Journal of Horticultural Research 2014, vol. 22(1): 123-129
DOI: 10.2478/johr-2014-0015

THE IMPACT OF THE BRASSICACEAE PLANT MATERIALS ADDED TO THE SOIL ON THE POPULATION OF *FUSARIUM SOLANI* (Mart.) SACC. AND *FUSARIUM OXYSPORUM* SCHLECHT

Urszula SMOLIŃSKA*, Waldemar KOWALCZYK
Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Received: March 31, 2014; Accepted: June 3, 2014

ABSTRACT

Soil with the spores of fungi in dormant stage from previous years infections belonging to the genus *Fusarium* caused significant loss of onion yield in Poland. The most important form of *Fusarium* inoculum in the field are chlamydospores, which may survive in soil for many years. There are no available methods to eradicate chlamydospores from soil environment. Inspired by data from the literature and own preliminary observation, we evaluated the effect of application of *Brassicaceae* plant material to the soil infested with *F. oxysporum* and *F. solani* isolates, which are pathogenic to onion. Obtained results showed that addition of fresh plant material from *B. juncea* and *B. alba* had no effect on amount of *Fusarium* propagules in the soil. Moreover amendment of infested soil with dry plants or milled seeds of *B. juncea* or *B. nigra* significantly stimulated the population of *Fusarium* in the soil.

Key words: *Brassicaceae* plants, biofumigation, *Fusarium* chlamydospores

INTRODUCTION

Fungi belonging to the genus *Fusarium* are among the most destructive pathogens of many arable crops worldwide. Several species of these fungi are infectious agents and cause significant losses in the production of onion. *Fusarium oxysporum* f. sp. *cepae* is particularly harmful and causes *Fusarium* basal rot which progresses from a slight discoloration to a total necrosis of the basal plate and bulb scales (Sintayehu et al. 2011; Taylor et al. 2013). In recent years, *Fusarium* disease of onion is become very common in Poland due to climate changes, with hot summers and heavy rainfalls in particular.

The main form of *Fusarium* inoculum in the field are chlamydospores (De Cal et al. 1997). They can survive in soil for many years (Couteaudier & Alabouvette 1990). Currently there is no method available for the eradication of chlamydospores from the field soil. Therefore, once the field is infested it becomes very difficult to use it for cultivation of onion. The methods of chemical fumigation are very expensive and are prohibited in many countries (Duniway 2002). Soil solarisation (solar heating) effective in many parts of the world but due to low temperate climate it is not possible in Poland.

One of the method to limit the population of plant pathogens from soil is biofumigation (Gamliel et al. 2000; Matthiessen & Kirkegaard 2006; Piekarska et al. 2010; Bohinc et al. 2012). 'Biofumigation' is a term used to describe suppression of soil-borne pests and pathogens by addition of organic material that possess allelopathic potential. Organic supplements from various sources were added to the soil for control of soil-borne pathogenic microorganisms. The detrimental effect of organic material on fungal pathogens was observed in the case of *Verticillium dahliae*, *Thielaviopsis basicola*, *Sclerotinia sclerotiorum*, *Phytophthora* sp., *Sclerotium cepivorum*, *Pythium* sp., *Rhizoctonia solani* (Chung et al. 2003; Lopez-Escudero et al. 2007; Bonanomi et al. 2007; Motisi et al. 2013).

*Corresponding author:
e-mail: Urszula.Smolinska@inhort.pl

Brassicaceae plants and *Brassica juncea*, in particular, are of special interest for used in biofumigation (Kirkegaard & Sarwar 1998). Plants belonging to this family contain significant levels of glucosinolate compounds. There are about 20 different types of glucosinolates commonly found in *Brassicaceae*. The types and concentration of these compounds vary between *Brassicaceae* species. During plant tissues destruction, in the presence of water, glucosinolates are hydrolysed by myrosinase enzymes. Myrosinase is stored in specialised myrosin cells, which are located separately within plant body. When the plant tissues are damaged by mechanical stress or microbial degradation, myrosinase decomposes glucosinolate, forming many products including isothiocyanates (ITC), thiocyanates, nitriles, oxazolidinethiones (Brown & Morra 1997). Isothiocyanates are the most toxic of several hydrolysis products and are known to have broad biocidal activity (Gimsing & Kirkegaard 2009). For example preparation Dazitol™, produced in the United States for broad spectrum control of soil born pests and diseases contains 4.37% isothiocyanates (Cao et al. 2007).

