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Quantitative PCR shows propagation of *Plasmodiophora brassicae* in Swedish long term field trials

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Abstract Clubroot (*Plasmodiophora brassicae*) is a serious soil-borne disease in brassica crops world-wide. We report on a time series of soil samples from Swedish long-term fertility trials started in 1957, 1963 and 1966, which were analyzed for the amount of *P. brassicae* DNA. The crop rotations included a brassica crop every 4 or 6 years. All experimental sites with a 4-year rotation of oilseed rape, except one with calcium carbonate in the soil profile, showed high (>1000 fg DNA g^{-1} soil) levels of *P. brassicae* DNA after 9, 11 and 12 rotations. In contrast, detectable levels (>5 fg DNA g^{-1} soil) of *P. brassicae* were found only at one of five sites with a 6-year rotation of spring oilseed rape. In years with high levels of *P. brassicae* DNA, low yield was reported and a subsequent decline in *P. brassicae* DNA in soil was observed. Different NPK (nitrogen/phosphorus/potassium) fertiliser regimes resulted in similar *P. brassicae* DNA levels. The robustness and reliability of the method applied was verified by analyses of soil from individual plots compared with a mixture of plots and by repeated analyses of selected samples, which showed that *P. brassicae* DNA remained stable during dry storage.

Keywords Long-term fertility field trials · Clubroot · Soil samples · *Plasmodiophora brassicae* · Quantitative PCR · qPCR

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Introduction

Clubroot in brassica crops, caused by the obligate endoparasite *Plasmodiophora brassicae* (Woronin), is recognised as a serious soil-borne disease associated with appreciable yield losses (Wallenhammar 1998; Strelkov and Hwang 2014). It is one of the economically most important diseases of cruciferous crops now found throughout oilseed rape and vegetable brassica growing areas world-wide (Dixon 2009a, 2014). Outbreaks of clubroot have been continuously reported in recent years in winter oilseed rape districts in southern Sweden and more frequently in oilseed rape fields in central Sweden, where production of this crop ceased temporarily due to clubroot 25 years ago (Wallenhammar et al. 2014). The survival structure, resting spores, are produced in large numbers inside infected roots can persist in the soil for up to 17 years (Wallenhammar 1996).

In the early 1950s, long-term soil fertility trials were established in Sweden to study the influence of mineral fertiliser on long-term productivity compared with the existing regime of circulating plant nutrients in manure (Carlgren and Mattsson 2001). Field experiments were established in the 1950s-1960s in different parts of the

country and comprised two crop rotations; one including a brassica crop and one including livestock manure, along with 16 combinations of manure and inorganic NPK (nitrogen/phosphorus/potassium) fertiliser. These fertilisers are compared in a 4-year rotation in southern Sweden and a 6-year rotation in central Sweden. In the early 2000s, after 11 and 12 rotations, spring oilseed rape crops were observed to be heavily infected by clubroot at several of the sites. Soil samples have been taken every 4th and 6th year in all crop rotations since the start of the experiments and stored dry.

Molecular methods based on polymerase chain reaction (PCR) permit rapid and accurate assessment of soil-borne pathogens. Quantification of plant pathogens in soil DNA extracts using real-time quantitative PCR assays has been described for a number of pathogens (e.g. Cullen et al. 2002; Lees et al. 2002; Ratti et al. 2004; Bilodeau et al. 2012). More recently, we developed and validated a real-time quantitative PCR (qPCR) method for detection of *P. brassicae* DNA in naturally and artificially infested soil samples (Wallenhammar et al. 2012). The protocol provides a robust and reliable technique for assessing this disease in soils and for predicting the risk of disease development in various cropping situations, and is now commercially available and used by Swedish farmers.

The objective of the present study was to use qPCR analysis to study changes in the concentration of *P. brassicae* DNA over time in archived soil samples. The hypotheses were that: (i) *P. brassicae* DNA concentration, as analysed in archived soil samples, increases to a peak in some years and then decreases in subsequent years; (ii) increasing rate of nitrogen (N) fertiliser decreases clubroot disease severity; and (iii) *P. brassicae* DNA remains stable during dry storage.

