University of Warmia and Mazury, Olsztyn, Poland

THE EFFECTIVENESS OF THE BIOLOGICAL CONTROL OF CLUBROOT (*PLASMODIOPHORA BRASSICAE*) IN BRASSICACEAE PLANTS

T.P. Kurowski, B. Majchrzak and E. Kowalska

Abstract

The study was performed in 2005–2008 to determine the effectiveness of biological and chemical control of clubroot in oilseed spring rape, white mustard, white kohlrabi and kale. Chemical control with the use of Altima 500 SC fungicide was found highly effective. Biological control also reduced the rates of infection with *Plasmodiophora brassicae*, although to a lower degree. EM-1 provided better disease control than Biochikol 020 PC. *Alternaria alternata* was isolated most frequently from the seeds of oilseed spring rape and white mustard in all treatments. Numerous *Fusarium avenaceum* colonies were also isolated from oilseed rape seeds, most of them from the not-treated combination (control), and the smallest number from crops protected with EM-1.

Key words: clubroot, spring rape, white mustard, white kohlrabi, kale, biological plant protection, chemical plant protection

Introduction

The risk posed by *Plasmodiophora brassicae* to Brassicaceae crops has increased recently. Clubroot caused by this pathogen is a common disease of vegetables from this family. However, the increase of oilseed rape and white mustard growing areas favors the epidemic occurrence of the disease on agricultural plants in some regions of Poland (Kurowski and Bruderek 2004, Kurowski et al. 2008, Korbas et al. 2009, Robak and Gidelska 2009). Several fungicides have been registered for clubroot control in horticultural crops, *inter alia*: thiram – Sadoplon 75 WP, tiophanate-methyl – Topsin M 70 WP, fluazinam – Altima 500 SC, dazomet –

Phytopathologia 52: 5–12
© The Polish Phytopathological Society, Poznań 2009
ISSN 2081-1756

Nemazin 97 FG, metham sodium – Nemasol 510 SC (Zalecenia... 2008), but there are no effective management strategies for the disease in agricultural crops.

The aim of this study was to test the effectiveness of biological control of clubroot (*P. brassicae*) in Brassicaceae crops, and to determine the effect of the applied control agents on the communities of fungi colonizing the seeds of oilseed spring rape and white mustard.

Material and methods

The experiment was carried out in the years 2005–2008 in microplots (1 × 2 m) located at the Experimental Station in Tomaszkowo near Olsztyn (N = 53°43'04", E = 20°25'06"). In August 2003, 1 kg of comminuted cabbage roots infected with *P. brassicae* were mixed with the surface soil layer in each microplot. In 2004 white mustard was sown in all microplots, and the degree of soil infestation was determined on mustard roots towards the end of the growing season. The index of infection with *P. brassicae* ranged from 58 to 66%. In the same microplots, clubroot severity was assessed on root systems of oilseed spring rape cv. 'Trend' and white mustard cv. 'Borowska' in 2005–2007, and on root systems of white kohlrabi cv. 'Titan' and kale cv. 'Średniowysoki Zielony Kędzierzawy' in 2006–2008.

In all four experiments, the first experimental factor was clubroot control in cruciferous crops, and the second – years of the study. Each experiment comprised four treatments in three replications, as follows:

1) control treatment (no protection against clubroot),

2) fungicide Altima 500 SC at the rate of 2 l/ha, applied to the soil surface and incorporated into the soil one day prior to sowing or planting,

3) EM-1 at the rate of 1 l/ha, applied to the soil surface and incorporated into the soil one week prior to sowing or planting,

4) seed dressing and seedling disinfection with Biochikol 020 PC by soaking in a 2.5% solution for 2 h.

According to Higa (US Patent... 1998) EM-1 was composed of approximately 70 species of microorganisms representing five groups, including: lactic acid bacteria (*Lactobacillus bulgaricus*, *L. casei*, *L. plantarum*, *Pediococcus halophilus*, *Propionibacterium freudenreichii*, *Streptococcus facealis*, *S. lactis*), phototrophic bacteria (*Chlorobium limicola*, *Chromatium okenii*, *Rhodopseudomonas sphaereoides*, *R. palustris*, *R. capsulatus*, *Rhodospirillum rubrum*), actinomycetes (*Nocardia asteroides*, *Micromonospora chalcea*, *Streptomyces albus*, *S. griseus*, *Streptoverticillium baldaccii*, *Rhodococcus rhodochrous*, *Rhodospirillum rubrum*), fungi (*Aspergillus japonicus*, *A. oryzae*, *Mucor hiemalis*) and yeasts (*Saccharomyces cerevisiae*, *S. lactis*, *Candida utilis*).