There is some information on the toxic effect of glucosinolate degradation products on *Fusarium* sp. (Ramirez-Villapuda & Munnecke 1987, 1988; Mayton et al. 1996; Smolinska et al. 2003; Burgieł 2005). The most harmful is 2-propenyl ITC which is found in high concentration in shoots and roots of mustard species: *B. juncea*, *B. carinata*, *B. nigra*.

The objective of this study was to determine the effect of soil amendment with *Brassicaceae* plant residues on population of *Fusarium* fungi, pathogenic to onion plants.

MATERIALS AND METHODS

Fusarium isolates

F. solani isolate C7 and *F. oxysporum* isolate C33 were obtained from diseased onions cultivated on the fields in Skierniewice and near Kalisz, respectively. Stock cultures were maintained on potato dextrose agar (PDA-Merck) at 5 °C. For this experiment, fungi were grown for 7-10 days on PDA at 25 °C.

Organic materials

The following material from *Brassicaceae* plants were used: mustard (*Brassica juncea* cv. Małopolska) and *B. juncea* (marked as II – from commercial source, unknown variety); white mustard (*Brassica alba*); meal from seeds of mustard (*B. juncea*) and black mustard (*B. nigra*). The seeds were sown in field microplots, and green plants were harvested at the beginning of flowering. Freshly harvested material was dried at room temperatures (20-25 °C) for about a week. The dry plants (stems without roots) were crushed into pieces of about 0.5 cm and kept in plastic bags for further use. During the experiments, fresh and dry plant material were used. In the treatment with seed meal, the seeds of *B. juncea* and *B. nigra* were milled for 3-5 s in a grinder.

Production of *Fusarium* chlamydospores

Fusarium chlamydospores were cultured according to Smith and Snyder (1971) method. The *Fusarium* isolates were grown on potato-dextrose agar (PDA-Merck) at 25 °C for about 10 days. After that mycelium with conidia were suspended in distilled water and added to the sterilised soil in an open, plastic container. The soil was allowed to dry for about 2 weeks at room temperature (about 20-25 °C). Then the soil was mixed, remoistened and dried for additional 4 weeks. During this period *Fusarium* conidia either converted into chlamydospores or died. Afterwards, the soil was crushed and kept in plastic bags until use. For each series of experiments a new portion of the soil infested with chlamydospores was prepared for each fungal isolate. The amount of *Fusarium* propagules was evaluated with dilution plate method on Komada medium (1975).

Effect of organic material on survival of *Fusarium* chlamydospores in soil

The experiment was conducted in the unsterilized, pseudopodsolic, sandy-loam soil of pH – 7.4; salinity – 0.16 $g \cdot dm^{-3}$ (NaCl); $N\text{-}NO_3$ – 18 $mg \cdot dm^{-3}$; P – 56 $mg \cdot dm^{-3}$; K – 57 $mg \cdot dm^{-3}$; Mg – 81 $mg \cdot dm^{-3}$; Ca – 1180 $mg \cdot dm^{-3}$ in laboratory conditions. Artificially infested soil with *Fusarium* chlamydospores (prepared as described above) was added in the proportion of 5 : 1 v/v, to the uninfested soil.

Afterwards, both soils were thoroughly mixed together with tap water to obtain humidity of about 60%. To 3 kg-portions of soil, 30 g (1% w/v) of *Brassicaceae* tissues (fresh or dry) were added. After mixing, the soil was divided into three portions, 1 kg each, and placed into plastic bags. In order to maintain an appropriate level of oxygen the bags were kept open. The soil was incubated at the room temperatures (20-25 °C) for 1 month.

The following supplements to soil infested with *F. oxysporum* C33 and *F. solani* C7 were applied:

1. control – no supplements
2. *B. juncea* (dry),
3. *B. alba* (dry),
4. *B. juncea* (fresh),
5. *B. alba* (fresh),
6. *B. juncea* II (fresh),
7. *B. juncea* (seed meal),
8. *B. nigra* (seed meal).

For each fungal isolate two series of experiment were prepared. The concentration of *Fusarium* propagules at the start of each experiment was slightly different, because for each series a new portion of the soil with chlamydospores was prepared. Hence, statistical analysis was done for each series separately. After 1 month the concentration of *Fusarium* propagules was evaluated using the method described below.

