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Relevance of verticillium wilt (*Verticillium longisporum*) in winter oilseed rape in the UK

by

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1.0 Abstract

This review was commissioned in response to the first confirmed cases of verticillium wilt in oilseed rape in the UK in 2007. The fungal pathogen involved, *Verticillium longisporum*, has been characterised by molecular and conventional methods and is distinct from the two common wilt pathogens *V. dahliae* and *V. albo-atrum*. It has larger spores, a greater DNA content in the nucleus and high specificity for *Brassica* species compared with *V. dahliae*. It is an important disease in oilseed rape in other European countries, particularly Sweden and Germany, and also affects vegetable crops in USA and Japan.

The disease cycle involves soil-borne microsclerotia that germinate to produce hyphae that invade root epidermal cells and progress across the root cortex and colonise the vascular tissue (xylem). The fungus spreads up the plant in the xylem in the spring, but symptoms only appear in the crop near maturity when numerous black microsclerotia are produced outside the vascular tissue. Symptoms cause premature ripening with yield losses up to 50% in Europe, though damage is affected by weather and varies from year to year. Verticillium wilt appears to be established and caused some crop loss in southern and central England. Its incidence ranged from 1-90% plants affected in fields monitored in 2007 and 2008.

There are some differences in susceptibility to verticillium wilt between winter oilseed rape cultivars, but levels of resistance in oilseed rape are limited and insufficient to prevent damaging attacks. New sources of resistance have identified in various other *Brassica* species, which, with further breeding work, could produce oilseed varieties with acceptable levels of resistance in future. There are no fungicide treatments available at present. Microsclerotia are thought to be capable of long term survival in soil (10-15 years), but the number of viable microsclerotia is likely to decline with time. Longer rotations with at least three years between brassica crops appear to be the most practical control strategy at present.

Priority should be given to: (i) establishing the occurrence of *V. longisporum* in oilseed rape in UK, (ii) developing soil tests to quantify inoculum, (iii) research to assist development of resistant varieties, (iv) identifying the conditions that favour infection and yield loss, and (v) determining the rate of decline in soil between susceptible crops, in order to inform rotation planning. Increased awareness of this new disease threat is required so that oilseed rape management strategies can be adjusted to safeguard future cropping.

2.0 Review

2.1 Introduction

Symptoms typical of verticillium wilt were apparent in some crops of winter oilseed rape in 2007. The disease is caused by the fungal pathogen *Verticillium longisporum* and is a significant problem in Europe, notably in Sweden, Germany, France and Poland. There have been several unconfirmed reports of the disease in recent years. The presence of this pathogen was confirmed for the first time in the UK by culturing from affected plants and by molecular diagnostic methods. Verticillium wilt poses a new threat to oilseed rape crops. The pathogen was first described as a new species in 1997 by Heale and co-workers at Kings College London, (Karapapa *et al.*, 1997). It is distinct morphologically from *Verticillium dahliae*, and has a more restricted host range. There is an established European literature on the pathogen and general guidance on key factors affecting its severity. Yield loss may be up to 50% in Sweden, but losses are known to be variable from year to year. The most serious problems are associated with short rotations. Few options are available to control the disease with an absence of resistant varieties and fungicides at present. There are long term consequences for oilseed rape cropping and yield with the appearance of this soil-borne pathogen in the UK. Management strategies are urgently required to minimise its effects. With this threat in mind, this project was instigated combining a literature review with further crop observations.

2.2 Objectives

The overall aim was to review the occurrence and importance of Verticillium wilt in UK oilseed rape crops and identify potential strategies to manage the disease.

Specific areas considered include:

1. Diagnostics for confirmation of plant infection and for quantification of soil-borne inoculum.
2. Cultivar resistance and tolerance – evaluation of existing and novel material under glasshouse and field conditions
3. Disease and yield loss relationships under UK conditions
4. Management strategies – to include effects of rotation, date of sowing and soil treatments
5. Novel approaches

There is a very extensive literature on *Verticillium* species and *Verticillium dahliae* in particular (Pegg and Brady 2002, Tjamos *et al.*, 2002). The genus is an important and variable group containing both plant and insect pathogens. The *Verticillium* stage is an asexual form characterised by spore-bearing conidiophores bearing several groups or whorls of phialides (verticils) at intervals. The phialides produce spherical heads of spores (conidia) in mucilage at their tips and these are termed 'wet spores'. Some species have a recognised sexual stage and these include *Nectria* species (Hypocreales, Ascomycotinia). The major plant pathogens are *V. dahliae* and *V. albo-atrum* and these have been in a section 'Nigrescentia'. Molecular analyses are being used to understand the relationships between *Verticillium* species and other genera (Pramateftaki and Typas, 2000; Tjamos *et al.*, 2002).

V. dahliae is a major pathogen world-wide with a very wide host range. It is important on cotton, tomato, aubergine, peppers, strawberry, potato, cucurbits, mint, woody ornamentals and fruit trees. *V. albo-atrum* has a less widespread distribution and generally has a lower maximum temperature limit than *V. dahliae*. *V. albo-atrum* is important on hops, tomato and potato (Heale, 2000). A third species, *V. tricorpus* causes mild symptoms on tomato and cotton. There is one necrotrophic species *V. theobromae*, that causes cigar-end rot of banana. It is not a vascular wilt disease. In addition, *V. fungicola* and *V. psaliotae* cause dry bubble, an important disease of commercial mushrooms.

The variation within and between species has been examined in some detail and is continuing (Collins *et al.*, 2005, Pegg and Brady, 2002, Tjamos *et al.*, 2002). Vegetative compatibility groups (VGC) have been used to examine variation within species, but these VGCs can now be subdivided into groups using molecular methods. The lucerne pathotype of *V. albo-atrum*, for example, is now recognised as distinct clonal population across Europe and the USA (Collins *et al.*, 2005, Heale, 2000).

Whilst much of the literature may contribute useful guidance for understanding and managing *V. longisporum*, the results from previous studies may well have to be adapted or modified for use in oilseed rape and other crops under UK conditions. This review concentrates on the literature relating to *V. longisporum*.

2.3 The pathogen

The nomenclature and taxonomy of *Verticillium* species are still subjects of active research and debate. In this review, the pathogen is referred to as *Verticillium longisporum*. This name was first proposed by Karapapa *et al.* (1997). Prior to this (and sometimes after 1997), the literature uses the names *Verticillium dahliae* or *Verticillium dahliae* var *longisporum* for isolates that are undoubtedly pathogenic to oilseed rape. It should be noted, however, that *Verticillium dahliae* (strict sense) also occurs in oilseed rape roots, but may cause little damage.

The main features of *Verticillium* species affecting oilseed rape and other crops in the UK are summarised in Table 1. These species differ morphologically and produce various types of resting bodies: microsclerotia, dark resting mycelium or chlamydospores.

Ordinary (short-spored or haploid) *Verticillium dahliae* causes wilt diseases in many crops and in many countries, including the UK, but they rarely affect cruciferous hosts. *V. dahliae* is an important pathogen in strawberries, potatoes, linseed and various trees and ornamental species. Isolates found in the upper stems of oilseed rape are largely restricted to cruciferous host plants and are very different from isolates from non-cruciferous hosts. *V. longisporum* isolates are amphihaploid (almost diploid) interspecific hybrids, with haploid *V. dahliae* as one of the parents (Clewes *et al.*, 2008). The important point is that in nature they are confined to cruciferous hosts. There are reports that other arable crops including cereals and some weed species may potentially be contributing to the survival and perhaps proliferation of *V. longisporum* (Johansson *et al.*, 2006, Zeise and von Tiedemann, 2002). However, the significance of this in the UK is not known.

The initial identification of *V. longisporum* (Karapapa *et al.*, 1997) was based on a study of *Verticillium* species from a range of plant hosts and geographic locations. They examined the first isolate named *V. dahliae* var *longisporum* that originated from horseradish (*Armoracia rusticana*) in North America (Stark, 1961) and an old isolate from Brussels sprouts (*Brassica oleracea* var *gemmifera*) in the Evesham area (Isaac, 1957). Oilseed rape isolates were mainly from Germany and France, but single isolates from Sweden and Poland were also examined. Two isolates from Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) originated from Japan. *V. dahliae* and *V.*

albo-atrum isolates from a range of hosts and countries were also included in the comparison.

