EFFICACY OF BACTERIAL AND FUNGAL BIOCONTROL AGENTS AS SEED TREATMENTS AGAINST *FUSARIUM OXYSPORUM* f. sp. *LACTUCAE* ON LETTUCE

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SUMMARY

Several antagonist strains of *Pseudomonas* spp., including *Pseudomonas putida*, and *Fusarium oxysporum* were evaluated on seeds of lettuce (*Lactuca sativa*) cv. Crispilla Blanca as treatments against *Fusarium oxysporum* f. sp. *lactucae* in comparison with standard chemical treatments and biological products available in commerce. Assessment of the efficacy of the seed treatments was done *in vivo* under controlled conditions in a glasshouse. Seed treatments with *Pseudomonas* strains showed in most cases a limited efficacy but were statistically comparable with those obtained by chemical treatments with Prochloraz and Thiram, in terms of infected plants and disease index. Seed treatments with *Fusarium oxysporum* strain MSA35 were slightly phytotoxic as they affected the germination rate of treated seeds. The efficacy of the tested seed treatments with biocontrol agents for controlling the pathogen was not totally satisfactory but amenable to improvements and combination with other strategies of seed sanitization.

Key words: Biocontrol, *Fusarium oxysporum*, lettuce, *Pseudomonas*, seed treatment.

INTRODUCTION

Fusarium oxysporum f. sp. *lactucae*, the causal agent of wilt disease of lettuce (Matuo and Motohashi, 1967; Hubbard and Gerik, 1993; Huang and Lo, 1998; Garibaldi *et al*., 2002; Matheron and Koike, 2003), is seedborne (Garibaldi *et al*., 2004), lettuce seeds being important for pathogen dissemination (Elmer, 2001; Pasquali *et al*., 2005). In Italy, *Fusarium* wilt has emerged as a major production problem in Lombardy (northwestern Italy) (Garibaldi *et al*., 2002), where lettuce is grown every year in the same soil. Symptoms were first observed on cv. Salad Bowl at thinning, when 30-day-old seedlings wilted.

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Since non-chemical treatments for seed dressing are used for a more sustainable agriculture (Groot *et al*., 2004), the use of heat (Nega *et al*., 2003; Schmitt *et al*., 2009; Kubota *et al*., 2012; Koch *et al*., 2010), resistance inducers (Schmitt *et al*., 2009, Tinivella *et al*., 2009, Koch *et al*., 2010), fungal (van der Wolf *et al*., 2012) and plant extracts (Van Der Wolf *et al*., 2008; Hashem *et al*., 2010; Koch *et al*., 2010) has been tested in the last years, also upon the demand of consumers and environmentalists, on vegetable seeds against seed-borne pathogens. Thus, non-chemical methods of disease management such as seed coating with antagonistic microorganisms has become a common practice (Harman, 1991).

Soil contains several potential biocontrol agents (BCA) such as *Pseudomonas* spp., *Trichoderma* spp. and non-pathogenic *Fusarium* spp. that have shown high antagonistic activity against several soil-borne pathogens (Srinivasan *et al*., 2009). Moreover, recent investigations on antagonistic microorganisms have shown promising results on seed treatments (Gilardi *et al*., 2005; Ugoji *et al*., 2006; Leisso *et al*., 2009; Schmitt *et al*., 2009; Tinivella *et al*., 2009; Koch *et al*., 2010).

This study was undertaken to evaluate the efficacy of lettuce seed treatments with five bacterial and two fungal strains against *F. oxysporum* f. sp. *lactucae*.

MATERIALS AND METHODS

Preparation of *F. oxysporum* f. sp. *lactucae* inoculum. The isolate FUSLAT 10 RB of *F. oxysporum* f. sp. *lactucae* was isolated from infected lettuce plants and maintained on streptomycin sulphate-containing PDA dishes and slants (Sigma-Aldrich/Fluka, Germany) at 8°C. The strain was grown in shaken cultures for 10 days on PDB (Sigma-Aldrich/Fluka, Germany) at 25°C under 12 h/day fluorescent light. The cell suspension was centrifuged, the pellet mixed with talc (1:2 w/w), distributed on clean paper, and stored at 25°C for 10 days, to dry it up and induce chlamydospore production. The chlamydospore concentration of inoculated talc was determined by suspending a sample in sterilized-deionized water and spreading a ten-fold serial dilution on streptomycin sulphate-amended PDA dishes.