Evaluation of *Fusarium* population in soil samples

The density of fungal propagules in the soil from the experiment described above were determined by the method dilution plating on the selective Komada medium for *Fusarium* species (1975). The medium contained: 2 g L-asparagine, 20 g D-galactose, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot H_2O$, 0.5 g KCl, 50 mg Fe(EDTA), 20 g agar per litre of distilled water. After autoclaving and cooling to about 40 °C, 0.5 g oxgall, 500 mg streptomycin sulphate, 1 g $NaB_4O_7 \cdot 10H_2O$, 1.25 g PCNB (pentachloronitrobenzene) were added. The pH was adjusted to 3.8.

The soil samples were taken from each plastic bags. There were three bags (replications) per one treatment. For each replication 10 g of soil was suspended in 100 ml of sterilised, distilled water. The suspension was shaken for 15 min and a 10-fold dilution series were prepared; 100 µl of the suspension was poured on one Petri plate with Komada medium. Suspensions of a selected dilution were plated on 3-4 Petri dishes. After 8 days of incubation at 25 °C, the colonies typical for *Fusarium* species were counted.

The experiment was repeated. Significance of differences between means was established by one-way analysis of variance and the Newman-Keuls test at $p = 0.05$.

RESULTS AND DISCUSSION

Obtained results showed that addition of fresh plant material from *B. juncea* and *B. alba* had no effect on the amount of *Fusarium* propagules in the soil (Figs. 1-4). Unfortunately, in other combinations the organic material increased the population of *Fusarium* in the soil.

The incubation of *F. oxysporum* C33 chlamydospores with *Brassicaceae* tissues gave different results depending on the type of material used. The highest number of *Fusarium* colonies (especially in the second series) was obtained from the samples where dry *B. juncea* tissues were added (Figs. 1 & 2). The biggest increase of fungal propagules was observed after the addition of milled seeds of black mustard to infested soil in the first series of experiment (Fig. 1). Also the addition of meal from *B. juncea* seeds stimulated increase of *Fusarium* population.

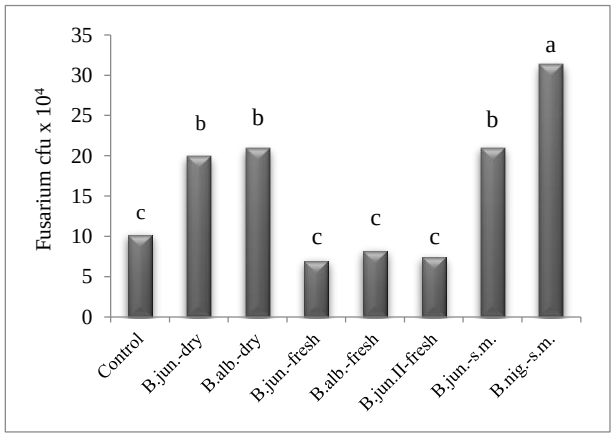

Fig. 1. Effect of soil supplementation with organic materials from *Brassicaceae* plants on cfu number of *F. oxysporum* C33 – series I in 1 g soil. Values marked by the same letter are not significantly different according to Newman–Keuls test ($p = 0.05$)

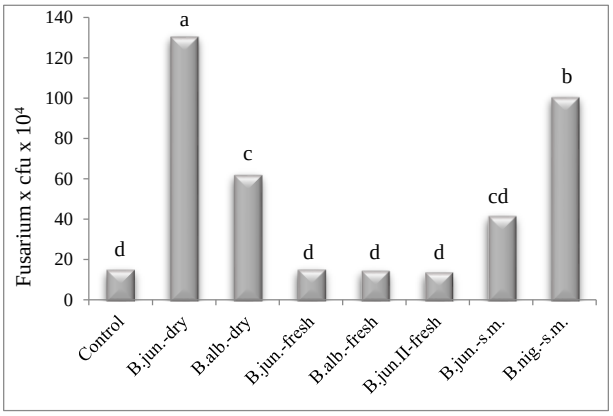

Fig. 2. Effect of soil supplementation with organic materials from *Brassicaceae* plants on cfu number of *F. oxysporum* C33 – series II in 1 g soil. Values marked by the same letter are not significantly different according to Newman–Keuls test (p = 0.05).

