IOBC / WPRS

Working Group „Integrated Control in Oilseed Crops“

OILB / SROP

Groupe de Travail „Lutte Intégrée en Culture d’Oléagineux“

Proceedings of the meeting

at

Soest, Germany

23 - 24 April, 2001

edited by

V.H. Paul & I. Föller

IOBC wprs Bulletin
Bulletin OILB srop Vol. 25 (2) 2002

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:

INRA – Centre de Recherches de Dijon
Laboratoire de recherches sur la Flore Pathogène dans le Sol
17, Rue Sully, BV 1540
F-21034 DIJON CEDEX
France

ISBN 92-9067-139-0

Preface

The working group „Integrated control in oilseed crops“ of the IOBC wprs held its 10th biannual meeting in Germany in Soest from April 23rd to 24th 2001. I would like to thank the local organisers Dr. Föller and Dr. Dapprich, who were helped by colleagues of their faculty, for the very successful organisation.

Through contacts established in the IOBC wprs meetings, two important projects have been established and worked on by members of the working group. One is the Project on the "Concerted Action on Biocontrol of Rape Pests" (Boris), which led to a follow up research project titled "Management Strategies for European Rape Pests" (MASTER).

In the general discussion of the last IOBC meeting three aspects have been discussed as beeing of main importance for oilseed rape in the near future:

- **Verticillium Wilt (*Verticillium dahliae*)**

  Verticillium Wilt is a very important disease in the UK. In Germany it has often been mixed up with Stem Canker (*Lepthosphaeria maculans/Phoma lingam*). With the establishment of an ELISA for *Verticillium*, it has been found in samples from different parts of Germany, that *Verticillium* is present far more often than expected. Therefore a monitoring of samples from all over Germany should be established. All present members of the IOBC have agreed to send samples for this purpose.

- **Stem Rot (*Sclerotinia sclerotiorum*)**

  Stem Rot is also one very important disease of oilseed rape. So far no variety with resistance against *Sclerotinia* has been established. Therfore more importance should be placed on the establishment of a biocontrol system against *Sclerotinia*. Some parts of this aspect are currently under investigtion in the UK. It is also of interest that one biological active agent (*Conyothirium minitans*) against *Sclerotinia* is on the market in Germany. It is planned to test this organism at the Department of Agriculture in Soest, under laboratory conditions, in regard to its suscebtibility to herbicides and fungicides.

- **Biocontrol of insect pests**

  As already mentioned, an EU Project has been granted for "Management Strategies for European Rape Pests". One of the aims of this project is to find biocontrol methods for the key species in oilseed rape, especially the cabbage stem flea beetle. Furthermore it is planned to establish a forcasting system for these key species. In this project different partners from Germany, UK and Poland will collaborate.

Now we can look forward to the next meeting, the 11th Biannual Meeting in Rothamsted, UK, in spring 2003.

Prof. Dr. Volker H. Paul
Convenor

List of participants:

Name	Adress
PD Dr. Wolfgang Büchs	Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Pflanzenschutz in Ackerbau und Grünland Messeweg 11/12 D - 38104 Braunschweig w.buechs@bba.de
Dr. Peter D. Dapprich	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest dapprich@mailso.uni-paderborn.de
Dr. Neal Evans	Crop and Disease Management IACR-Rothamsted Harpenden UK-Herts, AL5 2JQ neal.evans@bbsrc.ac.uk
Dr. Iris Föller	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest foeller@mailso.uni-paderborn.de
Dr. Volker Garbe	Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Pflanzenschutz in Ackerbau und Grünland Messeweg 11/12 D - 38104 Braunschweig v.garbe@bba.de
Dimitra Gkilpathi	Plant Pathology Department IACR-Rothamsted Harpenden, AL 52JQ, UK dimitra.gkilpathi@bbsrc.ac.uk
Dr. Franz-Ferdinand Gröblinghoff	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest grobl@mailso.uni-paderborn.de
Udo Heimbach	Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Pflanzenschutz in Ackerbau und Grünland Messeweg 11/12 D - 38104 Braunschweig U.Heimbach@bba.de
Dr. Michael Henneken	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest michas@mailso.uni-paderborn.de

Name	Adress
Huang Yongju	Plant Pathology Department IACR Rothamsted Harpenden Herts AL5 2JQ, UK yong-ju.huang@bbsrc.ac.uk
Dr. Malgorzata Jedryczka	Institute of Plant Genetics Polish Academy of Sciences Strzeszynska 34 PL-60-479 Poznan mjed@igrnov.igr.poznan.pl
Petra Kraus	Ludwig Boltzmann Institut für Biologischen Landbau und Angewandte Ökologie Arbeitsgruppe Agrarökologie Rinnböckstrasse 15 A-1110 Wien Petra_Kraus@mail.com
Yong Liu	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest yongliu@mailso.uni-paderborn.de
Aleksander Łukanowski	Katedra Fitopatologii Akademii Techniczno-Rolniczej ul. Kordeckiego 20 85-225 Bydgoszcz
Christer Nilsson	Forskningsledare / Senior Research Officer SLU, Dept Crop Science P.O.Box 44, SE-230 53 Alnarp, SWEDEN Christer.Nilsson@vv.slu.se
Prof. Dr. Volker H. Paul	Universität-GH Paderborn Labor für Biotechnologie und Qualitätssicherung Lübecker Ring 2 D-59494 Soest paul@mailso.uni-paderborn.de
Dr. Sarah A. M. Perryman	Department of Plant Pathology IACR-Rothamsted Harpenden Herts AL5 5SJ, UK. sarah.perryman@bbsrc.ac.uk
Prof. Czeslaw K. Sadowski	University of Technology and Agriculture Department of Plant Pathology Ks. Kordeckiego 20 PL - 85-225 Bodgoszcz fitopato@adm.atr.bydgoszcz.pl
Julie Steed	IACR-Rothamsted Harpenden UK-Herts, AL5 2JQ julie.steed@bbsrc.ac.uk

Name	**Adress**
Dr. Bernd Ulber	Institut für Pflanzenpathologie und Pflanzenschutz Universität Göttingen Grisebachstr. 6 D - 37083 Göttingen bulber@gwdg.de
Dr. Jonathan West	Plant Pathogen Interactions Division IACR – Rothamsted Harpenden AL5 2JQ, UK jon.west@bbsrc.ac.uk
Prof. Dr. G. A. Wolf	Institute of Plant Pathology and Plant Protection Göttingen University Grisebachstr. 6 D - 37077 Göttingen
Qinghua Zhao	Institute of Plant Pathology and Plant Protection Göttingen University Grisebachstr. 6 D - 37077 Göttingen qzhao@gwdg.de

Contents

Biological and Integrated Control of Diseases

Biological and Integrated Control

Supplement to the IOBC Meeting from May 31st to June 2nd, 1999 in Prague

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 1 - 12

Occurrence of fungal diseases on spring rape in Poland

Sadowski Czeslaw [1], Dakowska Sylwia [2], Lukanowski Aleksander [1], Jedryczka Malgorzata [2]

[1] *Dept. od Phytopathology, University of Technology and Agriculture, Kordeckiego 20, 85-225 Bydgoszcz, Poland*

[2] *Lab. of Plant Resistance to Diseases, Institute of Plant Genetics, Strzeszynska 34, 60-479 Poznan, Poland*

Abstract: For many years spring oilseed rape (*Brassica napus* subsp. *oleifera* forma *annua*) was cultivated in Poland in very small areas and from an economical point of view it was not regarded as an important crop. The dominating crop was the winter type of oilseed rape (*Brassica napus* subsp. *oleifera* forma *biennis*), which was the main source of domestic oil production for consumption and for technical purposes. However, for the last few years spring rape has become more important, especially in years with severe and long winters. Due to a low interest of farmers in growing spring rape in Poland, there have been no intensive breeding programmes or research on this crop. At present there are seven officially registered varieties, one of which is of Polish origin.

So far, there has been very little work on occurrence of diseases on spring cultivars in Poland. Since 1997 we have observed disease symptoms in several regions of spring rapeseed cultivation. The main aim was to evaluate the spectrum, incidence and severity of diseases on production fields and to compare disease levels on some officially registered varieties.

The dominating disease was dark leaf or pod spot caused by *Alternaria* spp. The disease was observed every year and in all regions. The symptoms were present on stems, leaves and pods. The seeds collected from plants were highly contaminated with pathogenic and saprophytic species of fungi from this genus. Another pathogen observed every year was *Peronospora parasitica*, the cause of downy mildew. It was usually present on leaves for the whole vegetative period, but its effect was not as damaging as that caused by dark pod spot. In two years, 1998 and 1999, there was quite a high incidence (DI up to 29,6%) of powdery mildew (*Erysiphe cruciferarum*). The disease symptoms were observed on leaves, main stems and side stems. The disease was not reported in 1997. The pathogens, *Sclerotinia sclerotiorum* (stem rot) and *Botrytis cinerea* (grey mould), were observed in lower or intermediate quantities in some years and/or regions only. *Leptosphaeria maculans*, the cause of stem canker and *Verticillium dahliae*, the cause of wilting and tracheomycosis, were noted very sporadically.

In 1999 and 2000 systematic observations were made on the five officially registered cultivars in three different growing regions: north-western, north-eastern and southern parts of Poland. The results varied depending on the year and region. The dominating disease was dark leaf or pod spot, followed by powdery mildew, grey mould and stem rot. The usual high number of pests was greatly reduced by high doses of pesticides, used routinely on experiment plots in contrast to some commercial fields.

Key words: spring oilseed rape, diseases. healthiness, Poland

Introduction

The basic oleic crop in Poland is winter oilseed rape. Mild winters in the last few years caused an almost complete lack of cultivation of other oleic crops. However, two severe winters, 1995/96 and 1996/97, and unfavourable conditions in winter and early spring caused frost damage of winter oilseed rape. In this situation spring oilseed rape was sown on some fields.

Favourable conditions during the growing season in 1996 caused the seed yield of spring oilseed rape from many plantations to be relatively high. Since then, there has been an increased interest in spring oilseed rape cultivation.

Usually in Poland the seed and oil yield of the spring type is about 30% lower than winter oilseed rape, but in appropriate weather conditions, spring rape can yield nearly as much as the winter type. The yield of spring oilseed rape depends mainly on the rainfall in spring and in the opinion of many authors, this is the reason why spring oilseed rape will not be competitive with winter form of this crop. However, it may be an alternative plant in the case of difficulties with winter rape sowing caused by late cereal harvesting or frost damage of winter rape on the large areas of its cultivation range. (Walkowski 2000). When the weather conditions and cultivation technology are optimal the yield level of spring oilseed rape in Poland is between 26 and 33 dt/ha and oil content is about 47-48% (Budzynski 1998, Musnicki and Tobola 1998).

Due to a low interest of farmers in growing spring rape in Poland, there have been no intensive breeding programmes or research on this crop. The first Polish variety of spring rapeseed was officially registered in 1998 by the Research Centre for Cultivar Testing (COBORU, Slupia Wielka). It is the hybrid cultivar, Margo, produced by ZDHAR Malyszyn. The other registered cultivars are of foreign origin: Star from Denmark, Bolero and Licosmos from Germany and Sponsor from Sweden.

The low interest of farmers in cultivation of spring rapeseed resulted in very little research on this form of the crop. Therefore until now there was very few data concerning diseases of the spring form of oilseed rape in Poland. In 1997 after the frost damage of large areas of winter oilseed rape, some varieties of spring oilseed rape, which were not officially registered in Poland, were permitted to be cultivated. For this reason there were no data about their economic value and susceptibility for pests and diseases in our conditions. It was expected that the spectrum of diseases would be similar to those found on winter rapeseed, but the disease incidence and disease severity could differ considerably (Jedryczka *et al.*, 1999). The aim of these investigations was to observe the spectrum and severity of fungal pathogens on plants of spring oilseed rape in natural infection conditions. The paper consists of two parts: the assessment of healthiness of cv. Star on several production fields in three different localities and the evaluation of fungal disease incidence and severity on five officially registered varieties of spring oilseed rape. Both investigations took place in three regions of intensive oilseed rape growing in Poland.

Material and methods

Occurrence of fungal pathogens on production fields in natural infection conditions.

Observations were made in three regions of cultivation of spring rape in Poland: 1) Kujawy (sum of rainfall from April to August is about 240 mm), 2) Zulawy and 3) Mazury (both with about 320 mm of rainfall). These observations were carried out in two periods: flowering and ripening phases. Each time 4x100 randomly taken plants from various parts of each field were analysed. The incidence (per cent) of infected plants, leaves, stems and pods and their severity of infection in per cent area or a specific scale was determined. Variety “Star” was cultivated on all analysed fields.

