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Isolate pathogenicity recognition of *Plasmodiophora brassicae* Wor. in different areas of Poland

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ABSTRACT

The aim of the research conducted in 2007-2009 was the evaluation of the pathogenicity of eight *Plasmodiophora brassicae* Wor. isolates collected from clubroot-infested white cabbage growing in different areas of Poland. Breeding materials from white cabbage resistant and susceptible to *P. brassicae* were used for this purpose. Cabbage seeds were infected by submerging them into a spore suspension. The screening of plant clubroot resistance using a nine-degree scale based on root symptoms was carried out at the eight-week-old seedling stage and at harvest time on a field infected by *P. brassicae* spores – called the 'death field'. Differences in the pathogenicity of the collected isolates were determined. Isolates from Szczecin, Ostromęczyn, Krojczyn and Maszkienice were defined as the most virulent. Plants of the 'Kilaxy' F_1 white cabbage cultivar confirmed resistance to all isolates, while plants of the susceptible HTM line from the 'Hitoma' F_1 white cabbage cultivar were characterised by the highest infestation level. The evaluation of adult plants in the field with high *P. brassicae* spore contamination confirmed seedling test results. Different susceptibility was observed between sub-lines from the 'Oregon 123' and 'Badger Shipper' cultivars with resistant genes. 'Oregon 123' sub-lines were less susceptible to applied isolates than 'Badger Shipper' sub-lines at both the seedling stage and during the field test. A double-stage plant screening to pathogen reaction caused the elimination of the most susceptible plants at the seedling stage. Plants with a certain resistance level were planted in the 'death field'.

Key words: clubroot, susceptibility, virulence, white cabbage

INTRODUCTION

Plasmodiophora brassicae, a soil-born obligate plant pathogen showing wide pathotype variation causes clubroot disease, which is a major threat to Brassica crops. Resistance breeding to such a pathogen is difficult, because it requires the introduction of several resistance genes to a new cultivar. The evaluation of various field populations with a wide range of pathogenicity of that pathogen from different areas of Poland is necessary.

MATERIAL AND METHODS

During cabbage harvest time in 2007, plants with clubbed roots were collected in different areas of Poland, particularly on large plantations. Seven isolates were obtained from an infested field of white cabbage from Miechów (M), Okrąg (O), Szczecin Dąbie (Sz), Żółwia Błoć (Z), Maszkienice (Ma), Krojczyn (K) and Ostromęczyn (Os). Isolate A was also included, initially belonging to the Department of Genetics, Plant Breeding and Seed Science of the University of Agriculture in Krakow and originating from central Poland (Tab. 1, Fig. 1). Samples of clubbed roots were cleaned and frozen for storage at -20°C.

The evaluation of the virulence of eight *Plasmodiophora brassicae* isolates was carried out based on standard objects of white cabbage breeding materials owned by the Department. Inbred lines

Table 1. Origins of *Plasmodiophora brassicae* isolates collected in 2007

Area	District	Isolate symbol
Miechów	Małopolskie	M
Maszkienice		Ma
Krojczyn	Kujawsko-Pomorskie	K
Szczecin Dąbie	Zachodniopomorskie	Sz
Żółwia Błoć		Z
Ostromęczyn	Mazowieckie	Os
Okrąg		O

derived from plant materials with known reactions to the pathogen were used as standard objects (Tab. 2). Lines from the 'Badger Shipper' and 'Oregon 123' cultivars obtained through self-pollination were the objects with resistant genes. Three sub-lines from each cultivar were selected for tests in 2008 and two lines in 2009. The HTM inbred line from 'Hitoma' F_1 white cabbage was used as a susceptible standard in each year, but was represented by two sub-lines in 2008 and only one sub-line in 2009. The white type 'Kilaxy' F_1 cultivar from Syngenta was used as a resistance standard in every tested year. The evaluation of cabbage resistance to clubroot was carried out in two stages; stage I – seeds inoculated by pathogen isolates were sown individually into multi-pot packs; after an eight-week period of plant growth, clubroot symptoms were evaluated; stage II – eight-week-old seedlings without clubroot symptoms were planted in the 'death field' and evaluated once more during harvest time (Tab. 3).

