CLUBROOT (*PLASMODIOPHORA BRASSICAE*) – A THREAT FOR OILSEED RAPE

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Abstract: Clubroot presents a serious threat to cultures of oilseed rape, especially if the share of this plant species in sowing structure is high. This is related to a long-lasting survival of pathogens resting spores in soil. The casual disease agent is *Plasmodiophora brassicae* Woronin – an obligatory, internal pathogen of *Brassicae* family which causes the formation of tumors on roots and hypocotyls. Plants with disease symptoms have a confined ability of nutrients' and water uptake and this may cause considerable losses in quantity and quality of potential yield. Monitoring performed in cultivation areas and the information obtained from farmers allowed to confirm a threat of disease occurrence in several voivodeships. At the same time there is a serious danger of the infection spread to other regions of oilseed rape crops.

Confining plant infection by clubroot pathogen may be obtained mainly by applying a correct crop rotation, and also by providing resistant varieties for cultivation. In the performed greenhouse experiment, reaction to the infection caused by *P. brassicae* of chosen winter oilseed rape cultivars was studied. The degree of plant infection was assessed at the growth phase of green bud. It was stated that cv. Mendel revealed significantly the lowest degree of infection, as compared to the other studied cultivars. The other tested varieties were infested in a differentiated degree, however these differences were mostly not confirmed statistically. The infection level was mainly dependent on places of soil derivation.

Key words: *Plasmodiophora brassicae*, clubroot; cultivars of oilseed rape, resistance, soil derivation

INTRODUCTION

Clubroot is a serious threat in oilseed rape crops, especially where the share of this plant in a sowing structure is considerable. In the recent years the interest of cultivating this species has increased and at the same time oilseed rape is regarded by many farmers as a plant that interrupts sowing wheat after wheat (Kurowski *et al.* 2008).

Clubroot has been known since XIII century. It commonly occurs worldwide, especially in humid, moderate climate regions, but also in tropical and subtropical zones (Agrios 2005). The main pathogen host plants are cultivated cabbage vegetables, oilseed rape, turnip like rape and mustard. This disease is also stated on numerous weeds of the cabbage family, but also on plants from other families, eg. poppy, knotgrass, red clover, some grass species (*Dactylis glomerata*, *Lolium perenne*) (Rimmer *et al.* 2007).

The causal agent of clubroot *Plasmodiophora brassicae* (Woronin) is an obligatory, internal pathogen from *Protozoa* kingdom, *Plasmodiophoromycota* phylum, *Plasmodiophoromycetes* class, *Plasmodiophorales* order (Fiedorow *et al.* 2008). It causes tumors of various size on top roots and their branching, and on plant hypocotyl. In the development of pathogen, a significant role is played by resting spores which can preserve their vitality in soil even for 20 years. Durability of spore structures arises due to an unique linking of chitin, protein and lipids (Rimmer *et al.* 2007) and this conditions their high resistance especially to unfavourable environmental conditions (eg. frost, drought, absence of host plant). Antagonistic organisms to this pathogen were not found in the soil environment (Borecki 2001).

Germination of resting spores is stimulated by specific host plant root exudates (Kowalski and Bochow 1996). Favourable conditions for clubroot development, apart from the high share of host plants in sowing structure, are moderate air and soil temperature (optimally ca 22–24°C) and high soil humidity, frequently related to non-regulated water relationships. Frequently it is stressed that the pathogen development is also related to high soil acidity. However, some cases of oilseed rape infection by *P. brassicae* were also recorded in soils with a neutral reaction or alkaline soils, if humidity, temperature and the level of inoculum were sufficient (Rimmer *et al.* 2007).

Tumors most frequently appear 4–6 weeks after infection. Multinuclear pathogen's plasmodia devoid of walls first colonize epidermal cells, then cambium and root

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vessels. Tumors appear mainly as a result of hypertrophy and their cells may be 5-fold larger comparing to healthy plant cells. Hyperplasia (excessive division of cells) plays here a lesser role (Borecki 2001). Excessive enlargement of cells and hastening their division is, among others, related to a high concentration of growth substances (indolo-3-acetic acid – IAA) in infected cells (Kryczyński 2005). Pathogenesis of *P. brassicae* relies on using organic substances produced as a result of plant photosynthesis for building-up plasmodia and inhibiting water uptake and nutrients by deformed root system, and roots devoid of cortical layer are easily infected by soil microorganisms. Water stress causes plant wilting, deficiency of nutrient substances and reddening of leaves. Plant growth is stunted, size of leaves reduced, diseased plants start flowering earlier and the yield is of inadequate quantity and quality (Borecki 2001; Manzanares-Dauleux *et al.* 2001; Jajor and Korbas 2008).