The highest incidence of fungal pathogens occurring on plants from production fields concerned black pod spot (*Alternaria* spp.), downy mildew (*Peronospora parasitica*) and powdery mildew (*Erysiphe cruciferarum*).

Peronospora parasitica severity was determined with the use of a six-degree scale (Sadowski 1987), where 0° = no disease symptoms on leaves; 5° = symptoms covers over

75% of the leaf area. *Alternaria* pod spot severity was estimated during flowering phase on four lower leaves (0-4°), and during ripening the per cent of pod and stem area with disease symptoms was noted. For estimation of *Alternaria* spp. incidence on leaves five-degree scale was used (Evens and Gladders 1981), where 0° = no spots on leaf; 4° = the spots cover over 50% of leaf area. Determination of *Alternaria* spp occurrence on stems and pods was carried out with the use of five-degree scale (Babadoost and Gabrielson 1979), where 0° = no disease symptoms; 4° = symptoms cover over 60% stem/pod area. For the evaluation of leaf infection with *Erysiphe cruciferarum*, the scale for estimation of *Peronospora parasitica* was used (0-5°). During the ripening phase, a six-degree scale was used to describe *P. parasitica* infestation on the whole plant (Penaud 1999), where 0° = no visible mycelium on the plant; 5° = stems brown or black. The results were converted into disease index (DI, max = 100%) using Townsend-Heuberger formula. Macroscopic diagnosis was verified in the laboratory using a microscope.

Occurrence of fungal pathogens on officially registred cultivars in Poland

The investigation was made in three regions of cultivation of spring oilseed rape: 1) Pomorze (north-west of Poland), locality: Rarwino; 2) Mazury (north-east), locality: Wrocikowo; 3) Malopolska (south), locality: Wegrzce. Four officially registered foreign cultivars: Bolero (P.H. Petersen, Germany), Star (DLF, Denmark), Sponsor (Svalöf, Sweden) and Licosmos (Deutsche Saatveredelung, Germany), and Polish cultivar Margo (ZDHAR Malyszyn) were observed in two seasons: 1999 and 2000. The observations in 1999 were made at the end of flowering (16-23 July). Due to a low disease incidence at this time of plant development, the observation in 2000 was made later, in the stage of green pods (1-7 August).

On each occasion, all fungal diseases present were noted. In the case of sclerotinia stem rot (*Sclerotinia sclerotiorum*) the observations concerned the percent of stem area with symptoms of the disease and the position of symptoms on stems in I-V scale, where I is stem base and V is the region of pods. In the case of black leaf and pod spot caused by fungal species from the genus *Alternaria* the observations concerned the infection of stems, leaves and pods. In each case both the per cent of plants affected and the area of symptoms were evaluated. Grey mould caused by *Botrytis cinerea* was noted as the per cent of stem and pod area infected. The per cent of stem area with symptoms of powdery mildew (*Erysiphe cruciferarum*) and verticillium wilt (*Verticillium* spp.) were also noted. In the case of blackleg or phoma leaf spot and stem canker (*Leptosphaeria* spp.), the per cent of infected plants was calculated. In addition, observations reported the per cent of plants with stem borers, aphids, damage of pods by birds and the per cent of pods infected with saprophytes.

Results

Occurrence of fungal pathogens on production fields in natural infection conditions.

During our research the most damaging pathogen noted on spring oilseed rape was *Alternaria* pod spot. It occurred every year in high intensity on all fields, and all regions of Poland. Disease symptoms were observed both on leaves, stems and pods. Higher infestation was noted in the regions with high rainfalls (Zulawy and Mazury). The incidence of *Alternaria* spp varied in the years investigated. Much more symptoms of the disease were noted in 1998 and 1999 (DI for leaves>30%, stems and pods >50%) than in 1997 (DI for leaves >20%, stem and pods >18%). Microscopic tests showed that the main species of *Alternaria* was *Alternaria brassicae*; *Alternaria brassicicola* was observed very rarely. The saprophytic species, *Alternaria alternata*, was commonly isolated (Fig. 1 and 2).

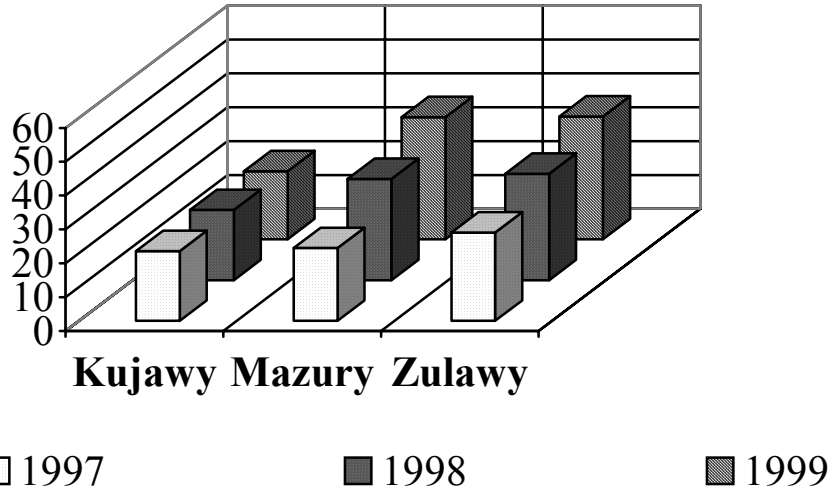


Fig. 1 *Alternaria* spp. occurrence on spring oilseed rape leaves in flowering phase (DI in %)

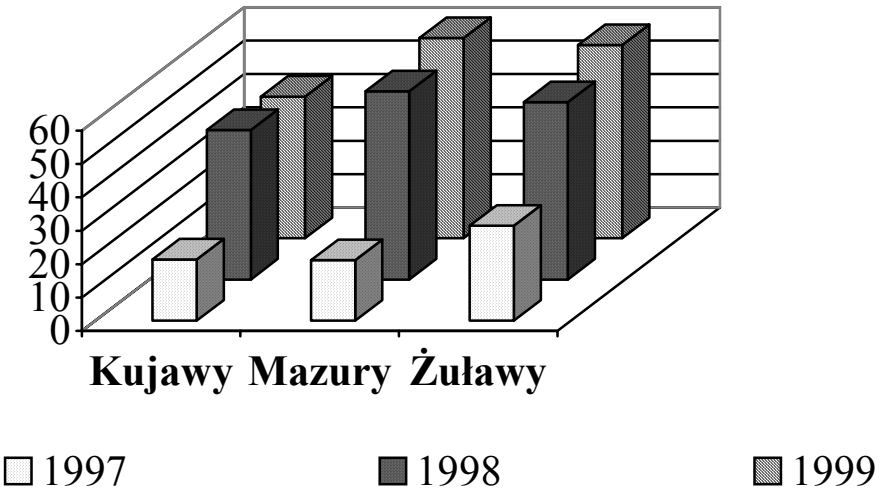


Fig. 2 *Alternaria* spp. occurrence on spring oilseed rape stems and pods in ripening phase (DI in %)

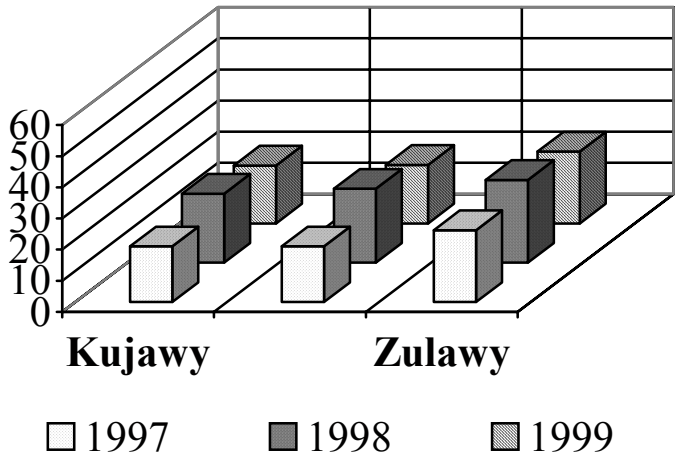


Fig. 3 Downy mildew occurrence on spring oilseed rape leaves in flowering phase (DI in %)

Every year downy mildew (*Peronospora parasitica*) was observed on leaves. DI in the flowering phase was from 17.9 to 26,6%. More symptoms was noted on Zulawy (DI 23,1-26,6). Differentiation in respective years was not so high like in the case of *Alternaria*. Slightly more symptoms were recorded in 1998 (Fig. 3).

Incidence of powdery mildew varied in respective years and regions. In 1997 it was noted in trace amounts, but in the next year the disease occurred in high intensity on all plantations. In the third year in the region of Kujawy its incidence was noted at a low level (DI about 6%), while in Zulawy and Mazury region DI was over 30% (Fig. 4 and 5). In the last period of vegetation its intensity was so high that on stems besides white-grey mycelium there were visible grey-black and then brown or black large spots on main and lateral stems and even on pods.

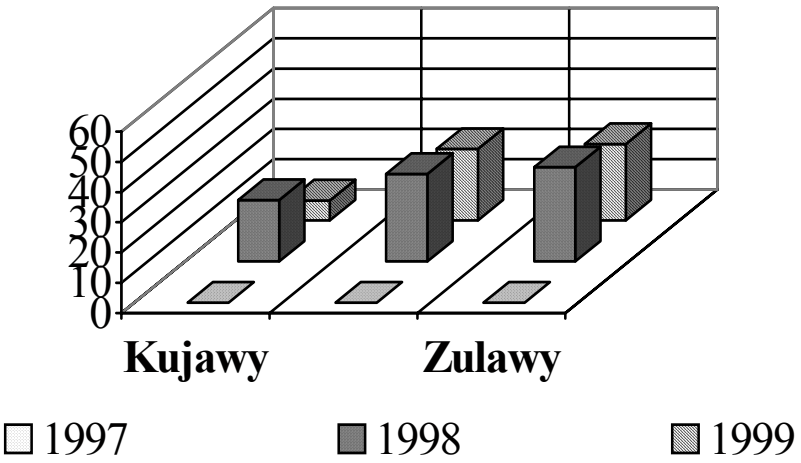


Fig. 4 Powdery mildew occurrence on spring oilseed rape in flowering phase (DI in %)

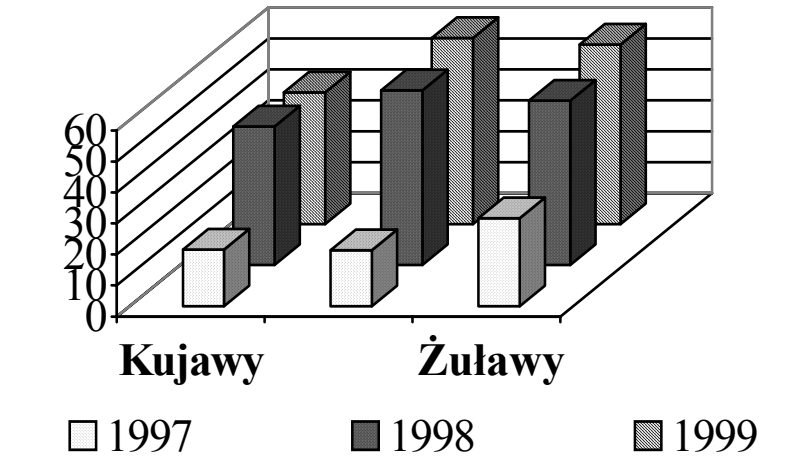


Fig. 5 Powdery mildew occurrence on spring oilseed rape in ripening phase (DI in %)

The other pathogens, such as grey mould (*Botrytis cinerea*), blackleg (*Phoma lingam*), sclerotinia stem rot (*Sclerotinia sclerotiorum*) and *Verticillium dahliae* were observed rarely or they were not present at all. There were a very few plants named as “early ripening”, which is often noted on the winter form of oilseed rape.

These observations are compatible with the results obtained by many authors who noted lower health status of winter oilseed rape cultivated in Zulawy region. In these specific environmental conditions the most dangerous pathogens for winter oilseed rape *are Phoma lingam*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria* spp. and early ripening complex. (Frence *et al.* 1991, Lewartowska *et al*. 1993, Sadowski and Zielinski 1995).