Materials and methods

Soil samples

The Swedish long-term soil fertility trials were initiated in 1957, 1963 and 1966 by the Department of Plant Nutrition at the Swedish University of Agricultural Sciences and are still ongoing. A complete description of the experimental sites and plans is given by Carlgren and Mattsson (2001). In brief, the 4-year rotation initially included barley (Hordeum vulgare), white mustard (Sinapsis alba), winter wheat (Triticum aestivum) and sugar beet (Beta vulgaris var. altissima). White mustard was replaced by spring oilseed rape (Brassica napus L) in 1986. The 6-year rotation comprises barley, oats (Avena sativa), white mustard/oilseed rape, winter wheat, oats and winter wheat.

The initial soil characteristics (Table 1) were analysed at the laboratory of the Department of Soil and Environment, SLU, Uppsala (Egnér et al. 1960) and these samples were then stored dry at room temperature (Table 1). In 1981 and 1996, lime was applied at any of the experimental sites with a pH value <6.1 (Fig. 1).

Soil was sampled each 4*th* or 6*th* year after harvest of the winter wheat crop following the brassica crop. The long-term fertility trials comprise several treatments and thus have only two replicates at each site (Carlgren and Mattsson 2001). To further reduce the experimental costs, the replicates are often bulked to one general sample. During selected years, however, all replicates ($n = 2$) were sampled and stored.

Samples from a treatment with annual application of 100 kg N, 15 kg P and 40 kg K ha^{-1} were chosen for DNA analysis. For the Ekebo site (M5), samples were also analysed from plots fertilised each year with: (i) 0 kg N, P and K; (ii) 150 kg N, 0 kg P and 0 kg K ha^{-1}; (iii) 0 kg N, 45 kg P and 120 kg K ha^{-1}; and (iv) 150 kg N, 45 kg P and 120 kg K ha^{-1}. Soils sampled in 2007 were chosen for the first round of analyses to get an indication of P. brassicae DNA concentration. This was followed by analysis of earlier and later samples in the time series showing presence of P. brassicae DNA.

DNA extraction and qPCR

The protocol developed by Wallenhammar et al. (2012) was used and qPCR analysis was performed in four replicates using the 7300 Real Time PCR System (Applied Biosystems, Foster City, United States). The amount of pathogen DNA was quantified using a standard curve generated by including reactions containing different amounts of a plasmid carrying the *P. brassicae* target sequence.

Influence of storage on *P. brassicae* DNA

The samples investigated in this study had been stored dry at room temperature for some years. An important issue was thus possible decomposition of *P. brassicae* DNA during storage. The first analysis using the protocol (Wallenhammar et al. 2012) was performed in 2006

Table 1 Initial characteristics of the soils at the 10 Swedish long-term fertility experiment sites (1957–1966) and pH (aq) measured in 1995 and 2007

Code	Site Name	Coordinates	Org. carbon %	Clay %	P-AL mg 100 g^{-1}	P-HCL mg 100 g^{-1}	K-AL mg 100 g^{-1}	K-HCL mg 100 g^{-1}	pH (aq) 1957	1995	2007
M1	Fjärdingslöv	54°24'N, 13°14'E	1.4	17	3.3	26	4.2	62	7.5	7.1	7.1
M2	Orup	55°49'N, 13°30'E	2.4	13	2.4	53	3.8	47	6.2	6.0	6.1
M4	Örja	55°53'N, 12°52'E	1.1	15	5.9	36	8.0	115	7.2	7.2	6.6
M5	Ugglarp	55°38'N, 13°25'E	1.5	8	4.1	38	4.1	36	6.6	5.7	6.0
M6	Ekebo	55°59'N, 12°52'E	3.1	14	6.7	37	5.4	56	6.8	6.1	6.1
E9	Högåsa	58°30'N, 13°14'E	2.4	10	4.4	33	10.7	43	5.9[1]	6.0	6.7
E10	Vreta Kloster	58°29'N, 13°08'E	2.1	50	6.7	41	19.4	368	6.7[1]	6.8	7.0
R94	Bjertorp	58°14'N, 13°08'E	2.2	30	4.6	38	12.4	242	6.4[1]	6.1	6.5
C7	Kungsängen	59°50'N, 17°40'E	2.1	56	3.7	56	14.0	440	7.1[2]	6.4[3]	6.4[4]
C8	Fors	60°20'N, 17°29'E	2.2	18	10.6	73	9.0	252	7.7[2]	7.5[3]	7.6[4]