There were several features that distinguished *V. longisporum* from other *Verticillium* species. *V. longisporum* was highly pathogenic to oilseed rape causing stunting and chlorosis within 15 days of inoculation of seedlings and death of plants in a month. *V. dahliae* isolates were not pathogenic to oilseed rape. *V. longisporum* isolates could be distinguished morphologically from those of *V. dahliae* by their elongate, irregularly-shaped microsclerotia compared with more compact, almost spherical microsclerotia of *V. dahliae* (Fig. 1 and Fig 2). The conidia (spores) of *V. longisporum* were longer (mean 7.9 μm) than those of *V. dahliae* (mean 4.4 μm).

The conidiophores of *V. longisporum* have mainly 3 phialides per node compared with 4-5 phialides per node on short conidiophores for *V. dahliae*. Staining of the nuclei indicated that *V. longisporum* was near diploid whilst *V. albo-atrum* and *V. dahliae* were both haploid. *V. dahliae* and *V. albo-atrum* produced extracellular polyphenol oxidase whereas *V. longisporum* did not. The inability to produce nitrate nonutilising (*nit*) mutants in *V. longisporum* has also been used as another feature to separate it from *V. dahliae* (Steventon *et al.*, 2002)

Three out of 21 PCT primers tested gave useful separation of the various *Verticillium* isolates using RAPDs (random amplified polymorphic DNA) (Karapapa *et al.*, 1997). A total of 37 different bands were identified for this selection of isolates. A small number of 'recombinant' isolates were examined; two produced spherical rather than elongate microsclerotia and two others produced both types of microsclerotia. The RAPD analysis supported previous work with RFLP (restriction fragment length polymorphism) polymorphisms on *V. dahliae*, *V. albo-atrum* and *V. longisporum* (Okloi *et al.*, 1994).

Three distinct groups were identified within these isolate studies: *V. dahliae*, *V. longisporum* and *V. albo-atrum*. The recombinant isolates formed a small group between *V. dahliae* and *V. longisporum*. The original Brussels sprouts isolate was unusual in having small spores and being weakly pathogenic to oilseed rape. As it was near diploid and closely related to *V. longisporum* and was probably a hybrid derived from *V. longisporum* that had lost some of its characteristics, including spore size and pathogenicity. Short-spored isolates of *V. longisporum* from France have been characterised, but no direct comparison was made with the UK short-spored isolate

(Steventon *et al.*, 2002a). Recent molecular studies have provided direct evidence that *V. longisporum* is a hybrid derived from *V. dahliae* and an unknown species (Clewes *et al.*, 2008).

Molecular studies have divided *V. longisporum* isolates into several groups, but one of these types is much more common and virtually all isolates from oilseed rape are of this type. Preliminary investigations of the first isolates from oilseed rape in the UK suggest that they resemble those found elsewhere in Europe (D Barbara, Warwick HRI, pers. comm.). Whether the short-spored type found on Brussels sprouts in the 1950s is still present in the UK is not known.

Primers were used to amplify part of the β -tubulin gene and non-coding region between two closely spaced 5S rRNA genes (IGR – an intergenic region). The presence of two sequence types in the nuclear genome of long-spored isolates compared with single sequences for haploid isolates was indicative of a hybrid species. (Clewes *et al.*, 2008 Karapapa *et al.*, 1997)

PCR primers have been identified that allow *V. longisporum* to be distinguished from other *Verticillium* species (Johansson *et al.*, 2006). A 340 base pair (bp) fragment was used by Steventon *et al.*, (2002a).

Nuclear SSU-rRNA (small subunit ribosomal RNA) primers have been used to amplify a 2.5 kb fragment in *V. longisporum* and a 1.65 kb fragment in both *V. dahliae* and *V. tricorpus* (Johansson *et al.*, 2002). Sequencing of the SSU-rRNA gene in isolates of various *Verticillium* species and *V. longisporum* from wild radish in Japan and oilseed rape in Germany identified a 839-bp (base pair) intron that was specific to *V. longisporum* (Karapapa and Typas, 2004). Specific primers for *V. tricorpus* are also available (Moukhamedor *et al.*, 1994).

Classification of isolates has been examined using RAPDs (Karapapa *et al.*, 1997), AFLP (amplification fragment length polymorphism (Clewes *et al.*, 2008)), RFLP (Restriction fragment length polymorphism) (Steventon *et al.*, 2002a) as well as by morphological and biochemical features (Karapapa *et al.*, 1997, Zeise and von Tiedemann, 2001). With an established literature on molecular variation and published primers, the development of PCR diagnostics tests in the UK should be relatively straightforward.

Under UK conditions, other species of *Verticillium* may be encountered in soil samples (Table 1). These include *V. nigrescens* that appears to be more prevalent in short rotations of oilseed rape (Mills *et al.*, 2009), *V. nubilum* and *V. tricorpus*. These species have characterised and classified using molecular techniques (e.g. Typas *et al.*, 1992). These may be weak pathogens on some crops and require further study to define their variation and pathogenicity.

Table 1. Major features of plant pathogenic *Verticillium* species in the UK

Species	Microsclerotia	Conidia*	DNA	Host range
<i>V. longisporum</i>	Elongate, irregular shape (50-70 µm)	Long spores ≥ 7µm (5-12.5 x1.66-3.4 µm)	Near diploid	Crucifers
<i>V. dahliae</i>	Spherical, (15-50 µm) compact	Short spores ≥ 5.5 µm (2.5-8 x 1.4 -3.2 µm)	Haploid	Very wide in broad-leaved crops and plants
<i>V. albo-atrum</i>	None – has dark resting mycelium	3.5-10.5 x 2-4 µm	Haploid	Wide especially hops, lucerne, Solanaceous crops
<i>V. tricorpus</i>	Microsclerotia elongated to spherical (60-85 µm), dark resting mycelium. Chlamydo spores 7.5-11 µm	3.5-10 x 1.5-3.5 µm	Haploid	Cotton, tomato
<i>V. nubilum</i>	Chlamydo spores 8.5-17µm Single or in chains of 2-6	4-10 x 2.5-3.5 µm	Haploid	Weak pathogen e.g. coiled sprout of potato
<i>V. nigrescens</i> (<i>Gibellulopsis</i>)	Chlamydo spores usually single 5.5-8 µm	4-8.5 x 1.5-2.5 µm	Haploid	Weak pathogen or saprophyte

* Spore sizes from CMI Descriptions

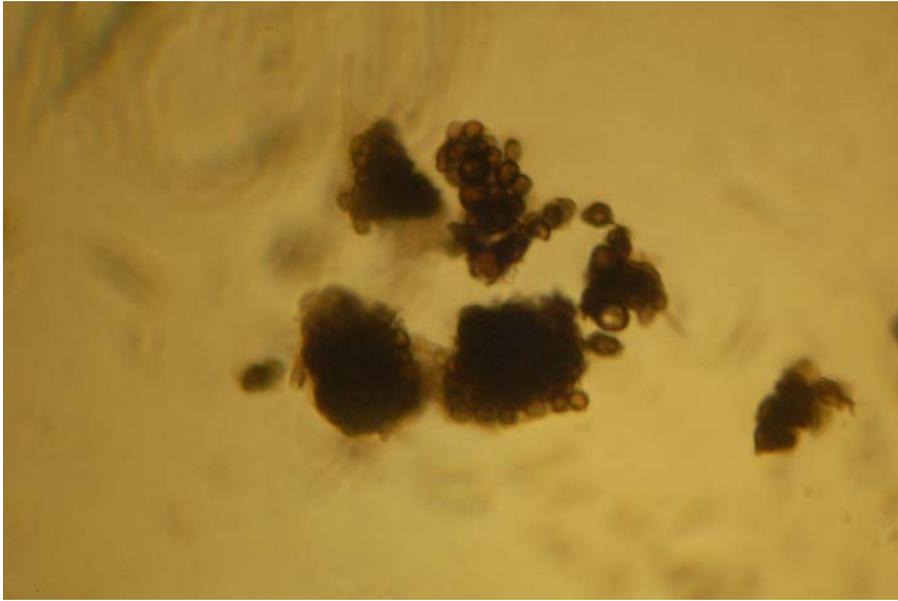


Fig. 1 Microsclerotia of *Verticillium dahliae* – note the globose shape.