Preparation of antagonistic bacterial and fungal strains inoculum. Five bacterial strains (FC6B, FC7B, FC8B, FC9B and FC24B) were isolated from recycled substrates of tomato soilless crops grown at Albenga (northern Italy), suppressive to tomato *Fusarium* crown rot (Srinivasan *et al*., 2009), that showed antagonistic activity against this disease. Bacterial strains were maintained on LBA dishes and slants (Sigma-Aldrich/Fluka, Germany) at 8°C. They were aslo grown in shaken cultures for 24 h on LB (Sigma-Aldrich/Fluka, Germany) at 25°C. The cell suspension was centrifuged and the pellet re-suspended in sterile deionized water. Concentrations of the resulting dilutions were determined at OD_{600} in a Lambda 35 UV/VIS spectrophotometer (PerkinElmer, Italy).

Antagonistic *F. oxysporum* strains 251/2 and MSA 35 (Agroinnova, University of Torino) isolated from *Fusarium*-suppressive soils, were used. The strains were grown in shaken cultures for 10 days on PDB at 25°C under 12 h/day fluorescent light. The culture suspensions were centrifuged, the pellet was mixed with talc (1:2 w/w), distributed on clean paper, and stored at 25°C for 10 days to dry up and induce chlamydospore production. The chlamydospore concentration of inoculated talc was determined as above specified.

Concentrations and application methods of the treatments, including antagonistic bacterial and fungal strains, are shown in Table 1.

Tested seeds and artificial inoculation with *F. oxysporum* f. sp. *lactucae*. Half of a lot of healthy lettuce seeds of cv. Crispilla Blanca was mixed with inoculated talc, to reach a concentration of 1×10^6 chlamydospores per gram of seed, then throughly mixed with the other half of the lot. The chlamydospore concentration of inoculated seeds was determined by a washing test, according to ISTA methods (Mathur and Kongsdal, 2003).

Reference treatments. In order to have a chemical reference for evaluating the efficacy of biocontrol treatments against *F. oxysporum* f. sp. *lactucae*, inoculated seeds were treated with a resistance inducer (acibenzolar-S-methyl: Bion 50, Syngenta Crop Protection, Switzerland) and two fungicides commercially available for seed treatment, i.e. prochloraz (Octave, Bayer Crop Science, Germany) and thiram (Pomarsol, Bayer Crop Science, Germany). Treatments with Serenade (Bayer Crop Science, Germany), Eko Seed (Nufarm, Italy), Mycostop (Bioplanet, Italy), Micosat (CCS Aosta, Italy), Remedier (Isagro, Italy), and Rizocore (Biofarm, Italy) were also included as reference biocontrol products. Commercial products, concentrations, biocontrol agents, and application methods used are reported in Table 1.

Efficacy of treatments on artificially inoculated seeds. Inoculated lettuce seeds were treated in a Hege 11 seed treater (Wintersteiger, Switzerland) using a modified container for small seed quantities. The products were sprayed in the seed treater (1 ml/g of inoculated seeds), then treated seeds where air-dried under a vertical-laminar flow chamber for 1 h.

Treated seeds were sown into 42 cell plug-trays each containing 7 litres of 1:3 (v/v) peat-perlite substrate [Perlite Agrilit3 (Perlite Italiana, Italy); Peat Tecno2, (Turco, Italy)], each plug-tray cell receiving one seed. Plug-trays were placed on glasshouse benches in a completely randomised design at temperatures between 26 and 28°C, relative humidity between 85 and 95%, and were watered daily for 40 days. A control with inoculated seeds and a control with non-inoculated seeds were also included. For each treatment and controls, four repetitions (three plug-trays each) were performed.

Germination rate was evaluated by counting the number of emerged plants 10 days after sowing. Weekly surveys for plants killed by *Fusarium* were carried out and the final fresh biomass from each repetition was determined. A 0-100 disease index was used by rating each plant from 0 to 4, where 0 was a healthy plant and 4 a dead plant, using the following formula:

$$\text{Disease index}_{0\text{-}100} = [(a\times25)+(b\times50)+(c\times75)+(d\times100)]/e$$

where "a" is the final number of plants rated "1", "b" the final number of plants rated "2", "c" the final number of plants rated "3", "d" the final number of plants rated "4", and "e" the number of emerged plants. *F. oxysporum* f. sp. *lactucae* infection on symptomatic plants was confirmed by isolation on Komada medium (Komada, 1975) and benomyl-spiked PDA dishes. Each trial was performed three times.

