PROJECT REPORT No. OS62

PRELIMINARY EVALUATION OF ROOT DISEASE OF WINTER OILSEED RAPE IN THE UK

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by

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Abstract

During the 2000/1 season, significant variability in the senescence of oilseed rape (OSR) stems was observed in Velcourt-managed fields. These symptoms tend to occur more in the southern part of the country, i.e. Kent and Wiltshire and in fields that have a history of OSR in the rotation. Roots of affected plants appeared blackened and stunted, very similar in appearance to take-all infected roots in cereals. Significant losses were observed at harvest from fields in an OSR rotation compared with adjacent virgin oilseed rape fields. The aims of this pilot project were to: a) determine the causal agent of any associated root disease, b) to determine the scale of the problem and c) to begin to identify solutions to address the problem.

Root samples were taken from matched pairs of virgin and rotational oilseed rape fields and sent for analysis at a number of sampling times; stem extension (March) and pod fill (May). Samples were visually inspected and incubated in moist chambers. After a period of 7 days, root samples were visually assessed and cultures were taken from any lesions present. A nematologist also assessed stem samples. Results from the March and May sampling times were inconclusive as nothing "exotic" or novel was found, either fungal or nematological.

As the season progressed the "blackened roots" observed in previous years did develop at a number of sample sites. In additional work outside that detailed in the project proposal, samples were taken at harvest at four sites. Visually infected roots appeared to be infected with the stem canker pathogen *Leptosphaeria maculans*. To verify this conclusion, root samples were placed in mesh covered plastic trays, placed outside and left under natural conditions. At weekly intervals, sections of stem from each site were observed under a binocular microscope and the development of any fungal structures was monitored. The only structures observed were pseudothecia (the sexual fruiting body) of the *L. maculans* pathogen. Progress was monitored using a development key (A - pseudothecia immature, asci and ascospores not differentiated; D – asci fully developed, ascospores fully differentiated and ready for release). Due to warm, dry weather, pseudothecial maturation was slow and did not occur until mid-late November. Isolations were done which confirmed that the fungus was *L. maculans*.

The results suggest that the saprophytic phase of growth of *L. maculans* may be more important than previously thought. In this study it would appear that the pathogen was able to colonise the root system of oilseed rape plants very rapidly at and around harvest time as the host plant underwent maturation and senescence. It is unlikely that such late season invasion would be responsible for any yield loss. Current knowledge of the stem canker pathogen life-cycle would suggest that the associated severe basal stem cankers would primarily cause yield loss earlier in the season. However, infected roots may act as a significant source of inoculum aiding carry-over of phoma/stem canker to the newly sown crop.

Summary

During the 2000/1 season, significant variability in the senescence of oilseed rape stems has been observed due to an unknown factor in a number of Velcourt-managed fields. Symptoms tended to occur more in the southern part of the England, i.e. Kent and Wiltshire. Roots of affected plants appeared blackened and stunted, very similar in appearance to take-all infected roots in cereals. The causal pathogen of any disease was never identified. Significant losses were observed at harvest from long-term rotational fields in comparison with adjacent virgin oilseed rape fields. During the 2001/2002 season, a pilot project was funded which aimed: a) to determine the causal agent of any associated root disease, b) to determine the scale of the problem, and c) to begin to identify solutions to address the problem.

Root samples were taken from matched pairs of virgin and rotational oilseed rape fields from sites situated throughout the main oilseed rape-growing region of England. Samples were sent by overnight courier to Rothamsted for analysis at a number of sampling times; stem extension (March) and pod fill (May). Firstly, samples were visually inspected, measured and any obvious damage or lesions was recorded. Sections of roots with damage/lesions were incubated in moist chambers (plastic sandwich boxes, 18 x 12 x 4 cm) on moistened filter paper (relative humidity ~100%) and were incubated at 15° C for seven days. Following incubation, root samples were visually assessed. A nematologist also assessed stem samples at this time. After incubation, root sections were observed under a stereo dissecting microscope and fungal pathogens were identified (if sporulation had occurred). If sporulation had not occurred, further isolations were made from fungal colonies onto antibiotic-amended potato dextrose agar (aaPDA). Petri dishes were incubated for 10 – 15 days (15° C), after which time colonies were observed using a stereo dissecting microscope and identification was made. However, results from the March and May sampling times were inconclusive as nothing “exotic” or novel was found, either fungal or nematological. All information from the March and May samples was placed on a database available at:

http://www.iacr.ac.uk/ppi/root/rootsurvey_sample1.xls (First "stem extension" sample)

http://www.iacr.ac.uk/ppi/root/rootsurvey_sample2.xls (Second "flowering" sample)