Cabbage seeds were inoculated by submerging them into a *P. brassicae* water spore suspension. The inoculum was prepared by macerating gall tissue obtained from diseased cabbage roots in a blender with distilled water. Later the resting spores were separated by filtration through eight layers of cheesecloth and centrifugation. Final spore concentration was adjusted with distilled water to 10^8 spore per 1 ml 30-50 seed samples per one object were drowned in 5 ml inoculum of the pathogen and kept in 19°C. After a 36-hour

Figure 1. Places of gathering of *Plasmodiophora brassicae* isolates in 2007

Table 2. White cabbage sub-line origin and virulence evaluation of collected *Plasmodiophora brassicae* isolates

Standard objects	Sub-line generation evaluation in years			
	2008		2009	
Badger Shipper	Sub-line I	S4	Sub-line I	S5
	Sub-line II	S2	Sub-line II	S5
	Sub-line III	S3		
Oregon 123	Sub-line I	S5	Sub-line I	S5
	Sub-line II	S4	Sub-line II	S5
	Sub-line III	S4		
HTM line	Sub-line I	S5	Sub-line	S4
	Sub-line II	S4		
Kilaxy F_1	Syngenta		Syngenta	

incubation period, the seeds were sown individually to multi-pot packs filled with substrate and grown in a phytotron chamber at 20°C under a light-dark cycle of 16:8 hours. At eight weeks after sowing, the root symptoms of each plant were graded on a scale of I to IX. The nine-degree scale based on root symptoms applied to screen plant susceptibility was simplified to a five-degree scale to improve this work: I – without visible disease symptoms, III – singular galls on lateral secondary roots, V – larger galls on lateral roots, VII – enlarged gall on the main and lateral roots, IX – large singular gall on root neck without root (Fig. 2).

The plants were removed from the pots and the soil was carefully shaken from the roots to protect them during screening time. Immediately after screening, cabbage plants in the I class and the III class were planted in the 'death field'.

A disease index was calculated according to individual estimation of plant infestation:

$$\mathbf{D} = \frac{\sum(n \cdot p)}{\sum n}$$

where: n – number of plants with the same class,
p – class of infestation I-IX.

The disease indexes of the tested objects were calculated according to plant susceptibility at the eight-week seedling stage and evaluated once more during harvest on the 'death field'. The clubroot

Table 3. Timetable of activities conducted in 2008 and 2009

Activities	2008	2009
Seed inoculation	16.04	15.04
Seed sowing into multi-pot packs	18.04	17.04
Seedling roots disease symptom evaluation	16.06	15.06
Planting on 'death field'	16.06	16.06
Harvest, disease screening and plant selection	13.10	20.10

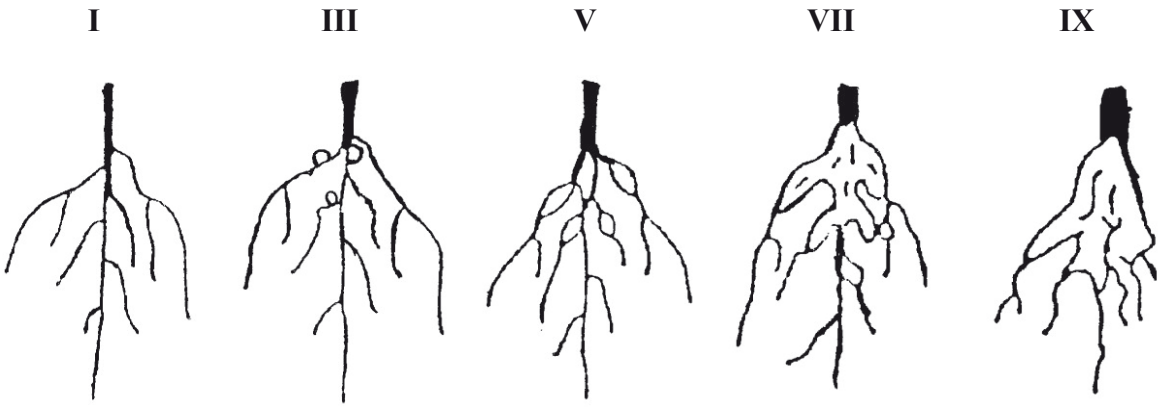

Figure 2. Nine-degree scale for screening cabbage root infestation of *P. brassicae*

