BIOLOGICAL SOIL MAPPING - INFESTTION BY *Plasmodiophora brassicae* AND SOIL CHARACTERISTICS

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ABSTRACT

Clubroot, *Plasmodiophora brassicae*, is a soil-borne pathogen that causes severe yield losses in many Brassica crops. The longevity of the spores makes them difficult to eradicate. The only practical way available to control clubroot development in oilseed rape rotations is to reduce the frequency of sensitive cultivars. Infection by the pathogen is favoured by edaphic environments such as acidic pH, low calcium content, poor soil structure, impeded drainage and high soil temperature. These environmental conditions vary within many arable fields, indicating that the prevalence of the pathogen may be patchy. A possible patchy presence in combination with great longevity makes it worthwhile mapping soil infection by *P. brassicae*. This study sampled 22 fields on commercial farms and analysed the soil for the presence of *P. brassicae* DNA using the qPCR technique. The level of infection varied considerably between and within fields in a patchy distribution and showed a low correlation with soil chemical characteristics. Mapping of *P. brassicae* using qPCR can be a useful management tool on farms with a history of intensive Brassica production and strong ambitions to avoid growing susceptible crops in areas of fields with a high risk of clubroot infection.

INTRODUCTION

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae*, is a soil-borne pathogen that results in severe yield losses in many Brassica crops. It is currently causing increasing problems in many Brassica-growing countries. Following infection by *P. brassicae*, large galls (or club-shaped swellings on the roots) are formed, reducing water and nutrient uptake by the plant. Severe infections can result in stunting, yellowing, wilting, premature senescence and, in winter oilseed rape (WOSR), reduced winter hardiness. Significant yield losses (>10%) can be expected when 20% of oilseed rape (OSR) plants are infected. In WOSR under Swedish conditions, the losses can reach 100% when infected plants

do not survive the winter. On a global scale, the losses have been estimated to range from 10 to 25%. The pathogen survives in the soil as resting spores, which have a half-life of about 4 years and remain viable for 18 years (Wallenhammar, 1996). The longevity of the spores makes them difficult to eradicate. The only practical way available in OSR rotations is to reduce the frequency of sensitive cultivars. In Brassica crops with higher economic margins, fertilisation with Calcium cyanamide can reduce the infection and loss (Dixon 2012). Partly resistant cultivars of cabbage are available and WOSR cultivars with some resistance recently became available to Swedish growers. However, the resistance is not complete, galls and spores are formed to some extent and the yield is reduced, particularly at high infection levels. An infection level of a few thousand spores per gram of soil is considered to have the potential to cause significant yield losses. Infection levels of >1 000 000 spores/g soil have been observed to cause 100% infection of OSR, which can reduce yield by 50% or more. This soil-borne pathogen can be transported and spread with soil on machinery or even by water and air. The disease was described by the Romans, was spread by European immigrants during the 19th century to eastern North America and Australia and lately moved into the prairie provinces of Canada, causing considerable problems in OSR production in e.g. Alberta (Strelkov & Hwang, 2014).

Soil infection by the pathogen is favoured by edaphic environments such as acidic pH (<6.8), minimal calcium (Ca) content, nitrogen in the form of ammonia, poor soil structure, impeded drainage (waterlogging) and soil temperatures above +15^{o}C. The fact that these environmental conditions vary within many arable fields indicates that the prevalence of the pathogen may be patchy. (Dixon 2014)

The severity of the clubroot pathogen and longevity of its spores, along with the lack of efficient control measures, make it important for farmers to obtain information on the infection status in a particular field when planning the crop rotation. The conventional method used by crop advisors to confirm and semi-quantify the presence of clubroot in a Brassica crop is to pull up plants and study the roots. However, this is often only done after stunted growth has been observed and soil compaction as a cause has been ruled out.

DNA-based methods that use PCR technology have been developed and used in practice, for example by scientists and food agencies, for over 30 years. Methods have also been developed to estimate the levels of clubroot infection in soils and a protocol to check the level of contamination of farm soils has been commercially available in Sweden for a few years (Wallenhammar et al., 2012; www.eurofins.se). This allows rapid on-site confirmation to be obtained when clubroot is suspected. It also enables a more proactive approach to mapping farms or fields for clubroot. The mapping approach proposed at present includes two steps. The first is to check the level of infection on a general field level and is

based on sampling with approx. 40 bulk sub-samples of topsoil along a W-transect on the field. The second step is geo-referenced point sampling, enabling a map and description of the distribution of clubroot pathogen in the field to be produced. This map can then be used to adjust the crop rotation within the field. For point sampling, 8-10 subsamples within a radius of 3 m are gathered either at each point in the field at which e.g. infection is suspected (low-lying areas, compacted areas, insufficiently drained areas) or systematically in a grid measuring 100 m x100 m, or less depending on crop. The aim is to identify areas where OSR can be grown with a low risk of clubroot infection.