Table 1

	Rarwino 16.07.1999					Rarwino 03.08.2000				
	Bolero	Licosmos	Margo	Sponsor	Start	Bolero	Licosmos	Margo	Sponsor	Star
Sclerotinia - % stem area	0,30	0,50	0,00	1,15	0,00	4,87	1,40	0,67	6,47	1,33
Sclerotinia - position	I-II	I	-	I	-	I,I-II	I,I-II	I,I-II	I,I-II	I,I-II
Alternaria - % inf. stems	0,28	0,07	0,04	0,22	0,26	7,20	14,47	7,07	9,67	8,87
Alternaria - % inf. pods	23,84	1,94	1,60	14,60	11,09	11,13	7,87	4,77	5,33	6,82
Alternaria - % pod area	3,49	0,69	0,63	4,31	2,54	5,03	7,53	4,28	6,67	5,73
Alternaria - % inf. leaves	5,00	4,80	0,00	9,65	10,35	-	-	-	-	-
Alternaria - % leaf area	1,12	0,83	0,00	2,04	1,65	6,00	3,23	2,11	4,07	2,20
Botrytis - % stem area	0,00	0,00	0,00	0,00	0,30	0,00	0,00	0,00	0,00	0,00
Botrytis - % pod area	1,86	0,30	0,20	8,88	1,11	4,47	4,87	1,49	1,20	3,47
Phoma - % inf. plants	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Erisiphe - % stem area	2,25	0,55	0,80	5,26	1,80	16,80	6,93	2,20	4,87	10,40
Erisiphe - % leaf area	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Verticillium - % stem area	0,00	0,00	0,00	0,00	0,00	1,87	2,87	1,80	3,80	0,87
Stem borers	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Aphids	0,00	0,00	0,00	0,00	0,00	0,11	0,20	0,12	0,11	0,07
Birds	0,00	0,00	0,00	0,00	0,00	8,60	2,33	1,20	4,40	6,20
Saprophytes - % pods	0,00	0,00	0,00	0,00	0,00	1,13	0,33	0,07	1,67	0,60

Table 2

	Wrocikowo 19.07.1999					Wrocikowo 07.08.2000				
	Bolero	Licosmos	Margo	Sponsor	Start	Bolero	Licosmos	Margo	Sponsor	Star
Sclerotinia - % stem area	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Sclerotinia - position	-	-	-	-	-	-	-	-	-	-
Alternaria - % inf. stems	0,22	0,16	0,04	0,37	0,22	14,80	17,20	19,73	28,89	26,67
Alternaria - % inf. pods	6,09	3,35	3,26	12,70	7,50	4,93	5,93	4,20	4,33	7,20
Alternaria - % pod area	2,26	1,23	1,60	4,87	3,49	7,93	7,13	8,47	12,53	14,07
Alternaria - % inf. leaves	30,50	25,80	30,50	37,10	36,00	-	-	-	-	-
Alternaria - % leaf area	6,35	8,15	8,45	13,10	8,75	0,00	6,00	6,00	0,00	9,73
Botrytis - % stem area	0,00	0,75	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Botrytis - % pod area	0,00	0,00	0,33	0,00	0,00	3,73	1,20	1,73	1,07	6,33
Phoma - % inf. plants	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Erisiphe - % stem area	0,00	0,00	0,00	0,00	0,00	29,91	30,40	28,80	26,40	24,13
Erisiphe - % leaf area	0,00	0,00	0,00	0,00	0,00	11,60	9,93	16,27	17,13	10,67
Verticillium - % stem area	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stem borers	0,01	0,30	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Aphids	0,00	0,12	0,20	0,10	0,57	0,00	0,00	0,00	0,00	0,00
Birds	0,00	0,00	0,00	0,00	0,00	2,53	1,64	3,47	1,24	4,20
Saprophytes - % pods	0,00	0,00	0,00	0,85	0,00	6,20	3,93	3,33	1,87	3,72

Occurrence of fungal pathogens on officially registered cultivars in Poland

The symptoms of fungal diseases on plants of different cultivars varied depending on the year and region. In general, the dominating disease was dark leaf or pod spot, followed by downy mildew, grey mould and stem rot. The detailed results are presented in tables 1-3 (locality: Rarwino, Wrocikowo and Wegrzce, respectively).

Sclerotinia stem rot (*S. sclerotiorum*) was observed mainly in the south of Poland in the region of Malopolska. The disease was absent in Mazury (north-east) and present to some extent in Pomorze (north-west). If the disease was present, it was observed on all cultivars. In 1999 the highest infection was observed on variety Margo, and in 2000 it was observed on variety Star. The lowest level of infection was observed on varieties Licosmos (1999) and Bolero (2000). In the stage of flowering the symptoms were usually restricted to the bottom of stems, but in the phase of green pods the symptoms were in many cases present on whole plants.

Table 3

	Węgrzce 23.07.1999					Węgrzce 01.08.2000				
	Bolero	Licosmos	Margo	Sponsor	Start	Bolero	Licosmos	Margo	Sponsor	Star
Sclerotinia - % stem area	7,60	5,58	11,20	9,26	7,20	4,33	10,00	8,80	7,60	10,67
Sclerotinia - position	I-IV	I-III	I-III	I-III	I-III	I,I-III	I,I-IIV	I,II,I-IV	I,I-III	I,II,I-IV
Alternaria - % inf. stems	0,21	0,24	0,34	0,33	0,29	12,64	14,33	4,87	16,00	9,33
Alternaria - % inf. pods	11,50	16,66	7,60	22,33	7,73	7,39	6,53	3,47	7,40	7,20
Alternaria - % pod area	5,44	5,58	4,00	0,86	4,54	2,98	3,60	1,11	3,83	3,55
Alternaria - % inf. leaves	-	-	-	-	-	-	-	-	-	-
Alternaria - % leaf area	-	-	-	-	-	0,00	0,00	0,00	0,00	0,00
Botrytis - % stem area	22,50	22,20	19,20	20,60	22,66	4,01	4,80	7,33	0,00	14,53
Botrytis - % pod area	26,41	26,53	25,60	27,60	25,66	5,16	2,96	0,60	4,53	13,93
Phoma - % inf. plants	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Erisiphe - % stem area	0,46	2,26	0,00	1,60	4,40	13,40	11,93	10,60	7,93	11,60
Erisiphe - % leaf area	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Verticillium - % stem area	0,00	0,00	0,00	0,00	0,00	0,00	1,07	0,40	1,47	1,07
Stem borers	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Aphids	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Birds	13,20	17,60	22,40	17,60	19,66	5,40	9,09	2,33	9,07	10,27
Saprophytes - % pods	0,00	0,00	0,00	0,00	0,00	5,03	7,84	0,27	4,84	13,03

Dark leaf or pod spot caused by fungi from the genus *Alternaria* was present in all regions, but the highest disease incidence and disease severity was reported from the north-east (Mazury). The highest disease scores were constantly obtained from varieties Star and Sponsor, and the lowest infection concerned varieties Licosmos, Bolero and sometimes Margo.

Grey mould (*Botrytis cinerea*) was observed on all varieties and in all regions, but mainly in the south of Poland. In contrast, powdery mildew was present mainly in the north, but also on all varieties. In north-east (Mazury) some symptoms of *Verticilium* were spotted. In all three regions and on all varieties no symptoms of phoma leaf lesions or stem canker were observed.

Usually in Poland the plants in production fields are highly infected by pests, if the fields are not sufficiently treated with insecticides. However, on plots of experiment stations of Research Centre for Cultivar Testing (COBORU) the fields were sprayed several times with insecticides and herbicides, and the number of stem borers are aphids was very low. When the pods were damaged by birds, they were also overgrown with saprophytic fungi.

Discussion

The results obtained here are only partially compatible with the common opinion that on spring and winter oilseed rape the same diseases are noted. The spectrum of diseases was similar to those found on winter rapeseed, but the disease incidence and disease severity could differ considerably (Jedryczka et al. 1999).

The most dangerous diseases of winter oilseed rape in Poland are: blackleg (*Phoma lingam*), sclerotinia stem rot (*Sclerotinia sclerotiorum*), black pod spot (*Alternaria* spp.) and disease complex called “early ripening”. Recently, in some regions of Poland, *Cylindrosporium concentricum* is a problem for farmers (Frencel et al. 1991, Sadowski 1987, Sadowski and Klepin 1991, Sadowski et al. 1995, Jedryczka et al. 1999, Karolewski and Weber 1992, Karolewski 1999, Lewartowska et al. 1993).

Our observations showed that the role of these diseases on the spring form of oilseed rape is completely different then on winter form of that crop. The highest incidence was noted in the case of dark leaf or pod spot (*Alternaria* spp.), downy mildew (*P. parasitica*) and powdery mildew (*E. cruciferarum*).

Disease symptoms were observed in high intensity on leaves in the phase of flowering and even more on stems and pods in the ripening phase. Disease occurrence was variable. A very high incidence of *A. brassicae* on pods was observed especially in 1998 and 1999; it was connected with preferable weather conditions. There were 70 days with the rainfalls in Mazury region in the period April – August. These conditions were preferable for infection and pathogen development. In 1997 there were 50 days with the rainfall and the temperature was higher than its normal mean value (calculated over many years). This probably caused the lower disease intensity in that year.

The proof for the important effect of rainfall frequency on *A. brassicae* occurrence is plant infection in Kujawy region. In 1997 and 1999, when the number of the days with rainfalls was (respectively) 57 and 47 – DI of the stems was 25-30.5% on average, but in 1998, when there were about 70 days with rainfall – the mean DI was 44.1%. Jalaludin and Kashem (1999) claim that the intensity of the disease may be high even when the temperature is lower (10-15°C) but on the one condition: air humidity must by very high.

The results obtained here are compatible with the reports of Marchegay *et al.* (1990), that frequent rainfalls and higher temperatures are preferable for pathogen development. Its intensity depends also on the amount of inoculum (Hong and Fitt 1995). The conidia may be easily transferred with the wind, which plays a much more important role than rain (Wadia *et al.* 1995). Winter oilseed rape was often cultivated not far from the spring form and that could be a reason for transferring the pathogen in all 3 regions of Poland.

Common occurrence of black pod spot in every year studdied and on all investigated experimental plots confirms the Canadian reports concerning its high severity on the spring form of oilseed rape (Tewari 1991, Pedras *et al.* 1999).

Black pod spot is also a big problem in England. Fitt *et al.* (1999) noted that in certain weather conditions, *Alternaria* spp. may occur on stems and pods in such high intensity that chemical control of this pathogen is required. Kennedy and Graham (1995) include *A. brassicae* and *A. brassicicola* to the group of the most dangerous oilseed rape pathogens. Damaged pods ripen sooner, which causes not only a lower seed yield but also lower quality. Ramsbottom and Thomas (1999) reported pod infection of about 30% in 1998.

There is differentiation of spring oilseed rape by varietal susceptibility to *Alternaria* spp. (Church and Fitt 1995, Klodt-Bussman and Paul 1995). When a new variety is being registered, its susceptibility to *A. brassicae* should be considered as one of the most important factors.

Sclerotinia sclerotiorum occurs on winter oilseed rape in Poland in some growing seasons in high intensity. It is a serious problem in Zulawy region (Frencel *et al.* 1991, Lewartowska *et al.* 1993, Sadowski *et al*. 1995, personal observations in 1999 r, information from Mr Wyczling M. Sc.).

Spring oilseed rape may be very susceptible on infection with ascospores in the spring (Jedryczka et al. 1999). These authors carried out investigations on the susceptibility of 17 varieties and races of spring oilseed rape using artificial infection. All of them were susceptible, including variety “Star”.

Every year on all plots, downy mildew (*P. parasitica*) was noted. It seems that the severity of this disease may be very serious and together with the other diseases it may decrease seed yield. Sadowski (1987) obtained similar results. Gladders (1991) claims that downy mildew is one of the most often noted pathogens of spring oilseed rape in England, but its occurrence does not always result in serious losses of seed yield. Harmfulness of this disease for winter oilseed rape was observed in Germany by Paul *et al.* (1991). The reaction of various varieties on it is very variable (Nashaat and Rawlison 1991, Klodt-Bussman and Paul 1995).

As far as powdery mildew is concerned, it occurrence was very differentiated. It was noted in its high intensity in two years, while in one year only in trace amounts. These observations are compatible with the results obtained by Penaud (1999) who claims that importance of this disease may vary depending on the region of cultivation.

During our investigation only a few symptoms of blackleg or stem canker (*Leptosphaeria maculans*/*Phoma lingam*) were observed. This is a very commonly occurring pathogen in all regions of winter oilseed rape cultivation, and causes the most important disease in European rape. It has been noted in Poland since the late 70s and nowadays it is recognised as one of the most dangerous pathogens. Very rare symptoms were observed on spring oilseed rape. Jedryczka et al.(1999) claim that the same pathogens may occur on this form of oilseed rape as on winter rape, but their intensity and severity may differ. That is why there must be more research conducted on the diseases of spring oilseed rape and its susceptibility to them.

The occurrence of grey mould (*Botrytis cinerea*) was noted in a low intensity. However, this disease is not important on winter oilseed rape in Poland. More symptoms are noted when there is high air humidity for a long time and when plants are damaged by pests.