In the studies by Wallenhammar (1998) a strict correlation was found between the degree of soil infestation, the level of plant infection and yielding of spring oilseed rape. It should be emphasized that losses are not usually uniform for the whole plantation, and this is a result of a differentiated level of *P. brassicae* soil infestation (Wallenhammar 1998).

At the moment of appearing of the disease on a plantation, the main preventing method of reducing losses is a break of oilseed rape cultivation during 7–10 years to the advantage of non-host plants (eg. cereals, potatoes, phacelia, beets). During this break it is necessary to control *Brassicaceae* weeds. The application of lime to the soil is also important, but directly prior to oilseed rape cultivation (Fiedorow *et al.* 2008). A high significance in obtaining a proper effectiveness depends on a form of calcium. In this case calcium cyanamide ($CaCN_2$) – 19.8% N, is frequently recommended (Naiki and Dixon 1987; Rimmer *et al.* 2007). According to Webster and Dixon (1991a) a higher concentration of calcium lowers the number of infections and the appearance of tumors, however this effect mainly depends on the amount of inoculum. So, there is a necessity of additional supplying of fertilization with macro- and microelements, because they improve a general plant condition, and also as boron they may hamper the disease development (Webster and Dixon 1991b). The important disease eradicating factors are soil structure and regulating water relations. Spreading of the pathogen to one or several neighbouring fields can be confined by abandonment of the application of some cultivation measures and by precise cleaning and disinfection of agricultural equipment. A condition necessary for undertaking of appropriate activities is a continuous and precise monitoring of oilseed rape crops (Jajor and Korbas 2008).

Intensive investigations are performed on resistance of cultivars to *P. brassicae* and on searching for resistance sources in all agronomicaly important *Brassicaceae* plants. Biological differentiation of this pathogen is very high (Robak 1991). Pathogen population consists of several dozens of pathotypes and this considerably negatively affects the investigation.

A number of Chinese cabbage (*Brassica rapa*) varieties were obtained using European turnips as resistance sources. Field populations of the pathogen have a wide variation of virulence. Resistance genes of *B. rapa* were widely studied using molecular markers. At least four independent loci were identified and mapped, which originated from European turnips (Hirai 2006). A very early reaction of resistant and susceptible varieties of *B. rapa* to the infection by *P. brassicae* was studied. Primary plasmodia were observed in hair roots both of resistant and susceptible cultivars, and secondary plasmodia were able to proliferate only in case of the last ones. After four weeks tumors only on non-resistant varieties were observed (Takahashi *et al.* 2006). Investigations on breeding of varieties with a possible durable resistance are immensely important. The remaining methods of confining clubroot, including cultivation are not effective enough (Tewari *et al.* 2005). Excessively extensive cultivation of oilseed rape in some regions of Germany, France and Scandinavia due to a frequent occurrence of clubroot causal agent has already led to reduction of production (Rimmer *et al.* 2007).

Cultivars resistant to *P. brassicae* are known from the Common Catalogue of Varieties of Agricultural Plant Species (CCA). There belongs the cultivar Mendel, which is a restored hybrid characterized by race-specific resistance to *P. brassicae*. Resistance of this variety derives from resynthesized line of *B. napus: B. rapa* ECD-04 x *B.oleracea* ECD-15 (Diderichsen and Sacristan 1996; Diderichsen *et al.* 2006). Resistance of plants should be used rationally to avoid spreading of new virulent pathotypes of the disease causal agent. Because of this they should be sown in places where the disease was present in the previous years (> 30% of diseased plants), but not applied as a prophylactic measure, that in cases when only the risk of clubroot occurrence exists (Diderichsen *et al.* 2006). It should be stressed that in the resistance strategy mixing of seeds of susceptible and resistant varieties destined for sowing on a given field is forbidden. At the present there are not many reports on breaking down resistance of field grown cultivar Mendel, however such evidence was recorded under experimental conditions (Diderichsen *et al.* 2003).