Statistical analysis. Data of all repetitions were pooled together for each trial and worked out statistically by one-way analysis of variance (ANOVA), using the SPSS-WIN software. Duncan's multiple range test was employed. $P<0.05$ was considered significant.

RESULTS

The effects of the treatments with chemical products, biological products, and BCAs on germination rate, infected plant infction, disease index, and fresh biomass obtained from artificially inoculated lettuce seeds are shown in Table 2. Inoculated seeds treated with *F. oxysporum* strain MSA35 had a germination rate statistically lower than those from the non inoculated and non treated control; on the other hand, inoculated seeds treated with Thiram and Serenade showed the highest germination rates among the treatments on inoculated seeds. Despite this behaviour on germination, plants from inoculated and treated seeds presented a fresh biomass statistically similar to that of non inoculated and non treated control throughout all trials.

Table 1. Treatments performed on lettuce cv. Crispilla Blanca seeds artificially inoculated with *Fusarium oxysporum* f. sp. *lactucae*.

Seed treatments with antagonistic strains			
Treatment	Strain	Concentration (CFU per g of seed)	Application method
FC6B	*Pseudomonas* sp. FC6B (EU836173)	1×10^7	Seed spray
FC7B	*Pseudomonas putida* FC7B (EU836174)	1×10^7	Seed spray
FC8B	*Pseudomonas* sp. FC8B (EU836171)	1×10^7	Seed spray
FC9B	*Pseudomonas* sp. FC9B (EU836172)	1×10^7	Seed spray
FC24B	*Pseudomonas* sp. FC24B (EU836173)	1×10^7	Seed spray
251/2	*Fusarium oxysporum* 251/2	1×10^7	Inoculated talc addition
MSA35	*F. oxysporum* MSA35	1×10^7	Inoculated talc addition

Chemical seed treatments				
Treatment	Commercial product	% a.i.	Concentration of a.i. applied per Kg of seed	Application method
Acibenzolar-S-methyl	Bion 50	50%	0.1 g	Seed spray
Prochloraz	Octave	50%	1.0 g	Seed spray
Thiram	Pomarsol	49%	9.8 g	Seed spray

Seed treatments with biological products			
Treatment	Biocontrol agent(s)	Concentration of commercial product per Kg of seed	Application method
Serenade	*Bacillus subtilis* QST 713	10 g	Seed spray
Eko Seed	*B. subtilis* BA41; *Streptomyces* sp. SB15; *Trichoderma harzianum* TH02; *Pseudomonas proradix* 10; *Glomus caledonium* GM24; *Glomus coronatum* GU53; *Gladius intraradices* GB67; *Trichoderma* spp.	2 g	Dry powder addition
Mycostop	*Streptomyces griseoviridis*	8 g	Seed spray
Micosat	*Streptomyces* spp. SB14; *G. coronatum* GO01; *G. coronatum* GU53; *G. caledonium* GM24; *B. subtilis* SR63; *Pseudomonas* spp. PM46; *Ulocladium* spp. UO18	2 g	Seed spray
Remedier	*T. harzianum* ICC 012 *Trichoderma viridae* ICC 080	2 g	Seed spray
Rizocore	*T. harzianum* mix of mycorrhyzal non specified strains	2 g	Seed spray

Artificial inoculation of lettuce seeds induced an infection rate of *ca.* 27.7% on the inoculated and non treated control 40 days post sowing. The infection rate of plants treated with acibenzolar-S-methyl, Prochloraz, Thiram, Eko Seed, Remedier, and Rizocore was statistically similar to that shown by the non inoculated and non treated control. Among the tested biocontrol agents, the treatments with *Pseudomonas* strains FC6B, FC7B, FC8B, FC9B, and the *Fusarium* strain 251/2 presented the same behaviour on plant infection (reduction of plant infection ranging from 57.8 to 76.2%). The treatment with the *Pseudomonas* strain FC9B reduced the plant infection about the 76.2% if compared with the inoculated and non treated control.