field described as the 'death field' was established eight years ago in RZD Prusy near Kraków. Harvest remains as well as soil from the field in Mydlniki near Kraków containing white cabbage plants with clubroot symptoms were scattered on the surface of the field. Cabbage seedlings with disease symptoms obtained from infected *P. brassicae* seeds have been planted there annually from that time. Clubbed plant roots are mixed with the soil for extra infectability by the pathogen from the 'death field', which is carried out after an autumn disease evaluation and harvest of the tested plants. As a result of this procedure the field was contaminated by a mixture of different *P. brassicae* pathotypes.

RESULTS AND DISCUSSION

The screening of root system infestation at seedling stage each year obtained from the inoculated *P. brassicae* spore seeds showed differences in cabbage plant susceptibility of standard objects. The differences were especially visible between the susceptible HTM line and the 'Kilaxy' F_1 cultivar that was resistant to *P. brassicae*.

Plants from the susceptible HTM line obtained from seeds inoculated by the Sz isolate were characterised with a high disease index of DI 7.2 in 2008 and 5.3 in 2009. A similar reaction to the pathogen was found in seedlings from the line of seeds inoculated by the Os isolate, resulting in a DI of 7.2 in 2008, and a DI of 5.3 in 2009 for the Ma isolate (Tab. 4). As predicted, these combinations also showed the lowest percentage of healthy plants with root systems without galls (18-42%) (Tab. 5). For the rest of the applied isolate infested plants in small degree of susceptible HTM line, DI was dependent on the year of testing and the isolate, and oscillated between 1.0-4.2.

Plants from the clubroot-resistant 'Kilaxy' F_1 cultivar obtained from seeds inoculated by eight *P. brassicae* isolates did not show disease symptoms at the seedling stage. The disease index was from 1.0 to 1.3 for each combination and was characterised by a very high percentage of resistant plants – 96-100%. 'Kilaxy' F_1 is one of the clubroot-resistant cultivars which were introduced to the market in 2005 and later by Syngenta Seeds B.V. The resistance to *B. rapa* does not cover all isolates of *P. brassicae,* however. The resistance will not work against all pathotypes and a small number of isolate infections was observed (Diederichsen et al. 2009). In the course of the current research, plants of this cultivar showed a resistance reaction to all eight isolates of the pathogen.

The Sz isolate from Szczecin Dąbie showed the highest pathogenicity in each year, and the mean DI was 3.5 in 2008 and 2.8 in 2009 (Tab. 4). In 2008, a similar pathogenicity was found for the Os isolate from Ostromęczyn, with a DI of 3.4, while the K isolate from Krojczyn has a DI of 2.8 in 2009. The Ma isolate from Maszkienice was less virulent in

Table 4. Disease index for standard objects evaluated in seedling test in 2008 and 2009

Standard objects	*Plasmodiophora brassicae* isolates used for seed inoculation															
	A		M		O		Sz		Z		Ma		K		Os	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Badger Shipper	1.1	1.1	1.0	1.2	1.5	1.0	3.8	2.8	1.6	1.2	1.1	1.5	3.0	5.1	1.9	1.3
Oregon 123	1.2	1.4	1.0	1.1	1.1	1.0	1.6	2.0	1.1	1.2	1.4	1.5	1.0	1.6	3.6	1.6
HTM line	1.3	2.3	1.0	1.0	3.2	1.2	7.2	5.3	1.2	2.1	3.0	5.3	4.2	3.6	7.2	1.5
Kilaxy F_1	1.3	1.0	1.0	1.0	1.0	1.0	1.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean	1.2	1.5	1.0	1.1	1.7	1.1	3.5	2.8	1.2	1.4	1.6	2.3	2.3	2.8	3.4	1.4

Table 5. Percentage of plants without disease symptoms of standard objects evaluated in seedling test in 2008 and 2009

