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Occurrence of *Plasmodiophora brassicae* in Finnish turnip rape and oilseed rape fields

Marika Rastas, Satu Latvala and Asko Hannukkala

MTT Agrifood Research Finland, Plant Production Research, Planta, FI-31600 Jokioinen, Finland

e-mail: marika.rastas@mtt.fi

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is a serious plant disease of cruciferous plants. A field survey of occurrence of clubroot in oilseed fields was conducted in Finland in 2007–2009 and in 1984–1989. At present, the disease is distributed throughout the oilseed cultivation area. Clubroot was found on average from 30% of fields, but its severity was low; fields with high numbers of infected plants and plants with severe symptoms were rare. According to the survey, cultivation frequency of cruciferous plants is the most important factor affecting clubroot occurrence and severity. Clubroot was found in soils with a wide range of pH-values (pH 5-7.6), but symptoms were most severe at low pH. According to the survey, and greenhouse and field trials, high temperature and moisture during the early growth period seem to favour disease development and can cause significant yield losses. In a survival trial, clubroot declined to close to zero after four years in the absence of host plants, but traces of the pathogen were still detectable after a 19-year trial period, making eradication of the pathogen very difficult.

Key words: clubroot, temperature, moisture, pH, crop rotation, yield

Introduction

Plasmodiophora brassicae Woronin is a soil-borne, root-invading obligate biotroph that causes clubroot disease worldwide in the Brassicaceae family; from the cultivated *Brassica* species to wild crucifers such as *Arabidopsis thaliana* L. (Karling 1968, Koch et al. 1991, Dixon 2009a, Kageyama and Asano 2009). Clubroot infection causes cellular hypertrophy in root cells and gall formation. Typical above-ground symptoms include yellowing of leaves and severe wilting. The combined effects of stunted, often rotten, roots, heavily compromised in their ability to obtain and regulate nutrients and water from the soil, and poor photosynthetic capacity of leaves, retard growth and can markedly reduce yield (Wallenhammar 1998, Dixon 2009a, Kageyama and Asano 2009, Hwang et al. 2011). The pathogen was recently placed into a novel phylogenetic supergroup [clade] of living organisms, Rhizaria (Bass and Cavalier-Smith 2009). *P. brassicae* is a representative of the kingdom Cercozoa (earlier Protozoa), belonging to the phylum Phytomyxea (earlier Plasmodiophoromycetes) and class Plasmodiophorida (Cavalier-Smith 1998).

Conventional chemical or cultural control measures against clubroot are generally uneconomic, impractical or not sufficiently effective (Ludwig-Müller 1999, Donald and Porter 2009). Liming to reduce the acidity of soil, sowing crops in the autumn when the soil is cooler and crop rotation have been applied in an attempt to manage the disease, but with little practical effect. Crop rotation is important in controlling the soil borne plant diseases. However, complete eradication of *P. brassicae* from soil is difficult to achieve in practice since the resting spores of *P*. *brassicae* can remain infectious for 20 years (Dixon 2009a). Also many common volunteer host plants, e.g. cruciferous weeds, can maintain viable inoculum in soil (Wallenhammar 1996). The development of resistant cultivars is now considered the most economic and efficient method for control of this disease.

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Cultivation of cruciferous oilseed crops started in Finland in the late 1970s and increased rapidly during the 1980s (Fig. 1). The main oilseed crop is summer turnip rape, *Brassica rapa* L. subsp. *oleifera* (DC.) Metzg. However, the cultivation of summer oilseed rape, *Brassica napus* L. subsp. *oleifera* (Moench) Metzg., is rapidly increasing in Finland due to new earlier cultivars and climate change (Peltonen-Sainio et al. 2009). Clubroot disease was recognized in cruciferous vegetables in Finland as early as the 1800s (Woronin 1878, Jamalainen 1936). It has been assumed that the disease came to Finland from Russia (Rainio 1930). According to observations made in the 1930s, clubroot was found from all regions where cruciferous plants were cultivated (Jamalainen 1936). At that time clubroot was already distributed as far north as 67°N and was most frequently observed in southern Finland, especially in the south-eastern regions. In a major survey conducted on vegetable farms in Finland in the late 1970s, clubroot disease was found in 81% of 101 communes examined and a total of 61% of the fields were infected (Linnasalmi and Toiviainen 1991). The prevalence of *P. brassicae* infestation in neighbouring Scandinavian countries is also well recognized (Wallenhammar 1996, Dixon 2009a).

The aim of this study is to describe the current clubroot disease situation in cruciferous oilseed crops in Finland based on field surveys made in 2007–2009. In this study the current clubroot situation is also compared to prevalence of the disease in the 1980s when there had been a rapid increase in growing cruciferous oilseed crops in Finland that changed *P. brassicae* from a limited vegetable cultivation pathogen into a large-scale field crop pathogen. The importance of temperature and soil moisture in the epidemiology of clubroot, as well as the survival of clubroot in the soil, are discussed in the light of the previously unpublished results from greenhouse and field trials carried out at the end of the 1980s. The prerequisites for maintaining sustainable oilseed production in the future are also discussed.

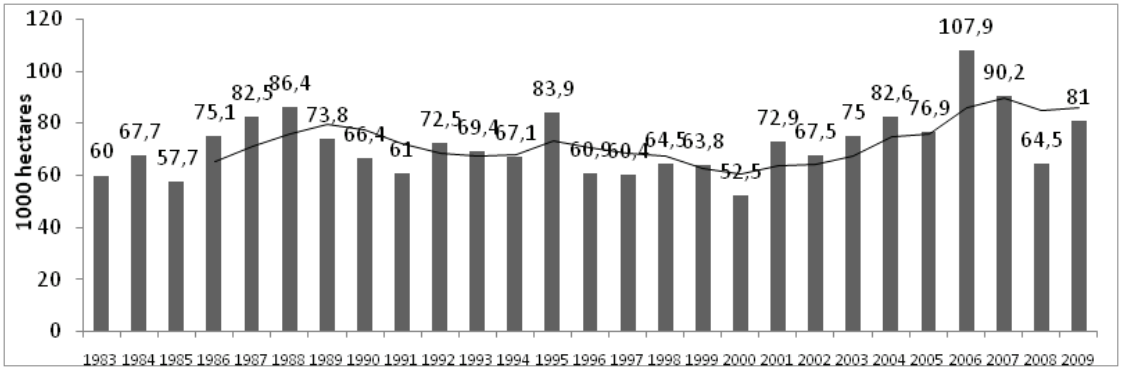

Fig. 1. Cultivation area of turnip and oilseed rape. Line shows a five year moving average of the cultivation area. Data from TIKE (the Information Centre of the Ministry of Agriculture and Forestry in Finland).