The aim of the present study was to demonstrate the new precision agriculture tool, 'Biological soil mapping of soil-borne pathogens', on a commercial farm.

METHODS AND MATERIAL

At Bjertorp farm in the Skaraborg County, Sweden, 22 fields were sampled, with 40 subsamples taken along a W-transect, in 2012. The samples were dried at room temperature and milled under aseptic conditions in a ball mill and sieved (mesh size 2 mm). The amount of *P. brassicae* DNA (plasmid DNA/g soil) was determined and the number of spores was estimated according to Wallenhammar et al. (2012). Information on the crop rotation in each field on the farm from 1970 to 2012 was supplied by the farmer.

Parts of field 7a ('field A') and 17a ('field B') were point-sampled in 2013. These field parts were chosen because the average level of past infection was high and the clay content varies considerably. The soil samples were analysed for the presence of *P. brassicae* DNA and some chemical characteristics, e.g. pH value and content of boron (B). The clay content had been determined and mapped earlier, after point sampling in a classical chemical soil mapping procedure combined with measurement in 2010 using the Mole instrument (Soil Company, Groningen, the Netherlands).

RESULTS AND DISCUSSION

The average level of club root infection varied considerably between the different fields, ranging from <5 to >700 fg DNA. The latter is equivalent to >140 000 spores/g soil. The lowest and highest levels of DNA were sometimes found in fields situated close to each other (Figure 1).It is important to bear in mind that 5fg DNA/g soil is equal to approx. 1000 spores/g soil and this level is generally considered sufficient to cause crop infection and result in significant yield losses.