Standard objects	*Plasmodiophora brassicae* isolates used for seed inoculation															
	A		M		O		Sz		Z		Ma		K		Os	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Badger Shipper	98	98	100	94	92	100	34	65	89	90	96	90	70	45	67	93
Oregon 123	96	89	100	98	93	100	89	66	97	90	92	89	100	75	47	80
HTM line	64	82	100	100	79	89	35	26	96	71	73	42	81	48	18	88
Kilaxy F_1	96	100	100	100	100	100	96	100	100	100	100	100	100	100	100	100
Mean	89	92	100	98	91	97	64	64	96	88	90	80	88	67	58	90

both years. Other isolates used for seed inoculation of standard objects showed low DIs from 1.0-1.7, but the M isolate from Miechów was the least virulent. Isolates with the highest pathogenicity level (Sz, Ma, O, K) also caused disease symptoms at the seedling stage in objects with genes resistant to *P. brassicae*.

Differences in susceptibility between sublines derived from the 'Badger Shipper' and 'Oregon 123' cultivars with genetic resistance to *P. brassicae* were observed in both years (Figs 3 and 4). Such a tendency was noticed between sublines I and II originating from 'Badger Shipper'. The disease index calculated for those lines was

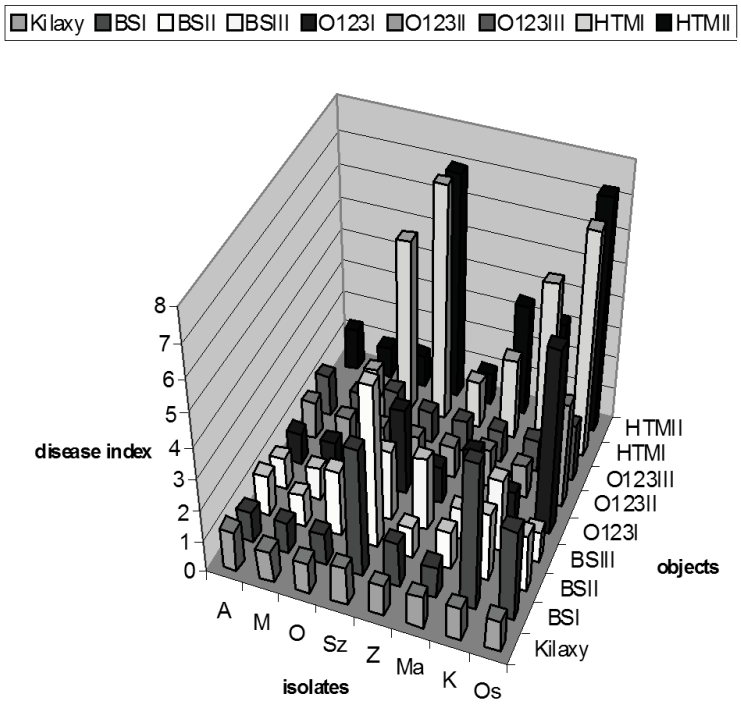

Figure 3. Disease index (DI) of standard objects in seedling test conducted in 2008

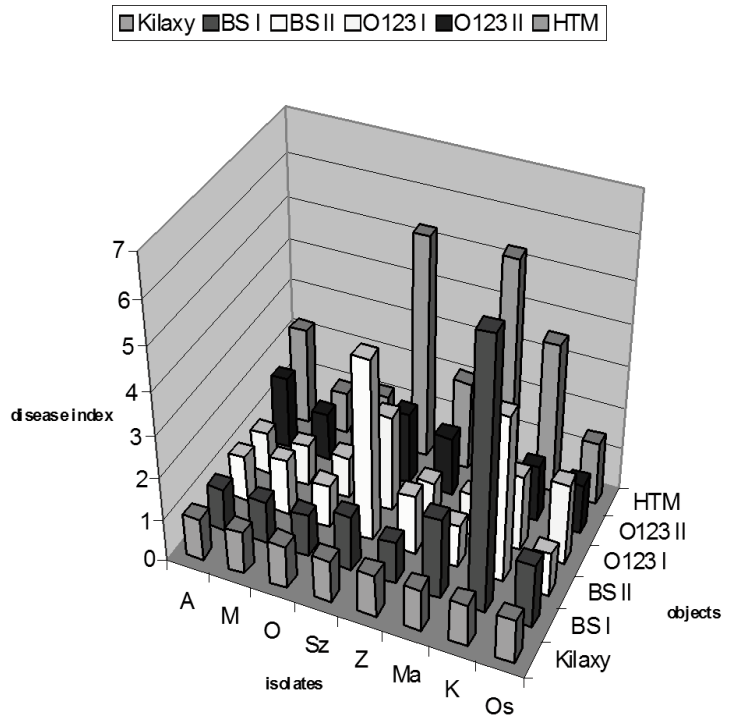

Figure 4. Disease index (DI) of standard objects in seedling test conducted in 2009