Materials and methods

Field survey and clubroot observation

A field survey was conducted in 1984–1989 and 2007–2009 to study the occurrence of clubroot. The survey was executed on private farmers' oilseed and turnip rape fields located within the main areas of rape cultivation in southern and western Finland between 60−63°N and 21−28°W. The main farming areas are

located in lowland with altitudes under 200 m, in the coast the absolute altitude is 100 m. Average annual rainfall in that area is 550-650 mm and main temperature 3−5°C (Drebs et al. 2002). Every year 30−181 fields were surveyed and an average sample of 24−186 plants per field was collected (Table 1.). All samples were taken from different field plots but the same farms were visited yearly. Sampling was conducted every year mainly during August, but a small proportion of samples were taken in late June or early September. The growth stage of plants at time of sampling was 75-99 according to the BBCH scale (Meier 2001). The plant samples were taken by pulling or digging plants and roots up from the soil. The plants were collected at even distances from a w-shaped route covering the field. The severity of symptoms was estimated visually from the collected plants using a scale of four classes (0 = healthy, no clubs observed, 1 = mild symptoms, only few clubs in lateral roots; 2 = moderate symptoms, considerable clubbing on lateral roots; 3 = severe symptoms, large clubs on lateral and main root) according to Williams (1966). The farmers provided the field background information. The pH values for each field were obtained from soil fertility analyses (Viljavuuspalvelu Oy) that farmers are obliged to have at five year intervals.

Table 1. The numbers of surveyed fields and average plant sample sizes.

Year	Field plots observed	Average sample size (plants / field plot)
1984	45	100
1985	49	79
1986	58	185
1987	118	128
1988	73	24
1989	30	119
2007	152	186
2008	181	177
2009	144	167

Effect of air temperature and moisture in greenhouse

A pot trial was carried out in 1988 to investigate the effect of temperature and soil moisture on clubroot. The growth substrate was a mixture of field soil (50% weight) and sterile sand (50% weight). Soil from a turnip rape field with 100 % of clubroot infested plants was used for the studies of clubroot. For healthy controls similar soil known to be free from the disease was used. Field soil, sand and fertilizer were carefully mixed in a concrete-mixer. The complete fertilizer used (Pellon Y-lannos 4, Kemira, Finland) provided N 420 mg , P 200 mg, and K 380 mg kg^{-1} soil.

One kg of field soil-sand mixture was put into one litre plastic pots. Ten seeds of turnip rape (*Brassica rapa* subsp. *oleifera*) cv. 'Emma' were sown in each pot at a depth of 2 cm. Each pot was placed in a separate plastic box to prevent contamination between neighbouring pots and greenhouse facilities. The pots were placed in four separate, small (2.5 m × 2.5 m) greenhouse compartments and the temperature was adjusted to 18 °C. Day length was 12 hours provided by standard greenhouse lamps. After emergence, when coty-

ledons were fully unfolded, the seedlings were counted and extra plants were removed so that finally seven plants were left in each pot.

After emergence the temperatures in separate greenhouse compartments were adjusted to 12, 16, 18 and 22 °C, respectively. The automatic control system allowed ±1 °C deviation from the adjusted temperature. The watering strategy for different treatments was applied simultaneously.

The soil moisture was adjusted to two extremes: wet or dry. Wet soil was watered initially to maximum water-holding capacity and this was maintained by daily watering so that the plastic boxes where pots were kept always contained at least 1 cm of free water at the base. In the dry conditions plants were watered only when they started to show slight symptoms of wilting. In one treatment the soil was kept constantly wet, and in another treatment constantly dry for the whole trial period from emergence to harvest. Four other treatments consisted of alternating wet and dry periods according to crop development. The crop development was divided into three periods: emergence to stem elongation, stem elongation to flowering and flowering to harvest. In two treatments the two first periods were either wet or dry and in the next two treatments two final periods were either wet or dry. Healthy controls were kept constantly either wet or dry (Table 2.). Watering treatments were randomized within six replicate blocks at each different temperature (greenhouse compartments).

Table 2. Watering strategies in a pot experiment in a greenhouse at 12, 16, 18 and 22 °C with clubroot-infested and healthy soil.

Soil wetness periods	Growth stage		
	Emergence to stem elongation	Stem elongation to flowering	Flowering to harvest
Infested soil 1	wet	wet	wet
Infested soil 2	wet	wet	dry
Infested soil 3	wet	dry	dry
Infested soil 4	dry	wet	wet
Infested soil 5	dry	dry	wet
Infested soil 6	dry	dry	dry
Healthy control 1	wet	wet	wet
Healthy control 2	dry	dry	dry

The experiment was started on 3 February and harvested when pods were fully developed on 17 March at 22 °C, 21 March at 18 °C, 28 March at 16 °C, and 4 April at 12 °C. At harvest the roots were washed under tap water and severity of clubroot symptoms was assessed visually using a scale of four classes according to Williams (1966). For the final analysis the two highest severity classes were combined to get an adequate number of cases in each disease class.

Effect of irrigation in field trials

The effect of soil moisture on clubroot incidence and severity was studied in field trials at Jokioinen (60°49' N, 23°29' E) in 1988 and 1989. The trials were established in a field heavily infested with clubroot and

at a nearby field only slightly infested with the disease. Soil in one half of each field was maintained wet by regular sprinkler irrigation (corresponding to 30 mm precipitation) during a two week period before onset of flowering. The other half of the field was not irrigated. Natural precipitation during the period in 1988 was 9.4 mm and in 1989 8.2 mm.

The turnip rape cv. 'Emma' was sown to give a seedling density of 400 seedlings m^{-2}. The crop was sown with a combine drill and fertilized with a commercial complete fertilizer (Normaali Super Y-lannos, Kemira, Finland) at 100 kg N ha^{-1}. Weed control was carried out before sowing with trifluralin (Super Treflan, Kemira, Finland, 2.0 l ha^{-1}). Insects were controlled according to needs with deltametrin (Decis 25 EC, Kemira, Finland). The plot size was 2 × 8 m^2 and there were four replicates for each treatment.

In 1988 there were 5 different sowing dates, 11, 16, 20, 25, 30 May, but all plots were harvested on 12 September. In 1989 the trial was sown on 22 May and harvested on 14 September. The clubroot symptoms were assessed in a similar way as in the greenhouse tests. All plots were harvested separately with a plot harvester. The seed yield was then dried, sorted and weighed and converted to kg ha^{-1} .