The present work aimed at assessing threat to winter oilseed rape by *P. brassicae* in different regions of cultivation in Poland.

MATERIALS AND METHODS

Monitoring of fields and questioning farmers in agricultural environment in Poland were conducted aiming at the identification of the most endangered regions by the occurrence of clubroot causal agent.

A greenhouse experiment was performed in the Institute of Plant Protection – National Research Institute in Poznań. Experimental material consisted of 9 winter oilseed rape cultivars most frequently cultivated in Poland in 2007, being present in the Polish National List of Varieties of Agricultural Plants and the cultivar Mendel (CCA). Varietals reactions to the infection by *P. brassicae* were studied. For this purpose the soil samples from five geographically distant localities in Poland were taken, in which the infection of plants by this pathogen was stated.

Then the soils samples were placed in pots marked with the letters a, b, c, d, e (Fig. 1). Then selected cultivars were sown in four replications for each locality. Two series of experiments were conducted. The names of cultivars are listed in the tables (Tables 1, 2). At the growth phase BBCH 50–51 (green bud) the evaluation of the degree of infected plants was carried out. To accomplish this, all the plants were dug out, the underground part was washed off and afterwards their infection was assessed. While recording plant disease symptoms a 9-degree scale was used where: 0 – lack of symptoms, 1– small, single tumors on secondary roots, 3 – larger tumors on secondary roots, 5–7 – enlarging tumors on secondary roots and the main root, 9 – a large single tumor on root neck, lack of roots (Robak 1991).

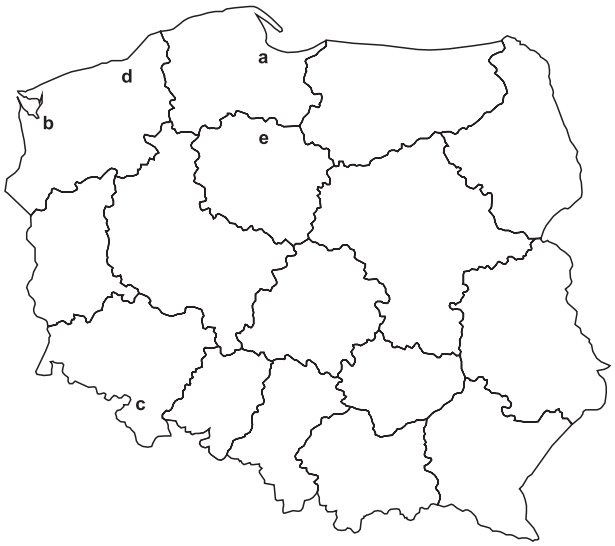

Fig. 1. Localities of soil derivation from oilseed rape plantations infected by *P. brassicae*

Index of disease infection was calculated according to Williams (1987). The results were compared statistically, using STATISTICA program. The analysis of experimental results was done according to double-direction classification model, using results of the two experimental series. It was accepted that the value of infection index is affected by the choice of variety (first factor), the soil (second factor) and that the interaction occurs between these factors. For comparing mean values of indexes a procedure of multiple comparison according to Tukey was applied (Ott 1984; Trętowski and Wójcik 1988).

RESULTS AND DISCUSSION

Performed monitoring of cultivated fields and the information obtained from farmers allowed to state endangerment by clubroot in the following voivodeships of the country: zachodnio-pomorskie, pomorskie, warmińsko-mazurskie, and a part of kujawsko-pomorskie, dolnośląskie, podkarpackie and lubuskie regions.

The analysis of variance performed for the greenhouse experiment allowed to accept that all considered factors very likely differentiated the degree of infection expressed by the value of calculated index. It was found that the studied varieties registrated in Polish National List of Varieties of Agricultural Plants showed a differentiated degree of susceptibility to the causal agent of clubroot on soil from different fields. The significance of interaction soil – cultivar confirmed a different varietal reaction to different (derived from other locations) soil samples. For particular soils, it was obtained much greater differentiation of indexes than for the varieties themselves. Maximal values of disease index was obtained for the soil samples (e) from northern part of kujawsko-pomorskie voivodeship and soil samples (d) from north-eastern region of zachodnio-pomorskie voivodeship. The lowest disease indexes were obtained for location (b) (north-western part of zachodnio-pomorskie voivode-

Table 1. Disease infection index of winter oilseed rape varieties infected by *P. brassicae* depending on different localities of soil derivation