4.0 and 5.1 for the Sz isolate. The DI was 5.8 for sub-line I from 'Oregon 123' for the Os isolate. In 2008, both 'Badger Shipper' sub-lines inoculated by the K isolate has a DI of 6.3 and 3.9 and a DI of 4.3 for the Sz isolate for the sub-line I BS. The calculated mean disease index showed a higher level of resistance for the applied isolates in inbred 'Oregon 123' lines than 'Badger Shipper' lines. However, the inoculation of clubroot resistant seeds of standard objects with less virulent isolates (A, Ma, O, Z) gave only incidental disease symptoms in seedling plants.

A different reaction in relation to some isolates observed between the 'Badger Shipper' sub-lines as opposed to the 'Oregon 123' ones may be the result of the interaction of resistant genes with different *P. brassicae* pathotypes. Reby and Michalik (2005) noted similar differences in reactions to *P. brassicae* isolates between inbred lines coming from those having clubroot-resistant gene cultivars. In their studies conducted in 2003-2004, three isolates of this pathogen collected from south and central areas of Poland were used. The results indicated a lower susceptibility reaction in the 'Oregon 123' sub-lines than the 'Badger Shipper' ones, especially to the isolate originating in central Poland (Reby and Michalik 2005). Laszczak et al. (2006) and others continuing the investigation in the next years obtained results that confirmed the differences in virulence amongst Polish isolates, reflected by genotype-dependent differences in the susceptibility of the screened host plants. The S_2 generation of 'Badger Shipper' were classified as resistant, moderately susceptible or susceptible, with results depending on the pathogen isolate. The S_2 generation of 'Oregon 123' and 'Kilaxy' F_1 plants were resistant to all pathotypes (Laszczak et al. 2006).

In Poland, the range of genetic variation of the pathogen was a subject of research conducted first by Nowicki in the 1970s, and later by Robak (1991) and Korbas et al. (2009). Robak (1991) noticed 10 different clubroot pathotypes in an analysed collection of *P. brassicae* samples from different areas of Poland. He also concluded that three of them, no. 4, 6, and 7, were dominant and prevalent in our country. A comparison of the locations of *P. brassicae* isolates obtained in 2007 to the occurrence of pathotypes of the pathogen in Poland as reported by Robak (1991) could explain the observed dissimilar reactions of 'Badger Shipper' and 'Oregon 123' to some of the isolates used in the current research. According to this information,

Table 6. Number of inoculated seeds, plant populations evaluated at seedling (st) and field tests (ft) of standard objects in 2008 and 2009

Standard objects	Year	Number of inoculated seeds	*Plasmodiophora brassicae* isolates																		Total plants evaluated at seedling stage	Total plants evaluatedon 'death field'	% of plants from inoculated seeds evaluated on 'death field'
			A		M		O		Sz		Z		Ma		K		Os						
			st	ft	st	ft	st	ft	st	ft	st	ft	st	ft	st	ft	st	ft					
Badger Shipper	2008	480	52	38	36	34	37	30	30	13	34	20	26	23	25	17	34	18	274	193	40
	2009	471	47	36	40	31	35	33	54	24	20	19	36	30	30	11	21	18	283	213	45
Oregon 123	2008	560	48	38	43	43	37	34	21	17	28	27	36	32	25	24	34	8	272	223	40
	2009	560	43	35	44	39	15	14	50	21	22	16	41	29	27	20	42	29	284	203	36
HTM line	2008	480	19	17	44	33	45	25	38	3	27	20	34	25	29	17	31	0	267	140	29
	2009	280	22	17	23	17	9	7	23	6	14	10	12	5	23	9	16	7	142	78	28
Kilaxy F_1	2008	280	27	19	31	20	26	20	26	19	29	18	21	20	20	18	25	17	205	151	54
	2009	280	25	19	27	14	8	5	27	14	23	18	29	18	21	16	30	16	190	120	43