Fig.2 Microsclerotia of *Verticillium longisporum* are more elongate than those of *V. dahliae*

2.4 Occurrence

V. longisporum has been reported as an important disease from Sweden since 1960 (Svensson and Lerenius, 1987). It is common in some oilseed rape growing areas in southern Sweden such as Skåne and western parts of Östergötland where problems are associated with short rotations or continuous cropping in the 1950's (Anon., 2006). It is also present in oilseed rape in Germany (Dunker *et al.*, 2008), France, Poland, southern Russia and Ukraine (Karapapa *et al.*, 1997). In Germany, verticillium wilt has increased rapidly since the 1980's (Dunker *et al.*, 2008). In vegetables, *V. longisporum* has been confirmed in chinese cabbage (*Brassica rapa* ssp. *pekinensis*) from Japan and cauliflower from California (Subbarao *et al.*, 1995). The isolates from cauliflower were found to be weakly virulent on Brussels sprouts and broccoli (Subbarao *et al.*, 1995).

Cases of verticillium wilt in Brussels sprouts in the Evesham area in 1956 and 1957 (Isaac, 1957) also involved *V. longisporum* (Karapapa *et al.*, 1997). However, the isolate examined was short spored rather than long spored and not strongly pathogenic to oilseed rape. The pathogen involved in the UK outbreak in Brussels sprouts is therefore quite distinct from those involved in oilseed rape crops in 2007 and 2008 (D. Barberra, Warwick HRI, pers. comm.).

Isolates from horseradish (*Armoracia rusticana*) from Germany and USA have also been classified as long-spored isolates of *V. longisporum* (Clewes *et al.*, 2008). They have been assigned to a separate subgroup by some authorities (Collins *et al.*, 2003).

Three isolates from oilseed rape in France examined by Steventon *et al.*, (2002) differed from German and Swedish isolates in having less DNA, short conidia and polyphenol oxidase activity. They also caused less disease when inoculated on to oilseed rape. They formed a distinct group in RFLP analyses, but were clearly more closely related to other isolates from oilseed rape than to *V. albo-atrum* or non-Brassica *V. dahliae* isolates (Steventon *et al.*, 2002).

2.5 Symptoms

It is difficult to diagnose verticillium wilt in the field until seeds start to ripen. Early symptoms that may appear at flowering include chlorosis of branches and leaves, or yellowing of one side of the leaf (Johansson, 2006). The last is not a reliable feature. If several leaves on the same plant were affected by one-sided yellowing, this would be more convincing than cases where only a single leaf is affected. Despite the name verticillium wilt, distinctive wilting symptoms are not usually seen. This may be due to the water-conducting (xylem vessels) being only partially colonised by the pathogen (Eynck *et al.*, 2007).

A vertical yellow or brown band extending up one side of the stem and into the branches is usually the most reliable symptom. This occurs whilst stems are still green and gradually darkens before surface layers decay or split to reveal the underlying grey microsclerotia (Fig. 3). The extent of the lesion can be explored by scraping away the surface of the affected band from soil level to the upper branches. This exposes the grey discolouration where the pathogen is present in the vascular tissue. Often only a small proportion of the stem circumference is affected so the vertical band and number of small branches affected is quite small. In some plants however, the whole circumference is affected and the whole plant ripens prematurely or dies. The extent of vascular colonisation can also be seen in transverse sections through the crown of the plant. There is formation of dark brown phenol-pectin deposits in the vascular system as a protective host reaction to invasion by the pathogen. This discolouration also occurs in other plant species affected by their own verticillium wilts, but it is very difficult to recognise early in oilseed rape (Johansson, 2006).

As the plant ripens and stems turn from green to yellow and brown, the verticillium symptoms become grey as microsclerotia are formed outside of the vascular tissue and in the pith. The microsclerotia are also formed within the roots where the affected tissues are grey in colour. Verticillium wilt is much easier to diagnose if phoma stem canker and other diseases are absent. Plants with verticillium wilt often have stem canker or phoma stem lesions present and these both cause blackening of roots and stems. Phoma stem lesions can also produce brown discoloration in vertical bands, but these do not extend over the whole length of the plant. The presence of pycnidia on phoma lesions is also a reliable feature that distinguishes them from

verticillium wilt. Stem canker usually causes black rather than grey discoloration within the roots.

The development of microsclerotia may continue after harvest, so that affected plants become more obvious in stubbles, as they turn grey-black.



Fig. 3 Symptoms of verticillium at harvest showing peeling of the stem epidermis and underlying black microsclerotia

In glasshouse experiments with artificial inoculation, *V. longisporum* causes yellowing of the cotyledons and leaves, some blackening of the leaf veins and stunted growth within 5 weeks (Karapapa *et al.*, 1997). These experiments were done in young seedlings with damaged roots, at relatively high temperatures (c 20°C) and with high inoculum. It is just possible such symptoms could occur in the field with early sown winter oilseed rape or late sown spring oilseed rape. Inoculation with spores (conidia) by a root dip method gave earlier and more severe disease symptoms than using microsclerotia added to soil (Dunker *et al.*, 2008).

Premature ripening of whole crops has been a feature of fields with high levels of verticillium wilt in southern England in 2007 and 2008. A range of problems can cause premature ripening in oilseed rape (e.g. severe stem canker, sclerotinia stem rot, phytophthora root rot, drought), but verticillium wilt should now be considered as a possibility.

There are leaf yellowing and wilting symptoms on vegetable brassicas (Koike et al., 2007). These can appear within 3-4 weeks of planting under Californian conditions. UK brassica growers need to be alert for signs of verticillium wilt and seek formal diagnosis of the disease.

2.6 Disease cycle (Fig.4)

The disease cycle has been investigated using microscopic and molecular techniques including green fluorescent protein (GFP) labelled isolates (Eynck *et al.*, 2007; Zhou *et al.*, 2006). Microsclerotia are thought to germinate and produce hyphae in response to root exudates as reported for *V. dahliae* (Mol and Scholty, 1995). Hyphae follow the root hairs to the root surface, where they grow over the surface along the epidermal cell walls and penetrate the epidermal cells. Host cell penetration is at the junction of epidermal cell walls or directly into epidermal cells by means of a thin penetration peg (Eynck *et al.*, 2007). Slight hyphal swelling is associated with this penetration process, but specialised infection structures such as appressoria are not involved. Zhou *et al.* (2006) reported penetration of root hairs and at the root tip, but this was not observed by Eynck *et al.* (2007).

After the fungus has invaded the roots in the root hair zone, it spreads across the cortex both inter- and intra-cellularly and then colonises individual vessels in the xylem. During growth within the root, no discolouration has been observed. Root infection is thought to occur in autumn in winter oilseed rape whilst soil temperatures are still warm (Fig. 4). The extent of further root infection in spring has not been established in winter oilseed rape, though it does occur in spring-sown crops. In winter oilseed rape, verticillium spreads up the stem within the xylem by means of hyphae and conidia during the period from stem extension to flowering. *V. longisporum* began to colonise the xylem three weeks after inoculation of young plants. It was restricted to individual xylem vessels and these became filled with hyphae. There was some spread of hyphae to adjacent xylem vessels via plasmodesmata.

Initially the pathogen is confined to the vascular tissue, but as the plant senesces, there is production of microsclerotia in the surrounding parenchyma tissues. The microsclerotia are produced first in the pith and then under the epidermis (Dunker *et al.*, 2008). The production of microsclerotia results in the characteristic grey or black discolouration of the stem tissues. This late development is a saprophytic phase of activity, contrasting with the earlier biotrophic phase during root colonisation.

Eynck *et al.* (2007) and Zhou *et al.* (2006) carried out their studies with isolates of both *V. longisporum* and *V. dahliae*. Plants were inoculated by root dipping when 10 days old and grown at 23°/20°C (14hr day/10 hr night). Symptoms of chlorosis and vein blackening were evident after 14 days and stunting was apparent after 21 days after inoculation with *V. longisporum*. *V. dahliae* was less successful than *V. longisporum* at colonising roots and there were no symptoms after inoculation with *V. dahliae*. Hyphae of *V. dahliae* spread randomly over the root surface and mainly intercellularly after root penetration. *V. dahliae* produced conidia around the root hairs, but no spores of *V. longisporum* formed outside the root (Eynck *et al.*, 2007). *V. dahliae* produced microsclerotia in the medium around the root whereas *V. longisporum* formed microsclerotia within the root cells. *V. dahliae* rarely reached the vascular tissues and hence it usually did not spread to the shoots and leaves. The restricted growth of *V. dahliae* was attributed mainly to inhibition of systemic growth rather than to root colonisation. The mechanisms involved are not well understood, but could include glucosinolates and other host-specific resistance to which *V. longisporum* is better adapted than *V. dahliae* (Eynck *et al.*, 2007).