Clubroot survival field trial

The survival of clubroot in infested soil was studied in a field trial at Jokioinen in 1992–2010. The experimental field was initially artificially inoculated in 1984 with soil and cabbage roots heavily infested with *P. brassicae*. In 1985–1990 the field was used for different clubroot experiments. By 1990 the soil was thoroughly contaminated. In 1991 the whole field was sown with turnip rape (*Brassica rapa* subsp. *Oleifera*) cv. 'Emma' to provide an even level of inoculum. In August 1991, 95% of sampled plants throughout the field had clubroot symptoms. In May 1992 a crop rotation trial was established on the field. The soil was cultivated three times with a rotary tiller prior to sowing to get resting spores of *P. brassicae* distributed as evenly as possible throughout the field. Four different crop rotation systems were carried out in the field from 1992 to 2010.

- Continuous turnip rape from year to year
- Three year rotation of spring wheat – barley – oats
- Continuous grass lay, no cultivation
- Open fallow, not cultivated, weeds controlled with appropriate herbicides

The experimental design was a randomized complete block with three replicates. Each plot was 8 × 8 m and there was a 4 m exclusion zone of grass lay between the plots to prevent soil movement among plots. Special emphasis was put on avoiding soil movement among plots during cultivation practices.

The crops in the trial were managed according to standard practices. Turnip rape and cereals were fertilized with commercial compound fertilizers (100 kg N ha^{-1}) at sowing. Turnip rape weeds were controlled annually with trifluralin applied just before sowing. For cereals, commercial herbicides were applied after emergence according to the manufacturer's recommendations. The grass lays were fertilized at the beginning of the growing season with commercial compound fertilizers (80 kg N ha-1). Additional N (100 kg ha^{-1}) was given after the first harvest, normally at the end of June. The grass lay was harvested twice per season. Open fallow was not fertilized. Weeds were controlled according to needs 2–3 times per season. At least one weed control per season was done using glyphosate.

The incidence of clubroot after each season was studied in a soil bioassay in a greenhouse. A soil sample from each experimental plot was taken after harvest during the latter part of September. The soil sample consisted of 10 subsamples taken from the soil surface to 15 cm depth and the total amount of soil was approximately 3 litres. Soil samples were stored at 6 °C in plastic containers until testing. Bioassays were carried out in a greenhouse during the following year from February to April. For the bioassay, turnip rape seedlings (cv. 'Emma') were grown in sterile sand in plastic seed trays placed in plastic boxes. In one plastic box there was space for a seed tray of 35 individual seedlings. After emergence, when seedlings were 2–3 cm high, each of the 35 seedlings was inoculated with 10 ml of soil water suspension prepared from each field sample. The soil water suspension was made by mixing one litre of soil from each field sample with one litre of tap water and then preparing a 10-fold dilution which was used in inoculation. However, in 1995 the inoculation was done with 1:1 soil water suspension due to very low infection level in all treatments.

After inoculation the sand was kept moist by keeping a 1 cm layer of free water at the bottom of the plastic boxes containing the seed trays. The temperature in the greenhouse was constantly +18 °C. Day length was 12 hours provided by standard greenhouse lamps. The seedlings were grown for 5 weeks. Thereafter plants were taken up, roots were washed and the numbers of plants with clubroot symptoms were recorded. Each year the bioassay was repeated 2 or 3 times for each field sample. The level of infestation was assessed as percentage of infected plants.

Statistical analyses

The statistical analyses were performed using SAS software for windows version 9.2 through SAS Enterprise Guide version 4.3 (SAS Institute Inc. Cary, NC, USA). For the effect of pH, a one-way analysis for variance was performed using the ANOVA procedure. For the disease incidence and effects of cultivation frequency in the field survey and effect of temperature and soil moisture on the disease incidence in the greenhouse and field trials, the data were analysed using the LOGISTIC procedure. Odds ratios for the risk of disease incidence were used to compare statistical significance of different classification factors in trials and field survey (Allison 1999, Lehtinen et al. 2007). The effects of irrigation and level of clubroot infestation on yield in the field trial were studied by performing analysis of variance using SAS GLM procedure (Littell et al. 1991).

Results

Clubroot occurrence and symptom severity in the field survey

The survey of clubroot occurrence was conducted in 1984−89 and 2007−2009. There was no obvious difference in clubroot occurrence between the different cultivation regions (Fig. 2) and the distribution of clubroot was quite similar in the 1980s and 2000s. However, in the 1980s no clubroot was found in the northernmost regions surveyed. Also in 2007−2009 the most severe clubroot outbreaks were absent from those regions.

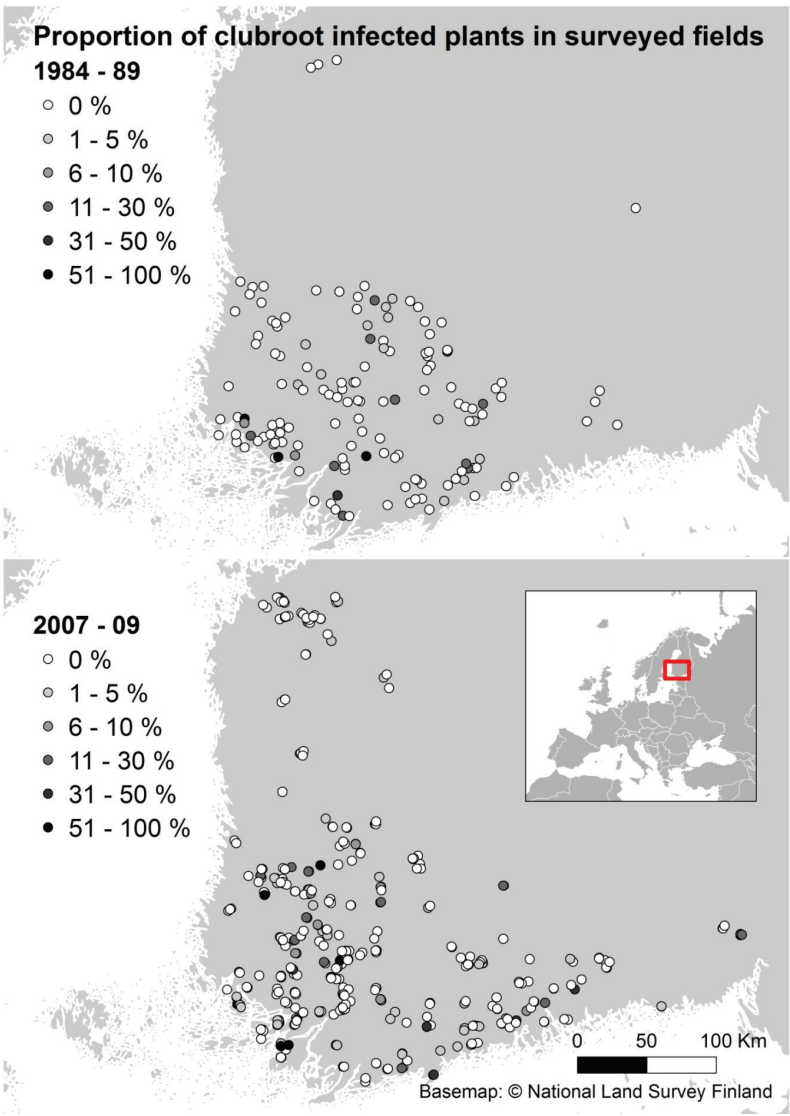

Fig. 2. The occurrence and severity of clubroot in field surveys conducted in 1984-1989 and 2007-2009.