Table 7. Disease index for standard objects evaluated on 'death field' in 2008 and 2009

Standard objects	*Plasmodiophora brassicae* isolates used for seed inoculation															
	A		M		O		Sz		Z		Ma		K		Os	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Badger Shipper	1.2	2.5	1.8	1.3	3.0	2.6	3.2	3.6	3.0	2.2	1.5	4.1	2.3	7.4	1.3	3.0
Oregon 123	1.9	1.6	1.2	2.4	1.9	1.6	1.9	4.4	4.5	2.3	2.4	2.8	1.4	3.4	1.0	1.5
HTM line	9.0	9.0	9.0	9.0	8.9	9.0	9.0	9.0	9.0	5.0	9.0	8.2	9.0	8.8	9.0	9.0
KilaxyF_1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mean	3.3	3.5	3.3	3.4	3.7	3.6	3.8	4.5	4.4	2.6	3.5	4.0	3.4	5.2	3.1	3.6

'Oregon 123' was resistant to pathotypes 4 and 7 as opposed to 'Badger Shipper', which was resistant to pathotypes 6 and 8.

There is a lot of information about the very high biological differentiation of *P. brassicae.* The genetic variation in the virulence of this pathogen has long been recognised in Europe and was the most troublesome problem in the breeding of a clubroot-resistant cultivar. Several researchers have written about those significant differences in pathogenicity existing both amongst the field populations of *P. brassicae* and in field isolates (Manzanares-Dauleux et al. 2000, Rocherieux et al. 2004, Diederichsen et al. 2009).

The field collections of resting spores may consist of mixtures of virulent pathotypes. One pathotype may be dominant and easily identified; another pathotype may occur at a very low frequency, infecting only the plants of susceptible cultivars. Since field isolates of this pathogen are possibly a mixture of genotypes, a single spore isolation technique is sometimes used to obtain genetically homogeneous populations of the resting spores (Hirai 2006).

In the studies conducted, the number of seedling plants with healthy root systems planted on the 'death field' in each year of testing was significantly lower in relation to the estimated seedling and it was dependant on the isolate used for seed inoculation as well as the level of object resistance (Tab. 6). Of the seedlings from the resistant 'Kilaxy' F_1 cultivar, 151 units in 2008 and 120 in 2009 could be planted. Fewer healthy plants were planted from the 'Badger Shipper' and 'Oregon 123' lines; depending on the year, the number ranged from 193 to 223, which was 36-45% of the inoculated seeds, respectively. The fewest plants on the 'death field' were from the susceptible HTM line: 140 in 2008 and 78 in 2009, only 28-29% of the tested seeds, respectively. The gradual death of susceptible plants due to *P. brassicae* was observed during the growing period on the field, with high contamination of resting spores from different clubroot pathotypes. The occurrence was the most visible in the susceptible HTM line. An almost complete lack of HTM plants with healthy roots was observed during harvest time in both years. The disease index calculated for most isolates was from 8.2 to 9.0 (Tab. 7). Plants from the HTM line obtained from seeds inoculated by the Z isolate from Żółwia Błoć with a DI of 5.0 and 30% healthy plants in 2009 were the exception to that rule (Tabs 7 and 8). All 'Kilaxy' F_1 plants planted on the 'death field' with extra soil-born infection had roots without disease symptoms during harvest time in both years of the experiment, confirming the cultivar's resistance to *P. brassicae*.