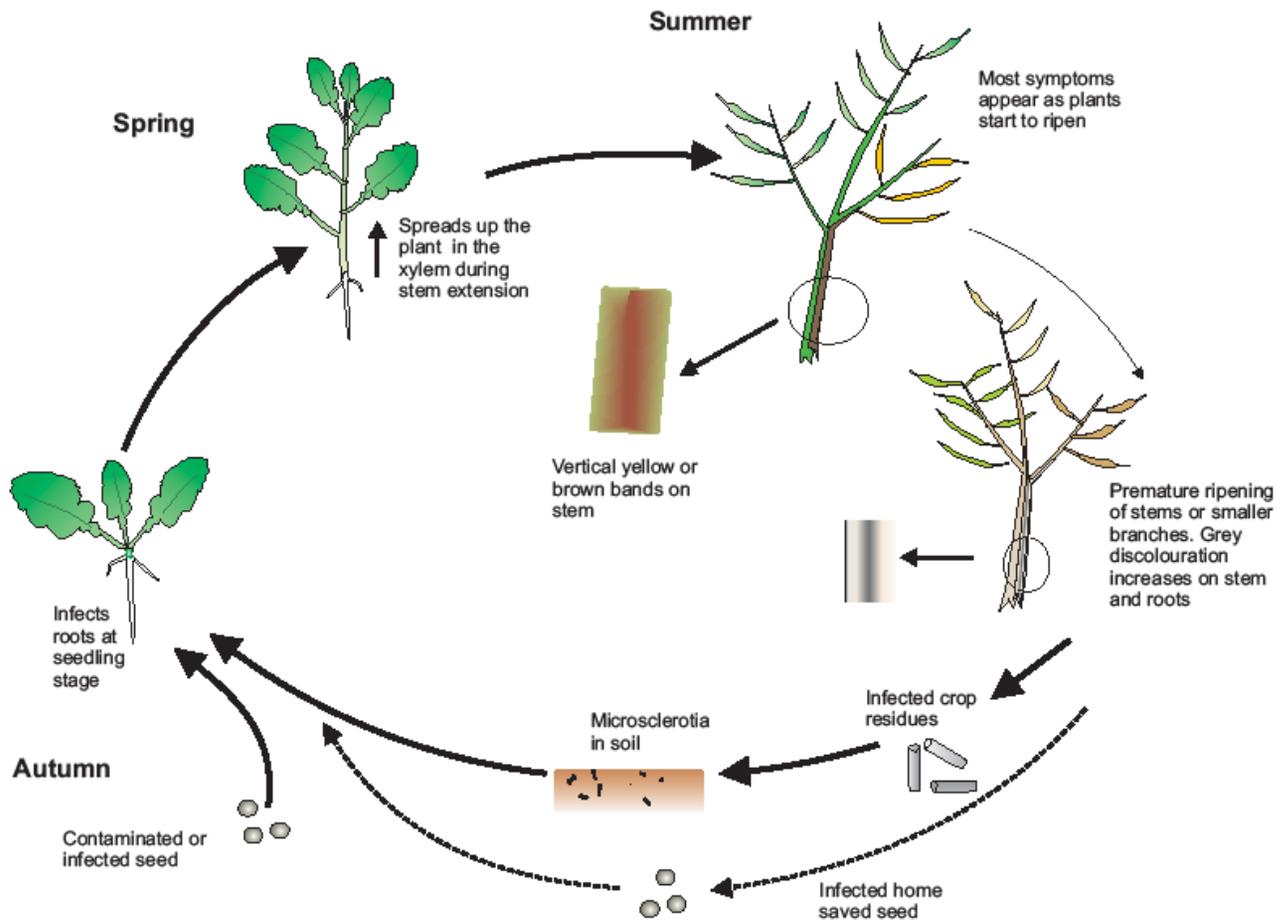


Fig. 4. Disease cycle of verticillium wilt in winter oilseed rape

2.7 Yield loss

As verticillium wilt causes premature ripening, significant effects on yield are expected. Severely affected crops may mature up to 14 days earlier than healthy plants (Svensson and Lerenuis, 1987). Small plot experiments in Sweden showed seed yield was reduced by 44% (Svensson and Lerenuis, 1987) when 96% of plants were affected (55% severely affected). This may underestimate the losses as 'untreated' plots still had 25% plants with verticillium wilt (13% heavily infected). They also noted a 3% decrease in oil content and an increase in seed chlorophyll from 15 to 23 ppm. Thousand seed weight was decreased by 24%. Yield losses were higher when beet cyst nematodes were also present.

Yield losses in Sweden are generally cited as being up to 50% (Eastburn and Paul, 2007), but these losses vary considerably from year to year (Anon., 2006). It is considered to be the most important disease on oilseed rape in Sweden.

Studies in Germany reported by Dunker *et al.*, (2008) quantified yield loss in experiments with natural infection or artificial inoculation using single plants and plots. In inoculated plots, the yield effects were small (c 10% decrease) and non-significant in 2003 although verticillium affected 54% of plants and symptoms were not severe (rating 0.57 on 0-2 scale). In 2004, there were slightly lower levels of verticillium wilt (maximum 34% plants affected, severity 0.16) and no significant yield effects in the inoculated plots. The differences between 2003 and 2004 were attributed, in part, to higher temperatures that led to earlier systemic spread of the pathogen in the shoots. A feature of these experiments was the very late shoot colonisation, which was quantified by ELISA tests.

The field results contrasted with inoculated single plant studies where up to 80% yield loss was recorded. Disease assessment methodology is important (Steventon *et al.*, 2002 b) and a 1-9 scale can be used to assess disease severity (Table 2). Yield loss has been shown to occur when the disease rating is more than 5.

Root dip inoculation gave more severe disease than microsclerotia inoculation and caused stunting with reduced shoot and root growth. These effects were more pronounced on the susceptible cv. Falcon than on the moderately susceptible cv. Talent (Dunker *et al.*, 2008).

Table 2: Assessment key for assessment of Verticillium wilt (from Dunker *et al.*, 2008)

Score	Description
1	None
3	<u>Low infestation:</u> streaky discolouration of the stem; sporadic striping of epidermis; microsclerotia in up to 25% of the epidermis; stem pith or single branches completely colonised with microsclerotia.
5	<u>Medium infestation:</u> epidermis easy to detach from the stem, up to 50% of the stem or stem pith colonised with microsclerotia.
7	<u>Strong infestation:</u> complete discolouration of the stem; epidermis broken into fibres; up to 75% of the stem material colonised with microsclerotia.
9	<u>Severe infestation:</u> premature dying; complete discolouration of the stem with microsclerotia in >75% of stem material.

2.8 Host Range

There have been few studies on the host range of *V. longisporum*. The most detailed study was carried out by Johansson *et al.* (2006) in Sweden. Seedlings of various crop and weed species (Table 3) were inoculated with three different isolates of *V. longisporum* at the cotyledon to first true leaf stage using a root dip method with a conidial suspension. Plants were then grown in sterilised potting soil (80% peat, 20% clay) in a greenhouse with 18 hour light (23°C) and 8 hours dark (18°C), and assessed weekly for symptoms until crop maturity. The presence of *V. longisporum* in plants was confirmed by PCR tests.

Stunting was evident in oilseed rape from an early stage and plants were 20% shorter at maturity. Charlock plants were more severely affected than oilseed rape (41% shorter on inoculated plants). Inoculated plants of both charlock and oilseed rape showed leaf chlorosis and vascular discolouration (Johansson *et al.*, 2006).

Table 3: Symptoms on inoculated plants in host range experiment (from Johanssen *et al.*, 2006)

Species		Stunting	Leaf chlorosis	Microsclerotia produced
Barley	<i>Hordeum vulgare</i>	0	0	0
Wheat	<i>Triticum aestivum</i>	+	0	+
				(lower leaves)
Oats	<i>Avena sativa</i>	+	0	++
				(lower leaves)
Oilseed rape	<i>Brassica napus</i>	++	Yes	++++
Charlock	<i>Sinapis arvensis</i>	++++	Yes	++++
Pea	<i>Pisum sativum</i>	0	0	+
Red clover	<i>Trifolium pratense</i>	0	0	0
Sugar beet	<i>Beta vulgaris</i>	0	0	0
Barren brome	<i>Bromus sterilis</i>	0	0	0
Blackgrass	<i>Alopecurus myosuroides</i>	0	0	0
Cleavers	<i>Galium aparinis</i>	0	0	0
Scentless mayweed	<i>Matricaria odora</i>	+++	0	+

+ 1-10%, ++ 10-20%, ++++ >50%

Microsclerotia were produced in large numbers on oilseed rape and charlock in the roots and lower stems. Some microsclerotia were produced in the leaves of wheat, scentless mayweed and oats (but not in the roots) after inoculating leaves at the late booting or flowering stages. There were no significant effects on thousand seed weight in seed harvested from these experiments apart from one isolate of *V. longisporum* on oilseed rape (Johansson *et al.*, 2006).