[a] Application of lime: 1 t ha^{-1} CaO applied in 1981 and 2 t ha^{-1} CaO in 1996 at sites M2, M5 and M6. 2 t ha^{-1} CaO were applied in 1981 at sites M1 and M4. 3 t ha^{-1} CaO were applied at site R94 in 2000

[b] The experiment started in 1966

[c] The experiment started in 1963

[d] Values from 1993

[e] Values from 2005

and those samples are stored dry in the soil bank at the Swedish University of Agricultural Sciences, Skara. Five of these samples were selected for repeated analyses, in 2012 and 2014.

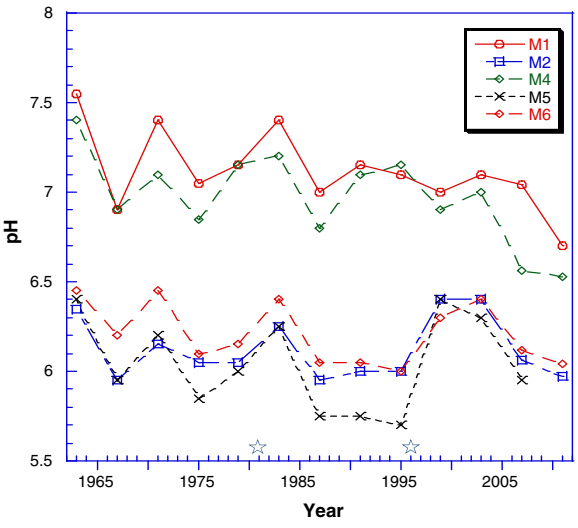

Fig. 1 Changes in soil pH in the Swedish long-term fertility experiments at sites M1–M6. ☆ Application of lime: 1 t ha-1 CaO applied in 1981 and 2 t ha-1 CaO in 1996 at sites M2, M5 and M6. 2 t ha-1 CaO applied in 1981 at sites M1 and M4

Statistical analysis

The influence of the time of storage on the *P. brassicae* DNA concentration was analysed using one-way ANOVA and Fisher LSD test with $P = 0.05$ significance limit. Statistical analysis was made using the STATISTICA 12 software (StatSoft Inc.)

Results

Plasmodiophora brassicae DNA was found in soil sampled in 2007 at four of five experimental sites in the 4-year rotation (Table 2) and at one of five experimental sites in the 6-year rotation (Table 3). The highest amounts of *P. brassicae* DNA were found in 2007 in the 4-year rotation at experimental sites in southern Sweden. In the 6-year rotation, a level of *P. brassicae* DNA exceeding the quantification limit (i.e. >5 fg g^{-1} soil) was observed only at one site, Bjertorp (R 94). Furthermore, when analysing samples from 2013 in the 6-year rotation, *P. brassicae* DNA was detected only at this same experimental site (Table 3).