As the season progressed the “blackened roots” observed in previous years did develop at a number of sample sites. In additional work outside that detailed in the project proposal, samples taken at or immediately after harvest at four sites, were sent to Rothamsted for inspection. Infected roots appeared to be infected with the stem canker pathogen *Leptosphaeria maculans*. In order to verify this conclusion, root samples were placed in mesh covered plastic trays, placed outside and left under natural conditions. At weekly intervals, sections of stem from each site were observed under a stereo dissecting microscope and the development of any fungal structures was monitored. The only structures observed were pseudothecia (the sexual fruiting body) of the *L. maculans* pathogen.

Progress was monitored using a development key (A - pseudothecia immature, asci and ascospores not differentiated; D – asci fully developed, ascospores fully differentiated and ready for release).

Due to warm, dry weather experienced during late autumn, pseudothecial maturation was slow and did not occur until mid-late November. However, when fully mature, sections of stem were inverted over fresh sterile distilled water agar petri dishes and were left overnight. During this period, ascospores were released from the pseudothecia. The resultant single spore colonies were re-isolated onto aaPDA petri plates and incubated for 10 days. The single spore isolates observed confirmed that the fungus was *L. maculans*.

The results suggest that the saprophytic phase of growth of *L. maculans* may be more important than previously thought. It would appear that the pathogen was able to colonise the root system of oilseed rape plants very rapidly at and around harvest time as the host plant underwent maturation and senescence. It is unlikely that such late season invasion would be responsible for any yield loss. Current knowledge of the stem canker pathogen life-cycle would suggest that the associated severe basal stem cankers would primarily cause yield loss earlier in the season. However, infected roots may act as a significant source of inoculum, aiding carry-over of phoma/stem canker to the newly sown crop.

Control/recommendations to industry

Infection by *L. maculans* on roots occurred, even though a robust foliar fungicide program was adhered to, thereby limiting the amount of inoculum initially. This raises the question whether a) there is a longer-term carry over of *L. maculans* from previous crops in the soil and b) if a seed treatment or soil incorporated fungicide may be more effective in controlling the pathogen through the prevention of initial infection.

Technical detail

I Introduction

The industry has become accustomed to the scale of variability in OSR senescence and takes a lot of this variation for granted. It is not until a close rotation OSR crop is compared with a field that has never grown OSR before, that you can begin to see the differences in root disease and hence premature senescence of plants. Upon inspection, roots of affected plants were blackened and stunted. At harvest, affected fields yielded 1 t/ha less than unaffected adjacent virgin oilseed rape crops

There are a number of pathogens that might cause root damage on oilseed rape, for example, stem canker (*Leptosphaeria maculans*), Verticillium (*Verticillium* spp.), Fusarium (*Fusarium* spp.), Rhizoctonia (*Rhizoctonia* spp.), Phytophthora (*Phytophthora* spp.) or other soil-borne pathogens of cruciferous plants. Stem canker (*L. maculans*) has recently been reported to infect roots of oilseed rape in Australia (Sosnowski *et al*., 2001). Verticillium is a genus with pathogens that infect many hosts. The newly described species *Verticillium longisporum* causes significant damage to oilseed rape crops in central and eastern Europe but has never been observed/described for the UK (Karapapa *et al*., 1997). If the observed diseased crops from last year are the first outbreaks of *Verticillium longisporum* in the UK this could have serious consequences for the UK oilseed rape industry. Another species, *Verticillium dahliae* has caused severe verticillium wilt on linseed in recent years in the UK. It is also interesting that this species and the species *V. albo-atrum* are pathogenic on hops, the crop that preceded the oilseed rape in the fields where the problem was diagnosed during last season. *Verticillium longisporum* has been isolated from Brussels sprouts in the UK, but whether this species has the ability to cause disease on oilseed rape has not been investigated fully. Rhizoctonia (*Rhizoctonia solani*) can cause severe losses in Canada (Verma, 1996). Fusarium and Phytophthora are also extremely damaging soil-borne diseases that are widespread throughout UK soils. Although infection of oilseed rape by these species is unusual, the potential for damage by one or more of these pathogens is possible, particularly if affected plants are stressed due to mineral deficiency.