At the end of the growing season, the entire plants were removed from the soil and their root systems were scored for the severity of clubroot symptoms with a three-point scale (Kurowski and Bruderek 2004), as follows: 1° – one or two galls formed on lateral roots, 2° – more than two galls formed on lateral roots or one small gall formed on the main root, 3° – at least one large gall formed on the main

root or a gall formed on the root crown. The results were expressed as an infection index according to formula of Townsend and Heuberger (Wenzel 1948).

The fungi that colonized the seeds of spring rape and white mustard were isolated with the modified method of Narkiewicz-Jodko (1986). The developing fungal colonies were transferred to potato-glucose agar slants, and the cultures were identified based on the relevant keys (Domsch and Gams 1972, Ellis 1971, Gams 1971, Kwaśna et al. 1991, Skirgiełło et al. 1979).

Results

Clubroot severity scores on oilseed spring rape were relatively low in 2005 and 2006 (12.7 and 17.9%, respectively), and substantially higher in 2007 (48.1%). The fungicide Altima 500 SC was found a highly effective tool for disease suppression (Table 1). The injury index of roots protected with the fungicide was as low as 5.6%, as compared to 46.4% in the control treatment. Both biocontrol agents also proved effective in the first and second year of the study, but in the third year, when the plants were severely infected, they were not able to suppress the disease development.

Table 1

Intensity of clubroot (*Plasmodiophora brassicae*) on the roots of cruciferous plants (infection index – %)

Plant	Year	Control	Altima 500 SC	EM-1	Biochikol 020 PC	Mean	LSD (p = 0.05)
Oilseed spring rape	2005	30.7	5.8	6.7	7.8	12.7	I – 2.48
	2006	38.7	5.9	7.8	19.0	17.9	II – 2.15
	2007	69.9	5.0	53.8	63.6	48.1	I×II – 4.31
	Mean	46.4	5.6	22.8	30.2		
White mustard	2005	32.4	17.6	19.1	20.0	22.3	I – 2.54
	2006	44.4	4.2	7.0	26.5	20.5	II – 2.20
	2007	38.0	1.5	18.8	49.5	27.0	I×II – 4.40
	Mean	38.3	7.8	15.0	32.0		
White kohlrabi	2006	94.8	0.0	45.8	42.6	45.8	I – 3.16
	2007	77.4	0.0	62.1	67.7	51.8	II – 2.74
	2008	19.9	0.0	11.9	14.9	11.7	I×II – 5.48
	Mean	64.0	0.0	39.9	41.7		
Kale	2006	55.1	1.1	34.8	54.7	36.4	I – 2.09
	2007	35.5	1.8	21.7	34.9	23.5	II – 1.81
	2008	10.6	0.0	7.1	9.0	6.7	I×II – 3.62
	Mean	33.7	1.0	21.2	32.9		

The infection rates of white mustard roots were comparable throughout the experimental period (Table 1). The average infection index ranged from 20.5% in 2006 to 27.0% in 2007. Among the tested control agents, Altima 500 SC proved most effective, followed by EM-1 and Biochikol 020 PC.

The root system of white kohlrabi was severely infected by *P. brassicae* in the control combination, and clubroot severity varied widely over the experimental period (Table 1), reaching the highest level in 2006 and the lowest in 2008. The disease did not occur on plants protected with Altima 500 SC. Biological control agents also suppressed disease development to a certain extent, but their effectiveness differed in particular years.

As regards kale, the highest incidence of clubroot was noted in the first year of the study, while the lowest – in the third year (Table 1). There were no significant differences in disease severity between the control combination and the Biochikol 020 PC treatment. Altima 500 SC provided an effective control of clubroot, and EM-1 reduced disease incidence by approximately 30%, as compared with the control combination.