The analysis of samples from 1971 onwards revealed a change in the level of *P. brassicae* DNA in the 4-year rotation at all experimental sites except Fjärdringslöv

Table 2 *Plasmodiophora brassicae* plasmid DNA (fg g^{-1} soil) and relative standard deviation (%) in archived soil samples collected in the Swedish long-term field fertility experiments (sites M1, M2, M4, M5, M6 and R94) 1971–2011

Year	M1 Fjärdingslöv	M2 Orup	M4 Örja	M5 Ugglarp	M6 Ekebo	R94 Bjertorp[c]
1971	<5	<5	n.d[a]	<5	<5	<5[c]
1975	<5	<5	n.d[a]	n.d[a]	<5	
1979	n.d[a]	<5	<5	<5	5 ± 40	<5[c]
1983	n.d[a]	<5	<5	<5	5 ± 40	
1987	n.d[a]	<5	<5	<5	7 ± 14	<5[c]
1991	n.d[a]	8684 ± 44	<5	372 ± 33	1110 ± 15	<5[c]
1995	n.d[a]	5 ± 6	<5	5 ± 2	5210 ± 4	
1999	<5	38,786 ± 3	9 ± 33	3063 ± 42	14,182 ± 14	20[c] ± 20
2003	<5	7907 ± 6	1200 ± 26	1370 ± 80	19,753 ± 4	
2007	<5	635 ± 48	138 ± 108	338 ± 8	1433 ± 61	252[c] ± 24
2011	<5	80 ± 9	5 ± 40	n.a[b]	37 ± 5	1012 ± 130

[a] *n.d* no detection, [b] *n.a* not analysed; field trial stopped in 2010, [c] Bjertorp 6-year rotation sampled in 1971, 1978, 1985, 1992, 2001, 2007 and 2013

(M1) (Table 2). The increase in *P. brassicae* DNA peaked at four of the experimental sites in the soil samples from 1999 and 2003. In general, there was an increase in the level of *P. brassicae* DNA of more than 1000-fold at the peak level and it differed by approximately 10-fold between sites M2 and M6 and sites M4 and M5. Interestingly, in 1991 the amount of pathogen DNA at sites M2, M5 and M6 was already high (range 372–8684 fg plasmid DNA g^{-1} soil). Similar patterns of change in *P. brassicae* DNA levels were observed in samples from treatments under different fertilisation regimes (Table 4).

At site M6, quantifiable levels of *P. brassicae* DNA were found in the samples from 1979 and at sites M2 and M5 in the samples from 1991. High levels of *P. brassicae* DNA were found in soil samples from 1999 and 2003 at M2, M5 and M6. Moreover, in 2002 low yield was reported at M2 (1450 kg ha^{-1}), M4 (1180 kg ha^{-1}), M5 (1360 kg ha^{-1}) and M6 (1050 kg ha^{-1}), whereas yield at M1 was higher, 2038 kg ha^{-1} (Table 5).

TThe high levels of *P. brassicae* DNA in samples from 2003 were followed by a substantial decrease (75–93 %) at all 4-year rotation sites sampled in 2007 and similar decreases (88–96 %) in the following 4 years to 2011 (Table 2). Similar patterns of change in *P. brassicae* DNA levels were observed in samples from treatments with different fertilisation regimes (Table 4).

To test the effect of mixing dried samples on quantification, the available replicates ($n = 2$) from experimental sites M2 (1991, 1999 and 2003) and M6 (1971, 1975, 1999) were analysed (Table 5). This test of accuracy was performed on two separate occasions for the two plot samples and the bulked samples. At site M2, yield in the two replicate plots was similar despite differences in *P. brassicae* DNA between these replicates (Table 5).

Table 3 Prevalence of *P. brassicae* DNA in soil (DNA fg g^{-1} soil) in the Swedish long-term fertility trials with oilseed crops in the crop rotation. 6th year of rotation, sampled 2007 and 2013

Code	Site Name	Coordinates	*P. brassicae* DNA (fg g^{-1} soil) 2007	2013
E9	Högåsa	58°30'N, 13°14'E	n.d	n.d
E10	Vreta Kloster	58°29'N, 13°08'E	n.d	n.d
R94	Bjertorp	58°14'N, 13°08'E	252 ± 24	1012 ± 130
C7	Kungsängen	59°50'N, 17°40'E	n.d	n.d
C8	Fors[1]	60°20'N, 17°29'E	n.d	n.d

n.d no detection of *P. brassicae* plasmid DNA

Table 4 *Plasmodiophora brassicae* plasmid DNA (fg g^{-1} soil) in archived soil samples collected in the Swedish long-term fertility experiment at Ekebo (site M6), 1987–2011, from treatments with different fertilisation regimes: No addition of NPK; 0 kg N, 45 kg P and 80 kg ha^{-1} $year^{-1}$; 150 kg N ha^{-1} $year^{-1}$, 0 kg P; and 150 kg N, 45 kg P, 80 kg K ha^{-1} $year^{-1}$. Relative standard deviation, ±%