If a root disease problem has developed in the UK, determination of the causal agent is of prime importance before any recommendations can be made for the effective control of the disease. Of the likely candidate diseases, root-based stem canker (*Leptosphaeria maculans*), verticillium, fusarium, phytophthora and/or rhizoctonia would have the potential to decrease yields substantially and would be an additional disease loss for the UK oilseed rape industry. For example, fusarium wilt has been observed on oilseed rape in Alberta, Canada in recent years and yield losses of up to 30% were estimated to have been caused by the disease (http://www.agric.gov.ab.ca/pests/ diseases/fusarium-wilt_canola.html). Similar levels of yield loss (up to 30%) were attributed to infection by *Rhizoctonia solani* in the same region of Canada in 1983 and 1984 (Sippell et al., 1985). Rhizoctonia has also caused disease on Canola in Australia (Khangura et al., 1999).

The aims of this pilot project were to: a) determine the causal agent of any associated root disease, b) to determine the scale of the problem and c) to begin to identify solutions to address the problem.

II Materials and Methods

a. Historical material

To investigate what may have caused the root disease observed in previous seasons, soil samples and available oilseed rape debris were collected from the site of the diseased field in Wiltshire (October 2001). The samples were sent to Rothamsted and tested using standard isolation techniques. Material was surface sterilised in 5% sodium hypochlorite solution, plated onto plain water agar and cultured at 20ºC for 4-6 days. Subsequent colonies were observed, recorded and transferred onto antibiotic amended potato dextrose agar (aaPDA). Transferred cultures were also incubated at 20ºC for 10-14 days and cultures identified.

b. "Virgin" non-rotational and rotational field samples

Velcourt managers identified a number of "virgin", non-rotational oilseed rape fields in close proximity to similar Velcourt-managed oilseed rape fields under normal rotational practices. The details of the sites are given in Table 1. Normal rotation field sites were carefully monitored for signs of disease/abnormal growth and later, differences in maturity and compared with the virgin oilseed rape crop throughout the season. A seed treatment experiment being done at Rothamsted in association with Bayer Crop Science was also another site. The pairs of fields (plus the site at Rothamsted) were sampled twice; at stem extension (Feb/March) and at late flowering (Early June). At each sampling time, fifty stems/roots were removed from each field (i.e. 100 stems per geographic location from the virgin and rotational fields) sampling 5 clusters of 10 plants in a W shape across the field. The lower 10 cm of stem and as much of the root as possible (i.e. at least 20 cm) were removed, excess soil was washed away using a pressure hose and samples were sent by "next-day-delivery" to Rothamsted for analysis.

Root samples were taken from matched pairs of virgin and rotational oilseed rape fields from sites situated throughout the main oilseed rape-growing region of England. Samples were sent to Rothamsted for analysis at stem extension (March) and pod fill (May). Samples were visually inspected, measured and any obvious damage or lesions were recorded. Sections of roots with damage/lesions were cut out, and incubated in moist chamber boxes at 15° C for a period of 7 days. A nematologist also assessed stem samples at this time point. After incubation, root sections were observed under a stereo dissecting microscope and fungal pathogens were identified (if sporulation had occurred). If sporulation had not occurred, further isolations were made from fungal colonies.

Infected sections of stem were surface sterilised in 5% sodium hypochlorite solution (for 2 minutes) and plated onto sterile distilled water agar and cultured at 20ºC for 4-6 days. Subsequent colonies were observed, recorded and transferred onto antibiotic amended potato dextrose agar (aaPDA). Petri dishes were incubated for 10 – 15days (15° C), after which time colonies were observed using a stereo dissecting microscope and identification was made.