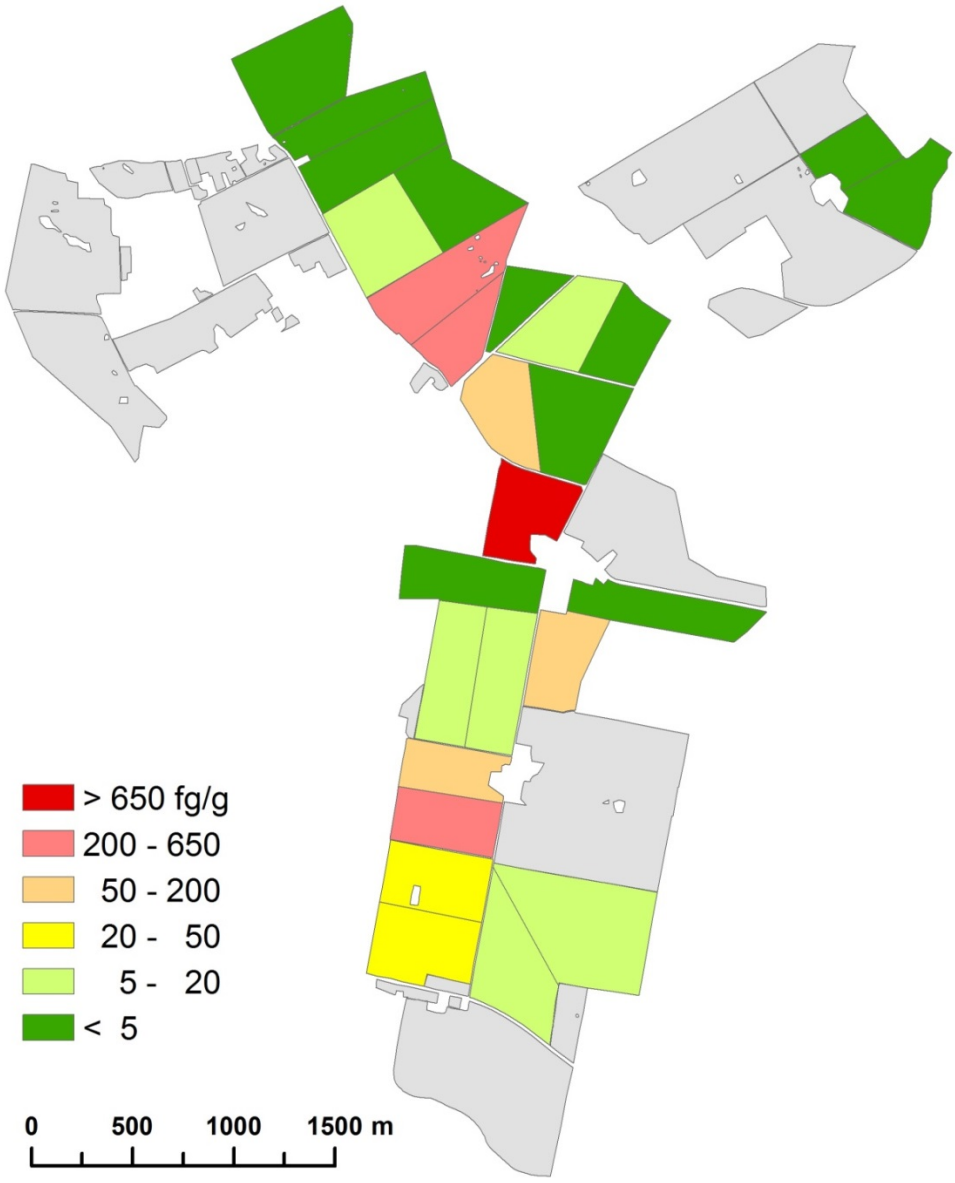

Figure 1. Map showing *Plasmodiophora brassicae* infection (DNA fg/g soil.) in fields of Bjertorp farm, Sweden. The sample analysed for each field was a bulk sample consisting of 40 subsamples.

The spores can survive in the soil for >15 years, which makes it relevant and meaningful to analyse the level of soil infection as part of routine crop management on the farm (Wallenhammar, 1996). The measured amount of DNA of *P. brassicae* indicates high amounts of spores (up to 140 000 spores/g soil) in the pooled 40 subsamples. This either means that one point in the field has 5.6 million spores/g, or that a larger area of the field has high amounts of spores.

High numbers of spores are formed after a year with a successful infection cycle, highlighting the risk of spread of the pathogen through transport of soil on machinery or via air currents (Strelkov & Hwang, 2014). The levels of infection also varied considerably within a short distance according to the point sampling of parts of fields A and B (Figure 2).