Verticillium dahliae was observed very rarely. The intensity of this pathogen in Poland is rather low (Zielinski and Sadowski 1995), but a high incidence and severity on winter oilseed rape was noted in 1998 and 1999 (Jedryczka *et al.* - unpublished data). In the last decade it has been noted at a higher intensity in the northern part of Europe (Kontowski et al. 1995) and is particularly dangerous in Sweden (Wallenhammar 1995). Chemical control is not possible, so the only control methods are good crop rotation and resistance breeding.

Conclusions

1. The same diseases may occur on the winter and spring form of oilseed rape but their intensity and importance can be completely different. Therefore the threat of pathogens on spring oilseed rape cannot be assumed to be the same as on the winter form
2. The most important threat for spring oilseed rape was black pod spot, caused by *Alternaria brassicae*. It occurred on all investigated plots in every year and in two years its incidence/severity was very high. Disease symptoms were visible during the whole of the growing season. The symptoms were observed on leaves of juvenile plants, stems and pods of mature plants. Very high infestation of pods suggests that more research on the effect of the pathogen on seed yield and its quality as well as oil quality should be conducted.
3. In two years the occurrence of powdery mildew (*E. cruciferarum*) on leaves, stems and pods was relatively high. It may be the threat for the spring form of oilseed rape. Downy mildew (*P. parasitica*) was noted only on leaves and its intensity was not high, so chemical control is not recommended.
4. Blackleg (*Leptosphaeria* spp.) - the most dangerous disease of winter rapeseed in Poland was noted very rarely and it had no practical importance.
5. Sclerotinia stem rot (*Sclerotinia sclerotiorum)* occurred mainly in the south, and in this region it could have some effect on seed yield.
6. Environmental conditions were important for fungal pathogen occurrence. There was less disease in the drier region of Kujawy, than in wetter regions of Zulawy, Mazury and Malopolska.
7. The disease incidence and severity on the five varieties studied, depended on the year and region. The dominating disease was dark spot, followed by powdery mildew, grey mould and stem rot. The usual high number of pests was much decreased by high doses of pesticides, used routinely on experiment plots in contrast to some plantations of farmers.

Acknowledgments

The authors are greatful to Prof. E. Gacek – the Director of the Research Centre for Cultivar Testing (COBORU, Slupia Wielka) – for the permission for the observations of spring oilseed rape cultivars on official trials. The authors would like to thank for the help of staff from experiment stations of Rarwino, Wrocikowo and Wegrzce.

References

Babadoost M., Gabrielson R.L. 1979. Pathogens causing *Alternaria* disease on *Brassica* seed crops in western Washington. Plant Dis. Reptr. 63: 815-820.

Budzynski W. 1998. Reakcja rzepaku na termin siewu i sposób odchwaszczania. Rosliny Oleiste 19 (1): 125-133.

Church V.J., Fitt B.D.L. 1995. Incidence and effects of diseases on seven winter oilseed rape cultivars in 1991/92 and 1992/93. IOBC/WPRS Bulletin 18 (4): 62-68.

Evens E. J., Gladders P. 1981. Diseases of winter oilseed rape and their control, east and south-east England 1977-1981. Proc. 1981 Brit. Crop Prot. Conf., Brighton, Vol. 2: 505-512.

Fitt B.D.L. Gladders P., Sutherland K. G., Turner J. A., Welham S. J. 1999. Epidemiology, forecasting and management of winter oilseed rape diseasea in the uk aus. 10th Intern. Rapeseed Congress, Canberra. New Horizons for Old Crop. Proc. CD-ROM, Doc. No. 4: 1-21.

Frencel I., Lewartowska E., Jedryczka M. 1991. The spectrum and severity of fungal diseases in field infections of winter oilseed rape in Poland. Integreated Control in Oilseed Crops. Proc. of the meetings at Rothamsted, UK, 1-3 March 1990: IOBC/WPRS Bulletin 14 (6): 137-140.

Gladders P. 1991. Priorities for research and development of diseases of oilseed in the United Kingdom. IOBC/WPRS Bulletin 14 (6): 2-4.

Hong C.X., Fitt B.D.L. 1995. Effect of infection conditions on the incubation period of dark leaf spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus)*. 9th Intern. Rapeseed Congress, Cambridge. Vol. 2: 595-597.

Jalaluddin M., Kashem M.A. 1999. Development of Alternaria blight in some advanced mutants of Brassica napus under field conditions. 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra. CD-ROM, Doc. No. 262: 1-10.

Jedryczka M., Lewartowska E., Dakowska S. 1999. Evaluation of susceptibility of spring oilseed rape to sclerotinia stem rot (in Polish). Rosliny Oleiste 20 (2): 659-668.

Karolewski Z. 1999. The occurrence of light leaf spot on winter oilseed rape in Western Poland in 1991-1996 and the characteristic of *Pyrenopeziza brassicae* isolates. Phytopathologia Polonica 18: 113-121.

Karolewski Z., Weber Z. 1992. Susceptibility of oilseed rape varieties on natural and artificial infection with *Phoma lingam* (in Polish). Rosliny Oleiste 14 (2): 219-225.

Kennedy R., Graham M. 1995. Infection on oil-seed rape by *Alternaria brassicae* under varying conditions of temperature and wetness. 9th Intern. Rapeseed Congress, Cambridge. Vol. 2: 601-603.

Klodt-Bussman E., Paul V.H. 1995. Investigations on natural disease resistance of 6 oilseed rape cultivars for minimizing application of fungicide in integrated production from 1992/93. IOBC/WPRS Bulletin 18 (4): 45-55.

Kontowski S., Horn R., Friedt W. 1995. Selection for resistance to *Verticillium dahliae* in rapeseed (Brassica napus) by the use of immunological techniques. 9th International Congress, Cambridge. Vol. 4: 1292-1294.

Lewartowska E., Jedryczka M., Frencel I., Górski P., Konopka W. 1993. Occurence of fungal diseases of winter oilseed rape in Zulawy region. (1987-1992) (in Polish). XXXIII Sesja Naukowa Instytutu Ochrony Roslin. Poznan: 156-161.

Marchegay P., Thorin N., Schiavon M. 1990. Effects of climatic conditions on the aerial liberation of spores of *Alternaria brassicae* (Berk) Sacc. and epidemiology of blackspot diseases in rape crops [biotic environment, abiotic environment]. Agronomie, V. (10): 831-839.

Musnicki Cz., Tobola P.1998. Reaction of double improved spring oilseed rape on sowing date (in Polish). Rosliny Oleiste 19 (1): 135-140.

Nashaat N.I., Rawlinson C.J. 1991. Resistance to downy mildew in *Brassica napus* ssp. *oleifera.* Bulletin IOBC/WPRS 14 (6): 166-173.

Paul V.H., Burhenne S., Gunzelmann A. 1991. Preliminary results of research on *Peronospora parasitica* (Pers. ex Fr.) Fr. in winter oilseed rape with special regard to the susceptibility of double-low cultivars. IOBC/WPRS Bulletin 14 (6): 174-180.

Pedras M.S.C., Zaharia I.L., Smith K.C., Gai Y., Ward D.E. 1999. *Alternaria* blackspot phytotoxins: new strategies for determing specific disease resistance traits. 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra. CD-ROM, Doc. No. 139: 1-8.

Penaud A. 1999. Chemical control and yield losses caused by *Erysiphe cruciferarum* on oilseed rape in France. 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra. CD-ROM, Doc. No. 327: 1-8.

Ramsbottom J.E., Thomas J.E. 1999. Latest findings on variety and fungicide interreaction. 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra. CD-ROM, Doc. No. 312: 1-8.

Sadowski Cz. 1987. An investigation on the occurrence and control of downy mildew on winter rapeseed. Proc. 7th Intern. Rapeseed Congress Poznań 5: 1097-1103.

Sadowski Cz., Klepin J. 1991. Effects of fungicides on the health and yield of oilseed rape. IOBC/WPRS Bulletin 14 (6): 272-281.

Sadowski Cz., Zielinski D., Klepin J., Zawislak K. 1995. The effect of agronomical practices on the healthiness of winter oilseed rape. 9th Intern. Rapeseed Congress, Cambridge. Vol. 4: 1228-1230.

Tewari J.P. 1991. Current understanding of resistance to *Alternaria brassicae* in crucifers. 8th Intern. Rapeseed Congress, Canada. Vol. 2: 471-475.

Wadia K.D.R., McCartney K.J., Doughty K.J., Kushwaha H.S. 1995. Studies on spore disperal of *Alternaria brassicae*. 9th Intern. Rapeseed Congress, Cambridge. Vol. 2: 607-609.

Walkowski T. 2000. Spring oilseed rape (in Polish). IHAR Poznan: 1-68.

Wallenhammar A.C. 1995. Prevalence of club root (*Plasmodiophora brassicae)* in a spring oilseed rape growing area in central Sweden. 9th Intern. Rapeseed Congress, Cambridge. Vol. 2: 586-88.

Zielinski D., Sadowki Cz. 1995. A preliminary study on *Verticillium dahliae* Kleb. in winter oilseed rape in Poland. 9th International Rapeseed Congress „Rapeseed today and tomorrow". Cambridge, UK 4-7 July, 649-651.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 13 - 19

Spectrum and severity of fungal diseases on spring oilseed rape in Russia

Malgorzata Jedryczka [1], Vladimir A. Nikonorenkov [2], Elena Gasich[3], Elzbieta Lewartowska [1], Ljudmila Portenko [2], Mark Levitin [3]

[1] *Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland*

[2] *All-Russian Research Institute of Rapeseed, Boevoy Proezd 26, 398037 Lipetsk, Russia*

[3] *All-Russian Institute of Plant Protection, Podbelski Rd. 3, 196608 St. Petersburg-Pushkin, Russia*

Abstract: Main oilseed crops in Russia are sunflower and soybean. Although the cultivation area of oilseed rape is low (*ca.* 300 thous. ha), it is regarded as an increasingly important oil crop with great perspectives. Spring type is predominating over the winter type (from 72 to 97% of area under oilseed rape, depending on the year). There are five main regions of spring rape cultivation: Volga (30%), Central region (15%), West Siberia (15%), East Siberia (6%) and Ural (5%) and the rest of fields are dispersed in other areas.

During the last five years (1996-2000), observations of plant healthiness were done semi-systematically in two regions of the European part of Russia: the North-Western region (near St. Petersburg) and central region (around Lipeck, *ca.* 600 km south of Moscow). Infected plant organs were collected and causal pathogens were isolated, identified and characterised. In the North-Western region the most damaging fungus was clubroot (*Plasmodiophora brassicae*) and in Central region the most damaging disease was Fusarium wilt (*F. oxysporum*). Several other diseases were also found: grey mould, downy and powdery mildew, Sclerotinia stem rot, Alternaria black spot and root rot complex caused by *Rhizoctonia solani* and *Fusarium* spp. A few diseases commonly observed on spring or winter rape in other regions of Europe, Canada or Australia were not found: light leaf spot, white leaf spot, Verticillium wilt and stem canker.

Introduction

The principle oilseed crop in Russia is sunflower. In 1999 the production of seeds from sunflower constituted 90.6% of the total production. The other important oilseed crop is soybean (5.4%). The area of sunflower occupied about 5.5 million ha and the soybean area was nearly 1 million ha.

In comparison to these two main oil crops, the area of rapeseed production in Russia is relatively small. However, in the last twenty years oilseed rape has become an increasingly important crop and there is a growing tendency in its breeding and cultivation. In the begining of 1990's it was cultivated on *ca.* 100 thousand hectares, which constituted only 0.1% of cultivation area of the Russian Federation. At the end of nineties the area increased *ca*. 2.7 times and it occupied about 276 thous. ha (0.27 %).

Oilseed rape is grown in many regions, but the largest ones are Volga (30%), North Caucasus (25%), Central region (15%), West Siberia (15%) and East Siberia (6%) (Fig. 1, Tab.1). The area of oilseed rape is cultivated mainly with the spring form of rapeseed (*Brassica napus* subsp. *oleifera* forma *annua*). In 1991-1999 the area of spring oilseed rape increased from 94 to 268 thousands hectares, constituting 72-97% of total cultivation of oilseed rape in Russia. Depending on the year, the area of winter oilseed rape ranged from 8

to 52 thousand ha. Winter oilseed rape is grown mainly in North Caucasus and Kaliningrad regions (Proizvodstvo 1998).

