Mini paper 7
Side effects of pesticide applications

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Brassica crops may be infested and infected by a wide range of pest and pathogen species. For example, as many as 50 species of insect from several orders (Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera) are considered to be *Brassica* pests. Some of these species are specialists in that they colonise plants from the Brassicaceae and close relatives, whilst others, such as *Myzus persicae* are generalists and colonise hosts from a range of plant families. Plant pathogens are *Brassica* specific, with none of the main fungal, viral, or bacterial species listed elsewhere in the report having host species outside cruciferous plants. *Brassica* crops are also invariably challenged by pressure from weed species, which are from a diversity of plant families.

According to EU DIRECTIVE 2009/128/EC, Integrated Pest Management (IPM) '*means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment*'. Whilst farmers and growers endeavour to take a 'holistic' approach to crop production and the need for a holistic approach is also implied by IPM, it is unlikely that many producers and their advisors consider the impact of any one management action on the 'whole crop'. For example, since pyrethroid insecticides are relatively cheap and effective against many species of Lepidoptera, they may be used as a product of first choice for caterpillar control. However, because pyrethroid insecticides also have a broad spectrum of activity, they are likely to kill non-target species that they contact, including natural enemies that may be contributing to the suppression/control of other pests. It is unlikely that many growers take account of this (or have sufficient information to know how to take account of this) when deciding which treatment to apply. In field trials during the last 10 years at Warwick Crop Centre in the UK, the 'side effects' of pyrethroid insecticides have led to larger numbers of *Thrips tabaci*, *Myzus persicae* and *Delia radicum* in pyrethroid-treated plots than in insecticide-free control plots (Rosemary Collier, unpublished data). Similar results were obtained in field experiments done by the Julius Kühn-Institute in Germany, when the insecticide Spinosad was used intensively to control caterpillars. In the Spinosad-treated field plots infestation by the cabbage aphid *Brevicoryne brassicae* was much higher than in the untreated plots (Hommes & Herbst, 2014). Thus, for the control lepidopteran larvae, the use Bt products might be more economic: the higher prices for Bt products might be compensated for by the reduced need for treatments against secondary pests. Other research has shown that the broad spectrum insecticide Spinosad, which is also approved for use on organic crops in some countries, may have adverse effects on non-target and often beneficial species (e.g. Viñuela, *et al.*, 2001).

Whilst the 'side-effects' of insecticides on natural-enemies that are insects are relatively easy to understand, the side-effects on pest insect control of the fungicides used to control plant pathogens may be less tangible. However, many pest insects are subject to natural infection by entomopathogenic fungi, which at certain times of the year may cause epizootics leading to a significant decline or 'crash' in the pest population. Some fungicides developed as plant protection agents certainly affect entomopathogenic fungi (Majchrowicz & Poprawski, 1993), although little is known about their impact in commercial crops. The same may be true of herbicides (Kos & Celar, 2013). The reduction of microbial antagonists of pests by pesticide applications was described for pollen beetle: infestation of pollen beetles with the microsporidian parasite *Nosema meligethi* occurs at endemic levels outside the rapeseed growing areas but is absent from areas where rapeseed is grown (Hokkanen 2008; Yaman 2007). Nosema infection lowers the fecundity and lifespan of pollen beetles, and cause high overwintering mortality. It is assumed that frequent insecticide treatments kill diseased (weakened) individuals more effectively than healthy ones, thus practically 'curing' the population of *Nosema* disease (Hokkanen 2008).

Non-pesticidal methods of control are not exempt from side-effects and, for example, the use of fine mesh netting to exclude pest insects such as *Delia radicum* from crops such as swede are likely also to impact on beneficial species, in ways that as are yet unmeasured. The removal of weeds, whether with herbicides, mechanically or by methods using heat is also likely to impact on natural enemies whether through increased mortality or as a result of a reduction in plant/floral diversity. Obviously the various methods of increasing plant diversity within and around crops (field margins containing flowers, beetle banks, companion planting, undersowing, intercropping) are to a certain extent all methods of 'replacing' the ecosystem services provided by weeds. However, if poorly managed, then at least some of these approaches can have the same adverse competitive effects on crops as weeds. The vision put forward by policy makers is for crop protection to rely less heavily on broad-spectrum pesticides and to replace these with selective methods of control such as host plant resistance, conservation biocontrol, introductions of biocontrol agents and applications of selective pesticides. To get the maximum impact from these approaches, it will be increasingly important to treat crop production/protection in a more holistic way and to be mindful of interactions and side-effects. This requires further and continuing research to develop these approaches and to identify adverse interactions with other management activities. As, for some time to come, pesticides are likely to be some of the key tools in the 'pest management' tool box it will also be important to increase general understanding of how to obtain the greatest benefit from them and avoid adverse side-effects. There is already quite a large amount of information available generally on the impacts of pesticides on non-target species, for example the US EPA ECOTOX database http://cfpub.epa.gov/ecotox/quick_query.htm which is a source for locating single chemical toxicity data for aquatic life, terrestrial plants and wildlife. This database provides a huge source of information from published studies but it does acknowledge limitations. It attempts to be comprehensive, but searches do not locate all relevant literature. In addition, the time lag from conducting a literature search, acquiring the publication and encoding it into the ECOTOX database can be up to, or exceed, six months. For this reason, the database managers also suggest that users conduct searches of the most recent publication year to ensure they capture data that has not been entered into the ECOTOX database. The managers also advise that the original scientific paper should be consulted to ensure an understanding of the context of the data retrieved from the database. Another source of information is provided by the IOBC Pesticide Side Effect Database http://www.iobc-wprs.org/ip_ipm/IOBC_Pesticide_Side_Effect_Database.html. The aim of this database is to compile the effects of plant protection products on beneficial arthropods and the data have been extracted from three major sources:

- IOBC Joint Pesticide Testing Programs (JPTP), organized by the Working group in the 1980s and 1990s. The results of the 1st and 2nd testing programme, where the methods used were not totally fully developed according the IOBC standards, have not been retained.

- IOBC Bulletin, proceedings of the working group meetings, from the end of the JPTP till 2008.
- DAR (Draft Assessment Report) - Public version, edited by the different rapporteur member state in the context of the registration process of the active substance at the European Level, available on the EFSA website.

Each data entry is linked to a reference that can be consulted for further details. Again the database has limitations in that the interpretation of the results, which is closely dependent on the test methods used, the formulation and the doses assessed, the test organisms used and the context of use of the product, are left to the user. With both databases there is quite a long way to go to make this information meaningful to and useable by farmers and growers.

Integrated Pest Management and the use of introduced biocontrol agents is much more highly-developed for crops grown in greenhouses (e.g. tomato and cucumber) than for field vegetable crops. This has happened partly because of the extreme effects of pesticide resistance in protected environments and because of the need to use insect pollinators in some cases. In addition, it has been feasible because of the high value of these crops per unit area grown, which means that growers are able to spend a relatively high amount on crop protection. Because of this considerable need to use biocontrol agents and the economic feasibility of doing so, much more research has been focused on side-effects and interactions, both between pesticides (when used as a last resort) and biocontrol agents or between biocontrol agents. There is for example, good information/databases on the internet offered by the producers of beneficials e.g. http://side-effects.koppert.nl or http://sideeffect.biobest.be. The greenhouse 'system' is relatively simple, in that apart from a small number of pest species and introduced organisms, biodiversity is low, so there is relatively little complexity in the interactions and side-effects when compared with crops grown outdoors on a field scale. The 'climate' within greenhouses is also 'stable'. Therefore, the development of analgous IPM strategies for *Brassica* crops presents a significantly greater challenge to researchers, advisors and growers alike.

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Mini-Paper 8

Clubroot in *Brassica*

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Clubroot is a very serious disease of Brassicaceae species worldwide, affecting especially *B. oleracea* (cabbage), *B. napus* (oilseed rape) and *B. rapa* (turnip). Infected plants present roots with bulbous masses of golf-ball-size galls thus disturbing water and mineral plant nutrition. Plants with clubbed roots show wilting, stunting, yellowing and premature senescence. The disease impacts quality as well as yield in the different *Brassica* species. In some cases, the field can be entirely destroyed. This disease is caused by the obligate biotrophic protist *Plasmodiophora brassicae* that can survive in soil as resting spores up to 18 years or more following an infected crop. Clubroot development is favoured by high soil mixture, soil warm temperatures (18°C-25°C) and low soil pH (<7).

The life cycle of the soil-borne *P. brassicae* consists of two phases. In the primary asymptomatic phase germinated resting spores infect the root hairs. The secondary phase occurs in the cortex and the stele of the hypocotyl and roots of infected plants. This secondary phase is associated with hyperplasia (uncontrolled cell division) and hypertrophy (abnormal cell enlargement) of plant host cells, resulting in the formation of root galls leading to development of clubs that obstruct nutrient and water transport (Kageyama & Asano 2009).

Detection of the pathogen is complex. The pathogen cannot be cultured in the laboratory, and traditional testing of soil for *P. brassicae* has been based on plant bioassays (soil baiting). Although PCR-based tests have now been developed to detect the pathogen in soil, results can be inconsistent due to effects of soil type on the detection and quantification methods. *P. brassicae* populations show high diversity but few data are available concerning inter- and intra-genetic variation and their distribution in Europe (and elsewhere).