Plants from the 'Badger Shipper' and 'Oregon 123' sub-lines screened during harvest time differed in resistance levels (Figs 5 and 6). Generation S_3 – S_5 sub-lines evaluated in 2008 according to *P. brassicae* isolate had a DI from 1.0 to 6.0, while

Table 8. Percentage of plants without disease symptoms of standard objects evaluated on 'death field' in 2008 and 2009

Standard objects	*Plasmodiophora brassicae* isolates used for seed inoculation															
	A		M		O		Sz		Z		Ma		K		Os	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Badger Shipper	97	81	79	81	63	75	49	60	61	81	80	55	72	20	87	71
Oregon 123	88	92	96	79	89	90	88	57	48	80	81	75	93	70	100	89
HTM line	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0
KilaxyF_1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Mean	71	68	69	65	63	66	59	54	52	73	65	58	66	48	72	65

sub-lines of the S_5 generation screened in 2009 showed a wide scale of DI, from 1.0 to even 9.0. Fewer differences in plant reaction to the pathogen were observed between 'Oregon 123' sub-lines than 'Badger Shipper' sub-lines in both years. Plants from sub-lines 'Oregon 123' screened on the 'death field' at harvest time were also less susceptible to the pathogen than those belonging to 'Badger Shipper' sub-lines.

Reby and Michalik (2005) used the same double-stage method for screening clubroot susceptibility reactions of cabbage plants in their research. After seedling-stage screening, only resistant seedlings were transplanted on the same 'death field' as in the current study for the purposes of observing adult plant reactions to the pathogen.

Manzanares-Dauleux et al. (2000) described the testing of cabbage plants for resistance to clubroot using a field method in which sowing was done on a seed bed in spring, then the plants were transferred to a commercial cabbage field badly infested with a natural inoculum of *P. brassicae* after five to

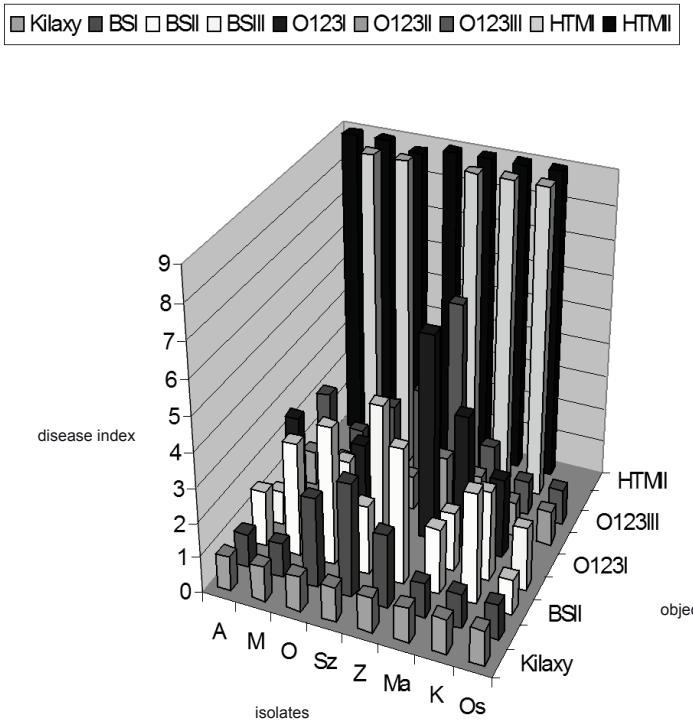

Figure 5. Disease index (DI) of standard objects in 'death field' test conducted in 2008

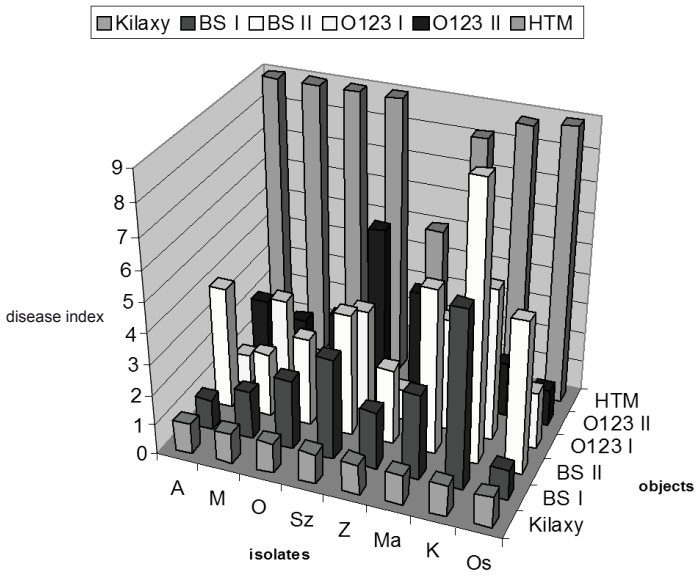

Figure 6. Disease index DI of standard objects in 'death field' test conducted in 2009