The survey data were split into three periods, 1984−1986, 1987−1989 and 2007−-2009, for the periodical analyses of clubroot occurrence. By 1984−1986 the area of oilseed crops in Finland had gradually increased to 60 000–75 000 hectares (Fig. 1). During that period clubroot was found from 16% of the fields (Table 3). During the 1987−1989 period the area increased to 86 000 hectares (Fig. 1). During that period the proportion of infested fields increased to 22%. The statistical risk (odds ratio) for clubroot occurrence had grown 1.5 fold in comparison to that for the 1984−1986 period. Also the risk for occurrence of heavily infested fields (more than 10% of plants showing symptoms) was 2.5 fold (16.7% of fields) in comparison to other survey periods. Between 1990 and 2005 the cultivated area of oilseed crops fluctuated between 60 000 and 80 000 hectares per year, suddenly increasing up to 108 000 hectares in 2006 (Fig. 1). During the period 2007−2009 the cultivated area fell back to 60 000 hectares (Fig. 1). However, the incidence of fields infested with clubroot in 2007−2009 was over 25% and the risk for disease incidence was 2 fold (p=0.026) in comparison to the period 1984−1986.

Table 3. Clubroot incidence and proportion of infected plants in oilseed and turnip rape field surveys.

Survey period	Clubroot infested fields %	Odds ratio vs. 1984 -1986	p for khi square	Severely infested fields %*	Odds ratio vs. 1984 -1986	p for khi square	Infected plants %**	t-value vs 1984 - 1986	p for t-value
2007 - 2009	25.4	1.8	0.026	5.7	1.5	0.869	3.5 b	1.55	0.121
1987 - 1989	22.2	1.5	0.552	16.7	2.5	0.013	5.5 a	2.81	0.005
1984 - 1986	15.8	-	-	5.2	-	-	1.6 c	-	-

* The fields with over 10% of infected plants.

** A statistically significant difference is indicated by a letter indicating the grouping in Tukey's test, $p < 0.05$.

The severity of clubroot symptoms was slightly lower in the 1980s than in the 2000s, except in 1988 (Fig. 3). The annual fluctuations in severity were quite high; especially in 1988 when clubroot occurrence was markedly higher than on average. This was most likely due to the increase of cultivation area of oilseed crops and favourable weather conditions for the disease. According to weather data from the surveyed areas, June was quite warm in 1988 (average temperature 16.4 °C) and precipitation was 50 mm, which is the average level for the survey period (Table 4). June was warm also in 1986 (16.5 °C), but the average precipitation sum was only 18.8 mm for that period.

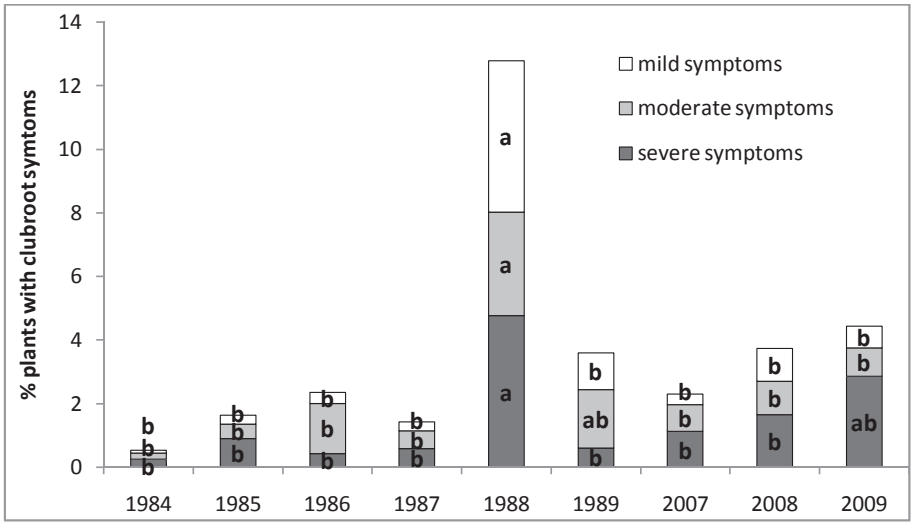

Fig. 3. Symptom classes in all surveyed fields. The letters indicate statistical significance according to Duncan's multiple range test ($p < 0.05$) within each class.

Table 4. Average weather conditions in the surveyed areas during the survey periods.

	Temperature °C					Rainfall mm			
	May	June	July	August		May	June	July	August
1984	12.6	13.6	15.1	14.0	1984	36.2	68.1	109.7	51.0
1985	8.2	13.3	15.4	15.5	1985	43.4	43.9	65.8	92.3
1986	10.6	16.5	16.4	12.9	1986	43.5	18.8	79.8	112.6
Average 1984-1986	10.5	14.4	15.6	14.2	Average 1984-1986	41.0	43.6	85.1	85.3
1987	7.6	12.6	14.8	11.6	1987	41.6	83.9	61.0	102.0
1988	11.0	16.4	19.2	13.9	1988	36.8	50.0	102.5	98.6
1989	10.3	15.5	16.5	14.0	1989	34.9	48.7	67.2	90.0
Average 1987-1989	9.6	14.8	16.8	13.2	Average 1987-1989	37.7	60.9	76.9	96.9
2007	9.9	14.6	16.3	16.2	2007	59.6	50.6	106.9	74.4
2008	9.7	13.8	16.2	13.9	2008	11.8	99.5	57.2	111.6
2009	10.9	13.4	16.3	15.3	2009	30.5	48.5	81.3	53.9
Average 2007-2009	10.2	13.9	16.3	15.1	Average 2007-2009	34.0	66.2	81.8	80.0
Survey period average	10.2	14.3	16.7	14.4	Survey period average	39.4	55.8	75.8	84.6

The effect of pH on clubroot incidence

In 2007−2009 clubroot was found in fields with pH values from 5 to 7.6. All of the severe outbreaks (more than 10% of plants showing symptoms) were in fields with pH values of 6.7 or lower. A negative correlation between pH and proportion of diseased plants per field was established (r=-0.01181, r^2=0.0132 P=0.0139). When fields were divided into two classes according to pH value, under and above 6.5, there was only a minor difference in clubroot occurrence between the groups: 28% of the fields with low pH and 23% of the fields with higher pH were infected. However, the proportion of infected plants per field was significantly higher in low pH soils (Table 5). When comparing only infected field plots, 17.6% of plants were infected at low pH over the years and 7.8% were infected at high pH. When examining only infected fields, also 9.5% of plants had severe symptoms (large cankers in a main root) at low pH, which was over double the amount as at higher pH (Table 5). The difference was similar also for moderate symptoms, but no significant difference between the two classes was recorded for mild symptoms.