The International Clubroot Working Group (ICWG) organises frequent meetings, usually attached to major plant pathology conferences and events, and attracts clubroot researchers from around the world, covering host resistance, pathogen variation, diagnostics and disease management. Abstracts are published. In Europe, a formal set of differential lines for pathotyping is recognised (the ECD series). Another set is currently used in France by major experts: the set of Somé *et al.*. Standardised test methods are defined. Results obtained are collated by asking researchers to submit any outcomes of tests carried out using ECD seed provided by the Warwick Gene Bank (UK). However, such tests are sporadic and not part of a coordinated survey. Currently, there is some pathotype survey work in France, Poland and Czechoslovakia. In France, a recent survey carried out by GEVES, CETIOM, INRA, UCATA and breeders (Limagrain, Serasem, Syngenta and NPZ) in 70 locations has shown the presence of 6 pathotypes (P1 to P6) over the 8 described. Pathotypes P1 to P3 were the most prevalent and a high proportion of pathotypes overcoming the resistance of the reference variety ('Mendel') was observed. In this study, a protocol was set up to study resistance of oilseed rape varieties to these pathotypes for variety registration purposes (Orgeur *et al.*, 2015). To provide more information on the population dynamics of *P. brassicae,* an ongoing European survey is proposed.

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Potentially, this could be carried out in conjunction with the ICWG, and could build on the experiences of other survey initiatives such as those for potato blight (www.euroblight.org) or the cereal rusts (www.wheatrust.org) where genetic variation (e.g. by SSR profiling) and/or pathotyping is carried out. The results would provide insights on genetic structuring of pathogen populations at the international, national and regional levels. Euroblight is supported by agrochemical companies and potato breeders and indicates the importance of the results for IPM strategies. Wheat rust studies are supported by national governments and international funding (Borlaug Rust Initiative), emphasising the importance of detecting new pathotype emergence for the security of cereal cultivars. In the case of clubroot, which affects both high value vegetable brassicas and major arable crops as oilseed rape, a combination of plant breeder and EU funding might be appropriate. By characterising and understanding the dynamics of clubroot pathotypes, improved deployment of available resistances could be achieved.

Genetic resistance to clubroot is a major issue for integrated management of the disease (Diederichsen *et al.*, 2009). Several sources of resistance, major genes and Quantitative Trait Loci have been described in several *Brassica* crops, mainly in *B. napus, B. oleracea and B. rapa,* revealing both isolate-specific and isolate-non-specific resistances (Manzanares-Dauleux *et al.*, 2000; Diederichsen *et al.*, 2009). However, whatever the *Brassica* species, only very few commercial clubroot resistant varieties are currently available.

In the UK, France and Germany, clubroot has become a significant problem in oilseed rape production (Burnett *et al.*, 2013; Jestin and Orgeur, 2014). The first resistant cultivars (e.g. Mendel, Mendelson, and, later Cracker, with the same resistance source) were effective in reducing the disease, but resistance-breaking strains started to predominate in fields where the cultivars had been used more extensively, rendering the resistance much less useful. Thus, resistance associated with the Mendel variety was overcome in different locations in France (Jestin and Orgeur, 2014; Orgeur et al., 2015). Other resistance sources are being introduced into oilseed breeding programmes, but the most effective deployment of different genetic resistances will depend on a thorough understanding of pathogen population dynamics at the field level. Some guidelines have been developed in the UK to combat clubroot in oilseed rape fields and maximise the benefits of currently available resistance (e.g. HGCA Topic Sheet No 110, from www.hgca.com). Growers are advised not to use resistant cultivars unless a field risk exists, and to avoid the build up of clubroot by delaying sowing if possible, keeping the pH above 7, and extending oilseed rape rotations to at least 1 year in 3, and preferably 1 year in 5.

Since 2012, in France, farmers are encouraged to complete a clubroot survey on the web in order to monitor the status and the spread of the disease (see map and reporting system on www.cetiom.fr). Major agronomic (choice of variety, cultural practice) and prophylaxis recommendations are also given, including advice about how to perform a bioassay to detect clubroot in the field (www.cetiom.fr/colza/cultiver-du-colza/maladie/hernie).

Lateral flow devices are being developed in the UK, primarily targeted at the high value brassica vegetable production sectors, to indicate field risk. The project, which is ongoing (see www.hdc.org.uk, Project No CP099a, Validation of the Clubroot lateral flow in UK commercial *Brassica* cropping systems) aims to provide growers with an easy to use inexpensive device to judge risk before land rental agreements are made, or to indicate the need for liming to reduce risk if field choice is limited.

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Improved and less costly diagnostic techniques are thus aiding the potential for integrated disease management, but options for deploying genetic resistance are still limited, and agrochemical options are currently not available in Europe.

Biocontrol products based on beneficial microorganisms seem to be at least partially effective under controlled conditions but they need to be optimized, especially at formulation level to be useable at field level. Their use for vegetable *Brassica* plantlets before transfer to the field may be an interesting approach for the near future.

Liming remains a relatively costly option, especially for the rapeseed crop, though in the UK "Limex", a by-product of sugar beet processing, is frequently used on arable soils. Soil steaming may also used by vegetable brassica growers but is very expensive.

Clubroot is an important disease in all European regions and in all vegetable and arable *Brassica* crops. This is a good example of a *Brassica* sanitary issue which should be handled at the European level, with coordination of research and development of alternative solutions with all *Brassica* crop experts.

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