial growth was inhibited to a relatively small extent, while conidial and chlamydospore germination were highly susceptible to inactivation by isothiocyanates (Smolinska et al. 2003). The results obtained under our experimental conditions show that biofumigation is not applicable on crops susceptible to the same formae speciales of *F. oxysporum* affecting Brassica species used for biofumigation, because in the presence of Fusarium yellows of the biocidal plants, the inoculum potential will increase in the soil, with possible negative effects on the crops grown. When brassica crops resistant to Fusarium yellows were used for biofumigation, *F. oxysporum* populations tended to decrease. For this reason, Brassica crops resistant to Fusarium yellows should be grown where biofumigation is applied.

Our results have been obtained by working on pots and with relatively short crop cycles, 30–35 days in comparison to those used in the field, which vary from 55 to 106 days (Lazzeri et al. 2004). Since biofumigation could be an interesting soil disinfestation method for short-cycle intensive crops, such as salad and other crops grown for processing, which are repeated 4–5 times in the same soil, it will be interesting to verify the behaviour of biocidal plants in the presence of other soil-borne pathogens (i.e. *Rhizoctonia solani*), which affect both the biocidal plants and the crops grown. Growers should consider using different crops in rotation for biofumigation, instead of using and incorporating in the soil the same

crop repeatedly. This practice might reduce the above hazards of disease increase. Our study shows a possible negative drawback when biofumigation is applied with a very short time of cultivation. Moreover, it suggests that further studies taking into consideration other pathogens, potentially affecting both biocidal as well as crop plants (i.e. *Rhizoctonia solani*) should be carried out. The concept that Brassicas used as biofumigants should not host the pathogen/pest of interest was discussed by Matthiessen and Kirkegaard (2006) and addressed in the case of nematodes (Stirling and Stirling, 2003; Pattison et al. 2006). Our results show that there is not one simple solution for soil disinfestation (Katan 2000). Extension services should be aware of the potential risks of biofumigation in order to prevent them.

Acknowledgements This work was supported by a grant from the Italian Ministry for Environment, Land and Sea. The fellowship of Lu Pingxiang was co-funded by the Italian Ministry for University and Research, the University of Torino and the Italian Ministry for Environment, Land and Sea. The authors thank Guido Martano for the skilful technical support given during the trials and Dr. Mattia Sanna for help in statistical analysis. The critical reviewing of the manuscript by Prof. Jaacov Katan and Prof. Abraham Gamliel is gratefully acknowledged.

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