V. longisporum has been reported on winter and spring types of both swede and turnip rape. Pathogenicity is restricted to cruciferous plants (Zeise and von Tiedemann, 2002). Oilseed rape appears to be much more severely affected than other *Brassica* species that have been rated in order of susceptibility: pak choi (*Brassica rapa* spp. *chinensis*) cauliflower (*Brassica oleracea* var *botrytis*) and broccoli (*Brassica oleracea* var *italica*) (Zeise and von Tiedemann, 2002).

No disease was found after inoculation of strawberry (*Fragaria x ananassia*), linseed (*Linum usitatissimum*), pea (*Pisum sativum*), lupin (*Lupinus* sp.) and broad bean (*Vicia faba*), but *V. longisporum* was weakly virulent, but did cause yield loss, on potato (*Solanum tuberosum*) and on tomato (*Lycopersicon esculentum*) (Zeise and von Tiedemann, 2002).

The literature on the host range of *V. longisporum* is limited and further investigations are required to determine the susceptibility of a wider range of weed hosts and to quantify microsclerotial production in cereals and other major crops. The range of weed species to examine should reflect their occurrence and importance in UK crops.

2.9 Survival

There is general consensus that microsclerotia can survive for long periods in soil, probably more than 10 years (Anon., 2006). Severe verticillium wilt can occur in oilseed rape even after 8-10 years without oilseed rape cropping (Eastburn and Paul, 2007). There do not appear to be any published data for rates of decline of soil populations of microsclerotia of *V. longisporum*. Microsclerotia of *V. dahliae* are known to survive for many years, regardless of rotations (Huisman and Ashworth, 1976). Factors such as soil moisture and temperature interactions may be more significant than crop rotations in influencing survival of microsclerotia (Huisman and Ashworth, 1976). Current work funded by the British Potato Council on verticillium wilt in potatoes also indicates that *V. dahliae* is capable of long term survival and is difficult to manage even with a one year in six rotation of potatoes (PC project number R/238). Survey data from Germany has highlighted the benefits of extending oilseed rape rotations from one year in three to one year in four for *V. longisporum* (Kreye *et al.*, 2006). As it has a narrower host range than *V. dahliae*, management by using crop rotation maybe more effective than for *V. dahliae*

Rates of decline are fundamental for rotational planning and should be a priority for future research.

2.10 Quantification of inoculum in soil

There is limited information on the levels of inoculum of *V. longisporum* in arable soils. Data are usually expressed as the number of colony forming units (cfu) per gram of soil. These are likely to be mainly microsclerotia.

Soil samples from 9 fields in Southern Sweden have been tested using agar medium (Johansson *et al.*, 2006; Harris *et al.*, 1993) normally used to test soils for *V. dahliae*. *V. longisporum* can be identified by microscopic examination of the colonies on the

agar plate. Sampling was done in fields of winter oilseed rape cv Banjo in spring 2003 and after they were harvested in autumn 2003 and spring 2004. The number of colony forming units ranged from 1 to 24 during oilseed rape cropping, 1-12 cfu in autumn 2003 and 0-48 cfu in spring 2004. Verticillium wilt incidence in the fields ranged from 4% plants affected to 72% and was not correlated with inoculum density in spring 2003. However, even 1 cfu/g gave up to 48% plants affected. In 2 of these 9 fields, *V. dahliae* was more numerous than *V. longisporum* and it was confirmed at 7 out of the 9 sites. Johansson *et al.*, (2006) also reported that up to 165 cfu/g of *V. longisporum* had been recorded after an oilseed rape crop in 2002.

There were changes over time in the number of microsclerotia colony forming units in the 9 fields. However, the changes were not consistent given an expectation that soil populations might be expected to increase after the production of new microsclerotia in diseased plants. Soil inoculum decreased between spring 2003 and spring 2004 at 6 sites, but increased substantially at two sites. It is possible that not all the microsclerotia had been released from crop residues and/or dispersed within the soil profile. Soil sampling studies should therefore be carried out over longer periods to determine rates of decline for microsclerotia and to track the effects of susceptible and non-susceptible crops in the rotation.

The agar plate method used only very small quantities of soil (0.18g soil/plate) so sampling and mixing of soil samples is very important. In future, quantification of soil populations on extracted DNA may be a more reliable technique. These could allow larger soil samples to be tested. As with other soil-borne pathogens, the presence of inoculum may not relate linearly to disease in the crop. Nevertheless, disease - inoculum relationships will need to be established as soil testing could be an important management tool for planning rotations.

Interactions with other soil micro-organisms, soil factors including moisture status and temperature, and cropping history may all affect host infection and disease development. For *V. longisporum* in particular, the timing of the final disease assessment is very critical as more than threefold increases in incidence can occur within 4 days when close to harvest (Algotsson, 2004). In future work, assessments relating to soil-borne inoculum may need to be done at harvest or even post-harvest on stubbles to ensure that full disease expression has been recorded.

2.11 Seed transmission

The occurrence and importance of seed transmission is not known, but transmission of *V. longisporum* via seed is a distinct possibility, given its similarities with *V. dahliae* and *V. albo-atrum* (Maude, 1996). Whilst there could be low levels of true seed-borne infection, contamination of seed stocks with soil or dust containing microsclerotia may also be involved.. Once the distribution and variation in *V. longisporum* is established in the UK, it may be possible to explain how and when the pathogen was introduced (Collins *et al.*, 2005). Testing of seed from infected crops is an area requiring further investigation. If seed transmission is likely to be important then specific seed testing procedures may need to be developed. In the short-term, untreated seed from infected crops should not be used.

2.12 Introduction and spread

As *V. longisporum* is soil-borne and present in crop residues, any activities that move or transfer soil or residues have potential to distribute inoculum within and between fields and farms (Pegg and Brady, 2002). Wind, surface or drainage water, farm machinery and the muddy boot are all likely to contribute to dispersal. In most cases, the amount of inoculum transferred will be small and several cropping cycles may be required before there is any significant crop infection. The occurrence of patchy distributions of verticillium wilt would be of interest and for interpreting how the pathogen was introduced. Field reports suggest that *V. longisporum* is already well-established in some fields and this suggests it has been present for many years. Widespread occurrence would be readily explained if there had been introduction with the seed over many years. A localised distribution would suggest very specific introductions. Survey data are required to quantify the occurrence of *V. longisporum* on UK farms so that the need for hygiene measures can be considered. If verticillium wilt is already widely distributed, hygiene may give little economic benefit.

2.13 Factors affecting disease development

Temperature

The effects of temperature for infection and disease development are not well-defined for *V. longisporum*. Seasonal differences in disease development have been related to

warmer, dryer conditions from flowering (May) until harvest (Dunker *et al.*, 2008). In Germany, temperatures from May until harvest are thought to have influenced final disease severity (Dunker *et al.*, 2008; Soesanto and Termorshuizen, 2001). Soil temperatures of 15-19°C are favourable for infection by *Verticillium* spp. (Eastburn and Paul, 2007) but soil moisture also needs to be adequate. With predicted increases in soil temperature of 2°C under climate change (Evans *et al.*, 2008, Hulme *et al.*, 2002), there is potential for infection to occur later in the autumn and earlier in the spring (Smith and Gladders, 2009).

Soil moisture

On Brussels sprouts, verticillium wilt was more severe in a wet season than a dry season (Isaac, 1957). Dry conditions delayed the onset of wilt symptoms, presumably by impairing root infection. Soil moisture content is thought to be critical for infection to occur, though this is not well defined for *V. longisporum*. At crop maturity, drought stress (combined with high temperatures) is likely to increase disease severity and yield loss (Eastburn and Paul, 2007). Further investigation of soil moisture factors and yield loss could include treatments with crop covers so that rainfall is restricted in some treatments.