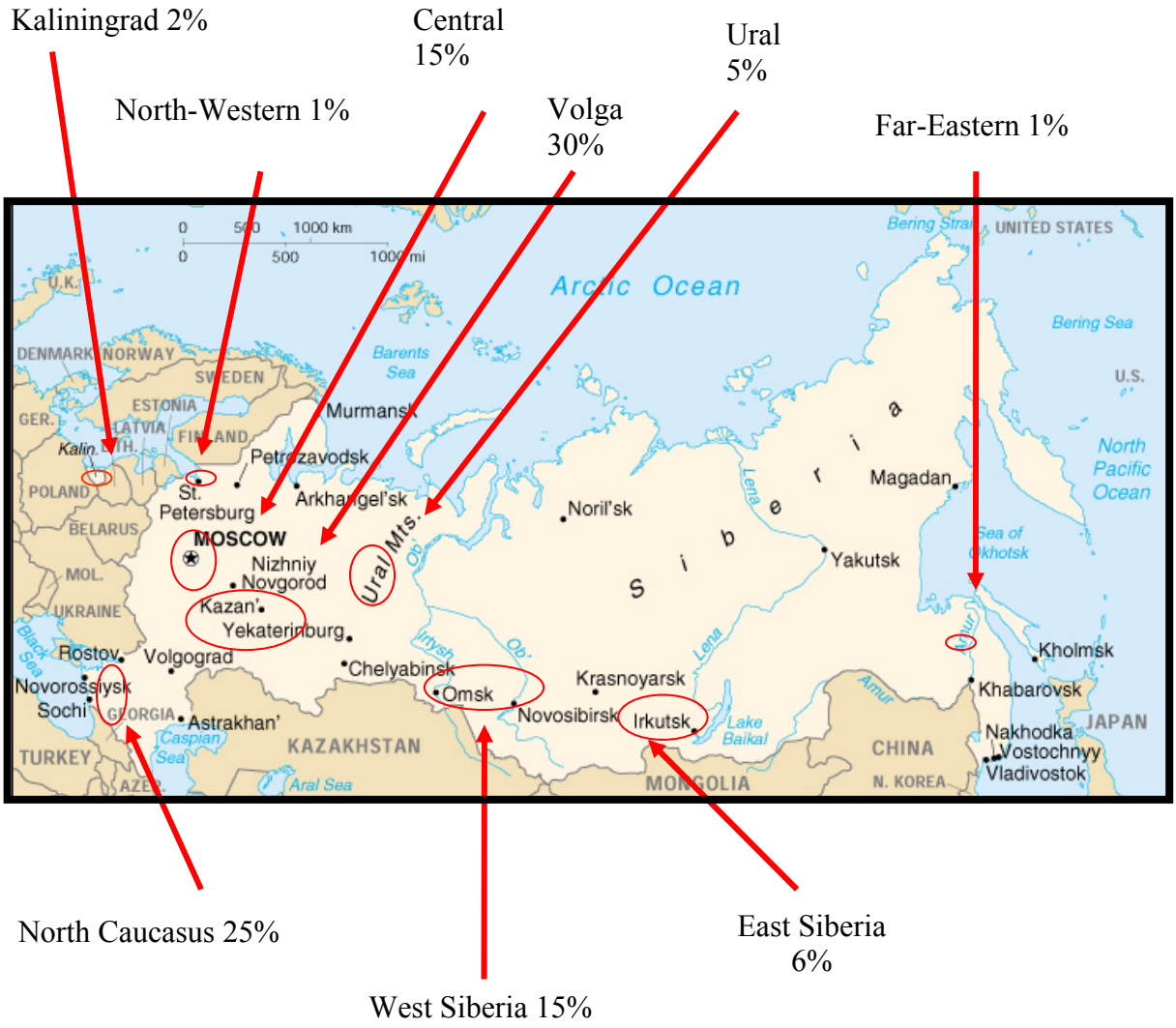


Fig. 1. Main regions of rapeseed cultivation in Russia

Spring oilseed rape is mainly grown in the Central region (85-100% of spring form over winter form), Volga region - 95-99% and Ural - 98-100%. In West and East Siberia only spring rape is cultivated. The yield of spring oilseed rape in 1991-99 years fluctuated between 4.2 to 8.8 dt/ha. The largest yields of spring oilseed rape were in Republic Tatarstan (6.7-10.2 dt/h) and Tumen region (6-10.4 dt/ha).

Between the years 1998 and 1999 the increase in oilseed rape production was as high as 25%. In 1998, oilseed rape was grown on 198.4 thousand ha, whereas in 1999 the area was 247.7 ha. The increase of area of oilseed rape was observed in all regions of Russia. The largest increase of area was in Volga and Central regions and Siberia. The increase in the area of oilseed rape was due to the cultivation of the spring form. The percent area of a spring form in 1998 was 75%, and in 1999 it had increased to 95.2%.

During the last 25 years the yield of spring oilseed rape oscillated between *ca.* 5 and 7 dt/ha. The total production of oilseed rape in 1999 was 135 thousand tons, which was 8% higher than in 1998. The increase of the total production of oilseed rape was due to the

increased cultivation of the spring form. Production in 1999 was 1.6 times higher than in 1998, increasing from 76 thousand tons to 120 thousand tons. In the same time the production of the winter form decreased 3 times (from 49 to 15 thousand tons).

Table 1. Total sowing area and average yields of oilseed rape (spring and winter form) in Russia

Region of rapeseed cultivation	**1986-1990**		**1991-1995**		**1998**	
	thou. ha	**dt/ha**	**thou. ha**	**dt/ha**	**thou. ha**	**dt/ha**
North-Western	2.1	3.9	0.6	4.1	0.1	2.3
Central	54.2	7.6	39.0	4.8	31.9	4.3
Volga	55.3	6.2	60.0	10.8	53.5	6.3
North-Caucasus*	27.4	15.9	28.1	14.5	60.1	10.0
Ural	27.5	4.6	14.8	5.4	11.3	1.8
West-Siberian	50.6	5.2	15.6	9.8	29.7	5.6
East-Siberian	47.4	4.1	17.6	4.1	7.9	3.7
Far-Eastern	1.4	3.7	0.4	2.4	0.1	2.5
Kaliningrad*	3.2	8.2/14.4*	2.2	-/9.7*	3.9	7.0/8.7*
TOTAL	**269.1**	**5.4/15.2***	**178.3**	**5.9/12.1***	**198.5**	**4.2/9.4***

* mainly winter rapeseed

There are very optmistic prognoses that the area of oilseed rape cultivation in Russia can be increased to as much as 2.5-3.0 million hectares (Artiomow 1989, Garejev 1996), however it will mostly depend on the profitability of the crop in relation to other oilseed crops and cereals. The avarege yield of spring rape in Central-Chernozem region around the city of Lipetsk can reach 19 dt/ha (Artiomov *et al.* 2000), and with well balanced nutrition it can be as high as 27 dt/ha (Savenkov and Shevtchenko 1999).

There are several climatic zones in Russia. Main oil crops, like sunflower or soybeans, are very popular and widely grown in warmer climatic conditions, but the area of spring oilseed rape may increase in colder climatic zones.

The production of sowing materials is not regarded as a limitting factor. The regions of oilseed rape cultivation are located in various climatic zones that differ very much in respect to temperature range, rainfall and soil fertility. In result of this, the cultivars of oilseed rape have different characters, appropriate to specific weather and soil conditions. In 2000 there were 46 cultivars of spring oilseed rape officially registered and grown in various regions of Russia and nearly half of them (23) were of Russian origin.

As a consequence of high variation between cultivars and climates, there is also a high variation of pathogens attacking the crop. However, generally, due to low intensity of oilseed rape cultivation the average incidence of diseases is lower than that in other countries with higher intensity of rapeseed production.

Material and methods

The observations of plant healthiness were done for five years (1996-2000) in two regions of the European part of Russia: the North-Western region (near St. Petersburg) and Central region (around Lipeck, *ca.* 600 km south of Moscow). The observations were done semi-systematically every year and the observations concerned all fungal diseases present. Each time the description included disease symptoms on various parts of plants, stage of plant development and disease severity (different scales according to different evaluators).

The causal pathogens were isolated from infected plant tissues and identified. The fungal cultures were placed on several different agar and liquid media and the culture morphology, growth rate, sporulation and/or metabolite production were observed.

Results

In the northern region the most damaging disease noted was clubroot, caused by the fungus *Plasmodiophora brassicae*. Although most of investigated fields were apparently free of disease, some of them were severely contaminated (up to 100 % of plants). The disease was very damaging in 1999. The symptoms were characteristic for the disease; the fungus caused deformation and big tumors on roots (Photo. 1). The disease had a strong effect on seed yield.

Photo 1. Close-up of strong symptoms of *Plasmodiophora brassicae* on spring oilseed rape in the region of St. Petersburg

The second important disease was dark spot caused by fungi from the genus *Alternaria* (mostly *A. brassicae*). The symptoms were observed every year but the disease severity was intermediately high. The symptoms were present on leaves, stems and pods. The spots were of dark brown colour and on leaves they were concentric and surrounded by the yellow halo.

Every year, especially in the spring and early summer, symptoms of *Peronospora brassicae* were observed on older leaves of spring rape plants. The symptoms were present every year, but the severity of disease was rather low.

Other diseases observed were stem rot (*Sclerotinia sclerotiorum*) and root rot. Stem rot caused by *S. sclerotiorum* was observed in lower parts of stems and on root necks of adult plants in a flowering stage. In several cases the symptoms were observed in the rosette stage. The fungal mycelium was visible on the outer parts of the stems and it was easy to disconnect the upper part of such plants from the root system. The symptoms were similar to that of *Rhizoctonia*, but the mycelium was abundant. Due to strong contamination of lower parts of stems it can be deduced that the infection process was started from sclerotia in the soil.

Root rot could be found every year, but the disease severity was not very high. The main effect on plant infection was connected with a high sowing rate. The disease was caused by a complex of different fungal species, mainly from the genus *Fusarium* and *Rizoctonia*. The main *Fusarium* species belonged to *F. avenaceum*, *F. oxysporum*, *F. culmorum* and *F. equiseti* (Gasich and Levitin 2000).

In 1998 a few isolates belonging to *Phoma* sp. were obtained from spring rape plants in the seedling stage. The fungi were isolated from dark brown spots on cotyledons and symptomless hypocotyl. The same plants were also highly damaged by insects from the genus *Psylliodes*. The isolated fungi were cultivated *in vitro* on several agar and liquid media and no melanin pigments were observed. The fungi did not produce sirodesmins characteristic for *Phoma lingam/Leptosphaeria maculans* (Kachlicki *et al.* 2001), so they apparently belong to *Phoma* species other than *P. lingam*.

Grey mould, caused by *Botrytis cinerea* was found every year, but with low intensity. The symptoms were observed on stems and leaves, but the severity of infection was not high. In the stage of green pods it was possible to find plants infected with *Erysiphe cruciferarum,* but the severity of infection was not very strong. The symptoms could be found on stems and leaves, but not on pods. The pods before the harvest were highly contaminated with saprophytes (*Cladosporium* sp., *A. alternata,* etc.).

The graphic presentation showing the spectrum, occurrence and severity of all fungal diseases observed on spring rape in the North-Western region of Russia is shown in Figure 2.

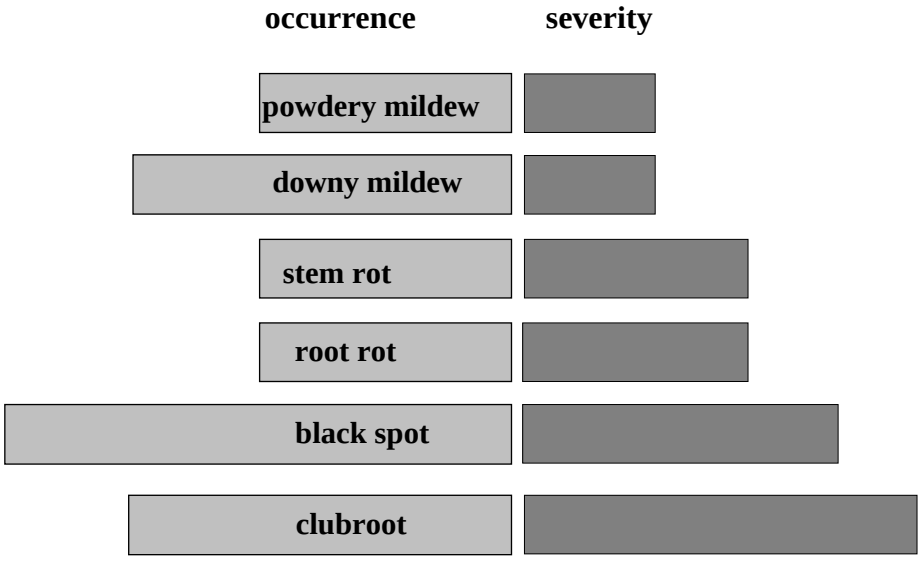


Fig. 2. Diseases of spring oilseed rape in northern Russia (St. Petersburg)

Photo 2. Plants of spring oilseed rape damaged by *Fusarium oxysporum*, observation from Central-Chernozem region around Lipetsk

In the Central region the most damaging disease was Fusarium wilt, caused predominantly by the species *Fusarium oxysporum* (Portenko and Nikonorenkov 1998). The symptoms varied from no germination, through strong retardation of plant growth and development, to wilting and premature drying of plants (Photo.2). There were strong symptoms of tracheomycosis with visible browning of vascular tissues and in many cases abundant sporulation was present on outer parts of the bottom fragments of stems. The bodies contained orange to pink masses of conidiospores. Further observations under the microscope showed numerous macroconidia.