Table 1: Velcourt farms sampled and oilseed rape root sampling times for the 2001/2 oilseed rape root disease study.

Velcourt Farm	**1st Sample**	**2nd Sample**
Green Drove, Wiltshire	11/12 March 02	20/21 May 02
Gedgrave, Woodbridge	11/12 March 02	20/21 May 02
Lees Farm, Andover	11/12 March 02	20/21 May 02
Hawarden Farms, Canterbury	11/12 March 02	20/21 May 02
George Farm, Stamford	11/12 March 02	20/21 May 02
Barkers, Rickinghall	11/12 March 02	20/21 May 02
Coneys, Lincs	18/19 March 02	27/28 May 02
Fitzroy Farming, Nuneaton	18/19 March 02	27/28 May 02
Haverholme, Lincs	18/19 March 02	27/28 May 02
Carrington, Boston	18/19 March 02	27/28 May 02
Cornbury Park, Charlbury	18/19 March 02	27/28 May 02

c. Post-harvest sample

In addition to the work detailed in the proposal, one extra sampling was done from a small selection of the sites used during the study. Material was collected from four sites after harvest and sent to Rothamsted for analysis. The dried, senescent material was examined using a stereo dissecting microscope and any infection noted. Material was then placed in the bottom of free-draining plastic seedling trays (25 roots/stems), covered with a plastic mesh (to prevent material blowing away during windy weather) and placed on the roof of a building at Rothamsted open to the natural elements. Development of fungal structures was monitored on a weekly basis using a stereo dissecting microscope. The only structures observed were pseudothecia (the sexual fruiting body) of the *L. maculans* pathogen. Progress was monitored using a development key (A-D: A - pseudothecia immature, asci and ascospores not differentiated; D – asci fully developed, ascospores fully differentiated and ready for release). When fully mature, sections of 10 stems per site were inverted over fresh sterile distilled water agar petri dishes and were left overnight. After four days incubation at 15 ° C, the resultant single spore colonies were re-isolated onto aaPDA petri plates and incubated for a further ten days (15 ° C).

III Results

a. Historical material

Material from the Wiltshire site was in a poor condition and was observed to be fairly well rotted. Consequently, very little was isolated from the stems/root debris. Nothing pathogenic could be isolated from the material, probably because of overgrowth by saprophytic fungi.

b. "Virgin" non-rotational and rotational field samples

Over the two sampling times, 1754 root samples were measured, examined and incubated. However, results from the March and May sampling times were also inconclusive as nothing “exotic” or novel was found, either fungal or nematological. All information from the March and May samples was placed on a database (Appendix 1 and 2). A general observation from both sampling dates was that the roots of both the "virgin" sites and the rotational sites were surprisingly clean with very little damage/infection. Damage that was observed appeared to be mechanical or physiological rather than disease related. Data from both sampling dates can be seen in Appendix 1 and Appendix 2.

c. Post-harvest sample

The “blackened roots” infected roots appeared to be infected with the stem canker pathogen *Leptosphaeria maculans*. Often infected root systems were associated with a typical basal stem canker lesion. Pseudothecial maturation was slow and did not occur until mid-late November due to warm, dry weather experienced during late autumn. Stems were observed to develop typical pseudothecia, the majority of which were A-type pseudothecia which developed on the surface, whereas occasionally B-type pseudothecia were observed to develop just beneath the surface of the root epidemal cells. Of the 10 stems from each of the four sites from which single spore isolations were made, all 40 were infected with *L. maculans* (Table 2).

Table 2. Single spore isolates of *Leptosphaeria maculans* produced from Oilseed rape root material from four UK sites during the autumn of 2002. Isolates were made from ascospores produced from pseudothecia which developed under natural conditions at Rothamsted 9/8/02 - 22/11/02.