Table 2

Fungi isolated from seeds of oilseed spring rape in 2005–2007

Species of fungus	Control		Altima 500 SC		EM-1		Biochikol 020 PC		Total
	d	n	d	n	d	n	d	n	
Acremoniella atra			1						1
Acremonium tubakii		1							1
Alternaria alternata	41	70	67	116	38	126	63	182	703
Alternaria brassicae				3	6	4			13
Alternaria brassicicola	1							3	4
Botrytis cinerea		8				2		9	19
Cladosporium cladosporioides						12	5		17
Epicoccum purpurascens		2				2		7	11
Fusarium avenaceum	32	82	4	77		6	2	52	255
Fusarium equiseti			2						2
Fusarium oxysporum	1								1
Fusarium poae	1								1
Mortierella alpina							1		1
Penicillium spp.	2	3	7	23	11	61	11	30	148
Rhizopus nigricans	10	31		23	91	61	14	7	237
Trichothecium roseum						29		31	60
Non-sporulating fungi	4	6	5	6	19	24	3	2	69
Sum	92	203	86	248	165	327	99	323	1 543

d – disinfected seeds, n – non-disinfected seeds.

The tested control agents had an indirect effect on the health status of oilseed spring rape and white mustard seeds. A phytopathological analysis revealed quantitative and qualitative changes in the fungal communities colonizing seeds of the investigated crop species.

A total of 1543 fungal colonies were isolated from oilseed spring rape seeds (Table 2). The lowest number of cultures was obtained from the control combination (295), higher number from chemically protected plants (334), and the highest from plants protected with the biological methods (422 and 492, following application of Biochikol 020 PC and EM-1, respectively). The predominant species was *Alternaria alternata* (45.6% of all isolates). *Fusarium avenaceum* (16.5%) was also abundant, accounting for 38.6%, 24.3%, 12.8% and 1.2% of all isolates in the control, Altima, Biochikol and EM-1 treatments, respectively. Other relatively abundant fungi were *Rhizopus nigricans* (15.4% of all isolates), *Penicillium* spp. (9.6%), non-sporulating fungi (4.5%) and *Trichothecium roseum* (3.9% obtained only from non-disinfected seeds in biological control treatments).

A total of 1785 fungal cultures were isolated from white mustard seeds (Table 3). The lowest number of colonies was isolated from the control combination (380), and the highest from chemically protected plants (503). Following the ap-

Table 3

Fungi isolated from seeds of white mustard in 2005–2007

Species of fungus	Control		Altima 500 SC		EM-1		Biochikol 020 PC		Total
	d	n	d	n	d	n	d	n	
Alternaria alternata	93	221	161	187	102	230	110	204	1 308
Alternaria brassicae	1								1
Alternaria brassicicola							2		2
Arthrinium sphaerospermum							2		2
Aspergillus sp.							3		3
Botrytis cinerea								1	1
Cladosporium cladosporioides	2	1							3
Epicoccum purpurascens	4	26	2	5	4	13	2	39	95
Fusarium avenaceum							1		1
Fusarium chlamydosporum						2			2
Fusarium sporotrichioides							2		2
Mucor hiemalis	2								2
Penicillium spp.	4	1	11		2		3		21
Rhizopus nigricans		19		50		36	76	52	233
Trichothecium roseum				84					84
Non-sporulating fungi	6		3		6		10		25
Sum	112	268	177	326	114	281	211	296	1 785

d – disinfected seeds, n – non-disinfected seeds.

plication of the biocontrol agents EM-1 and Biochikol 020 PC, 395 and 480 fungal cultures were obtained, respectively. The prevailing fungal species was *A. alternata* (73.3% of all isolates). *Rhizopus nigricans* (13.1%) and *Epicoccum purpurascens* (5.3%) were also relatively abundant, mostly on non-disinfected seeds. *Trichothecium roseum* accounted for 4.7% of all isolates, but it was obtained only from non-disinfected seeds of plants treated with Altima 500 SC.

Discussion

Clubroot occurs every year on cruciferous crops worldwide, causing substantial yield losses. The disease control is dependent on the development of effective management strategies. It seems possible that the biological method that effective in preventing the growth of other pathogens could also reduce the incidence of *P. brassicae*.

Research results show that the pathogen is extremely dangerous to cruciferous plants, since its resting spores may remain dormant in the soil for many years. In the present study, the average injury index over the experimental period in the control combination ranged from 33.7% (kale) to 64.0% (kohlrabi). However, the severity of disease symptoms varied greatly in particular years, depending on the precipitation during the period when *P. brassicae* zoospores invaded the host's root system, as clubroot spread is favored by wet weather (Nowicki 1984, Robak 1991, Wallenhammar 1996, Strelkov et al. 2006, Ministry of Agriculture...).