Other diseases, like dark spot or downy mildew were commonly found every year, and they were regarded as intermediately damaging. Dark spot was commonly observed on leaves, stems and pods. The causal agents were identified as *Alternaria brassicae* and *A. brassicicola* (Portenko 1997). The pods of spring rape were highly contaminated by *A. alternata* and other saprophytes. The contamination of leaves by *Peronospora parasitica*, the cause of downy mildew, was quite high, especially in 2000, when spring and early summer were cold and rainy.

Sclerotinia stem rot, clubroot and grey mould did not occur frequently. The symptoms of root rot were predominantly caused by *Rhizoctonia solani*, but fungi from the genus *Fusarium* were also found in the complex (Nikonorenkov *at al*. 1997). During our studies no symptoms of *Phoma lingam/Leptosphaeria maculans* were noticed on plants. The results of the observations of fungal diseases of spring oilseed rape in the Central-Chernozem region near Lipetsk are presented in Figure 3.

In both regions no symptoms of light leaf spot (*Pyrenopeziza brassicae*), white leaf spot (*Pseudocercosporella capsellae*) and Verticillium wilt (*V. dahliae, V. longisporum*) were found on spring rapeseed plants.

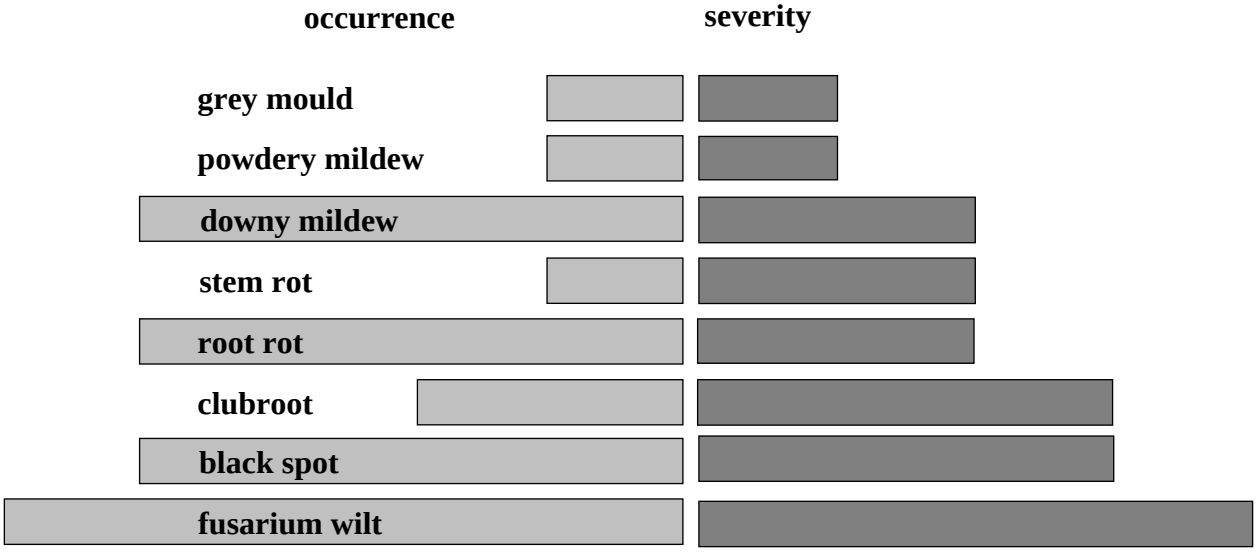


Fig. 3. Diseases of spring oilseed rape in central Russia (Lipetsk)

References

Artiomov I.V. (1998). Raps. Moskva.

Artiomow I.V., Karpachev V.V., Savenkov V.P., Nikonorenkov V.A. (2000). Stan i prognoza uprawy rzepaku w Rosji oraz szczegolne przypadki stosowania nawozow fosforowych i potasowych w rzepaku jarym. *Zbilansowane nawozenie rzepaku – aktualne problemy. Poznan,* 2000: 241-243.

Garejev R.G. (1996). Raps – kultura vysokogo ekonomicheskogo potenciala. Kazan.

Gasich E.L., Levitin M.M. (2000). Micromycetes on oilseed rape in Leningrad region (in Russian). *Proceed. International Meeting on Oilseed Rape. Lipetsk*, 18-20.07.2000: 65-68.

Kachlicki P., Lewartowska E., Jedryczka M., Gasich E.L., Levitin M.M., Bochkaryeva E.B. (2001). Charakterystyka metabolitow grzyba *Phoma lingam* porazających rzepak na terenie Rosji. *Materialy XI Konferencji "Grzyby mikroskopowe – badania genetyczne i molekularne nad patogenami roslin i ich metabolitami", Warszawa,* 3.04.2001: 28-32.

Nikonorenkov V.A., Portenko L.G., Karpachev V.V. (1997). Bolezni rapsa. *Kormo-proizvodstvo,* 1997, vol. 5: 42-44.

Portenko L.G. (1997). Vidovoy sostav vozbuditeley chernoy pjatnistosti rapsa i jego sorodichey v Tsentralnom Chernozemie. *Materiali vserossiskoi konferentsii "Nauchnoje nasledie P.P. Semenova-Tjan-Shanskogo i jego rol v razvitii sovremennoi nauki. Lipetsk*, 1997, vol. 2: 80-81.

Portenko L.G., Nikonorenkov V.A. (1998). Fusarioznoje uvjadanije rapsa. *Mikologija i fitopatologija, B3,* vol. 32: 56-60.

Proizvodstvo semian rapsa i rapsavogo masla w Rossii. Moskva, 1998.

Savenkov V.P., Shevtchenko A.V. (1999). Osobennosti adaptivnogo ispolzovanija mineral-nych udobrienij pod jarowoj raps v uslovijach lesostepi Centralno-Chernozemnogo regiona. *Tes. Dokl. Mezhregion. Konf. Voronez*.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 21 - 29

Biology of *Leptosphaeria maculans* ascospore release in England and Poland

J.S. West[1], M. Jedryczka[2], P.K. Leech[1], S. Dakowska[2], Y.-J. Huang[1], J. M. Steed[1], B.D.L. Fitt[1]

[1] IACR - Rothamsted, Harpenden, AL5 2JQ, UK; [2]Institute of Plant Genetics, 60-479 Poznan, Poland

Abstract: Experiments over three growing seasons in the UK (1997/98-1999/2000) and two seasons in Poland (1998/99-1999/2000) showed that air-borne ascospores of *Leptosphaeria maculans* were mainly present from autumn (September/October) to spring (April/May). Populations of *L. maculans* in the UK and Poland differed from each other; in the UK most isolates belonged to A group (Tox^+), whereas in Poland most isolates belonged to B group (Tox^0). In both countries, ascospores were first released in the autumn, after rain and when the mean daily temperature had decreased to below 15°C. In England, phoma leaf spotting appeared 14-25 days after ascospores were first detected in the autumn; in Poland leaf spotting was uncommon. In 1999/2000 in England, the timing of infection by the two species appeared to differ; leaf lesions in the autumn were predominantly attributed to the A/Tox^+ species, while the proportion of leaf spots attributed to the B/Tox^0 species increased in mid-winter. In England, very low numbers of ascospores were detected on wet days in August and September (<4 spores per m^3 per day). The first spore releases attributed to stubble of the current year's harvest were detected from mid-September to early October, with the majority of spores detected in the period between late October and late December. Ascospores continued to be released throughout the winter, depending on rainfall, but with numbers declining to the background level by late spring. In Poland, the beginning of ascospore release in the autumn was in late September (in 1998) and in mid-October (following a particularly hot, dry summer in 1999). Although most spores were released in the autumn in Poland, the incidence of phoma leaf spot was very low. Ascospores were occasionally detected in low numbers in the winter, when temperatures were oscillating around 0°C and when snow cover was absent. Ascospores were again trapped in large numbers in the spring (in April 1999, late March 2000) and even the summer in Poland (in July 2000). In England, there was a diurnal periodicity in spore release in the absence of rain, with many more spores released during the day, when the temperature was higher than at night. In Poland, most ascospores of *L. maculans* were observed late in the morning, when temperatures were rising, but the humidity was still high. In controlled conditions, most spores were released from mature pseudothecia at 15°C, but many were still released at 20°C and at temperatures as low as 5°C.

Keywords: *Leptosphaeria maculans*, stem canker, ascospore, epidemiology, disease prediction

Introduction

Leptosphaeria maculans is a serious pathogen of oilseed rape (*Brassica napus*) causing blackleg in young plants, and crown cankers and stem lesions in mature plants (West *et al*., 2001). The pathogen survives on crop debris, releasing ascospores (in the autumn in Europe), which infect leaves of oilseed rape plants to produce leaf lesions (phoma leaf spot). The fungus is able to grow biotrophically from the leaf lesions to the stem (Hammond *et al.*, 1985). Early appearance of stem cankers can often lead to the development of severe symptoms by harvest, resulting in yield loss (Sun *et al.*, 2001). However, the pathogen

population has been shown to comprise at least two different species, A or Tox^+ and B or Tox^0, which differ in colony morphology, pigment and toxin production (Koch *et al.*, 1989; Williams & Fitt, 1998). Recently, these groups have been renamed as *Leptosphaeria maculans* and *Leptosphaeria biglobosa*, respectively (Shoemaker & Brun, 2001). However, the B group is a very polymorphic group, probably including more than one species (Rouxel *et al.*, 1994) and the description of *L. biglobosa* is based on a few isolates taken from *Brassica juncea* (Somda *et al.*, 1996). It is not yet clear whether the description *L. biglobosa* covers all of the B-group population so the two groups of *L. maculans* are described as A/Tox^+ and B/Tox^0 in this paper. The B/Tox^0 species is considered to be less damaging; for example, in Canada prior to the introduction of the A/Tox^+ species, yield losses were very slight (Gugel & Petrie, 1992). There are many similarities in the biology of the two species. The population structure of *L. maculans* differs greatly between that in England, which has a mixture of both species, and that in Poland, which for the last several years was predominantly (almost exclusively) the B/Tox^0 species (Jedryczka et al., 1999). This paper reports a comparison of spore release and crop infection in the two countries to try to understand reasons for the differences in population structure and to obtain new information about the biology of *Leptosphaeria maculans*.

Materials and methods

Spore trapping, disease assessment and weather data collection in the UK

Ascospore numbers in the air were estimated from numbers of ascospores counted each day from a Burkard spore sampler (Burkard Manufacturing Company Ltd., Rickmansworth, UK), operated from late August to June in the 1997/98, 1998/99 and 1999/2000 seasons. The sampler was located outdoors at IACR - Rothamsted, and was surrounded by 8 trays, each ≈ 0.5 m^2 and containing ≈100 infected oilseed rape stems, which had been collected at the end of the previous season and kept outdoors.

Disease assessments were made in crops of winter oilseed rape, located ≈2 km from the Burkard spore sampler, in the 1997/98, 1998/99 and 1999/2000 seasons. Four 9 m^2 plots each sown with cultivars Capitol or Lipton in late August, were located in a field of oilseed rape plants (various cultivars) sown at the same time. The NIAB rating for resistance to stem canker for Capitol was 6 and that for Lipton was 5 (Anon., 1997). Areas adjacent to the plots were inoculated each season (in early September) with infected winter oilseed rape stem base debris (*ca*. 200 stems per field), which had been collected at the end of the previous season and kept outdoors. The plots did not receive any fungicide applications but were treated with pesticides and fertiliser according to local commercial practice. Each month 25 plants, sampled at random from each plot, were assessed for incidence and severity of phoma leaf spot in 1997/98 and 1998/99 when lesions caused by the A/Tox^+ and B/Tox^0 species were not differentiated.

In 1999/2000 the incidence of phoma leaf spots caused by A/Tox^+ or B/Tox^0 species of *L. maculans* was assessed separately on plants in these plots (cvs Capitol and Lipton) in December, February, March and April. On each occasion, five plants were taken at random from each plot and lesions on these plants were identified as belonging to the A/Tox^+ or B/Tox^0 species by their visual appearance (Toscano-Underwood *et al.*, 2001). Meteorological data were collected using an automatic meteorological station situated in the same fields as the plots in each season.