The disease index calculated on the visible symptoms shown by plants from inoculated and non treated seeds was *ca*. 20.7%. Treatments with Mycostop, Micosat, and the *Fusarium* strain MSA35 were the only ones that yielded a disease index statistically higher than that determined on the non inoculated and non treated control. Among the experimental biocontrol agents, seed treatment with *Pseudomonas* strain FC9B reduced the disease index by about 79.7% if compared with the inoculated and non treated control; the other treatments with *Pseudomonas* strains and the *Fusarium* strain 251/2 reduced disease index from 62.3 to 72.5%.

DISCUSSION

In this study, the bacterial and fungal strains tested showed some antagonistic activity against *F. oxysporum* f. sp. *lactucae* when used as dressing on lettuce seeds. The present *in vivo* evaluations allowed an improved analysis of the effect of BCAs agents against *F. oxysporum* as a seed-borne pathogen of lettuce plants, if compared to our previous *in vitro* experiments (unpublished information) this because the actual assessment of *Fusarium*-induced symptoms on plants from inoculated seeds was included. Indeed, the detection under glasshouse conditions of fusariosis symptoms in the non inoculated and non treated controls, suggests that the seed lot was already infected, even if our previous *in vitro* evaluations did not report it

Table 2. Effect of treatments with chemical products, biological products, and biocontrol agents on lettuce cv. Crispilla Blanca seeds artificially inoculated with *F. oxysporum* f. sp. *lactucae* 40 days after sowing under controlled conditions in a glasshouse. Mean of three trials.

Treatment	Germination rate (%)	Infected plants (%)	Disease index (%)	Fresh biomass (g)
Non inoculated and non treated control	80.9 ± 3.4 b*	1.5 ± 0.7 a*	0.5 ± 0.3 a*	160.1 ± 25.7 a*
Inoculated and non treated control	75.4 ± 3.5 ab	27.7 ± 9.6 e	20.7 ± 9.6 c	121.0 ± 38.9 a
Acibenzolar-S-methyl	71.0 ± 4.8 ab	11.3 ± 7.6 a-d	8.1 ± 6.7 ab	149.8 ± 9.7 a
Prochloraz	75.4 ± 4.4 ab	2.3 ± 1.4 ab	1.1 ± 0.5 a	143.3 ± 38.9 a
Thiram	75.9 ± 2.2 b	7.8 ± 1.2 a-d	5.2 ± 0.7 ab	139.8 ± 31.9 a
Serenade	75.9 ± 6.2 b	12.7 ± 1.7 bcd	9.5 ± 2.3 ab	140.3 ± 60.9 a
Eko Seed	69.8 ± 8.7 ab	11.3 ± 2.6 a-d	7.1 ± 3.2 ab	132.1 ± 18.2 a
Mycostop	73.3 ± 9.8 ab	17.9 ± 8.4 d	12.2 ± 6.7 b	136.9 ± 17.7 a
Micosat F	73.4 ± 9.6 ab	16.4 ± 6.5 cd	12.6 ± 4.7 b	139.6 ± 6.5 a
Remedier	73.5 ± 7.5 ab	9.4 ± 2.4 a-d	5.7 ± 2.3 ab	150.7 ± 22.0 a
Rizocore	72.5 ± 5.2 ab	11.3 ± 6.4 a-d	7.8 ± 4.9 ab	141.5 ± 37.3 a
FC6B	75.0 ± 5.2 ab	11.7 ± 7.5 a-d	7.8 ± 5.1 ab	173.9 ± 31.4 a
FC7B	73.4 ± 4.3 ab	8.4 ± 3.6 a-d	5.7 ± 3.1 ab	138.9 ± 35.8 a
FC8B	71.4 ± 8.7 ab	9.4 ± 5.6 a-d	6.7 ± 4.1 ab	120.4 ± 18.8 a
FC9B	73.6 ± 1.8 ab	6.6 ± 4.7 abc	4.2 ± 3.7 ab	158.3 ± 61.3 a
FC24B	71.2 ± 7.4 ab	12.8 ± 2.6 bcd	8.3 ± 2.6 ab	158.6 ± 24.8 a
251/2	69.8 ± 5.7 ab	10.6 ± 6.0 a-d	7.7 ± 4.4 ab	131.3 ± 12.0 a
MSA35	63.2 ± 5.3 a	13.2 ± 6.8 cd	10.2 ± 6.0 b	118.3 ± 25.4 a

* Duncan's multiple range test ($P < 0.05$).