Among the investigated crop protection agents, Altima 500 SC was found highly effective. Application of this fungicide reduced disease severity to a level non-significant for crop yield, and only single plants showed symptoms of 2° and 3° infection. The high effectiveness of Altima 500 SC in controlling clubroot disease has been reported by Kurowski and Bruderek (2004), and Kurowski et al. (2008).

The biocontrol agents EM-1 and Biochikol 020 PC also reduced root infection rate with *P. brassicae*, but their efficacy was considerably lower, in comparison with Altima 500 SC. The relatively high disease-suppressive potential of effective microorganisms (EM) with respect to agricultural and horticultural plants has been well documented. According to Primavesi (1998) and Tokeshi et al. (1997) EM products exhibit a wide range of activities, of which increasing soil microbial biodiversity and making selected compounds readily available to plants are most important to maintain a good sanitary state of the crop.

Chitosan contained in Biochikol 020 PC has been demonstrated to improve plant health. In contrast to typical fungicides, Biochikol 020 PC not only inhibits the growth of pathogens but also stimulates disease resistance in plants (Stössel and Leuba 1984, Benhamou and Theriault 1992, Pośpieszny 1997, Wojdyła and Orlikowski 1997). However, in this experiment Biochikol 020 PC showed the lowest efficacy against clubroot.

The agents applied to control clubroot indirectly affected the abundance and species composition of fungal communities colonizing the seeds of cruciferous

crops. The species *A. alternata* was isolated most frequently from oilseed spring rape and white mustard seeds. *Fusarium avenaceum*, *R. nigricans*, *T. roseum*, *Penicillium* spp. and non-sporulating fungi were also relatively abundant in some years. According to some authors (Richardson 1996, Majchrzak et al. 2002), these fungi are most common on seeds of cruciferous plants. The rates of fungal colonization of seeds varied substantially among treatments. Both EM-1 and Biochikol 020 PC (although to a lower extent) significantly reduced *F. avenaceum* populations on oilseed spring rape seeds, but these highly promising results require further confirmation.

Streszczenie

SKUTECZNOŚĆ PREPARATÓW BIOLOGICZNYCH W OCHRONIE ROŚLIN KAPUSTOWATYCH PRZED KIŁĄ KAPUSTY (*PLASMODIOPHORA BRASSICAE*)

W latach 2005–2008 badano przydatność preparatów biologicznych i chemicznych do zwalczania kiły kapusty w rzepaku jarym, gorczycy białej, kalarepie białej i jarmużu. Chemiczna ochrona roślin kapustowatych fungicydem Altima 500 SC okazała się bardzo skuteczna. Zastosowanie preparatów biologicznych również wpłynęło, choć w mniejszym stopniu, na zmniejszenie porażenia roślin przez *Plasmodiophora brassicae*. Lepsze wyniki uzyskano po zastosowaniu preparatu EM-1 niż Biochikolu 020 PC. Z nasion rzepaku jarego i gorczycy białej uzyskanych ze wszystkich kombinacji doświadczenia izolowano głównie *Alternaria alternata*. Z nasion rzepaku jarego uzyskano również dużą liczbę kolonii *Fusarium avenaceum* – najwięcej z kombinacji kontrolnej, a zdecydowanie najmniej z chronionej preparatem EM-1.

Literature

Benhamou N., Theriault G., 1992: Treatment with Chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Physiol. Mol. Plant Pathol. 41: 33–52.

Domsch K.H., Gams W., 1972: Fungi in agricultural soils. Longman, London.

Ellis M.B., 1971: Dematiaceous Hyphomycetes. CMI, Kew, Surrey.

Gams W., 1971: Cephalosporium-artige Schimmelpilze (Hyphomycetes). Fischer, Jena.

Korbas M., Jajor E., Budka A., 2009: Kiła kapusty (*Plasmodiophora brassicae*) zagrożeniem dla rzepaku. Streszcz. 49. Ses. Nauk. Inst. Ochr. Rośl.: 126.

Kurowski T.P., Bruderek A., 2004: Próba ochrony rzepaku ozimego przed kiłą kapusty (*Plasmodiophora brassicae* Wor.) przy użyciu fungicydu Altima 500 SC. Progr. Plant Prot. / Post. Ochr. Rośl. 44, 2: 893–895.