Furthermore, in 1999/2000, 32 winter oilseed rape plants (cv. Lipton) with two fully expanded leaves, each growing in a 13 cm diameter plastic pot, were planted in a bed of sand,

outdoors at Rothamsted in September 1999. Debris of oilseed rape plants (≈ 300 stems), collected at harvest in 1999 and incubated in natural conditions, were placed on the surface of the sand around the potted plants to supplement naturally occurring air-borne ascospores of *L. maculans*. The number of leaves bearing lesions attributed to the A/Tox$^+$ or B/Tox^0 species by visual appearance (Toscano-Underwood *et al.*, 2001) were assessed each month from October to February.

Spore trapping, disease assessment, and weather data collection, in Poland

Ascospore numbers in the air were estimated from numbers of ascospores counted each day from a Burkard spore sampler, operated constantly from 28 September 1998. The sampler was located outdoors at the grounds of the Institute of Plant Genetics, PAS. The Burkard spore trap was surrounded by 12 trays, each ≈ 0.25 m^2 and containing about 60-70 infected oilseed rape stems, which had been collected at the end of the previous season and kept outdoors.

Disease assessments were made in crops of oilseed rape, located *c.* 20 km from the Burkard spore sampler. In the 1998/1999 season three 75 m^2 plots were each sown with cultivars Capitol or Lipton in late August. The field experiment in the 1999/2000 season occupied a larger area; it comprised 100 m^2 plots with four replicates of cv. Capitol and Lipton. The plots were inoculated at the end of September each season with debris of winter oilseed rape stems with symptoms of infection by B/Tox^0 *L. maculans*. Random isolations of the causal fungus from stems proved that the debris were contaminated exclusively with B/Tox^0 isolates. The debris had been collected at the end of the previous season and kept outdoors. In September 1998, each individual plot was inoculated with 150 stem fragments (*ca.* 8 cm long), i.e. 2 fragments per 1 m^2. In September 1999 less inoculum was applied than the previous year: 1 stem fragment per 1 m^2, i.e. 100 stems per plot. The sample plots did not receive any fungicide applications but were treated with pesticides and fertilizer according to usual practice in Poland. Each month 25 plants, sampled at random in each sample plot, were assessed for incidence and severity of all diseases present. The last observation (before harvest) was made on 50 plants (1999 harvest), or 100 plants (2000 harvest). The observations from September to March recorded the incidence of leaf spotting in the rosette stage. Observations from April recorded symptoms at successive, adult stages of plant development. The disease incidence relates to stem lesions, evaluated according to a scale from 0 to 9, where 0 is a healthy plant and 9 is a dead plant. Meteorological data were collected using an automatic meteorological station situated about 5 km from the field-site.

Effect of temperature on ascospore release in controlled conditions

Naturally infected stem debris from untreated plots of winter oilseed rape, grown at Rothamsted, was collected after harvest (July 1998) and incubated outdoors for development of pseudothecia. Four sections (2 x 1 cm) of the debris, bearing mature pseudothecia and dried at room temperature, were fixed to 9 cm Petri-dish lids using a layer of petroleum jelly. These Petri-dishes were placed in unlit incubators at 5, 10, 15 or 20°C. After a period of acclimatisation, the stubble sections were sprayed with water, which was at the same temperature as the incubator. After 4 hours, any spores that had been released into the base of the Petri-dish, were suspended in 2.5 ml of aqueous 0.1% Tween and counted using a haemocytometer slide. The experiment was repeated six times, with new stubble sections used each time.

Results

Spore release, leaf spotting and weather in the UK

There was a background level of < 4 spores per m^3/day, in all three seasons, during late spring and summer (attributed to spore release from old stubble, i.e. over one year old). There was an increase in spore numbers in late September/ early October after rain and when the mean temperature fell below 15°C (the first 'new' spore release from pseudothecia on stubble harvested in July). Most spores were released in the period from late October to late December. Spore release generally commenced within one hour of rain following a dry period. There was a diurnal periodicity to release from moist stubble, although the numbers of spores released were very small in the absence of rain. Leaf spotting appeared on oilseed rape leaves 14-25 days after the ascospores were first released and incidence increased to a maximum within a few weeks. In the autumn (October to December) of 1999, the majority of infected leaves had lesions typical of the A/Tox^+ species (Table 1). Lesions associated with the B/Tox^0 species became more frequent in mid-winter (January and February, 2000), although the majority of lesions were caused by the A/Tox^+ species.

In England, the majority of leaf infections were caused by A/Tox^+ *L. maculans* (75% of lesions and 72% of affected leaves). In October, 88% of affected leaves had lesions attributed to A/Tox^+ *L. maculans*, but this figure reduced to only 53% in January, then increased slightly in February. The proportion of A/Tox^+ leaf lesions was 69% and 67% in December and February, increasing to 78% or more from March onwards.

Table 1. Changes in the occurrence of A/Tox^+ and B/Tox^0 species of *Leptosphaeria maculans* in leaves of winter oilseed rape in different months of 1999/2000 at Rothamsted, England.

Month of assessment	Number of lesions on leaves of 20 field-grown plants		Number of leaves with one or more lesions, of each type, on 32 potted plants	
	A/Tox^+	B/Tox^0	A/Tox^+	B/Tox^0
October	–	–	49	7
November	–	–	27	6
December	146	65	42	16
January	–	–	28	25
February	251	124	30	14
March	235	67	–	–
April	236	32	–	–
Total	868 (75.1%)	288 (24.9%)	176 (72.1%)	68 (27.9%)

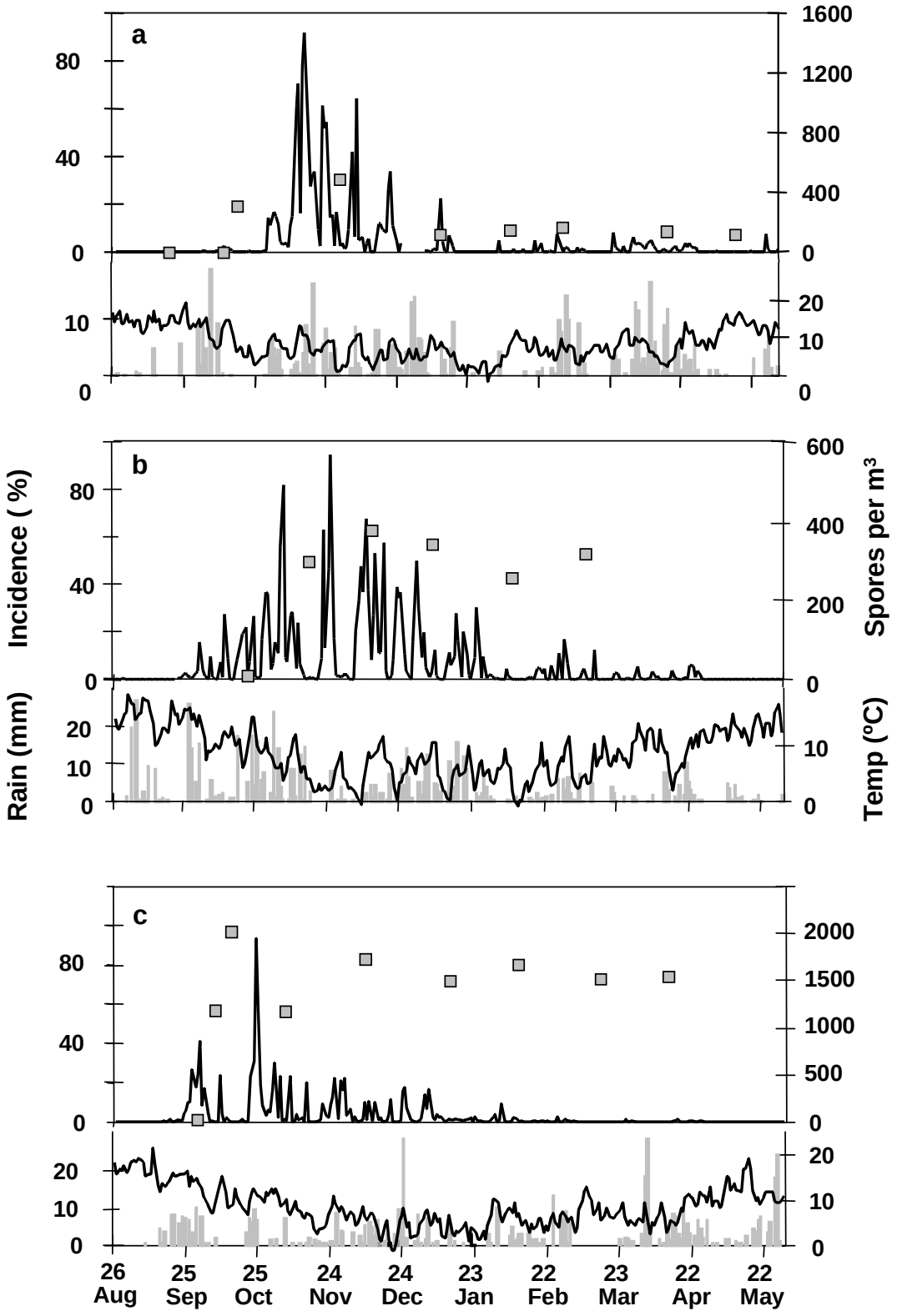


Figure 1. Changes with time in the numbers of airborne ascospores of *Leptosphaeria maculans* (line on upper axis) and incidence (% plants affected) of phoma leaf spots (squares) in untreated winter oilseed rape (cv. Lipton) in relation to daily rainfall (vertical bars) and temperature (line on lower axis) at Rothamsted in 1997/98 (a), 1998/99 (b) and 1999/2000 (c).

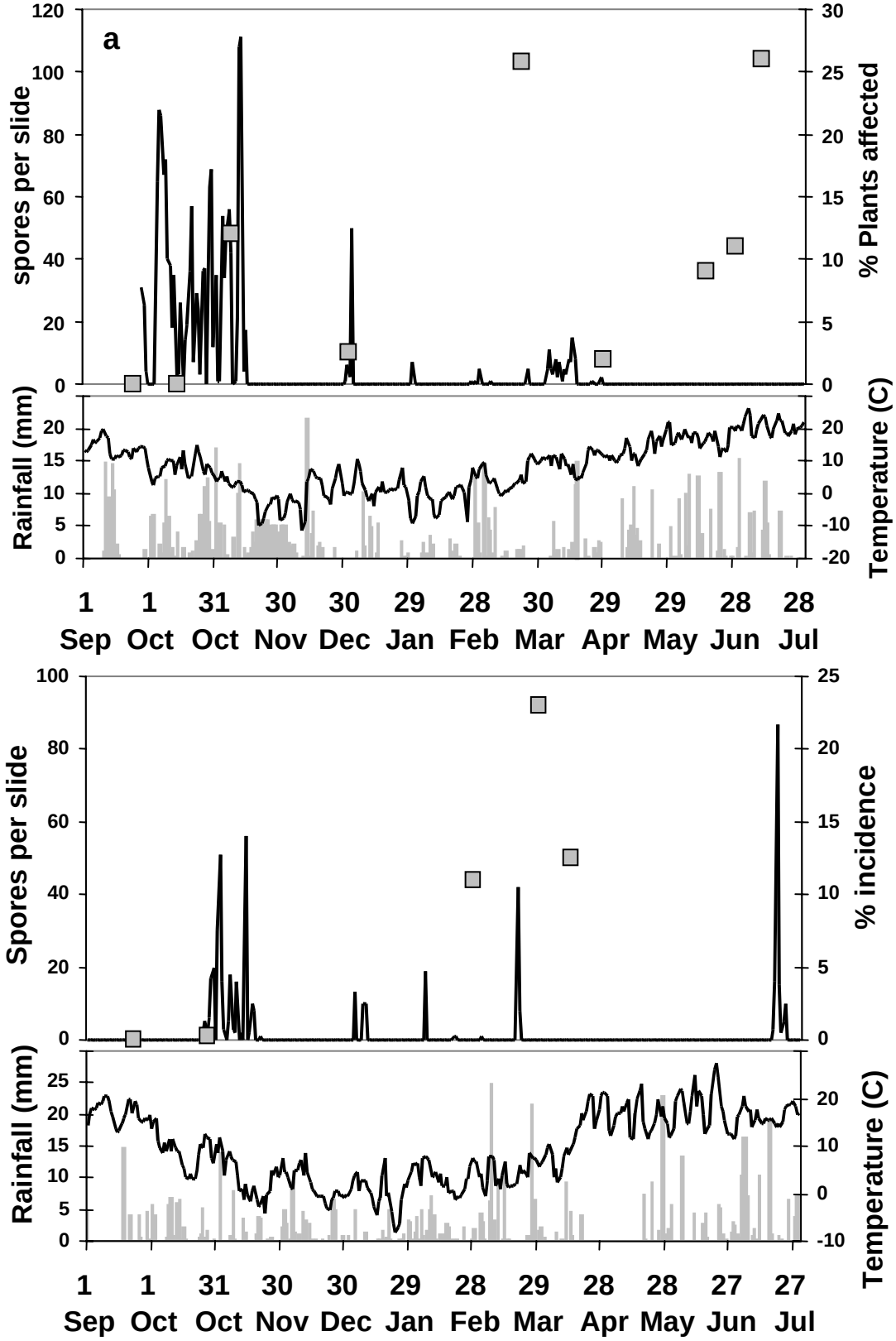


Figure 2: Changes with time in the numbers of airborne ascospores of *Leptosphaeria maculans* (line on upper axis) and incidence (% plants affected) of phoma leaf spots (squares) in untreated winter oilseed rape (cvs Capitol & Lipton) in relation to daily rainfall (vertical bars) and temperature (line on lower axis) at Poznan in 1999/99 (a), 1999/2000 (b).