six weeks. The plant reaction to the pathogen was examined in autumn; this method of testing was not very reliable because of the influence of environmental conditions. In most research on breeding for resistance, disease was induced by artificial inoculations, either to supplement natural infection or to replace them completely. The goal is to develop methods which would give consistent results that could be used to predict field performance of plant resistance (Robak and Gabrielson 1988).

As far as clubroot disease is concerned, several inoculation techniques have already been developed. The most popular of them are the soil inoculation method and the dipping method. These methods or their modifications are widely used in plant breeding and research. Comparative studies on the influence of different inoculation procedures indicate substantial differences between the obtained results. One of the biggest problems in evaluating *Brassica* lines for resistance to clubroot disease has been the inconsistency of results from test to test and between researchers (Robak 1991).

CONCLUSIONS

1. Differences in the pathogenicity of *P. brassicae* isolates collected from different areas of Poland were observed. Isolates from Szczecin, Ostromęczyn, Krojczyn and Maszkienice were defined as the most virulent.
2. Susceptibility plant screening conducted in both years on eight-week-old seedlings showed distinct differences in reactions to the pathogen between the susceptible HTM line and the resistant 'Kilaxy' F_1 cultivar. They were more visible in the case of seed inoculation with more virulent isolates.
3. The evaluation of adult plants in a field with high *P. brassicae* spore contamination confirmed seedling test results. 'Kilaxy' F_1 plants confirmed a resistance while plants of the susceptible HTM line were characterised by the highest infestation level.
4. Different susceptibility was observed between sub-lines from 'Oregon 123' and 'Badger Shipper' with resistant genes of *P. brassicae*. These differences were more visible according to field screening. 'Oregon 123' sub-lines were less susceptible to applied isolates than 'Badger Shipper' sub-lines at the seedling stage and in the field test.
5. A double-stage plant screening to the pathogen caused the elimination of the most susceptible plants at the seedling stage. Plants with a certain resistance level were planted on the 'death field'. This enabled a more precise and restrictive plant object estimation.

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OKREŚLENIE PATOGENICZNOŚCI IZOLATÓW *PLASMODIOPHORA BRASSICAE* WOR. WYSTĘPUJĄCYCH NA TERENIE POLSKI

Streszczenie: Celem badań prowadzonych w latach 2007-2009 była ocena patogeniczności ośmiu izolatów *Plasmodiophora brassicae* Wor. pobranych z porażonych kiłą roślin kapusty

głowiastej białej pochodzących z różnych rejonów Polski. Do tego celu użyte zostały materiały hodowlane kapusty głowiastej białej odporne i podatne na *P. brassicae*. Nasiona kapusty inokulowano poprzez ich moczenie w zawiesinie zarodników przetrwalnikowych patogena. Ocenę podatności roślin na kiłę prowadzono w oparciu o 9-cio stopniową skalę porażenia korzeni roślin w stadium ośmiotygodniowej rozsady i podczas jesiennego zbioru roślin na polu zainfekowanym *P. brassicae* tzw. 'polu śmierci'. Określono różnice w patogeniczności zebranych izolatów. Najbardziej wirulentne izolaty pochodziły ze Szczecina, Ostromęczyna, Krojczyna i Maszkienic. Kapusta głowiasta biała odmiany 'Kilaxy' F_1 potwierdziła swoją odporność na wszystkie izolaty. Natomiast rośliny podatnej linii HTM pochodzącej z odmiany 'Hitoma' F_1 charakteryzowały się najwyższym stopniem porażenia. Obserwowano różnice w podatności pomiędzy subliniami pochodzącymi z mających geny odporności odmian 'Oregon 123' i 'Badger Shipper'. Mniej podatne na zastosowane izolaty zarówno w teście rozsadowym jak i w polu były sublinie 'Oregon 123' niż sublinie 'Badger Shipper'.

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