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

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### 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 – Sadoplón 75 WP, tiophanate-methyl – Topsin M 70 WP, fluazinam – Altima 500 SC, dazomet –

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 faecalis*, *S. lactis*), phototrophic bacteria (*Chlorobium limicola*, *Chromatium okenii*, *Rhodospseudomonas sphaereoides*, *R. palustris*, *R. capsulatus*, *Rhodospirillum rubrum*), actinomycetes (*Nocardia asteroides*, *Micromonospora chalicea*, *Streptomyces albus*, *S. griseus*, *Streptovercillium 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   |       |
| <i>Acremonia atra</i>               |         |     | 1             |     |      |     |                  |     | 1     |
| <i>Acremonium tubakii</i>           |         | 1   |               |     |      |     |                  |     | 1     |
| <i>Alternaria alternata</i>         | 41      | 70  | 67            | 116 | 38   | 126 | 63               | 182 | 703   |
| <i>Alternaria brassicae</i>         |         |     |               | 3   | 6    | 4   |                  |     | 13    |
| <i>Alternaria brassicicola</i>      | 1       |     |               |     |      |     |                  | 3   | 4     |
| <i>Botrytis cinerea</i>             |         | 8   |               |     |      | 2   |                  | 9   | 19    |
| <i>Cladosporium cladosporioides</i> |         |     |               |     |      | 12  | 5                |     | 17    |
| <i>Epicoccum purpurascens</i>       |         | 2   |               |     |      | 2   |                  | 7   | 11    |
| <i>Fusarium avenaceum</i>           | 32      | 82  | 4             | 77  |      | 6   | 2                | 52  | 255   |
| <i>Fusarium equiseti</i>            |         |     | 2             |     |      |     |                  |     | 2     |
| <i>Fusarium oxysporum</i>           | 1       |     |               |     |      |     |                  |     | 1     |
| <i>Fusarium poae</i>                | 1       |     |               |     |      |     |                  |     | 1     |
| <i>Mortierella alpina</i>           |         |     |               |     |      |     | 1                |     | 1     |
| <i>Penicillium</i> spp.             | 2       | 3   | 7             | 23  | 11   | 61  | 11               | 30  | 148   |
| <i>Rhizopus nigricans</i>           | 10      | 31  |               | 23  | 91   | 61  | 14               | 7   | 237   |
| <i>Trichothecium roseum</i>         |         |     |               |     |      | 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<br>500 SC |     | EM-1 |     | Biochikol<br>020 PC |     | Total |
|-------------------------------------|---------|-----|------------------|-----|------|-----|---------------------|-----|-------|
|                                     | d       | n   | d                | n   | d    | n   | d                   | n   |       |
| <i>Alternaria alternata</i>         | 93      | 221 | 161              | 187 | 102  | 230 | 110                 | 204 | 1 308 |
| <i>Alternaria brassicae</i>         | 1       |     |                  |     |      |     |                     |     | 1     |
| <i>Alternaria brassicicola</i>      |         |     |                  |     |      |     | 2                   |     | 2     |
| <i>Arthrinium sphaerospermum</i>    |         |     |                  |     |      |     | 2                   |     | 2     |
| <i>Aspergillus</i> sp.              |         |     |                  |     |      |     | 3                   |     | 3     |
| <i>Botrytis cinerea</i>             |         |     |                  |     |      |     |                     | 1   | 1     |
| <i>Cladosporium cladosporioides</i> | 2       | 1   |                  |     |      |     |                     |     | 3     |
| <i>Epicoccum purpurascens</i>       | 4       | 26  | 2                | 5   | 4    | 13  | 2                   | 39  | 95    |
| <i>Fusarium avenaceum</i>           |         |     |                  |     |      |     | 1                   |     | 1     |
| <i>Fusarium chlamydosporum</i>      |         |     |                  |     |      | 2   |                     |     | 2     |
| <i>Fusarium sporotrichioides</i>    |         |     |                  |     |      |     | 2                   |     | 2     |
| <i>Mucor hiemalis</i>               | 2       |     |                  |     |      |     |                     |     | 2     |
| <i>Penicillium</i> spp.             | 4       | 1   | 11               |     | 2    |     | 3                   |     | 21    |
| <i>Rhizopus nigricans</i>           |         | 19  |                  | 50  |      | 36  | 76                  | 52  | 233   |
| <i>Trichothecium roseum</i>         |         |     |                  | 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.

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*Accepted for publication: 5.05.2009*