Focus Group SOIL-BORNE DISEASES

Mini-paper - *Monitoring of soil-borne pathogens (fungi, protists and nematodes) and soil tests*

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Introduction

Maintenance of food security is a key EU policy driver. The future expectation of crop production is high as domestic production of protein, vegetable oil and energy increases within EU under the confounding pressures of sustainable intensification, reduced and sustainable pesticide application (91/414/EEC; 128/2009/CE), and a changing climate. Soil borne pathogens, of which several are persistent, for example, virus-vector nematodes (Taylor et al., 1994) and sclerotial pathogens, frequently depress yield and reduce crop quality. Although motile within soil many fungal, protists and nematode pathogens are effectively sedentary and rely on external factors to move such as through adherence to crop debris and machinery (Boag, 1985) and via irrigation water or run-off after rainfall events (Baxter et al., 2013).

Monitoring is the cornerstone and one of the key principles of effective integrated pest management (IPM). IPM cannot be implemented effectively without accurate estimates of target fungal and nematode abundance; assessment of presence/absence of natural control e.g. fungal control of cereal cyst nematode (*Heterodera avenae*); or without reliable assessments of crop damage and its effects on yield. Furthermore, long-term baseline data should be available to act as a direct comparator of field collected data, especially important where environmental conditions alter subtlety, for example, a changing climate. Initial implementation of IPM strategies by inexperienced farmers may result in crop damage thus it is important to have sufficient underpinning baseline data available.

The amount and frequency of monitoring required for decision making depends upon the crop and its pathogens. Almost invariably, uniformity of pathogen infestations does not occur, so it is essential to take a representative sample that overcomes the lack of uniformity. Also, it is important to make a representative survey of a field in the least amount of time.

The resolution of monitoring is dependent upon background knowledge of the a) target crop; b) biology of the pathogen; c) main soil factors suitable for pathogen development and d) environmental factors e.g. weather conditions. There are three recognized monitoring methodologies namely: absolute, relative and population indices.

Absolute methods estimate pathogen population densities as a level per unit of cropped area (area of soil used for growing crops) or as a percentage of the sampling units affected. Examples of such methods include direct visual counts per plant or per crop row or per unit of area. The advantages of such methods are their broad range of applicability across pathogen taxa and are less influenced by spatial patterns and changes in pathogen behavior and sampling efficiency.

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Furthermore, data generated by such monitoring typically results in easier prediction of potential crop damage. However, this is offset by the (human) resource intensive nature of the method, for example, a skilled taxonomist may typically only process 3-4 nematode samples per day (minimum 200 nematodes per sample identified to species). However, molecular methods (Donn et al., 2012) have increased sample throughput.

Relative methods estimate pathogen population activity per unit of effort or time but not expressed as units of the cropping area. A distinct advantage of these methods is they yield more data compared to absolute methods given the same effort, are less time-consuming, and are easier to establish and implement. However, the efficiency of these methods are impacted by numerous biological and environmental factors such as pathogen behavior, diurnal activity, weather conditions, the crop habitat being sampled, and variations in the way the methods are deployed; requires more information to relate relative estimates to potential crop damage. As above for nematodes this is constrained by sample throughput.

Population indices estimate frequency of pathogen infestations which indirectly reflect the size of the pathogen population. Examples of this technique include percentage of plants infested or diseased, percentage of defoliation, percentage of damaged fruits, visual ratings of root or foliage injury, and crop quality scoring. Such methods are easy to implement and are not resource intensive. Also there are direct links to crop yield losses and quality issues. However, as indices typically condense a lot of disparate data into a single value they are typically not used as the sole decision making tool for management control and should be incorporated with a decision support system framework. Sensitivity of potato cultivars to tobacco rattle virus vectored by species of free-living nematode belonging to *Paratrichodorus* and *Trichodorus* are assessed using a symptom severity index (Dale and Solomon, 1988).

Soil sampling

Key to monitoring is appropriate sampling for target pathogens. Soil borne pathogens have a heterogeneous, i.e. patchy distributed within fields even at small scale (Nielsen et al., 2010). Thus the choice of sampling technique for field testing is a vital consideration and can be pathogen specific.