Table 5. The effect of pH on clubroot severity in infected fields.

	pH < 6.5		pH > 6.5			
	Average %	SD	Average %	SD	Propability	Tukey*
Infected plants	17.6	23.01	7.8	15.62	0.0107	*
Mild symptoms (%)	3.1	4.40	2.1	3.42	0.1744	
Moderate symptoms (%)	5.0	7.30	2.1	3.58	0.006	*
Severe symptoms (%)	9.5	18.19	3.6	12.02	0.0363	*

*A statistically significant difference in Tukey's studentized range test ($p < 0.05$) is indicated by an asterisk.

The effect of crucifer cultivation frequency

Cultivation frequency of cruciferous plants had a significant effect on clubroot incidence according to the survey conducted in 2007−2009. If cruciferous crops were grown in the same field plot less than two years apart, clubroot was recorded in 53.6% of the fields (Table 6). After five to six years since the last cruciferous crop, clubroot was found from 19.7% of the fields. The statistical risk for clubroot outbreak was 6 fold after 1−2 years break and still 5 fold after 3−4 years break when compared to the field with a break of seven years or more. Along with increased clubroot outbreaks, frequent cultivation of crucifers led to a higher proportion of infected plants and increased severity of symptoms. A total of 12.3% of all observed plants were infected after 1−2 years from the last cruciferous crop and after seven years the infection rate was under 1%. When infected fields only were examined, 15% of the plants had severe disease symptoms when crucifers were cultivated within 1−2 years (Fig. 4). The proportion of severe symptoms was half of that after 3−4 years. The cultivation frequency did not have as drastic an affect on the prevalence of mild and moderate symptoms as on severe symptoms.

Table 6. The effect of cultivation frequency on clubroot incidence and proportion of infected plants.

Years from last cruciferous crop	Number of fields (proportion of all fields, %)	Clubroot infested fields %	Odds ratio vs. >7	p for khi square	Severely infested fields %*	Odds ratio vs. >7	p for khi square	Infected plants in fields %**	t-value vs. >7	p for t-value
1-2	28 (7,7)	53.6	5.98802	<0.0001	20	11.999	0.0168	12.312 a	4.26	<0.0001
3-4	6 6 (18,1)	43.7	4.65116	0.0041	16.9014	9.762	0.0235	7.3751 a	3.45	0.0006
5-6	2 2 2 (61,0)	19.7	1.46843	0.008	5.24017	2.654	0.2077	1.9027 b	0.61	0.5442
>7	48 (13,2)	14.3	-	-	2.04082			0.7869 b	-	-

* The fields with over 10% of infected plants

** A statistically significant difference is indicated by a letter indicating the grouping in Tukey's test, $p < 0.05$.

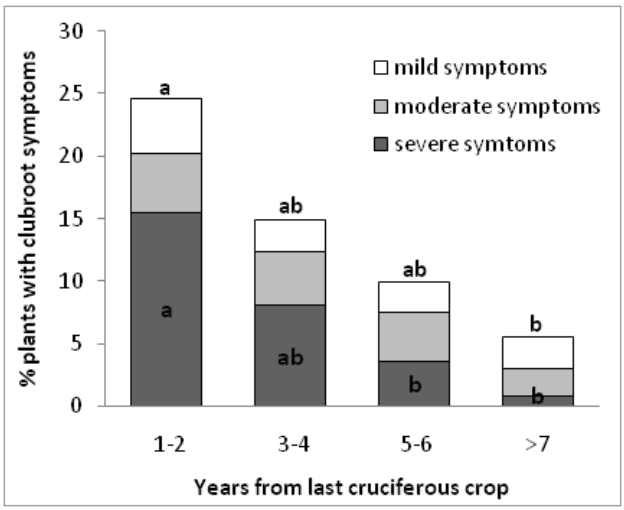

Fig. 4. The proportion of clubroot infected plants and disease severity in infected fields in a field survey done in 2007-2009. The letters indicate statistical significance according to Duncan's multiple range test ($p < 0.05$). The letters for the proportion of infected plants are above the bars and for the severe symptoms in the bar. There were no statistically significant differences within mild and moderate symptoms.

Clubroot survival in soil

The decline of clubroot inoculum in naturally infested soil started in fallow, cereal and grass plots within the first four years (Fig. 5). Also the turnip rape plots showed a decline in infection potential in this period. In fallow and cereal fields the clubroot level settled close to zero in 4 years and in grass the inoculum eradication took 6 years, but the difference between the infection levels in different treatments was minimal. In continuous turnip rape cultivation, after a rapid decline in infection level during the first four years, the infection level ranged from 10 to 80%, but the trend was rising. The average infection level in 1992-1995 was 63%, in 1996-1999 37%, in 2000-2003 49%, in 2004-2007 59% and in 2008-2010 66%, while in other treatments the infection level stayed under 10% in the time periods after 1996. Clubroot was detectable through the whole 19-year-trial period also in treatments without host plants, but only in trace amounts.