Site code	Stem number	No. of A-type colonies	No. of B-type colonies
VE-OSR-1B	2	6	0
VE-OSR-1B	3	6	0
VE-OSR-1B	4	4	0
VE-OSR-1B	8	5	0
VE-OSR-1B	10	6	0
VE-OSR-1B	13	6	0
VE-OSR-1B	16	6	0
VE-OSR-1B	17	6	0
VE-OSR-1B	19	5	0
VE-OSR-1B	25	6	0
VE-OSR-8A	10	5	0
VE-OSR-8A	16	5	0
VE-OSR-8A	19	6	0
VE-OSR-8A	25	5	0
VE-OSR-8A	26	6	0
VE-OSR-8A	27	6	0
VE-OSR-8A	28	5	0
VE-OSR-8A	30	5	0
VE-OSR-8A	31	6	0
VE-OSR-8A	32	6	0
VE-OSR-9B	4	6	0
VE-OSR-9B	5	0	5
VE-OSR-9B	8	6	0
VE-OSR-9B	9	6	0
VE-OSR-9B	10	0	5
VE-OSR-9B	12	6	0
VE-OSR-9B	15	6	0
VE-OSR-9B	18	5	0
VE-OSR-9B	29	5	0
VE-OSR-9B	30	6	0
VE-OSR-10A	5	5	0
VE-OSR-10A	7	7	0
VE-OSR-10A	8	5	0
VE-OSR-10A	16	6	0
VE-OSR-10A	25	3	2
VE-OSR-10A	26	0	6
VE-OSR-10A	27	6	0
VE-OSR-10A	30	6	0
VE-OSR-10A	31	6	0
VE-OSR-10A	32	0	6

IV Discussion

The results suggest that the saprophytic phase of growth of *L. maculans* may be more important than previously thought. In this study it would appear that the pathogen was able to colonise the root system of oilseed rape plants very rapidly at and around harvest time as the host plant underwent maturation and senescence. As very little infection was found at either the stem extension or flowering sample dates and infected roots were often associated with typical basal stem cankers at harvest, it can be suggested that the "root problem" observed during this study was an extension to the stem canker lifecycle, as yet unreported in the UK. Sosnowski *et al*. (2001) reported that *L. maculans* was associated with premature senescence in Australia and that affected plants have stunted, blackened roots and demonstrated that *L. maculans* was able to infect roots of oilseed rape directly through wounded roots. Sosnowski *et al*. (2001) suggested that the infection did not develop from crown (basal) stem canker but that the disease could also be soil-borne. The authors of this report suggest that (in the UK) this is probably not the case. If soil-borne, presumably the pathogen would be present all of the time and at higher levels in the "rotational" field samples. One would expect significantly more *L. maculans* infection earlier in the season and more infection in rotational field samples in comparison to "virgin" oilseed rape field samples, and this was not the case. Although our current knowledge of the life-cycle does not include a soil-borne section and long-term survival structures have never been reported for this species, only systematic testing of soil samples from possible infected UK fields will determine whether our current view of the life-cycle is correct or not.

In terms of control/prevention, control of phoma leaf spotting and prevention of the development of subsequent stem cankers would minimise colonisation of the root system. The results suggest that late season colonisation would not have been the primary cause for yield loss. It would seem more likely that severe basal stem cankers would cause yield loss earlier in the season and the authors would suggest that the primary concern of the grower should be the control of initial phoma leaf spotting to prevent subsequent stem canker and possible root infection. However, infected roots may play an important role in the life-cycle by acting as a significant source of inoculum aiding carry-over of phoma/stem canker to the newly sown crop. The upper (above soil) parts of the oilseed rape plant are fleshy and tend to rot easily following senescence. In contrast, root material is highly lignified and tends to persist in soil for a longer period of time following senescence. If this is the case, some form of stubble management may be required to reduce inoculum potential for future crops.

Control/recommendations to industry

Infection by *L. maculans* on roots occurred, even though a robust foliar fungicide program was adhered to, thereby limiting the amount of inoculum initially. This raises the question whether a) there is a longer-term carry over of *L. maculans* from previous crops in the soil and b) if a seed treatment or

soil incorporated fungicide may be more effective in controlling the pathogen through the prevention of initial infection.

V References

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VI Appendices

Appendix 1: Stem extension sample date Excel file, available at:
http://www.rothamsted.ac.uk/ppi/root/rootsurvey_sample1.xls

Appendix 2: Flowering sample date Excel file, available at:
http://www.rothamsted.ac.uk/ppi/root/rootsurvey_sample2.xls