Agronomic factors

Surveys of crops in Germany indicated that crop rotation was the most important factor affecting the occurrence of verticillium wilt (Kreye *et al.*, 2006). There were no obvious effects of agrochemical inputs. Conservation tillage gave slightly more verticillium wilt than ploughing so this is a factor to consider in UK investigations. Extending the growing season from a standard 45 weeks (315 days) by 1-3 weeks increased substantially both disease incidence and microsclerotial populations in soil (Kreye *et al.*, 2006). The extended season was achieved mostly by earlier sowing and this may have increased plant colonisation by *V. longisporum* in autumn whilst soil temperatures were high. Delayed sowing may reduce the risk of severe autumn infection, but a balance must be drawn to ensure that oilseed rape plants are still well established.

Survey data on agronomic factors and verticillium wilt are extremely limited. Data from CropMonitor should provide information on the factors affecting verticillium in the UK, when data from sufficient seasons have been accumulated.

Inoculum

Disease incidence increased with the amount of inoculum though not linearly between the rates tested (Dunker *et al.*, 2008). For a given level of inoculum, there can be significant seasonal differences in disease development and severity. Johansson *et al.*, (2006) did not find a correlation between inoculum density and disease incidence in the field. The range of inoculum density tested in these experiments may have been too high to establish good correlations with disease.

In California, 10 microsclerotia per gram of soil could cause 50% wilt incidence in cauliflower (Xiao *et al.*, 1997). There was some aggregation of inoculum in 5 out of 12 fields investigated, but the other 7 fields showed no spatial patterns (Xiao *et al.*, 1997).

Inoculum-disease relationships require further investigation. There are many factors that influence the germination of microsclerotia and the development of disease. Nevertheless the aim should be to identify critical thresholds of soil inoculum that can produce damaging levels of verticillium wilt.

2.14 Control

Chemical control

Fungicides may have activity against *Verticillium* species *in vitro*, but there is a significant challenge to use them to protect crops for long periods. MBC fungicides have been used to control Verticillium wilt of tomatoes. There are opportunities to test fungicidal seed treatments to protect young seedlings against root infection and to target treatments in spring when the pathogen is colonising and spreading within the vascular tissue.

Chemical fumigation or heat treatment of soil is unlikely to be used in arable rotations because of cost. Chemical options are now rather limited, because use of methyl bromide is no longer permitted (O'Neill, 2008). HDC-funded work on control of soil-borne *V. dahliae* and other fungal pathogens provides some UK data on potential treatments, though these might be more relevant to high value horticultural crops (O'Neill, 2008).

Seed treatments

Kinsey (2009) reported very effective control of seed-borne *V. dahliae* in spinach with a treatment called GoSeed™. It also showed activity against some other seed and soil-borne pathogens. Details of the treatment were not given, though it is approved in the USA for organic use. Fungicidal seed treatments with activity against *V. longisporum* may be able to prevent transmission by seed-borne infection or seed contamination. A greater challenge would be to protect seedlings against soil-borne infection. This might be possible given a short window after sowing when soil temperatures were high enough for root infection to occur. If the temperature window extends over several weeks then roots may be unprotected against infection.

Resistant varieties

The development of resistant varieties is critically important for control of verticillium wilt as there are few control methods available other than crop rotation. A single dominant gene has given resistance to verticillium wilt in tomato varieties. The *Ve* gene gave resistance to Race 1 of *V. dahliae*, but has now been overcome by Race 2. Race 2 isolates were less pathogenic than Race 1 isolates on susceptible varieties. However, crosses between Race 1 and Race 2 isolates have occurred naturally (termed 'parasexual crosses') and this has changed the pathogenicity of isolates (Heale, 2000). The *Ve* locus has been found to consist of two closely linked genes that encode for cell-surface like receptors (Happstadius *et al.*, 2003). Polygenic resistance to verticillium wilt in oilseed rape is more likely to be durable.

Plant breeders have been trying to produce resistant varieties for many years and differences in susceptibility have been identified. However, the level resistance is not adequate to give good control under field conditions and specific recommendations based on resistance have not been made. The varieties Express and Talent have some resistance, whilst Falcon is susceptible. The resistance available in oilseed rape (*Brassica napus*) appears to be rather limited and plant breeders are now introducing better resistance from other *Brassica* species including *Brassica oleracea* and wild species (*B. incana*) (Happstadius *et al.*, 2003). The most promising sources of resistance from *B. oleracea* were crossed with winter turnip rape (*B. rapa*) to produce resynthesized rapeseed lines for crossing to advanced oilseed rape breeding material (Happstadius *et al.*, 2003). In Germany, the cauliflower cv. Sernio showed good resistance to *V. longisporum* and the resistance appeared to restrict spread and development of the pathogen in the plant (Debode *et al.*, 2005). This resistance is of

interest to cauliflower breeders and might be used by oilseed rape breeders as well. The resistance mechanisms involved have not been established. New sources of resistance have also been identified in cabbage and in *B. rapa* (Rygulla *et al.*, 2007). Four different chromosome regions with quantitative trait loci (QTL) have been identified, with the two major QTL regions being on the C-genome. (Note *Brassica napus* is an interspecific hybrid between *B. rapa* (genome AA, 2N =20) and *B. oleracea* (genome CC, 2n=18)). The resistance is therefore likely to have originated from *B. oleracea* (Rygulla *et al.*, 2008).

The aim is to combine several resistance loci to produce resistant genotypes. Mapping populations have been produced and molecular markers need to be identified to assist the selection of resistant loci (Rygulla *et al.*, 2007, Rygulla *et al.*, 2008).

2.15 Novel approaches

Broccoli residues (*B. oleracea* var *botrytis*) were very effective at reducing number of microsclerotia in soil in the Salinas Valley of California (Koike and Subbarao, 2000) whereas cauliflower residues were not effective. Residues were incorporated after normal harvesting (c. 6 kg/m²) and effects were comparable to chemical fumigation, and improved vigour and head weight. In laboratory experiments, fresh residues were more effective than dry ones and worked best if temperatures were above 25°C (Koike *et al.*, 2000). Variation in the effectiveness of crop residues has been reported in relation to soil type (Debode *et al.*, 2005). They also reported that lignin may be one of the key components in crop residues that affected the activity of different crop residues against microsclerotia. Both perennial ryegrass and maize residues reduced microsclerotial viability. Volatiles from Indian mustard (*Brassica juncea*) killed microsclerotia *in vitro*, but had little effect in the field (Debode *et al.*, 2005). Residues from various crops, rates of incorporation, temperature, moisture and sealing of treated areas are all factors that are likely to affect the effectiveness of these treatments.

This technique may be useful for vegetable brassica growers in the UK, if verticillium wilt develops in their crops. Soil temperatures appear to be critical for the effectiveness of incorporated residues so the technique may only be effective in the UK during the summer months when temperatures are high. However, treated areas might be covered with polythene if temperatures are becoming rather low in autumn.

Evaluation of the technique for high value crops in the UK should be considered for research funding in future.

Bio-fumigation relies on incorporation of plant residues. Special selected Brassicas including mustard varieties are available and merit further investigation (Kirkegaard and Sarwar, 1998, and Sawar and Kirkegaard, 1998). The periods when these crops can be grown and incorporated may make them difficult to include in winter cereal/winter oilseed rape rotations as the mustards are not winter hardy. There will remain difficulties in achieving good control with both chemical treatments and amendments because oilseed rape will be able to root beyond any treated zone into untreated soil (Giotis *et al.*, 2009).

Organic amendments including composts (Lazarovits *et al.*, 2000, Noble and Coventry, 2005) and crops grown to produce large quantities of leaf material *in situ* have been studied against a range of pests and pathogens. Many of these studies targeted a single disease though there should be a wider focus in future so that broad-spectrum activity can be identified. If control of one problem aggravates another pathogen, the treatment may have limited value. With concerns about 'replant' problems in short oilseed rape rotations, broad-spectrum activity against major and minor pathogens would be advantageous to yield (Mills *et al.*, 2009). Interestingly, *V. nigrescens* (one of the soil fungi that increased under short rotations of oilseed rape (Mills *et al.*, 2009)) may be a hyperparasite as well as a weak pathogen (Cook and Baker, 1996). Experiments on mint showed that inoculation with *V. nigrescens* 5-9 days before inoculation with *V. dahliae* substantially reduced wilt symptoms and pathogen propagules in the plant (Melouk and Horner, 1975). The mode of action of soil amendments often relies on changing the soil microflora so that natural antagonists and competitors suppress the pathogen (Cook and Baker, 1996; Giotis *et al.*, 2009). The addition of a biological control agent to the compost has worked well against sclerotia of the Allium white rot pathogen (*Sclerotium cepivorum*) (Coventry *et al.*, 2006). This approach combining composts and biological control agents should be considered in future work against *Verticillium* species.