Year	Fertiliser	(kg ha^{-1} $year^{-1}$)		
	N 0 P 0 K 0	N 0 P 45 K 80	N 150 P 0 K 0	N 150 P 45 K 80
1987	<5	<5	10 ± 50	2665 ± 10
1991	n.d.	<5	<5	<5
1995	72 ± 0	216 ± 19	2737 ± 23	7078 ± 7
1999	<5	20,217 ± 35	<5	36,663 ± 11
2003	67,731 ± 10	323 ± 3	33,838 ± 13	21,099 ± 9
2007	33 ± 27	112 ± 17	25 ± 12	m.s[a]
2011	<5	16 ± 25	<5	20 ± 5

[a] missing value

Analysis of the influence of storage time on detected amount of *P. brassicae* DNA in 2012 and 2014 in five samples first analysed in 2006 revealed that the amount had changed significantly in two samples during 6 or 8 years of storage (Table 6).

Discussion

The qPCR method provides new possibilities to analyse the presence of pathogen DNA in archived samples and to re-evaluate observations in long-term field experiments. We examined clubroot disease development over time using a qPCR assay on archived soil samples from two series of crop rotation experiments with brassica crops running for 42 years. The results confirmed the first hypothesis, that *P. brassicae* DNA concentration in soil increases, reaches a peak, and then decreases as a consequence of successful infection of OSR crops, club formation followed by degradation of spores in the soil. The oilseed crops in the 4-year rotation were heavily infected with *P. brassicae*, recorded by field personnel as high number of plants showing clubroot disease symptoms, at two experimental sites (M6 and M2) in 2002. This outbreak of clubroot was accompanied by an increase in *P. brassicae* DNA in soil, followed by a rapid decline in the *P. brassicae* DNA level (Table 2).

The qPCR results also showed that *P. brassicae* inoculum was already present in amounts (5 fg g^{-1}) corresponding to more than 3000 spores per g soil at three of five sites in the 4-year crop rotation in 1991, 12 years (or three Brassica crops) before considerable yield losses were observed in 2002 at sites M2 and M6 were attributed to clubroot (Table 5). This shows the significance of early detection of the pathogen and of analysing the level of *P. brassicae* DNA before deciding on the frequency of brassica crops in a specific field.

At experimental sites M2 and M6, the amount of *P. brassicae* DNA reached a peak in 1999, so severe clubroot infection is likely to have occurred in 1998, after a spring oilseed crop had been grown for the 11th time. The substantial increase in *P. brassicae* DNA was probably a consequence of clubroot occurrence already in 1990. There were considerable yield reductions in 2002 at M6 and M2, but at M2 also in 1998. In 2000, the yield at site M1, with no detection of *P. brassica* DNA, was 2038 kg ha, i.e. 30 % and 48 % higher than at sites M2 and M6, respectively. Clubroot as the cause of yield loss was first confirmed at site M6 in 2002, indicating the problem for growers of detecting clubroot in field crops without systematic assessment. As pointed out in the previous study (Wallenhammar et al. 2012), there is a risk of >10 % crop losses in susceptible crops with *P. brassicae* DNA concentrations in soil ranging from 5 to 200 fg plasmid DNA g^{-1} soil (corresponding to 3000 to 130 000 spores g^{-1} soil). As a consequence of significant yield losses in 2002, spring oilseed rape was replaced in the experimental rotation with an oat crop.