Using a diagonal transect across a field is applicable for general surveys. For some pathogens a standardized pattern such as a 'W' is often considered to be ideal. A 'W' pattern consisting of 30-40 subsamples (soil cores) generating a composite sample is suggested for a general survey of the field (Wallenhammar et al., 2012). However for free-living nematodes, a W pattern with samples taken at random points (Marshall et al., 1998) with a minimum of 70 sub-samples is required and ideally one composite sample per hectare. Whilst sampling pattern has been explored for many pathogens, sample interdependence, i.e. the minimum spatial distance required to ensure that any two samples are independent from each other has been poorly studied. Webster and Boag (1992) produced the seminal study on the application of geostatistical analysis such as kriging to ascertain the minimum sample distance required for cyst nematodes. For potato cyst nematode, considerable research has been conducted in the Netherlands to optimize sampling methods (Been and Schomaker, 2000).

Notwithstanding the optimum sampling strategy, ensuring the correct sample size is important to fully exhaust the potential for pathogen detection. For example, the traditional standard soil sampling unit for extraction of nematodes is 200 g (Flegg and Hooper, 1970) which has recently been confirmed in a molecular context (Wiesel et al., 2015). Such a large sample size conflicts with the volumes of soil that DNA extraction kits typically use, e.g. 0.5-10.0 g. Furthermore, DNA extraction directly from soil can be confounded by the presence of inhibitory factors though methods are being developed to overcome this barrier (Brierley et al., 2009).

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Spatial distribution maps

A more precise mapping of the spatial distribution of the pathogen infestation can be an efficient way to employ adequate crop management schemes more site-specific. By use of precision agriculture (PA) techniques and Geographic Information Systems (GIS), it is possible to keep track of and present infestation levels of soi lborne pathogens in a way that is easy to grasp and enables counter measures such as variable–rate application of lime or micro-nutrients.

Fungal and protist pathogens where monitoring is available

Recently, the top 10 fungal pathogens of perceived economic and/or scientific importance were published (Dean *et al*., 2012). Whilst *Botrytis* and *Fusarium* species were present it was surprising that neither *Verticillium* nor *Phytophora* were considered of significant importance given their prevalence across Europe associated with a range of mainstream crops. This result may be an artefact of the survey method used.

Turnip and oilseed rape suffer from a range of severe soil borne pathogens as clubroot (*Plasmodiophora brassicae)*, Sclerotinia stem rot and *Verticillium* wilt. Pea production is limited due to outbreaks of rot root caused by *Aphanomyces eutheiches*, and production of sugar beet encounters problems with soil borne diseases, particularly *Aphanomyces cochloides,* and *Rhizoctonia solani.* Take- all (*Gaumannomyces graminis*) is the most important and the least managed disease of wheat and barley, largely becacuse chemical controls are difficult to implement and no resistant cultivars are available. It is estimated that half of the UK wheat crops are affected and that they suffer yield losses of 5-20 %.

Crop rotation is based on market prices. The possibility to predict and evaluate the infestation level of major critical soil borne pathogens is crucial to in practice optimize crop rotation and efficient use of "break crops". In practice, pea cropping in moist soil conditions have led to problems with *A. eutheiches* and the acreage is reduced in recent years, mean while the acreage of faba bean (*Vicia faba)* has increased. A "new" pathogen (*Phytophthora pisi*) infecting peas and faba beans was recently detected in Sweden. Clubroot caused by the protist *Plasmodiophora brassicae*, is spreading throughout European oil seed rape (OSR) production areas, e g in Germany, Poland, Sweden, UK. The pathogen, previously known as the most economically important disease in vegetable brassica crops, is now rapidly spread in OSR.