There are fungal mycoparasites with potential to control *Verticillium* in soil. *Microsphaeropsis ochracea* has shown good activity against fungal structures or propagules with pigmented cells (Carisse *et al.*, 2002). There have been some promising studies against *V. longisporum in vitro*, suggesting potential to reduce

microsclerotial populations in soil (Stadler et al., 2008). *Talaromyces flavus* is another biocontrol fungus that might be included in screening studies against *V. longisporum* (Spink and Rowe, 1989). *T. flavus* gave 67-76% control of verticillium wilt of aubergine (caused by *V. dahliae*) in field tests (Marois et al., 1982).

Micro-organisms in the root zone or within the roots have potential to affect pathogen activity and host susceptibility. In Sweden, endophytic bacteria with inhibitory activity against *V. longisporum* *in vitro*, suggesting identified in the winter oilseed rape cultivars Express (tolerant) and Libraska (susceptible) (Granér et al., 2003). Diverse microbial populations have also been identified in different seed stocks, and cross inoculation of bacterial populations from cv. Express to cv. Libraska gave beneficial effects. There is potential to use micro-organisms as seed treatments for biological control of *V. longisporum* or induction of systemic resistance (Granér et al., 2003).

3.0 Disease and yield loss relationships under UK conditions

3.1 Disease outbreaks

The first confirmed cases of verticillium wilt in the UK were found in late June/early July 2007 at sites on Romney Marsh, Kent on cv. Castille sown on 31 August 2006 and near Hereford on cv. Barrel sown 1 September 2006. There was more verticillium wilt at the Romney Marsh than at the Hereford site although diagnosis at the latter was more difficult because of high stem canker incidence. There was some variation in disease incidence across the fields, but verticillium wilt was easy to find throughout the crops. This distribution suggested that the pathogen had been present, but undetected, for some years. Subsequently, some oilseed rape stems collected by ADAS from experiments in the same field in 2005 were found to have verticillium wilt. Following publicity about these initial cases, further infected crops were reported by crop consultants. Some samples of stubble were provided to confirm the identification. These were mainly from south-east England.

In 2008, as part of the project reported here, single winter oilseed rape crops on the farms with the initial outbreaks were inspected at monthly intervals from early stem extension onwards for symptoms of verticillium wilt. At the Romney Marsh farm the 2008 crop cv. Es Astrid was sown on 7 September 2007 after winter wheat in 2007 and winter oilseed rape in 2006. A few plants with yellowing on one side of the leaves were noted on 25 May at the late flowering stage in a second field of cv. Es Astrid

sown on 9 September 2007, but it was not confirmed that verticillium was present in these plants. This second field subsequently showed only low levels of verticillium (<1% plants affected) pre-harvest. Stem symptoms were first detected on 30 June at Romney Marsh and 26 June (more affected by 7 July) at the Hereford site, almost identical to dates of the 2007 records. The incidence pre-harvest was 15% at the Hereford site and 56% plants were affected in Kent in 2008 (Table 4).

Table 4: Occurrence of verticillium wilt on the two farms with initial outbreaks in 2007 and 2008.

Site	Plants affected		% premature ripening*	
	2007	2008	2007	2008
Romney Marsh, Kent	32.0	56.0	6.0%	14.2%
Hereford	10.5	15.0	Trace	Trace

*severity scores may be underestimates as plants with both verticillium and severe stem canker or sclerotinia have been excluded

Further cases of verticillium wilt were confirmed from samples and on field visits during 2008 and there have been other unconfirmed reports from crop consultants. There was greater awareness of verticillium wilt after publicity about the 2007 outbreaks. Diagnosis was easier in many cases because stem canker was less prevalent than usual in 2008, although phoma stem lesions also produce vertical brown stripes on the stem. Several new cases were identified because the whole crop ripened prematurely, whereas neighbouring fields were still green. Verticillium wilt appears to be common in southern, eastern and central England, though its incidence in individual crops is very variable. There have been no substantiated cases reported from the East Midlands northwards.

The farms in Kent and Hereford where outbreaks occurred in 2007 were monitored from stem extension onwards. No early symptoms were found and stem symptoms were only evident in late June at crop maturity. The incidence was 15% at the Hereford site and 56% plants were affected in Kent in 2008. This suggests that verticillium wilt is well established on some farms. A severely affected crop near Bedford (c. 90% plants affected) was sampled at maturity and yield components were assessed on 50 plants in relation to wilt severity. Yield was reduced by about 60% where the whole plant had ripened prematurely.

3.2 Pathogenicity tests

Pathogenicity tests with UK isolates of *V. longisporum* on oilseed rape have been completed with eight isolates including three from each the original outbreaks in Romney Marsh and Hereford have been inoculated using the root dip technique with 1×10^6 spores/ml (Karapapa *et al.*, 1997) on seedlings (2-leaf stage) of cv. Castille under heated glasshouse conditions. A spore drench with 20 ml of the spore suspension to another three newly potted seedlings was also used. Seedlings were assessed for wilt symptoms over a five week period. The first signs of leaf yellowing were found 26 days after inoculation. The yellowing increased during the following 7-10 days, affecting the three oldest leaves. The mean percentage leaf area affected by yellowing and plant fresh weights have been used to define pathogenicity (Table 5).

Table 5: Pathogenicity test results with UK isolates of *Verticillium longisporum*.

Isolate	% leaf yellowing			Plant fresh weight (g)		
	Root dip	Root drench	Mean	Root dip	Root drench	Mean
Hereford 1	65.8	25.8	45.8	26.7	30.5	28.6
Hereford 2	65.0	40.0	52.5	22.0	29.4	25.7
Hereford 3	63.3	75.8	69.6	20.9	23.2	22.1
Romney Marsh 1	72.5	10.8	41.7	22.3	25.5	23.9
Romney Marsh 2	72.5	33.3	52.9	18.3	25.6	22.0
Romney Marsh 3	78.3	30.8	54.6	22.7	29.8	26.2
Bedford 1	70.4	26.7	48.5	18.2	22.9	20.6
Great Oakley, Essex 1	46.2	32.5	39.4	23.8	29.6	26.7
Untreated Control	0.0	0.8	0.4	26.2	26.8	26.5

Mean	59.6	30.7	45.0	22.3	27.0	24.7
SED	7.18		15.24	1.142		2.423
F test	<0.001		0.010	<0.001		0.032
	Interaction ns			Interaction ns		

There were more severe symptoms and lower fresh weights with the root dip technique. All isolates were pathogenic as indicated by leaf yellowing symptoms.

There were no significant differences between isolates based on leaf yellowing but isolates did differ significantly in their effects on plant fresh weight (Table 5). Only the isolate from the Bedford site (where plant yield losses were estimated) significantly decreased plant weight compared with the untreated control. *V. longisporum* was reisolated from inoculated plants at the end of the experiment, thus fulfilling Koch's postulates.

3.3 Soil tests

Soil tests for *Verticillium* species were done in May 2008 by ADAS (using the selective agar medium routinely used for *V. dahliae*) on the Hereford and Kent fields investigated in 2007 and 2008, along with a Boxworth field cropped continuously with winter oilseed rape during 1988-2002 and again in 2005 and 2006 (17 winter oilseed rape crops in 21 years).

V. dahliae was found in four of the five samples, with particularly large numbers of propagules (30.9/g soil) in the soil from the field at Boxworth that had a history of intensive oilseed rape cropping (Table 6). Whilst it is believed that *V. dahliae* does not spread systemically in oilseed rape, it may have some deleterious effects.

Verticillium longisporum was found at low levels in soil samples taken from both fields with wilt problems in 2007 (i.e. after cropping) and at the Kent site prior to disease onset in 2008. The agar test is probably not sensitive enough for detection of *V. longisporum*, given that moderately severe verticillium wilt developed on these sites with very low propagule numbers. The results do indicate that *V. longisporum* is present soils and can be detected.

Table 6: Soil tests for *Verticillium* spp. in spring 2008.