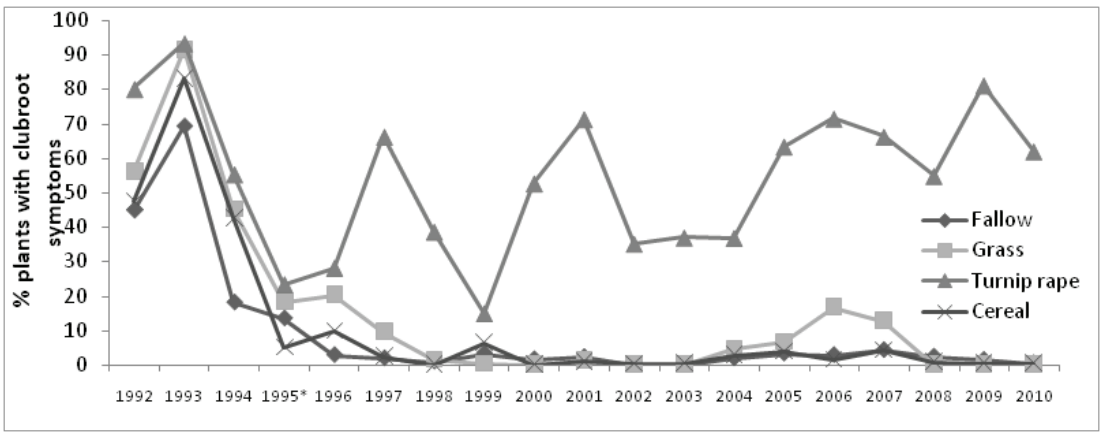

Fig. 5. The proportion of plants showing clubroot symptoms in a greenhouse bioassay. *In 1995 a 1:1 soil water suspension was used.

The effect of temperature and soil wetness on clubroot in the greenhouse

The air temperature had a very significant effect on clubroot incidence in infested soil. No disease was detected in healthy soil at any temperature. At 12 °C risk for clubroot symptoms was almost 40 fold lower than at 22 °C (Odds ratio 0.026, $p< 0.0001$). At 16 °C and 18 °C the risk was 10 (Odds ratio 0.107, $p=0.0089$) and 5 (Odds ratio 0.199, $p=0.0608$) fold lower than at 22 °C, respectively (Fig. 6).

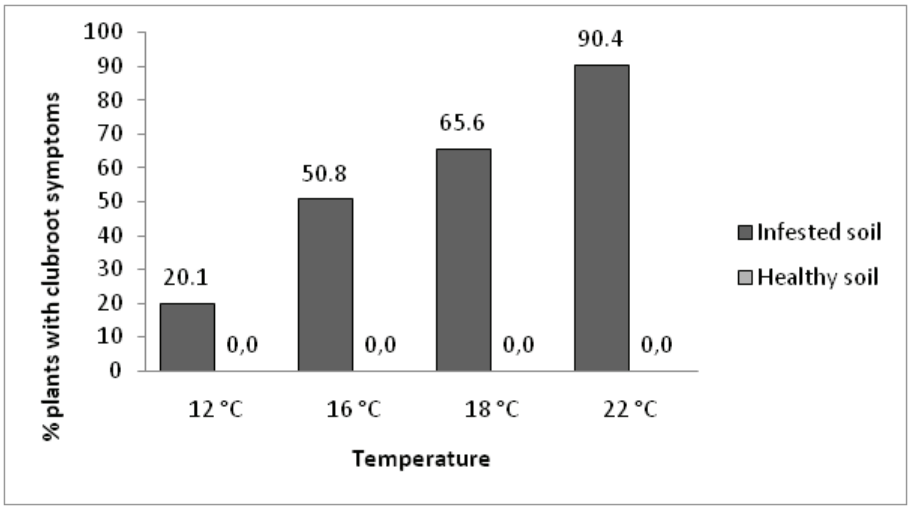

Fig. 6. Effect of temperature on clubroot incidence in plants grown in infested and healthy soil.

Soil wetness clearly increased clubroot incidence. Both the duration and timing of the wet period had a statistically significant effect on symptom development. The risk for clubroot symptoms when the period before flowering was dry was less than half of that when soil was wet prior to flowering (Odds ratio 0.633, $p= 0.0069$). The risk for clubroot symptoms when there were at least two dry periods during the season was also less than half of that when there were two or more wet periods during the season (Odds ratio 0.413, $p<0.0001$) (Fig. 7).

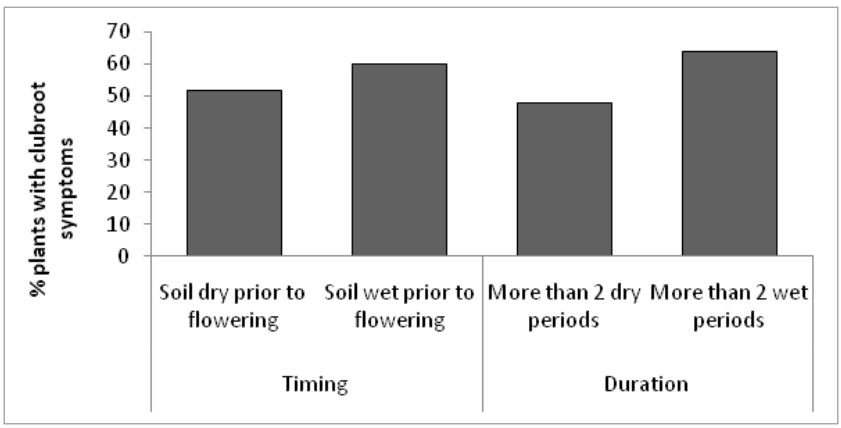

Fig. 7. Effect of timing and duration of wet and dry periods on the incidence of clubroot

The effect of timing and duration of wet and dry periods on clubroot incidence was different at different temperatures. At 12 °C the risk for clubroot development was two times higher in dry soil prior to flowering in comparison to wet soil (Odds ratio 1.934, p=0.0667). At 16 °C and 18 °C the risk of clubroot infection in dry soil prior to flowering was half of the risk in wet soil (Odds ratios 0.398, p=0.0015 and 0.463, p=0.0127). At 22 °C, where disease incidence was highest, soil moisture had no effect on clubroot risk (Odds ratio 0.623, p=0.3485) (Fig. 8).

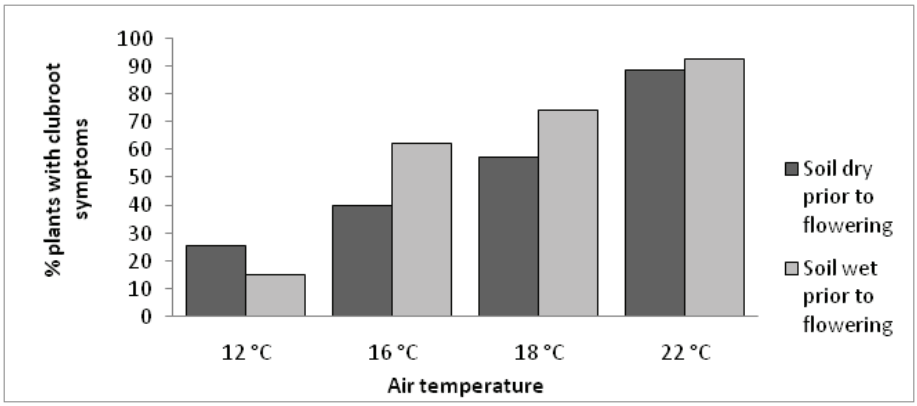

Fig. 8. Effect of temperature and soil moisture prior to flowering on the incidence of clubroot symptoms.