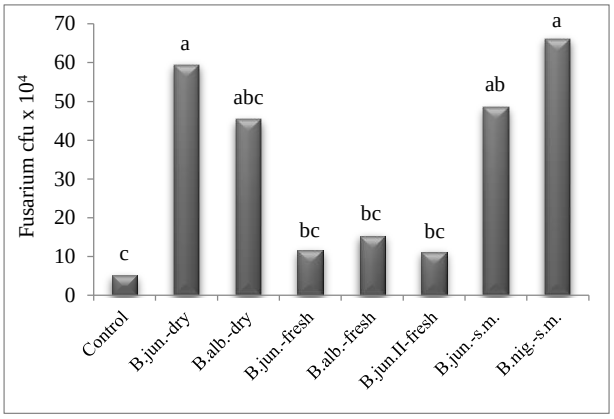

Fig. 3. Effect of soil supplementation with organic materials from *Brassicaceae* plants on cfu number of *F. solani* C7 –series I in 1 g soil. Values marked by the same letter are not significantly different according to Newman–Keuls test (p = 0.05).

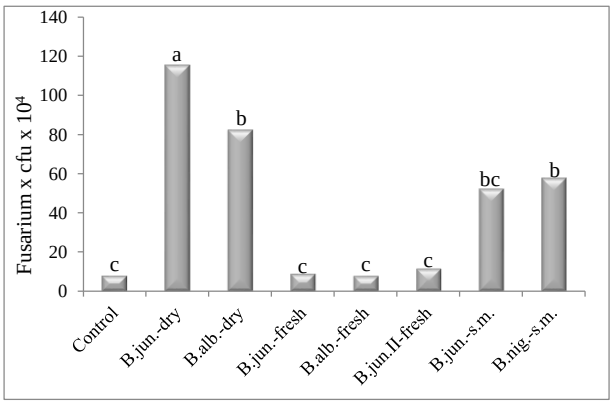

Fig. 4. Effect of soil supplementation with organic materials from *Brassicaceae* plants on cfu number of *F. solani* C7 – series II in 1 g of soil. Values marked by the same letter are not significantly different according to Newman–Keuls test (p = 0.05).

The incubation of *F. solani* C7 chlamydospores with plant material also gave different results depending on the type of material used (Figs. 3 & 4). The most significant increase of *Fusarium* in the soil was observed after the addition of dry shoots of *B. juncea*, both in the first and in the second series of the experiment. The increase in the second series (Fig. 4) was almost twice as large when compared with the first one (Fig. 3). The addition of milled seeds of mustard and black mustard also significantly increased number of *F. solani* C7 propagules. Therefore, in the case of both *Fusarium* isolates similar tendency were observed.

The soil used in this experiment was not sterilised. The level of indigenous, saprotrophic population of *Fusarium* in this soil was about 100-200 cfu (colony forming units) per gram. To make the count easier (and for limitation of mistakes), the soil used in the experiments was infested with high number of chlamydospores: the introduced *Fusarium* population was more than 10^5 cfu per gram of soil. As a result, by comparison with the initial population of the *Fusarium*, an assumption can be made, that almost all colonies counted on selective medium came from introduced *Fusarium* isolates, rather than of the indigenous population of this fungi occurring in the soil. Fungal colonies growing from soil samples almost without exemptions had the morphology corresponding to the *Fusarium* isolate introduced.

In both series the addition of fresh material of *B. juncea* and *B. alba* did not affect population of *Fusarium* (Figs. 1 & 2). In the previous work, we demonstrated that chlamydospores of *F. oxysporum* f. sp. *lycopersici* exposed to volatiles released from decomposed tissues of *B. juncea* and *S. alba* completely lost their viability (Smolinska 2000). The observation was confirmed using pure chemical compounds (the same are released from these plants): 2-propenyl, ethyl, phenylethyl, benzyl isothiocyanates (ITC) (Smolinska et al. 2003). On the basis of these observations an assumption can be made that reduction in pathogen population resulting from the application of *Brassicaceae* plant material are likely achievable, because chlamydospores are sensitive to glucosinolate degradation products, especially isothiocyanates.

However, results of current experiment showed that obtaining such an effect in soil conditions is not possible. The most important in direct effect is the amount of specific isothiocyanates which target on the pathogen. It is associated with many factors such as concentration of these active compounds in plant tissues and soil conditions (humidity, pH, compaction and others). In this experiment, the amount of isothiocyanates produced in the soil was not sufficient to decrease fungal population. Isothiocyanates had been lost from the soil by either volatilisation or microbial degradation. Furthermore, it cannot be ruled out that analysed *Fusarium* isolates exhibited relatively low sensitivity to these compounds.