Site	Site	Propagules/g soil	
		<i>V. dahliae</i>	<i>V. longisporum</i>
Romney Marsh	Wheat after 2007 wosr crop with 32% Verticillium wilt	6.5	0.4
Romney Marsh	Wosr crop monitored in 2008	3.6	0.1
Hereford	Wheat after 2007 wosr crop with 10.5% Verticillim wilt	5.1	0.6
Hereford	Wosr crop monitored in 2008	0.0	0.0
ADAS Boxworth	Field with 17 wosr crops in 21 years	30.9	0.1

3.4 Yield loss from verticillium wilt

Individual plants were selected on 3 July 2008 from a small area within a crop of winter oilseed rape cv. Castille near Bedford showing premature ripening symptoms. The growth stages ranged from seed green-brown mottled to black (GS 6,4-6,7) depending on the severity of disease. About 90% of plants showed varying degrees of premature ripening and distinctive symptoms of verticillium wilt on the upper stems and branches, whilst similar crops in adjacent fields remained green. A total of 50 single plants were collected. Individual plants were cut off at ground level and pods were placed in a large brown envelope and allocated a reference number. Stem base diameter and the percentage of the plant affected by premature ripening recorded. After thorough drying, the numbers of pods, seeds and the oven dry weight of seed from each plant were recorded. Data were analysed using Genstat.

Total seed weight per plant was significantly related to percentage of plant dead from verticillium. However, the relationship was masked by natural plant to plant variation and disease-related reduction in seed weight appeared to occur almost exclusively at 100% plant dead.

Stem diameter had a significant positive effect on the number of pods, seed number and total seed dry weight and seeds per pod (63.8%, 536% and 56.6% variance accounted for, respectively), thousand seed weight and seeds per pod were less affected (12.9% and 11.1% variance accounted for). There was no significant relationship between stem diameter and verticillium wilt severity. Hence, stem diameter was used as a co-factor in the analysis.

Verticillium wilt had a significant negative effect on the number of pods, seeds per pod, thousand seed weight and total seed weight per plant (Table 6). The largest effect was on total seed weight and this accounted for 22.5% of variance (Fig. 5), compared with 10-18% of variance accounted for by the other components. The scatter of plant yield datapoints in Fig. 5 reflects, in part, the variation due to stem diameter. By combining the factors of verticillium wilt severity and stem diameter, 66.6% of the variance was accounted for (Table 7).

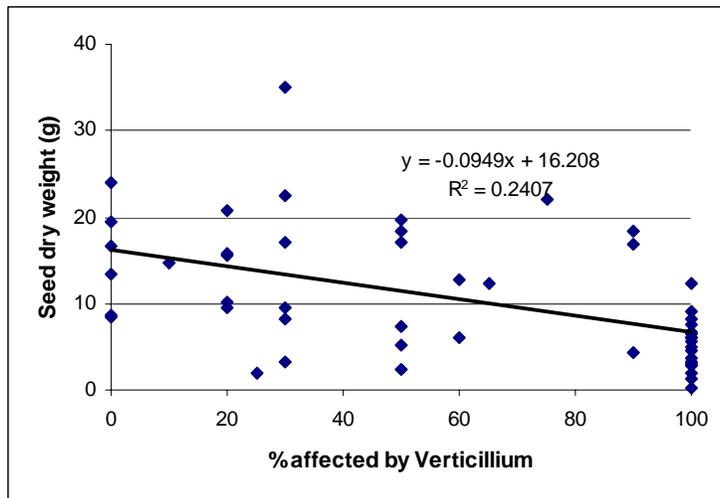


Fig. 5. Relationship between seed weight per plant and severity of verticillium wilt

Table 7: Relationships between verticillium wilt severity, stem diameter and yield components, Bedford 2008.

Response variate	Seed dry weight (g)	TSW (dry, g)	No. seeds	No. pods	No. seeds/pod
F pr.	<0.001	0.002	<0.001	<0.001	0.005
% variance accounted for	66.6	20.7	60.8	70.4	17.1
standard error (estimated)	4.26	0.491	1078	71.4	2.84
Stem diameter t pr.	<0.001	0.022	<0.001	<0.001	0.033
% Verticillium t pr.	<0.001	0.021	0.003	0.001	0.040

The fitted line for regression analysis of disease severity and yield indicated that the dead plants had a 60% loss of yield.

Thus where crops have premature ripening caused by verticillium wilt, some loss of yield is likely to occur. The extent of yield loss will depend on the severity of verticillium symptoms as damage appears to occur mainly where symptoms are severe.

4.0 Conclusions

The overall conclusion from this review is that verticillium wilt poses a significant threat to yield in oilseed rape and possibly other *Brassica* crops in the UK. It is likely to be more damaging in the warmer production areas of southern, eastern and central England, where higher soil temperatures will be more favourable for infection and disease development than further north.

The farms in Kent and Hereford where initial confirmed outbreaks occurred in 2007 had different fields affected in 2008. These observations, together with other samples and reports received in 2007 and 2008, suggest that verticillium wilt is well-established in oilseed rape on some farms in southern, eastern and central England. It is already causing some premature ripening and hence yield loss. Pathogenicity of UK isolates from winter oilseed rape has been demonstrated.

Contact has been made with researchers in the UK and in Europe as part of preparing the literature review. There are no simple solutions and Sweden is still coping with the legacy of very short oilseed rape rotations that were used in the early 1950s. There are no claims for fungicidal control or resistant varieties, though breeders now have useful sources of resistance to work with.

For the immediate future management of verticillium wilt is therefore likely to be largely dependent on longer rotations.

There is an urgent need to establish the occurrence of verticillium wilt in the UK and to guide growers on suitable rotations and management strategies. The priorities for further research are set out in 4.1.

4.1 Recommendations

1. Determine the occurrence of verticillium wilt in oilseed rape in the UK. This can be achieved using existing Defra-funded surveys in CropMonitor, *ad hoc* samples and monitoring of specific farms.
2. Develop diagnostic tests to quality soil-borne inoculum of *Verticillium longisporum* and *V. dahliae*. Quantitative PCR tests are favoured so that

samples can be processed quickly and all levels of inoculum can be quantified. Diagnostic tests for plants are required to overcome disease identification problems in plants prior to crop maturity and to assist research on disease development.

3. Establish the economic importance of verticillium wilt in the UK. Compare natural field infection and inoculated scenarios. As drought stress may increase yield loss, experimental studies should include crop cover treatments that restrict rainfall. Establish threshold levels of *V. longisporum* inoculum that cause yield loss.
4. Investigate the effects of temperature in host infection and disease development using both winter and spring oilseed rape, to define periods of infection in autumn and spring.
5. Work with plant breeders to establish the genetic basis of resistance and identify markers that breeders can use to select novel resistance from Brassicas. Develop methodologies for screening and evaluation of varieties for resistance to verticillium wilt and, in due course, include resistance ratings on the HGCA Recommended List.
6. Establish a network of reference sites for monitoring changes in soil population of *V. longisporum*. The aim should be to identify rates of decline and the effects of different rotations on soil population dynamics. Soil samples from other projects may be useful, where DNA extracts are available.
7. From survey samples, identify factors that affect the occurrence of verticillium wilt. This could include agronomic factors such as date of sowing, establishment method, variety, seed rate, rotation and environmental factors (temperature, rainfall) as well as location, soil type and agrochemical inputs. Investigate the significance of weeds and non-brassica crops as hosts of *V. longisporum*
8. Establish an isolate collection of *Verticillium* species recovered from oilseed rape and other Brassicas. Use this collection to investigate the variation within *V. longisporum*. Examine samples for the presence of *V. dahliae* in the stem and

carry out pathogenicity tests on isolates that are recovered from upper stem. It would be appropriate to investigate disease-yield loss relationships if pathogenic isolates are identified.

9. Define optimum rotations to manage verticillium wilt and quantify the benefits of modifying the date of sowing for disease management and yield.
10. Work with agrochemical manufacturers to identify potential chemical control treatments.
11. Evaluate soil amendments and biological control agents for control of verticillium wilt.
12. Investigate the importance of seed-borne infection and seed contamination by *V. longisporum*. Provide for development of seed testing methods and appropriate guidance on using home-saved seed if problems are identified.
13. Maintain close liaison with other sectors with interests in Brassica crops, particularly HDC for vegetable brassica growers.
14. Technology transfer to increase awareness of verticillium wilt should be given high priority using publications, electronic media, technical events and conferences. An HGCA Topic Sheet should be produced summarising current knowledge.

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