Detection and prediction

Bioassay based tests of clubroot and pea rot root were offered in Sweden for many years by SW Seed AB, and thus farmers got used to plan crop production according to the prevalence of soil borne pathogens. As these services were closed down several years ago, there was an explicit need to develop specific and quantitative detection of low levels of plant pathogens in naturally infested soil samples. In the joint program BioSoM (Biological Soil Mapping) run by SLU (the Swedish Agricultural University) in collaboration with several stakeholders a quantitative PCR analyse method was developed for *P. brassicae*. The analyses are offered by two commercial laboratories in Sweden. DNA-based methods for the detection of soil fungal pathogens such as *A. eutheiches*, *A.cochlioides*, *V. dahliae/ V-longisporum* and *Rhizoctonia solani* (e.g., Banno et al., 2011; Debode et al., 2011; Mordadi et al., 2014, Wei et al., 2015, Budge et al., 2009) are described in literature and are in the pipeline for use in practice. One of the limitations of these DNA-based techniques is that commercially-available DNA extraction kits can only process small soil sample volumes (usually 0.25-1.00 g and, exceptionally, up to 10 g of soil). There is debate whether such limited amounts are representative for a whole field.. Therefore, extraction of the fungal propagules from soil should precede DNA extraction and may increase sensitivity of the assays.

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Survey of methods offered:

In France *A. eutheiches* has drawn much attention in recent years. A predictive test is based on a bioassay of field soils is offered by four commercial laboratories in France and Belgium. Interpretation of the results and recommendations are available a **http://www.arvalis-infos.fr/file/galleryelement/pj/5f/41/b9/25/qdn_protea_sud_2012_7-annexes6346188539893610538.pdft**
In Sweden a predictive test based on a bioassay is offered by Findus R&D. Interpretations of the results and recommendations are presented. For canning peas fields exceeding a certain disease index are excluded for contracts.

Analyses for *P. brassciae* are offered in Sweden by Eurofins Sweden Testing AB and Scanbi Diagnostics. Interpretations and recommendations are provided by BioSoM at http://www.slu.se/en/departments/soil-environment/research/precision-agriculture-and-pedometrics/biological-soil-mapping-biosom1/.

International collaboration - Take-all

Collaboration with the Swedish BioSoM research group and the South Australia Research and Development Institute (SARDI) that offers the advisors and grain producers a soil testing service which is based on a series of DNA analyses for the major soilborne fungal pathogens in Australia including management recommendations. The analyses can be used to predict the need of treating the seed with a proper fungicide.

Nematode pathogens

Nematodes are a major constraint on agricultural production. Plant parasitic nematodes have been calculated to consume approximately 10% of the world's global agricultural output, causing economic losses valued at over $125 billion each year (Chitwood, 2003). Large scale monitoring of selected pathogenic nematodes occurred nationally for many European countries mostly during the 1970/1980s: Belgium (de Waele and Coomans, 1983), Bulgaria (Choleva et al., 1985), Finland (Kurppa, 1985), France (Scotto la Massese, 1985), Italy (Roca and Lamberti, 1985), Netherlands (Seinhorst and van Hoof, 1982), Norway (Stoen and Markussen, 1985), Poland (Szczygiel and Bezeski, 1985), Spain (Bello and Arias, 1979), Sweden (Eriksson and Banck, 1985), Switzerland (Klinger and Vallotton, 1985), United Kingdom (Alphey and Boag, 1976; Boag and Neilson, 1996), Yugoslavia (Ivezic, 1985). However as the European taxonomic skillbase declined (Andre et al., 2001), monitoring reduced, primarily to field scale with the notable exception of potato cyst nematode which is subject to regulatory legislation (2007/33/EC) at national level.

Important European nematodes

Numerous nematode pathogens occur across Europe. *Meloidogyne* species have a wide host range but are important pathogens of horticultural crops, vegetables and potato. Potato cyst nematode is a specialist pathogen of potato though one of the two species can be managed by use of resistant or partly-resistant cultivars. Beet cyst nematode a major pathogen of sugar beet is effectively managed through long rotations and adoption of resistant sugar beet varieties. *Longidorus* and *Xiphinema* are within Europe a key pathogen of the viticulture industry though careful selection of variety aids management of the problem. *Paratrichodorus* and *Trichodorus* species have a wide host range and are key pathogens to potato, carrot, parsnip, peas, sugar beet and blueberry. *Pratylenchus* species can be pathogenic to soft fruit, potato, root vegetables and horticultural crops. Although *Ditylenchus dipsaci* (stem nematode), an EPPO A1 listed quarantine pest, is a growing problem in Switzerland, Germany, the Netherlands and Scandinavia, no systematic geographic monitoring for this species is in place. As with all

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quarantine organisms regulatory bodies across Europe will be vigilant for the present of this and other species in imported plant material.