Variety/soil		Soil (a)	Soil (b)	Soil (c)	Soil (d)	Soil (e)
		Factor 2: soil				
		3.50	2.27	2.42	5.45	5.70
		LSD (0.05) = 0.542; LSD (0.01) = 0.776				
Californium (Cal)	Factor 1: variety	3.70	1.96	2.87	5.50	5.76
Cabriolet (Cab)		3.70	2.67	2.26	5.98	5.26
Bazyl (Baz)		4.14	2.00	2.49	4.58	7.35
Libomir (Lib)		4.93	2.22	3.13	5.90	5.61
Kaszub (Kas)		3.94	2.82	2.17	6.11	6.00
Ontario (Ont)		3.39	2.68	2.51	7.05	5.98
Pomorzanin (Pom)		4.10	2.56	4.24	7.20	6.75
Extrem (Ext)		3.82	3.46	2.16	6.78	6.81
Bojan (Boj)		3.29	2.27	2.22	5.20	7.42
Mendel (Men)		0.00	0.03	0.12	0.14	0.02
LSD (0.05)		1.991	1.761	1.867	2.587	1.899
LSD (0.01)		2.315	2.047	2.170	3.007	2.207

Table 2. Mean disease infection index of oilseed rape by *P. brassicae*

No.	Variety	Infection index (mean for 5 soil)	Difference between value of infection index in reference to the variety Mendel
1	Californium (Cal)	3.96	3.90*
2	Cabriolet (Cab)	3.97	3.91*
3	Bazyl (Baz)	4.11	4.05*
4	Libomir (Lib)	4.36	4.30*
5	Kaszub (Kas)	4.21	4.14*
6	Ontario (Ont)	4.32	4.26*
7	Pomorzanin (Pom)	4.97	4.91*
8	Extrem (Ext)	4.60	4.54*
9	Bojan (Boj)	4.08	4.02*
10	Mendel (Men)	0.06	–

* significant differences at level $\alpha = 0.01$

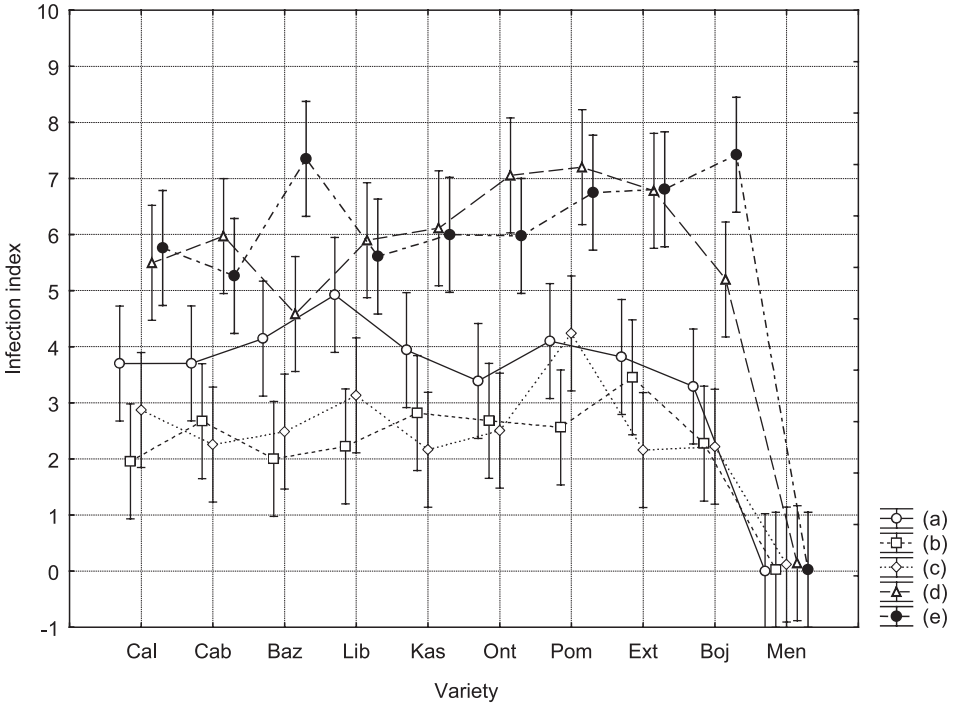

Fig. 2. Reaction of winter oilseed rape cultivars to infection by *P. brassicae* depending on soil derivation locality. Vertical bars indicate 95% confidence intervals

ship) and (c) (dolnośląskie voivodeship – the Valley of Kłodzko). This was probably related to the fact, that soil samples derived from different locations showed a different inoculum potential (Rimmer *et al.* 2007). In case of listed pairs of soils (d) and (e) a significant differentiation of disease infection index was not observed, similarly as for soils (b) and (c), however the remaining soil pairs highly significantly differed between themselves with the respect of plant infection degree (Table 1, Fig. 2).