Following the addition of dry plant material or seed meal a significant increase of both *F. oxysporum* C33 and *F. solani* C7 populations has been observed (Figs. 1-4). This increase depended on the kind of *Brassicaceae* additions used in the experiments. In the case of using dry plant materials these results can be easily explained. It is known that during drying of *Brassicaceae* plants the cells are damaged and glucosinolates were degraded. So, dry plants could act only as a source of mineral nutrients for microorganisms, and stimulated the chlamydospores germination and proliferation of *Fusarium*.

It was much more difficult to explain the increase of *Fusarium* population after addition of seed meals of *B. juncea* and *B. nigra*. It is known that these materials contained glucosinolates, which released toxic compounds during degradation (Brown & Morra 1997). Detrimental effect of mustard (*B. juncea*) ground seed meal toward *Rhizoctonia solani* was observed by Chung et al. (2002). Motisi et al. (2013) showed that incorporation of the crushing mustard leaves and stems into the soil significantly enhanced the possibility of control of primary infection of sugar beet by *R. solani*. The toxic effect of volatile compounds produced from *Brassica napus* seed meal significantly decreased survival and inoculum potential of *Aphanomyces euteiches* oospores (Smolińska et al. 1997). However, Mazzola et al. (2001) in greenhouse trials observed suppression of apple root infection caused by *Rhizoctonia* spp. regardless of regardless of glucosinolate content in *B. napus* seed meal added. Analogically to our work, they observed stimulating effect on *Pythium* sp. population after the application of *B. napus* seed meal. Results obtained in our work showed that if the amount of biologically active compounds present in plant tissues is not sufficient, organic carbon and another nutrients devoid toxic effects, may serve as a source of food and to increase *Fusarium* population.

However, despite the fact that in this work it did not result in a reduction of *Fusarium* population caused by addition of fresh *Brassicaceae* plant material, benefits of using green manure in agricultural practise are unquestionable. Biofumigation might also be attributed to the proliferation of antagonistic to pathogenic fungi microorganisms. Increase of suppressivity of soil caused by addition of organic material is due to the result of interactions between antagonistic microorganisms and pathogens, via competition, hyperparasitism or antibiosis (Borneman & Becker 2007). For example, Klein et al. (2011) showed 80% reduction of disease incidence and severity of root rot in cucumber plants inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum*, when seedlings were planted in the soil 2-34 months after soil supplementation with material of *Brassica oleracea* var. *italica* (broccoli).

In our experiment, incorporation of *Brassicaceae* plant materials to the field soil infested with *F. oxysporum* isolate C33 and *F. solani* isolate C7, fungi pathogenic to onion plants, stimulated reproduction of these fungi. However, we could not rule out that after application of fresh plant material with high concentration of glucosinolate and in different soil conditions, the decrease in chlamydospores survival may take place.

REFERENCES

Bohinc T., Ban S.G., Ban D., Trdan S. 2012. Glucosinolates in plant protection strategies: a review. Arch. Biol. Sci. 64: 821-828. DOI: 10.2298/ABS1203821B.

Bonanomi G., Antignani V., Pane C., Scala F. 2007. Suppression of soilborne fungal diseases with organic amendments. J. Plant Pathol. 89: 311-324.

Borneman J., Becker J.O. 2007. Identifying macroorganisms involved in specific pathogen suppression in soil. Annu. Rev. Phytopathol. 45: 153-172.

Brown P.D., Morra M.J. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. Adv. Agron. 61: 167-231.

Burgieł Z.J. 2005. Research on possibilities of utilization of chosen Brassicaceae plants in protection of cucumber against damping-off caused by Rhizoctonia solani Kühn and *Fusarium culmorum* (W.G. Smith) Sacc. Acta Agrobot. 58: 171-178. [in Polish with English abstract]

Cao A.C., Zhang W.J., Liu J.H. 2007. Progress in the alternatives to methyl bromide in soil disinfestations. Plant. Prot. 33: 15-18. DOI: 10.3969/j.issn.0529-1542.2007.01.005.

Chung W.C., Huang J.W., Huang H.C., Jen J.F. 2002. Effect of ground *Brassica* seed meal on control of *Rhizoctonia* damping-off of cabbage. Can. J. Plant Pathol. 24: 211-218.