Pre-plant testing and detection

The lack of national scale monitoring during the last thirty years exposes Europe to infestations of new nematode species as a direct consequence of climate change (Neilson and Boag, 1996) and an unknown pathogen burden exacerbated in the short term by removal of approved nematicides (91/414/EEC). The move to field scale monitoring rather than national scale moves the burden of responsibility from policymakers to individual landowners and growers to test soil prior to crop planting. Such pre-plant tests are only available in European member states that have the nematological infrastructure to process large sample numbers and a dissemination pipeline between researcher and farmer. One example which has been in operation for 10+ years occurs in Scotland, where farmers through agronomy companies submit soil samples for nematode testing prior to planting of potato, soft fruit and root vegetables. Results determine whether the selected crop and/or the choice of a resistant cultivar is made, thereafter the nematode data is passed to the supermarket multinationals who make decisions on nematicide application thus determining which branding the product can be sold e.g. "green" label. Other nematode services are provided in the UK mainly by SRUC, SASA and FERA. In the Netherlands commercial laboratories process nematode samples and run advisory services based on the Dutch nematode scheme (Molendijk and Mulder, 1996) and decision support systems like NemaDecide (Been et al., 2007). Following Eurofins acquisition of a Dutch commercial laboratory, nematode analyses will be offered as a new service for Swedish farmers and the former Swedish nematode laboratory at SLU has been acquired by a private company offering analyses.

With the continuing decrease of taxonomic skills in nematology across Europe, DNA based tests are being developed for use by non-specialists to inform the agricultural industry whether a specific nematode pathogen is present. DNA tests take two forms, a presence/absence test using standard PCR methods or a more sophisticated qPCR test that quantifies the presence of the tested pathogen. Such tests are rapid and have high sample throughput compared to classical tests. Europe has been at the forefront of the development of nematode molecular diagnostics with tests developed for *Meloidogyne* (Ziljstra, 2000), *Longidorus* and *Xiphinema* (Hubschen et al., 2004), *Paratrichodorus* and *Trichodorus* (Duarte et al., 2011; Roberts et al., in prep), *Pratylenchus* (Waeyenberge et al., 2000) and PCN (Adams et al., 2009).

Exemplar grower led demand for a monitoring service – Austria

Vegetable growers in the most fertile area of Austria grow peas, beans, sweetcorn, spinach, cabbages, carrots, onions and lettuces. In addition, potatoes, sugar beet, soybeans, corn, winter wheat and durum wheat are components of the rotation.

The main local soil-borne disease problems are *Thielaviopsis* (peas, carrots, beans), *Rhizoctonia* (potatoes, sugar beet, peas), *Sclerotinia* (beans, carrots, onions), *Fusarium* (peas, onions, sweetcorn, winter wheat), *Colletotrichum* (potatoes), *Pythium* (spinach, peas). *Globodera* spp. (carrots, potatoes, sugar beet), *Xanthomonas* (cabbages, beans) and *Rhizomania* (spinach, sugar beet).
Growers would like a monitoring system which provides a more detailed and precise information than current bioassay based tests in order to suppress the above named pathogens and to enhance the natural beneficial root microbiome.
Potential achievable goals are as follows:

- The growers take representative soil samples from their fields and provide seed samples from all crops in the planned rotation (once every 5 - 6 years, depending on the crop intervals) for relevant testing.
- Bioassays shall be made with the soil samples and seeds provided.

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- After a relevant period (perhaps several weeks) DNA fingerprinting analyses of the root-associated microbiome (= rhizodermis-associated) of the young seedlings/plants in the bioassays is conducted.
- Both qualitative and quantitative DNA fingerprinting results has the potential to i) enable a good risk assessment of root-associated pathogen infestations to be provided and ii) be a potential indicator of natural antagonist density and the disease suppressive potential for a given rotation period.