(unpublished information). It is important to consider that, when antagonistic *Fusarium* strains are used, they may displace pathogenic *Fusarium* species rather than eradicate infections already present in the seeds (Gilardi *et al*., 2005), so the whole analysis should be addressed at determining the presence of pathogenic *Fusaria* on the seeds. Anyhow, if *F. oxysporum* is external to the seeds as it was the case in this study, seed disinfection can contribute to reduce the spreading of the pathogen (Garibaldi *et al*., 2004).

Treatments with *Pseudomonas* FC6B, FC7B, FC8B, and FC9B strains showed interesting results on pathogen control, but their performance was still variable throughout the trials. Treatments with *Fusarium* strains 251/2 and MSA35 showed some antagonistic activity, but to a lower rate if compared with the bacteria-based treatments. Future experiments should consider testing for microflora assessment on treated seeds, for a more precise determination of the BCAs concentration. The main modes of action of BCAs, such as competition (Alabouvette *et al*., 2006), should be also studied for defining the actual interaction between them and the target pathogen. Comparing the germination rates from all the performed treatments through the trials, the low values from *Fusarium* MSA35 treatment could suggest phytotoxicity on treated lettuce seeds.

Despite the different levels of efficacy against *F. oxysporum* f. sp. *lactucae* shown by all the treatments tested in this study, it is important to point out that the efficacy of any seed treatment against a seed-borne pathogen is strongly influenced by the application method. So, future studies should consider different concentrations of fungicides and resistance inducers as well as different application methods to define a better reference for the performance of treatments with BCAs.

Since BCAs treatments did not offer a complete disinfection of inoculated seed, they could be integrated with other strategies for enhancing pathogen control. Physical methods such as aerated steam (Schmitt *et al*., 2009) can be used if done before applying BCAs, this for assuring a successful colonization of the seeds by the antagonist(s). At the same time, treatments with BCAs could be transferred to other crops for conventional and organic farming due to their activity against seed-borne pathogens and those already present in the soil. More research should be linked to understanding the interactions among plant, pathogens, antagonists, environmental conditions and farm practices (Alabouvette *et al*., 2006). The result of such integrated approaches could become a tool for deciding on the correct intervention at the farm level.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 311875 "TESTA - Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health". The authors thank Prof. Dr. Ulrich Gisi for the scientific support.

REFERENCES

Alabouvette C., Olivain C., Steinberg C., 2006. Biological Control of Plant Diseases: The European Situation. *European Journal of Plant Pathology* **114**: 329-341.

Elmer W.H., 2001. Seeds as vehicles for pathogen importation. *Biological Invasions* **3**: 263-271.

Garibaldi A., Gilardi G., Gullino M.L., 2002. First Report of *Fusarium oxysporum* on Lettuce in Europe. *Plant Disease* **86**: 1052.

Garibaldi A., Gilardi G., Gullino M.L., 2004. Seed transmission of *Fusarium oxysporum* f.sp. *lactucae*. *Phytoparasitica* **32**: 61-65.

Gilardi G., Tinivella F., Gullino M.L., Garibaldi A., 2005. Seed dressing to control *Fusarium oxysporum* f. sp *lactucae*. *Journal of Plant Diseases and Protection* **112**: 240-246.

Groot S.P.C., Van Der Wolf J.M., Jalink H., Langerak C.J., Van Den Bulk R.W. , 2004. Challenges for the production of high quality organic seeds. *Seed Testing International* **127**: 12-15.

Harman G.E., 1991. Seed treatment for biological control of plant disease. *Crop Protection* **10**: 166–171,

Hashem M., Moharam A.M., Zaied A.A., Saleh F.E.M., 2010. Efficacy of essential oils in the control of cumin root rot disease caused by *Fusarium* spp. *Crop Protection* **29**: 1111-1117.