Chung W.C., Huang J., Jen J. 2003. Control by *Brassica* seed pomace combined with *Pseudomonas boreopolis*, damping-off of watermelon caused by *Pythium* sp. Can. J. Plant Pathol. 25: 285-294.

Couteaudier Y., Alabouvette C. 1990. Survival and inoculums potential of conidia and chlamydospores of *Fusarium oxysporum* f.sp. *lini* in soil. Can. J. Microbiol. 36: 551-556.

De Cal A., Pascual S., Melgarejo P. 1997. Infectivity of chlamydospores vs microconidia of *Fusarium oxysporum* f.sp. *lycopersici* on tomato. J. Phytopathol. 145: 231-233.

Duniway J. 2002. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology 92: 1337-1343.

Gamliel A., Austerweil M., Kritzman G. 2000. Nonchemical approach to soilborne pest management – organic amendments. Crop Prot. 19: 847-853.

Gimsing A., Kirkegaard J. 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev. 8: 299-310.

Kirkegaard J., Sarwar M. 1998. Biofumigation potential of brassicas. Plant Soil 201: 71-89.

Klein E., Katan J., Gamliel A. 2011. Soil suppressiveness to *Fusarium* disease following organic amendments and solarization. Plant Dis. 95: 1116-1123. DOI:10.1094/PDIS-01-11-0065.

Komada H. 1975. Development of selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8: 114-125.

Matthiessen J.N., Kirkegaard J.A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci. 25: 235-265.

Mayton H.S., Olivier C., Vaughn S.F., Loria R. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86: 267-271.

Mazzola M., Granatstein D.M., Elfving D.C., Mullinix K. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. Phytopathology 91: 673-679.

Motisi N., Poggi S., Filipe J.A.N., Lucas P., Doré T., Montfort F., Gilligan C.A., Bailey D.J. 2013. Epidemiological analysis of the effects of biofumigation for biological control of root rot in sugar beet. Plant Pathol. 62: 69-78. DOI: 10.1111/j.1365-3059.2012.02618.x.

Lopez-Escudero F.J., Mwanza C., Blanco-Lopez M.A. 2007. Reduction of *Verticillium dahliae* microsclerotia viability in soil by dried plant residues. Crop Prot. 26: 127-133. DOI: 10.1016/j.cropro.2006.04.011.

Piekarska A., Bartoszek A., Namieśnik J. 2010. Biofumigation as an alternative method of crop protection. Ecol. Chem. Eng. S. 17: 527-547. [in Polish with English abstract]

Ramirez-Villapuda J., Munnecke D.E. 1987. Control of cabbage yellows (*Fusarium oxysporum* f.sp. *conglutinans*) by solar heating of field soils amended with dry cabbage residues. Plant Dis. 71: 217-221.

Ramirez-Villapuda J., Munnecke D.E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f.sp. *conglutinans* and other organisms. Phytopathology. 1988. 78: 289-295.

Sintayehu A., Fininsa C., Ahmed S., Sakhuja P.K. 2011. Evaluation of shallot genotypes for resistance against fusarium basal rot (*Fusarium oxysporum* f.sp. *cepae*) disease. Crop Prot. 30: 1210-1215. DOI: 10.1016/j.croppro.2011.04.011.

Smith S.N., Snyder W.C. 1971. Germination of *Fusarium oxysporum* chlamydospores in soil favorable and unfavorable to wilt establishment. Phytopathology 62: 273-277.

Smolinska U., Knudsen G.R., Morra M.J. 1997. Inhibition of *Aphanomyces euteiches* f.sp. *pisi* by volatiles produced by hydrolysis of *Brassica napus* seed meal. Plant Dis. 81(3): 288-292.

Smolinska U. 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. J. Phytopathology 148(6): 343-349. DOI: 10.1046/j.1439-0434.2000.00519.x

Smolinska U., Morra M.J., Knudsen G.R., James R.L. 2003. Isothiocyanates produced by Brassicaceae species as inhibitors of *Fusarium oxysporum*. Plant Dis. 87(4): 407-412.

Taylor A., Vagany V., Barbara D.J., Thomas B., Pink D.A.C., Jones J.E., Clarkson J.P. 2013. Identification of differential resistance to six *Fusarium oxysporum* f. sp. *cepae* isolates in commercial onion cultivars through the development of a rapid seedling assay. Plant Pathol. 62: 103-111. DOI: 10.1111/j.1365-3059.2012.02624.x.