If such information was shared between growers at regional and/or international level, it has the potential to create a "best practice" knowledge base for better control of soil borne disease, highlight risks and indicate positive management opportunities for disease-suppressive soil microbiomes. Such a mechanism may be a good opportunity for a pan-European operational group.

Future

Currently nematology based IPM across Europe, with the exception of the EU PCN directive, is *ad hoc* driven by individual growers. An effective way forward would be to integrate nematode data into decision support systems (DSS) and where possible precision agriculture methodologies. An example of the former where is NemaDecide (Been et al., 2007) is a decision support system for the management of potato cyst nematodes used in the Netherlands. A DSS for beet cyst nematodes (BCN-Watch) is in preparation for Swedish growers. An aspirational goal is to have a pan-European framework that includes biological data on the main nematode pathogens with associated biofumigant (see Biofumigation mini-paper), soils, climatic and crop data to develop a tool for European farmers to manage nematode populations. In order to have effective DSS supporting IPM as a preliminary step for National Action Plans (NAP) implementation in Europe, simple/low cost basic but high quality data could be collected by each Member State/Region in order to establish:

a) actual damage risk for target disease/susceptible crop combinations under defined different agronomic/climatic conditions;
b) the main risk factors and their interactions relevant to target crops.

A simple exemplar data-base structure for this type of data collection is given in Annex 1. Results of such monitoring would allow defined targets to be achieved for each combination of target organism/pesticide/crop/region and to identify metrics that describe the success of such strategies. Resultant data could be subsequently used to estimate the cost of special insurance schemes, such as mutual funds, i.e. farmer-managed no-profit insurance (Regulation EU No 1305/2013) to cover the risks associated with IPM implementation underpinned by the likely incidence of risk factors. Such a scheme has the potential to encourage greater IPM adoption by farmers.

Emerging technologies will be at the forefront of cutting edge IPM for soil-borne pathogens. Unmanned aerial vehicles with hyperspectral imaging platforms are in Germany and the United Kingdom being tested as potential tools to detect crop disease prior to the onset of visual symptoms. Linked to precision agriculture this would be a powerful tool to reduce inputs (pesticides) and maintain crop yields. The possible use of remote sensing techniques is also being discussed, analogous to the Copernicus satellite system which has land use and soil moisture detection capabilities.

As technologies improve, movement of diagnostics from non-specialists in the laboratory to so-called point-of-care diagnostics in the field operated by farmers is a legitimate prospect. Prototype technology development is under consideration in the United Kingdom and would empower landowners and growers to make immediate decisions on pathogen control rather than laboratory based non-specialists.

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Whilst fungal and nematode soil borne pathogens have been the focus of this paper, it is recognized that there are other soil borne pathogens that also deleteriously impact crops across Europe which are outwith the scope of this paper. Thus a pragmatic way forward should be the development of "crop toolboxes" that have IPM solutions for all pathogens of a particular crop. Furthermore, such toolboxes should wherever possible have minimal collateral impact on non-target organisms that are beneficial to the environment such as earthworms and the majority of the soil nematode community. Thus a component of any IPM toolbox should be a demonstration that soil health/quality is being maintained (see soil quality minipaper) which can be maintained by assessing a number of soil bioindicators (Ritz et al., 2009; Donn et al., 2012).
With the lack of resources to maintain national surveys of pathogens across EU member states, data is therefore lacking to act as a baseline comparator to detect shifts in pathogen burden and hence future crop risk. Thus data from long-term experiments or platforms are immensely valuable as they can be used for risk scenario modelling, epidemiological studies and efficacy of IPM measures. Such platforms exist in the United Kingdom (**http://www.hutton.ac.uk/about/facilities/centre-sustainable-cropping**) and Sweden (Jonsson et al., in preparation)
As stricter legislation is implemented on the application of synthetic chemicals to control pathogens, novel control measures need to be explored (see Biofumigation mini-paper). However many social and cultural barriers exist that constrain the use of new methodologies, for example, a lack of belief in product efficacy. Thus incentivisation schemes for farmers, perhaps via CAP, may be required to encourage adoption of holistic IPM measures for soil-borne pathogen control.

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