Kurowski T.P., Majchrzak B., Jaźwińska E., Wysocka U., 2008: Skuteczność fungicydu zawierającego fluazynam w ochronie rzepaku ozimego przed kiłą kapusty (*Plasmodiophora brassicae* Woronin). Progr. Plant Prot. / Post. Ochr. Rośl. 48, 1: 212–215.

Kwaśna H., Chełkowski J., Zajkowski P., 1991: Sierpik (*Fusarium*). In: Grzyby (Mycota). T. 22. Instytut Botaniki PAN, Kraków.

Majchrzak B., Kurowski T.P., Karpińska Z., 2002: Zdrowotność jarych roślin krzyżowych a grzyby zasiedlające ich nasiona. Acta Agrobot. 55, 1: 199–210.
Narkiewicz-Jodko M., 1986: Wartość siewna przechowywanego ziarna trzech zbóż w aspekcie fitopatologicznym. Zesz. Nauk. AR Wroc. Rozpr. 55.
Nowicki B., 1984: Zróżnicowanie biologiczne *Plasmodiophora brassicae* Wor. w Polsce oraz podatność uprawianych roślin krzyżowych na wykryte patotypy grzyba. Rozpr. Nauk. Monogr. SGGW-AR 34.
Pośpieszny H., 1997: Niektóre aspekty stosowania Chitozanu w ochronie roślin. Progr. Plant Prot. / Post. Ochr. Rośl. 37, 1: 306–309.
Primavesi A.M., 1998: Seed treatment with EM and micronutrients for controlling rice and maize diseases. In: Proceedings of Four International Conference on Kyusei Nature Farming "Effective Microorganisms for a Sustainable Agriculture and Environment", June 19–21 1995, Paris, France. Eds. J.F. Parr, S.B. Hornic. 140–144.
Richardson M.J., 1996: Seed mycology. Mycol. Res. 100, 4: 385–392.
Robak J., 1991: Zmienność patotypów *Plasmodiophora brassicae* Wor. występujących w Polsce i ich patogeniczność w stosunku do odmian i linii hodowlanych *Brassica oleracea*. Pr. Hab. Inst. Warzywn. 6.
Robak J., Gidelska A., 2009: Epidemiologia i nowe możliwości zwalczania *Plasmodiophora brassicae* – sprawcy kiły kapusty na roślinach kapustowatych w Polsce. Streszcz. 49. Ses. Nauk. Inst. Ochr. Rośl.: 127.
Skirgiełło A., Zadara M., Ławrynowicz M., 1979. Glonowce (*Phycomycetes*), Pleśniakowce (*Mucorales*), Kłębiankowe (*Endogonales*). In: Grzyby (Mycota). T. 10. Instytut Botaniki PAN, Kraków.
Stössel P., Leuba J.L., 1984: Effect of Chitosan, chitin, and some aminosugars on growth of various soilborne phytopathogenic fungi. Phytopathol. Z. 111: 82–90.
Strelkov S.E., Tewari J.P., Smith-Degenhardt E., 2006: Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Can. J. Plant Pathol. 28: 467–474.
Tokeshi H., Ales M.C., Sanches A.B., Havana D.Y., 1997: Control of *Sclerotinia sclerotiorum* with effective microorganisms. Summa Phytopathol. 23: 146–154.
US Patent 5707856. Microbiological method for disposing of organic waste materials. 1998. T. Higa. [www.patentstorm.us/patents/5707856/claims. html].
Wallenhammar A.C., 1996: Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol. 45: 710–719.
Wenzel H., 1948: Zur Erfassung des Schadenausmasses in Pflanzenschutzversuchen. Pflanzenschutz-Ber. 15: 81–84.
Wojdyła A., Orlikowski L., 1997: Chitozan w zwalczaniu grzybów odglebowych i nalistnych. Progr. Plant Prot. / Post. Ochr. Rośl. 37, 1: 300–305.
Ministry of Agriculture, Alberta, Canada. [www1.agric.gov.ab.ca].
Zalecenia ochrony roślin na lata 2008/2009. Część III. Warzywa, sady. 2008. Instytut Ochrony Roślin, Poznań.

Authors' address:
Prof. Dr. hab. Tomasz P. Kurowski, Prof. Dr. hab. Barbara Majchrzak, Elżbieta Kowalska M.Sc., Department of Phytopathology and Entomology, University of Warmia and Mazury, ul. Prawocheńskiego 17, 10-722 Olsztyn, Poland, e-mail: kurowski@uwm.edu.pl

Accepted for publication: 5.05.2009