Huang J.H., Lo C.T., 1998. Wilt of lettuce caused by *Fusarium oxysporum* in Taiwan. *Plant Pathology Bulletin* **7**: 150-153.

Hubbard J., Gerik J., 1993. A new wilt disease of lettuce incited by *Fusarium-oxysporum* f sp *lactucae* forma specialis nov. *Plant Disease* **77**: 750-754.

Koch E., Schmitt A., Stephan D., Kromphardt C., Jahn M., Krauthausen H.J., Forsberg G., Werner S., Amein T., Wright S.A.I., Tinivella F., Gullino M.L., Roberts S.J., Van Der Wolf J., Groot S.P.C., 2010. Evaluation of non-chemical seed treatment methods for the control of *Alternaria dauci* and *A. radicina* on carrot seeds. *European Journal of Plant Pathology* **127**: 99-112.

Kubota M., Hagiwara N., Shirakawa T., 2012. Disinfection of Seeds of Cucurbit Crops Infested with *Acidovorax citrulli* with Dry Heat Treatment. *Journal of Phytopathology* **160**: 364-368.

Komada H., 1975. Development of a selective medium for quantitative isolation ofFusarium oxysporum from natural soil. *Review of Plant Protection Research* **8**: 114-124.

Leisso R., Miller P., Burrows M., 2009. The influence of biological and fungicidal seed treatments on chickpea (*Cicer arietinum*) damping off. *Canadian Journal of Plant Pathology* **31**: 38-46.

Matheron M., Koike S., 2003. First report of fusarium wilt of lettuce caused by *Fusarium oxysporum* f. sp *lactucae* in Arizona. *Plant Disease* **87**: 1265.

Mathur S.B., Kongsdal O., 2003. Common Laboratory Seed Health Testing Methods for Detecting Fungi. 1st Ed. International Seed Testing Association, Bassersdorf, Switzerland.

Matuo T., Motohashi S., 1967. On *Fusarium oxysporum* f. sp. *lactucae* n. f. causing root rot of lettuce. *Transactions of the Mycological Society of Japan* **32**: 13-15.

Nega E., Ulrich R., Werner S., Jahn M., 2003. Hot water treatment of vegetable seed – an alternative seed treatment method to control seed borne pathogens in organic farming. *Journal of Plant Diseases and Protection* **110**: 220-234.

Pasquali M., Dematheis F., Gilardi G., Gullino M.L., Garibaldi A., 2005. Vegetative compatibility groups of *Fusarium oxysporum* f. sp *lactucae* from lettuce. *Plant Disease* **89**: 237-240.

Schmitt A., Koch E., Stephan D., Kromphardt C., Jahn M., Krauthausen H.J., Forsberg G., Werner S., Amein T., Wright S.A.I., Tinivella F., Van Der Wolf J., Groot S.P.C., 2009. Evaluation of non-chemical seed treatment methods for the control of *Phoma valerianellae* on lamb's lettuce seeds. *Journal of Plant Diseases and Protection* **116**: 200-207.

Srinivasan K., Gilardi G., Garibaldi A., Gullino M.L., 2009. Bacterial antagonists from used rockwool soilless substrates suppress *Fusarium* wilt of tomato. *Journal of Plant Pathology* **91**: 145-152.

Tinivella F., Hirata L., Celan M., Wright S.A.I., Amein T., Schmitt A., Koch E., Van Der Wolf J.M., Groot S.P.C., Stephan D., Garibaldi A., Gullino M.L., 2009. Control of seed-borne pathogens on legumes by microbial and other alternative seed treatments. *European Journal of Plant Pathology* **123**: 139-151.

Ugoji E., Laing M., Hunter C., 2006. An investigation of the shelf-life (storage) of *Bacillus* isolates on seeds. *South African Journal of Botany* **72**: 28-33.

Van Der Wolf J.M., Birnbaum Y., Van Der Zouwen P.S., Groot S.P.C., 2008. Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts. *Seed Science and Technology* **36**: 76-88.

Van Der Wolf J.M., Michta A., Van Der Zouwen P.S., De Boer W.J., Davelaar E., Stevens L.H., 2012. Seed and leaf treatments with natural compounds to induce resistance against *Peronospora parasitica* in Brassica oleracea. *Crop Protection* **35**: 78-84.

Received February 12, 2014
Accepted June 20, 2014