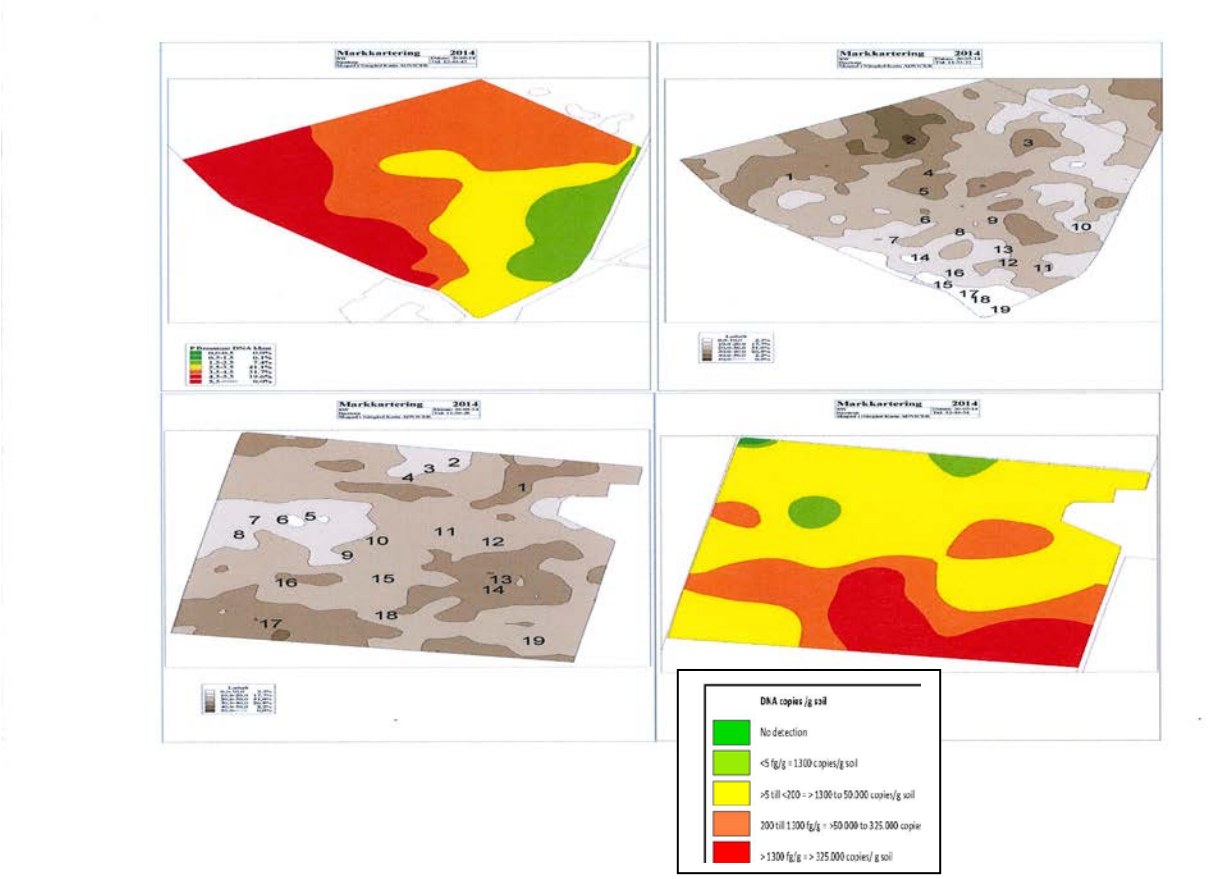

Figure 2. Interpolated amount of *Plasmodiophora brassicae* inoculum in parts of (a) field A and (d) field B. The sampling points are shown in the map of interpolated clay content for (b) field A and (c) field B.

Fields A and B had a relatively high average level of infection in samples taken along the W-transect (459 and 128 fg plasmid DNA/g soil, respectively). These amounts correspond to approx. 300 000 and 80 000 spores/g soil of *P. brassicae*, respectively. In the point samples from parts of the fields, the infection level ranged from <5 to 27 409 fg/g and from <5 to 7300 fg/g soil for field A and B, respectively. The point sampling sites were in parts of the field where the pH value and the clay content varied and where a patchy distribution of *P. brassicae* was suspected (Figure 2 a-d). The observed value of >27 000 fg DNA/g is high, although we have detected >50 000 fg DNA/g soil (unpublished data). The

patchiness of the pathogen incidence indicates that it could be informative to carry out directed point sampling if it is suspected that some soil conditions (e.g. soil water content) have varied during growth of a clubroot-susceptible Brassica crops. This may be especially relevant for cabbage producers and WOSR growers.

The main aim of this study was to describe and map the field and farm incidence of soil infection by the pathogen *P. brassicae*. However, the relationship between level of clubroot infection and soil characteristics is also of interest from a precision agriculture perspective, since the possibility to apply fertiliser and lime at variable rate within fields might have an effect on the development of clubroot infection. A high pH value and, in particular, a high Ca content are reported to inhibit development of clubroot (Dixon, 2014).
In this study, the pH showed low variation in the point-sampled areas (range 6.0 to 6.8). As could be expected, the lowest pH values were observed in samples from the parts of fields A and B with a lower clay content (<10%). The correlations between presence of *P. brassicae* and pH (Figure 3a) and Ca content (data not shown) were both low and not significant, expected since the variations in pH and Ca were low within these fields.