Patchy development of clubroot and the risk of infestation between plots might have affected the results obtained in this study in various ways, since the samples were not handled in the sterile way recommended in Wallenhammar et al. (2012). Patchiness has been observed between plots in the experiment (Table 5). The similar pattern of increase in pathogen DNA at sites M2, M4, M5 and M6 and the similar amount of *P. brassicae* DNA in the plotwise observations support the validity of the results. Results from the analysis before and after bulking of plotwise soil samples (Table 5), together with repeated analyses of dried samples 2006–2014 (Table 6), further confirmed that the observed levels are relevant and in accordance with the third hypothesis, i.e. that the concentration of *P. brassicae* DNA has remained stable enough over time during dry storage, and our observation of an important pattern in the epidemiology of *P. brassicae*. The observed reduction of *P. brassicae* DNA in stored samples was significant in some samples (Table 6) but not considered of sufficient

Table 5 *Plasmodiophora brassicae* plasmid DNA (fg g^{-1} soil) in archived soil samples from the Swedish long-term fertility trials at sites M2 (Orup) and M6 (Ekebo). A 50/50 (% *w*/w) mixture of duplicate soil samples from plots 44 and 58 was compared with individual samples from these plots. The calculated average for the 50/50 (% *w*/w) mix is also shown. In addition, plotwise yield data are presented for site M1 (Fjärdringslöv). OSR = oilseed rape

Site & Year	Plot sample	Results of analysis (fg g^{-1} soil)	Calculated average, (fg g^{-1} soil) for the mix 50/50 (%)	Harvest year	Yield of OSR kg ha^{-1}	Yield OSR kg ha^{-1}
M2 Orup						M1 Fjärd.
1991						
	44	2235		1990	1442	1743
	58	14,921			1517	1796
	Mix 44/58	8684	8578 (99 %)			
1999						
	44	772		1998	1190	2376
	58	88,350			1220	1820
	Mix 44/58	38,786	43,561 (112 %)			
2003						
	44	3534		2002	1434	2067
	58	17,377			1447	2006
	Mix 44/58	7907	10,455 (132 %)			
M6 Ekebo						M1 Fjärd.
1971				1970	1970	2080
	44	<5				
	58	<5				
	Mix 44/58	<5	<5 (–)			
1975				1974	1790	1540
	44	n.d				
	58	<5				
	Mix 44/58	n.d	<5 (–)			
1999						
	44	23,997		1998	1880	2376
	58	16,674			1706	1820
	Mix 44/58	14,182	20,335 (143 %)			
2003						
	44	25,828		2002	1030	2067
	58	15,824			1051	2006
	Mix 44/58	19,753	20,826 (105 %)			

magnitude to affect the general interpretation of the results. A previous report that DNA can be extracted from non-viable microorganisms after 140 years of storage of air-dried soil samples (Hirsch et al. 2013) confirms the conclusions.

The amount of *P. brassicae* DNA declined significantly in the 4 years following the peak, with a reduction of approx. 90–95 % for each consecutive 4-year period. Similarly, Peng et al. (2013) reported a 90 % reduction in spores with a 2-year break. The half-life of resting spores is estimated to be 3.6 years in central Sweden (Wallenhammar 1996) and 4.4 years in central Alberta (Hwang et al. 2013). In the present study, the half-life of *P. brassicae* DNA was approx. 1 year during the 8 years. Despite this rapid decay, the amount (>20.000 fg g^{-1}) observed in soil samples 1 year after the spring oilseed crop was equivalent to 4 million spores g^{-1} soil, so with a reduction of 90 % every 4 years, it would still take 16 years to reach a level of less than 1000 spores g^{-1} soil. The rapid decline in *P. brassicae* DNA raises

Table 6 *Plasmodiophora brassicae* plasmid DNA (fg g^{-1} soil) in archived soil samples collected and analysed in 2006 and in analyses repeated in 2012 and 2014

Year	Soil sample				
	No:92	No:300	No:334	No:96	No:312
2006	4,7[a]	3,9[a]	175[a]	341[a]	1108[a]
2012	4,3[a]	5.0[a]	22[b]	268[a]	936[ab]
2014	3,0[a]	<5	22[b]	282[a]	671[b]

[a] Different letters indicate statistically significant differences

questions about the condition and vitality of the surviving spores and indicates a need for more information about the mechanism causing suppressiveness and decline (Donald and Porter 2009).