At 12 °C and 22 °C the duration of a moist period had no effect on the risk of clubroot incidence (Odds ratios 0.718, p=0.3463, 0.607, p=0.3252). At 16 °C the risk for clubroot symptoms was more than four fold higher when soil was wet for longer periods in comparison to dry soil (Odds ratio 0.223, p<0.0001). At 18 °C the risk for clubroot during a long wet period was twice as high as for dry soil (Odds ratio 0.463, p=0.0124) (Fig. 9).

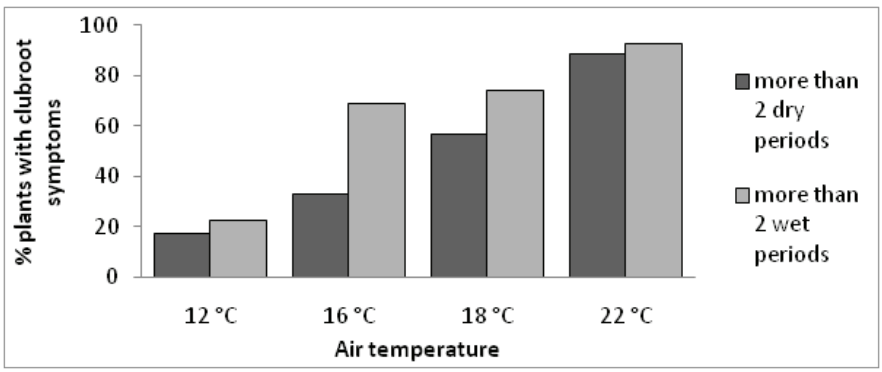

Fig. 9. Effect of temperature and duration of wet and dry periods during the growing season on the incidence of clubroot symptoms.

Effect of soil moisture on clubroot severity and crop yield

Increasing soil moisture by irrigation before flowering in field trials significantly increased clubroot severity, especially in soil severely infested with the pathogen. In severely infested irrigated soil the probability for severe clubroot symptoms was 70 fold higher (Odds ratio 71.4, $p<0.0001$) than in dry soil. In slightly contaminated soil the probability for severe clubroot symptoms was also significantly higher (Odds ratio 16.4, $p=0.0001$) in wet than in dry soil (Fig. 10).

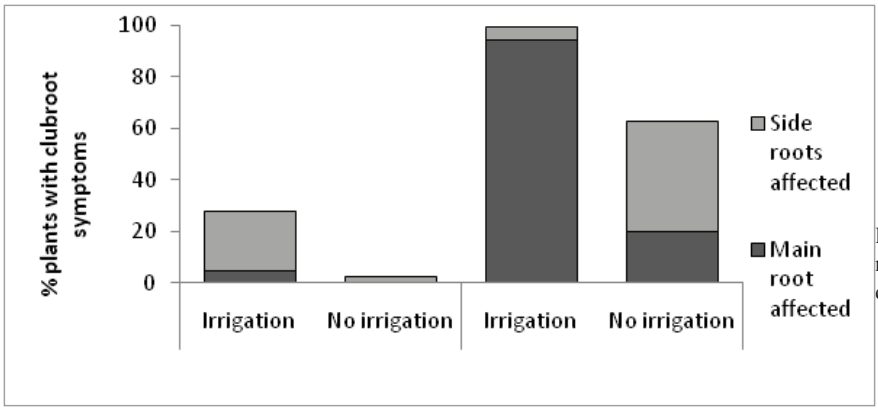

Fig. 10. The effect of irrigation on clubroot incidence in field trials.

The increased disease severity due to wet soil also seriously reduced the seed yield. In severely infested wet soil the yield was only 454 kg ha^{-1} while in dry soil the yield was 1444 kg/ha. In slightly infested soil irrigation increased yield from 1707 kg ha^{-1} to 1746 kg ha^{-1}, but the difference was not statistically significant (Fig. 11).

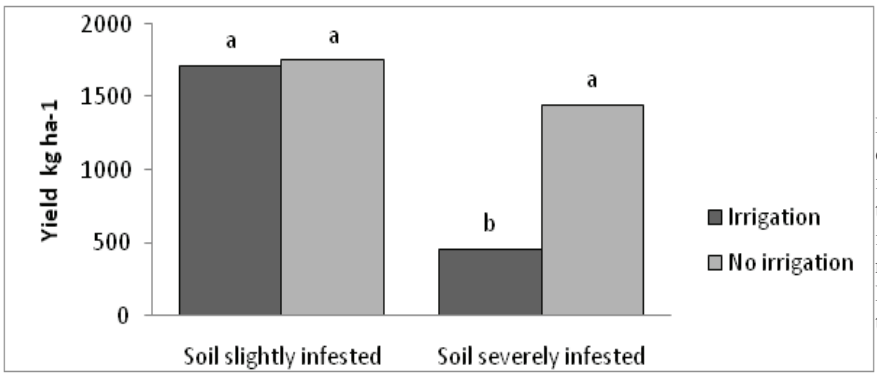

Fig. 11. The effect of clubroot incidence and irrigation on yield of turnip rape. The letters indicate statistical significance according to Duncan's multiple range test ($p < 0.05$).

Discussion

Clubroot occurrence

According to the survey, clubroot disease was distributed over the whole cultivated oilseed crop area in Finland. However, the average disease severity on the majority of fields was low. There seems to be a temporal correlation between an increase in oilseed area and clubroot occurrence, since the peaks in occurrence were in parallel with the increasing cultivated area of oilseed crops. Cruciferous crops were cultivated mainly as small-scale vegetable crops until the 1980s when the cultivation of oilseed crops increased. This might explain the lower occurrence of the disease in the northernmost oilseed areas, where vegetable cultivation has been less intensive. Previous studies on clubroot occurrence in Finland have been conducted on vegetable farms where the occurrence was markedly higher than in oilseed fields. In a survey conducted in 1974–1978, 61% of vegetable farms with cruciferous crops had clubroot (Linnasalmi and Toiviainen 1991). In Sweden, where cruciferous oilseed crops have been intensively grown since the early 1940s, clubroot is frequently observed in oilseed crops (Engqvist 1994, Wallenhammar 1996). In the study by Engqvist (1994), clubroot was found from 57% of soil samples from oilseed fields. Wallenhammar (1996) found clubroot from 78% of oilseed fields in the county of Örebro, Sweden. If the growing area of oilseed crops is still increased in Finland and susceptible crops are cultivated in short rotations, there is a high potential risk for severe disease outbreaks.