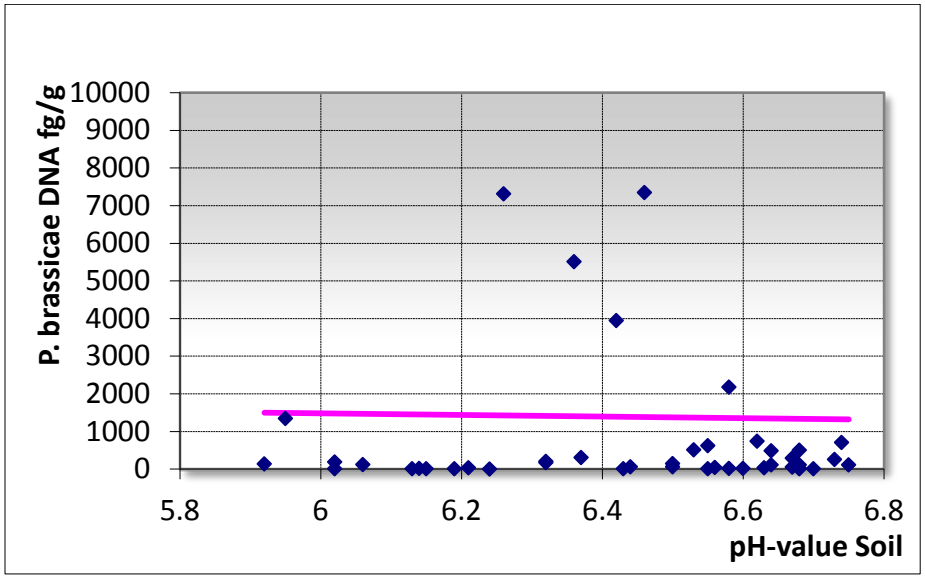

Figure 3. Correlation between soil pH value and *Plasmodiophora brassicae* infection in soils from fields A and B on Bjertorp farm.

Boron is reported to reduce infection of *P. brassicae* and is sometimes added when planting cabbage (Dixon, 2014). The soil B content in fields A and B was approx. 0.3-0.8 mg/kg soil, which is close to the level considered to indicate B deficiency in this type of Swedish soil (0,8 -1,0 mg B/kg; Swedish Board of Agriculture 2014). The lowest B content was observed in parts of the fields with

low clay content and the correlation observed between *P. brassicae* and B content was low (Figure 4).

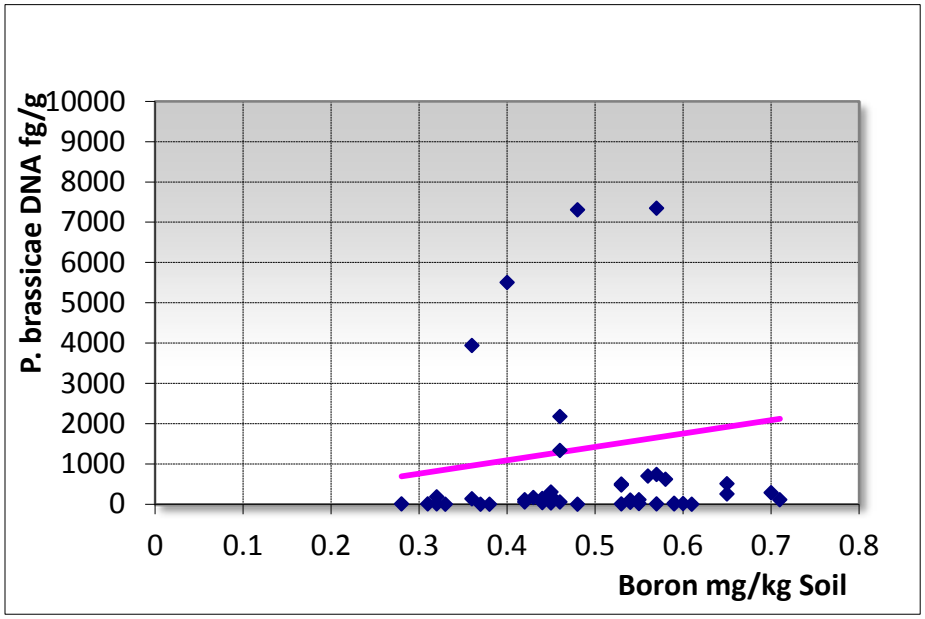

Figure 4. Correlation between content of boron in the soil and infection with *Plasmodiophora brassicae* in soils from fields A and B on Bjertorp farm.

Overall, no strong correlation between presence of clubroot and soil characteristics was observed. The most susceptible sites for *P. brassicae* infection within a field are often considered to be lower-lying parts, which carry a risk of wetter conditions, and areas that have been compacted by heavy machinery, where water is retained and fills up the pores, enabling the germinated spores of *P. brassicae* to swim to host plant roots (Strelkov & Hwang, 2014).

In conclusion, this study showed that soil mapping of *P. brassicae* can be an efficient management tool on farms with a history of intensive Brassica production and with strong ambitions to reduce the risk of growing susceptible crops in areas with a high risk of clubroot infection.

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