Plants of oilseed rape cultivar Mendel highly significantly differed with the respect of resistance to *P. brassicae* from the remaining varieties. Rare case of infection of Mendel cultivar by *P. brassicae* was observed, and the disease infection index was low and confined to the interval from 0.02 to 0.14 (Table 1). However this might indicate a possibility of breaking down resistance of this cultivar under the experimental conditions (Diderichsen *et al.* 2003). The remaining varieties did not significantly differ in resistance to the infection by the discussed pathogen. In spite of this, varieties may be classified in the order from those characterized by the highest disease infection index to characterized by the lowest index. In the performed experiment this order was as follows: Pomorzanin, Extrem, Libomir, Ontario, Kaszub, Bazyl, Bojan, Cabriolet, Californium (Table 2).

Analyzing varietal reactions for particular soils statistically confirmed differences between disease infection indexes observed only for soil (c), where the index for the variety Pomorzanin was significantly higher than related values for varieties: Cabriolet, Kaszub, Extrem and Bojan, and also on soil (e) where infection of varieties Bazyl and Bojan was higher in relation to variety Cabriolet (Table 1, Fig. 2).

CONCLUSIONS

Clubroot can present a serious threat to oilseed yielding in many regions of the country, especially in crops with a high share of oilseed rape in a sowing structure. To the localities most endangered with clubroot occurrence belong the voivodeships: zachodnio- pomorskie, pomorskie, warmińsko-mazurskie and a part of kujawsko-pomorskie, dolnośląskie, podkarpackie and lubuskie.

The level of pathogen's infection depends mainly on soil derivation (degree of soil infestation). In majority cases, no significant differences were found between the studied cultivars from the Polish National List of Varieties of Agricultural Plants considering the level of pathogen's infection. The cultivar Mendel (CCA) revealed a significantly high level of resistance to *P. brassicae* from various localities in Poland.

REFERENCES

Agrios G.N. 2005. Plant Pathology. Elsevier Academic Press, 922 pp.

Borecki A. 2001. Nauka o Chorobach Roślin. PWRiL, Warszawa, 379 pp.

Diderichsen E., Beckmann J., Schondelmeier J., Dreyer F. 2006. Genetics of clubroot resistance in *Brassica napus* 'Mendel'. Acta Hort. 706: 307–311.

Diderichsen E., Deppe U., Sacristan M.D. 2003. Characterization of clubroot resistance in recent winter oilseed rape material. Proc. 11th Intl. Rapeseed Congress, Copenhagen, Denmark 1: 168–170.

Diderichsen E., Sacristan M.D. 1996. Disease responses of resynthesized *Brassica napus* L. lines carrying different combinations of resistance to *Plasmodiophora brassicae* Wor. Plant Breed. 115: 5–10.

Fiedorow Z., Gołębniak B., Weber Z. 2008. Choroby Roślin Rolniczych. AR, Poznań, 208 pp.

Hirai M. 2006. Genetic analysis of clubroot resistance in *Brassica* crops. Breeding Sci. 56: 223–229.

Jajor E., Korbas M. 2008. Kiła kapusty (*Plasmodiophora brassicae* Wor.). p. 40–43. In: „Poradnik Sygnalizatora Ochrony Rzepaku" (F. Walczak, ed.). Inst. Ochr. Roślin, Poznań, 153 pp.

Kowalski K., Bochow H. 1996. Observations on the behaviour of *Plasmodiophora brassicae* resting spores in the presence of cruciferous and non-cruciferous plant root exudates. Acta Horticul. 407: 411–418.

Kryczyński S. 2005. Podstawy Fitopatologii. Fundacja Rozwój SGGW, Warszawa, 292 pp.