Disease severity and environmental conditions

It has been noted that environmental conditions, including moisture and temperature, greatly affect the development of clubroot disease (Dixon 2009b). This was also observed in this survey; clubroot symptoms were less severe in those years when the beginning of the growing season was dry. Warm temperatures, together with high or moderate precipitation during the early growing season, seem to increase disease severity. In the greenhouse and field trials, high temperature and moisture were also shown to increase the disease. It has been long recognized that moisture and relatively high temperature favour the development of clubroot disease (Monteith 1924). In the studies conducted in growth chambers with cabbage, chinese cabbage, mustard and radish, the optimal temperature for symptom development was 21–22 °C (Thuma et al. 1983). In a recent study with pak choi (*Brassica rapa* subsp. *chinensis)* the optimum for disease development was even higher, 25 °C (Sharma et al. 2011). High temperture was shown to favour disease development also in our studies with turnip rape. Thuma et al. (1983) also tested the effect of moisture on clubroot of radish under field conditions and noted that high moisture at the seedling stage promoted disease development. Since clubroot disease is favoured by higher temperature, climate change might lead to increased severity of clubroot in the future. The significance might be less dramatic if sowing can be done earlier in the spring, since the age of plants at the time of infection is an important factor affecting disease severity (Hwang et al. 2011).

In addition to temperature and moisture, soil properties and cultivation techniques also have an effect on clubroot disease outbreaks and severity. It was noted in the survey that low pH increases the amount of infected plants within a field plot and makes the symptoms more severe, but it does not necessarily increase the clubroot outbreaks. In this study clubroot was found in the fields with pH values from 5 to 7.6, but all severe outbreaks were at pH values under 6.7. The soil pH values obtained from the farmers for the survey may differ slightly from the actual values since the soil fertility analyses may have been done up to five years prior to the survey. Since the number of surveyed fields with pH information was large (n=440) the results can be considered, however, as indicative of the effect of pH on clubroot.

Heavily infected fields were also found for a wide range of pH values in the other surveys (Wallenhammar 1996, Strelkov et al. 2007). The effect of pH is dependent on spore concentration in the soil; at high spore densities the disease will occur even in alkaline soil (Webster and Dixon 1991, Murakami et al. 2002b). Liming has been shown to reduce spore density in soil and to affect symptom development (Webster and Dixon 1991, Murakami et al. 2002a). The effect of liming is dependent on both alkalinity and calcium content of the soil, which both have similar, but separate, effects on reducing clubroot infection and disease development (Dixon 2009b). However, as noticed in this survey and previous studies, high disease levels may occur also at pH values near neutral or even higher, and raising soil pH by liming is not a sufficient method for controlling clubroot on a large field scale (Wallenhammar 1996, Strelkov et al. 2007). Low pH makes conditions more favourable for canker development (Webster and Dixon 1991), which was seen in this study as an increased incidence of severe symptoms at lower pH values. This means that the pathogen is able to produce more spores in the field at low pH and that, in part, will enhance survival and dispersion of the pathogen.

Crop rotation and survival of clubroot in soil

According to the survey, the most important factor affecting clubroot occurrence and severity is the cultivation frequency. Frequent crucifer cultivation leads to increased risk of clubroot outbreaks, higher numbers of infected plants in the field and more severe clubroot symptoms. Thus, the low occurrence and severity of clubroot in the surveyed oilseed fields is most likely due to the good crop rotation practice. Of the surveyed fields only 7.7% were with a 1–2 year break in crucifer cultivation and 18.1% were fields with a 3–4 year break (Table 6). The significance of crop rotation was shown also by Wallenhammer (1996), where extending time from previous cruciferous crop and lower frequency of oilseed cultivation was shown to decrease the infestation of soil assessed by bio-assay. When cultivated area of crucifers is increased it might lead to a shortening of crop rotation intervals. This could explain why the highest disease occurrence was observed in the field survey in parallel with increasing oilseed cultivation area.

Since clubroot is a soil-borne pathogen, its survival in soil is essential for disease outbreaks. According to some studies, the resting spores of *P. brassicae* remain infective in soil for 20 years (Dixon 2009a). In the survival trial, the eradication of infectivity was evident after a two year break in crucifer cultivation, but clubroot was still detectable after a 19 year break. In a study by Wallenhammar (1996), clubroot was eradicated beyond detection level in 17.3 years. The small clubroot traces, observed in the survival trial, might be a result of contamination from the turnip rape plots regardless of the procedures carried out to prevent movement of soil from heavily infected plots to the other plots. However, the results indicate that the total eradication of clubroot from soil is difficult to achieve. There were no obvious differences between different treatments, but the eradication took slightly longer in grass than in open fallow or cereal cultivation. Some studies indicate that root exudates of some typical grass species like perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pretense* L.) stimulate germination of resting spores of *P. brassicae* (Friberg et al. 2005). The germination, in the absence of host plants, leads to reduction in the amount of viable spores in soil. However, this was not seen in this trial as a decrease in infection level. It seems that the reduction of spores by grass crops is inefficient in reducing clubroot since the use of non-cruciferous plants as bait crops was unsuccessful in previous greenhouse and field tests (Friberg et al. 2005, Friberg et al. 2006, Ahmed et al. 2011). Annual fluctuation in the infection level under continuous turnip rape cultivation was quite high. The reason for this might be that the bioassay method is affected by the environmental greenhouse conditions. The decline observed during the first years in the continuous turnip rape cultivation might be due to the change in turnip rape cultivars since the clubroot strains have been shown to have different virulences against different cultivars and host species (Some et al. 1996, Fähling et al. 2003, Strelkov et al. 2007).

The effect of clubroot on oilseed yield

According to the field trials, clubroot infection can lead to a marked reduction in oilseed crop yield, which makes the pathogen a significant risk for oilseed crop cultivation. The disease severity and yield reduction are highly dependent on the soil moisture, especially before the crop flowering stage. Significant yield losses that correlate with clubroot disease severity have been reported previously in oilseed (Wallenhammar 1998, Hwang et al. 2011). The yield losses make clubroot a serious economic threat to oilseed production.

The most important factor in controlling clubroot seems to be sufficient crop rotation, since liming and use of chemical control methods are uneconomical and not sufficiently effective for large-scale field cultivation. However, there is a high potential risk for severe disease outbreaks if the growing area for oilseed crops is increased and crop rotation intervals are shortened under pressure to increase the cultivation area. For effective disease control the development of clubroot resistant cultivars is urgently required since all currently available oilseed varieties are susceptible to clubroot.

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