Suppression of clubroot development by addition of different nitrogen fertilisers was observed by Nilsson (2014) in growth chamber experiments and was also reported by Dixon (2009b, c). In the present study, however, the pattern observed for changes in *P. brassicae* plasmid DNA was similar in all samples from the treatments at Ekebo (M5), where increasing amounts of N fertiliser were applied (Table 4). This contradicts the second hypothesis, that increasing rate of N decreases clubroot severity.

The results showed that the amount of spores found after a severe outbreak is 100–1000 times higher than that reported to be needed for infection (Diedrichsen et al. 2009; Tsushima et al. 2010). The high levels of *P. brassicae* DNA found in the archived soil samples indicate great potential for transmission of *P. brassicae* inoculum between fields via infected soil carried by farm equipment (Cao et al. 2009; Gossen et al. 2014; Strelkov and Hwang 2014). Wind erosion is another important transmission pathway, as substantial amounts of inoculum are present in wind-blown dust from infested fields (Rennie et al. 2015).

Soil pH and calcium concentration are important characteristics for interpreting the development of clubroot (Webster and Dixon 1991). The soil at site M1, where *P. brassicae* DNA was barely detected, has a sandy loam texture, with increasing clay and calcium carbonate concentration to 100 cm depth and a pH value of 8.0 at 50–100 cm depth (Carlgren and Mattsson 2001). These characteristics most likely supported the disease suppressive ability of that soil. The soil at site M4, where *P. brassicae* DNA increased at a lower rate, is a sandy clay loam with low calcium carbonate concentration throughout the profile, whereas the soil at the most disease-conducive experimental site, M6, lacks calcium carbonate and has a pH value of 5.7 at 50–100 cm depth (Carlgren and Mattsson 2001). The pH at sites M1 and M4 showed less variability than at sites M2, M5 and M6 and, moreover, lime has been applied twice at these sites, compared with once at M1 and M4 (Fig. 1). The properties of soils with a natural content of calcium carbonate need to be further studied. For example, Webster and Dixon (1991) studied the relationships between *P. brassicae*, calcium ions and pH and showed that increased calcium content reduced the number and severity of infections, depending on inoculum pressure. These findings were confirmed and extended by Dixon and Page (1998). Application of lime is regarded as a positive clubroot suppressive measure that should be carried out regularly in oilseed rape production. The correlation between pH value and disease incidence is, however, often found to be weak (Wallenhammar 1996; Strelkov et al. 2007; Gossen et al. 2013) and should probably be evaluated with regard to soil calcium concentration.

In conclusion, this qPCR analysis of stored samples from the Swedish long-term fertility trials revealed a substantial increase in *P. brassicae* DNA in soil during the years preceding destructive *P. brassicae* infection, followed by a rapid decline in subsequent years. The results confirmed the risk of clubroot proliferation associated with short-time rotations of Brassica crops. The low level of *P. brassicae* DNA recorded in the 6-year rotation indicates that a rotation with a period of 5 years between brassica crops is one of the most efficient measures in integrated control of clubroot (Wallenhammar 1999). Since the profitability of oilseed rape cropping is attractive, much shorter rotations are used in practice (Wallenhammar et al. 2014). For example, in southern Sweden a crop rotation with 3 years between brassica crops is common and even shorter intervals have been reported, especially in Canada (Peng et al. 2014). On the Canadian prairies, a break of 3 years in combination with resistant cultivars was suggested by Peng et al. (2014) as a strategy to manage fields infected with clubroot. Our studies in the Swedish long-term fertility trials indicate that a 3-year break between brassica crops is too short and that general recommendations should be replaced by field-specific recommendations based on analysis of *P. brassicae* DNA in soil.

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