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

Manzanares-Dauleux M.J., Divaret I., Baron F., Thomas G. 2001. Assessment of biological and molecular variability between and within field isolates of *Plasmodiophora brassicae.* Plant Pathol. 50: 165–173.

Naiki T., Dixon G.R. 1987. The effects of chemicals on developmental stages of *Plasmodiophora brassicae* (clubroot). Plant Pathol. 36: 316–327.

Ott L. 1984. An Introduction to Statistical Methods and Data Analysis. PWS Publishers, Boston: 583–591.

Rimmer S.R., Shattuck V.I., Buchwaldt L. 2007. Compendium of *Brassica* Diseases. The APS, St. Paul, 117 pp.

Robak J. 1991. Zmienność patotypów *Plasmodiophora brassicae* Wor. występujących w Polsce i ich patogeniczność w stosunku do odmian i linii hodowlanych *Brassica oleracea*. Rozprawa habilitacyjna. Instytut Warzywnictwa, 61 pp.

Takahashi H., Ishikawa T., Kaido M., Takita K., Hayakawa T., Okazaki K., Itoh K., Mitsui T., Hori H. 2006. *Plasmodiophora brassicae* – induced cell death and medium alkalization in clubroot-resistant cultured roots of *Brassica rapa*. J. Phytopathol. 154: 156–162.

Tewari J.P., Strelkov S.E., Orchard D., Hartman M., Lange R.M., Turkington T.K. 2005. Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can. J. Plant Pathol. 27: 143–144.

Trętowski J., Wójcik A.R. 1988. Metodyka Doświadczeń Rolniczych. WSRP, Siedlce, 215 pp.

Williams P.H. 1987. CrGC Resource Book Dept. of Plant Pathology. Univ. of WI, Madison. USA. PH. Williams, Hort Sci. 15, 802/1980/.

Wallenhammar A.C. 1998. Observations on yield loss from *Plasmodiophora brassicae* infections in spring oilseed rape. Z. Pflanzenkrankh. Pflanzensch. 105 (1): 1–7.

Webster M.A., Dixon G.R. 1991a. Calcium, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae.* Mycol. Res. 95 (1): 64–73.

Webster M.A., Dixon G.R. 1991b. Boron, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae.* Mycol. Res. 95 (1): 74–79.

POLISH SUMMARY

KIŁA KAPUSTY (*PLASMODIOPHORA BRASSICAE*) ZAGROŻENIEM DLA RZEPAKU

Kiła kapusty stanowi poważne zagrożenie w uprawie rzepaku szczególnie tam, gdzie udział tej rośliny w strukturze zasiewów jest duży. Jest to związane z możliwością przetrwania przez wiele lat tego patogena w glebie w formie zarodników przetrwalnikowych. Sprawcą kiły kapusty jest *Plasmodiophora brassicae* Woronin – bezwzględny, wewnątrzkomórkowy patogen z królestwa Protozoa. Poraża on przede wszystkim rośliny z rodziny kapustowatych i powoduje powstawanie narośli na ich korzeniach oraz części podliścieniowej. Rośliny z objawami choroby mają ograniczoną zdolność pobierania substancji pokarmowych i wody, co w konsekwencji może być przyczyną znacznych strat ilości oraz jakości ich potencjalnego plonu. Przeprowadzony monitoring pól uprawnych i uzyskane informacje w środowisku rolniczym pozwoliły na potwierdzenie zagrożenia ze strony sprawcy kiły kapusty w kilku województwach.

Ograniczenie porażenia roślin przez sprawcę kiły kapusty można uzyskać, przestrzegając przede wszystkim stosowania racjonalnego płodozmianu, a także zapewniając m.in. odpowiednią strukturę i odczyn gleby oraz wybierając do uprawy odmiany odporne. Wykonano doświadczenie szklarniowe, w którym na glebach z pięciu lokalizacji badano reakcję wybranych odmian

rzepaku ozimego na porażenie przez *P. brassicae.* W fazie zielonego pąka wykonano analizę stopnia porażenia roślin. Stwierdzono, że odmiana Mendel (CCA) charakteryzowała się istotnie niższym stopniem porażenia w porównaniu z innymi badanymi odmianami z Krajowego Rejestru Odmian Rzepaku. Odmiany te były porażane w zróżnicowanym stopniu, ale w większości nie zostało to potwierdzone statystycznie. Poziom porażenia zależał przede wszystkim od miejsca pochodzenia gleby.