Maturation of pseudothecia, spore release, leaf spotting and weather in Poland

In Poland, near Poznan (central-west part of the country), ascospores were already present in the air when spore trapping commenced on 28 September 1998. This relatively early spore release led to the appearance of phoma leaf spots (12% of plants affected, cvs. Capitol and Lipton) by 9 November, over one month after spores were first detected. The incidence of affected plants declined during the winter due to the shedding of frost affected leaves. However, the number of plants with phoma leaf spots increased with warmer weather in March 1999. In the following season, ascospores were first detected nearly one month later than the previous season, on 25 October 1999. As a result of late ascospore release, no phoma leaf spots had developed in the autumn, particularly as mean temperatures remained around or below 0°C from 11 November 1999. However, the incidence of phoma leaf spot increased in late winter and early spring, reaching 23% of plants affected by late March. An additional observation carried out in mid-April showed that the number of leaf spots had decreased, due to shedding of old and infected leaves. In both seasons, occasional spore releases during the winter, were associated with mild (above freezing) wet weather.

Effect of temperature on ascospore release in controlled conditions

In controlled conditions, most spores were released (in a 4 hour period) from mature pseudothecia at 15˚C (2.1×10^4), but many were still released at 20˚C (1.0×10^4), 10˚C (1.4×10^4) and at 5˚C (1.0×10^4).

Discussion

These experiments suggest that there are similarities in the seasonal pattern of ascospore release of *L. maculans* in England and Poland. In both countries, ascospores were first released in the autumn, following rain and when the average temperature had dropped to below 15°C. A drop in temperature to below 15°C was also associated with initial ascospore release in France, by Pérès *et al*. (1999). However, it is not clear whether a temperature decrease is a direct factor affecting spore release because controlled environment experiments showed that although more ascospores were released at 15°C, ascospores could be also released from mature pseudothecia at 20°C. Clearly rainfall is required for spore release but the prediction of first spore release has proved to be difficult, probably because many factors influence the maturation of pseudothecia. The numbers of ascospores released in England remained high until a general decline from January onwards, occasionally with a small number of spores released in warmer spring weather. As a result, phoma leaf spots develop in the autumn, and are often present throughout the winter and early spring. In Poland, a similar situation to England occurred in 1998/99 although there was large reduction in spore release and plants affected (visibly) during the cold mid-winter period. It can be presumed from the high numbers of ascospores present in the air at the end of September 1998, that the ascospore release may have started some time earlier. However, in 1999/2000 the late ascospore release, explained by a very long, hot summer of 1999, meant that no visible leaf spots had developed by the onset of freezing weather in November. However, infections are thought to have remained latent during the winter and developed into leaf lesions when warmer weather returned in late winter. In 2000 a large release of ascospores at the end of July may be explained as a result of a late maturation of pseudothecia, the previous autumn. Maturation was not completed by the early onset of winter. The following very warm spring caused drying of inoculum and a further suspension to the release of ascospores.

In England, many of the earliest leaf infections, in the autumn, were caused by the A/Tox$^+$ species rather than by the B/Tox^0 species. Johnson & Lewis (1994) had also shown a predominance of the A/Tox$^+$ species in the earliest leaf infections in the autumn in 1988/89

experiments in Norfolk, England. Toscano-Underwood *et al.* (2001) showed that the time period required for the appearance of symptoms, following infection by ascospores, was similar for the two species. This indicates that differences in the number of infections caused by the two species in the autumn is not due to differences in their rate of development. The B/Tox^0 epidemic in England appeared to increase from December (reaching 30.8% of lesions on leaves and 27.5% of infected leaves) rising to a peak in January (47% of infected leaves). Additionally, West *et al.* (2001) and Thürwächter *et al.* (1999) reported a predominance of the A/Tox$^+$species in isolates taken from the stem base, which presumably derived from early leaf infections. The predominance of the A/Tox$^+$ species in early leaf infections indicates a predominance of the A/Tox$^+$ species in early ascospore releases in the autumn. This could contribute to the major difference in population structure between western Europe, where the A/Tox$^+$ species predominates and where winter oilseed rape is grown, and central and eastern Europe where the B/Tox^0 species is more prevalent, and early infected leaves of winter oilseed rape may be shed due to frost (Jedryczka *et al.*, 1999; Volke, 1999). However, long, warm autumns and milder winters observed in recent years may have caused the recent increase in the A/Tox$^+$ population in Poland (Jedryczka *et al.* 1999; 2001). Recently, predominantly A/Tox$^+$ rather than B/Tox^0 strains were isolated from leaves (Karolewski 1999). Furthermore, in the spring and summer of 2001 very strong symptoms of stem canker were observed on several fields in different regions of Poland. It is presumed that the plants were infected by highly aggressive strains of *L. maculans* (A/Tox$^+$). Isolations are currently being made and identified. It seems that Poland is just experiencing a change of *L. maculans* population, in the direction towards an increasing presence of highly aggressive isolates.

A new simple technique for large-scale identification of ascospores as A/Tox$^+$ or B/Tox^0, by differences in germ-tube growth of germinating ascospores (Huang *et al.*, 2001) could be used to assess changes in the population structure between samples of airborne ascospores taken periodically throughout the autumn, winter and spring. A better understanding of seasonal changes in the population structure of *L. maculans* could improve disease control strategies.

Acknowledgement

This study was funded by the European Union (as part of the IMASCORE project, Fair contract CT96-1669; co-ordinator M-H Balesdent), the Perry Foundation and the UK Department of Environment, Food and Rural Affairs.

References

Anon., 1997. Oilseeds Variety Handbook. National Institute of Agricultural Botany, Cambridge. E & E Plumridge Ltd, Linton, Cambridge. 80pp.

Gugel R.K., Petrie G.A., 1992. History, occurrence, impact, and control of blackleg of rapeseed. *Canadian Journal of Plant Pathology* 14: 36-45.

Hammond K.E., Lewis B.G., Musa T.M., 1985. A systemic pathway for the infection of oilseed rape plants by *Leptosphaeria maculans*. *Plant Pathology* 34: 557-65.

Huang Y.J., Toscano-Underwood C., Fitt B.D.L., Todd A.D., West J.S., Koopmann B., Balesdent M.H., 2001. Effects of temperature on germination and hyphal growth from ascospores of A-group and B-group *Leptosphaeria maculans* (phoma stem canker of oilseed rape). *Annals of Applied Biology* (in press).

Jedryczka M., Kachlicki P., Lewartowska E., 2001. Changes in the population of *Leptosphaeria maculans* in Poland. *GCIRC Technical Meeting, Poznan, 5-7 June, 2001. Abstracts*: 38-39.

Jedryczka M., 2001. Severe stem canker of oilseed rape is present on farmers' fields in Poland. *GCIRC Technical Meeting, Poznan, 5-7 June, 2001. Abstracts*: 40-41.

Jedryczka M., Fitt B.D.L, Kachlicki P., Lewartowska E., Balesdent M.-H., Rouxel T., 1999. Comparison between Polish and United Kingdom populations of *Leptosphaeria maculans*, cause of stem canker of winter oilseed rape. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 106: 608-17.

Jedryczka M., Lewartowska E., Kachlicki P., Walczak F., 1999. Notes on blackleg of oilseed rape in Poland – general information and current research. *Blackleg News* 6: 20-26.

Johnson R.D., Lewis B.G., 1994. Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans*. *Plant Pathology* 43: 269-77.

Karolewski Z., 1999. Variability of *Leptosphaeria maculans* population. *Proceedings Polish Phytopathological Society Congress "Biodiversity in European Plant Pathology at the Turn of the Centuries", Poznan 7-9 September, 1999*: 78.

Koch E., Badawy H.M.A., Hoppe H.H., 1989. Differences between aggressive and non-aggressive single spore lines of *Leptosphaeria maculans* in cultural-characteristics and phytotoxin production. *Journal of Phytopathology* 124: 52-62.

Pérès A., Poisson B., Le Sourne V., Maisonneuve C., 1999. *Leptosphaeria maculans*: Effect of temperature, rainfall and humidity on the formation of pseudothecia. *Proceedings of the 10th International Rapeseed Congress, September 26-29, 1999, Canberra, Australia*.

Rouxel T., Gall C., Balesdent M.-H., 1994. Du polymorphisme au complexe d'espèces: combien d'agents pathogènes sont impliqués dans la nécrose du collet du colza? *Agronomie* 14: 413-32.

Shoemaker R.A., Brun H., 2001. The teleomorph of the weakly aggressive segregate of Leptosphaeria maculans. *Canadian Journal of Botany* 79: 412-419.

Somda I., Renard M., Brun H., 1996. Morphology, pathogenicity and isozyme variation amongst French isolates of *Leptosphaeria maculans* recovered from *Brassica juncea* cv Picra. *Plant Pathology* 45: 1090-1098.

Sun P., Fitt B.D.L., Steed J.M., Toscano-Underwood C., West J.S., 2001. Factors affecting development of phoma canker (*Leptosphaeria maculans*) on stems of winter oilseed rape (*Brassica napus*) in southern England. *Annals of Applied Biology* (in press).

Toscano-Underwood C., West J.S., Fitt B.D.L., Todd A.D., Jedryczka M., 2001. Development of phoma lesions on oilseed rape leaves inoculated with ascospores of A-group or B-group *Leptosphaeria maculans* (stem canker) at different temperatures and wetness durations. *Plant Pathology* 50: 28-41.

Thürwächter F., Garbe V., Hoppe H.H., 1999. Ascospore discharge, leaf infestation and variations in pathogenicity as criteria to predict impact of *Leptosphaeria maculans* on oilseed rape. *Journal of Phytopathology* 147: 215-22.

Volke B., (1999) *Leptosphaeria maculans*, der Erreger der Wurzelhals- und Stengelfäule an Raps: Verbreitung verschiedener Pathogenitätsgruppen in Europa, Quantifizierung des Befalls und Schadwirkung im Freiland. *PhD Thesis, University of Göttingen, Germany*, 116 pp.

West J.S., Kharbanda P.D., Barbetti M.J., Fitt B.D.L., 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathology* 50: 10-27.

Williams R.H., Fitt B.D.L., 1999. Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of winter oilseed rape. *Plant Pathology* 48: 161-75.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 31 - 42

Effects of temperature and incubation time on germination of ascospores of *Leptosphaeria maculans* and *Leptosphaeria biglobosa in vitro*

Yongju Huang[1], Bruce Fitt[1], Claudia Toscano-Underwood[1], Alan Todd[1], Jonathan West[1], Avice Hall[2], Malgorzata Jedryczka [3]

[1] IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK; [2] University of Hertfordshire, College Lane, Hatfield, Hertfordshire, AL10 9AB, UK; [3] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479, Poznan, Poland

Abstract: Ascospores of both *L. maculans* and *L. biglobosa* germinated on water agar at temperatures from 5 to 20°C. After 2 h of incubation on water agar, some *L. maculans* ascospores had germinated at 10 to 20ºC and some *L. biglobosa* ascospores had germinated at 5 to 20ºC. The percentages of both *L. maculans* and *L. biglobosa* ascospores that had germinated after 24 h of incubation increased with increasing temperature from 5 to 20ºC. The observed time (Vo_{50}) which elapsed from inoculation until 50% of the spores had germinated was shorter for *L. biglobosa* than for *L. maculans* ascospores. Germ tube length increased with increasing temperature for both ascospore groups. Germ tubes from *L. biglobosa* ascospores were longer than germ tubes from *L. maculans* ascospores at all temperatures tested, but the mean diameter of germ tubes from *L. maculans* ascospores (1.8 µm) was greater than that of those from *L. biglobosa* ascospores (1.2 µm) at 15 and 20ºC. The average number of germ tubes produced from *L. maculans* ascospores (3.8) was greater than that from *L. biglobosa* ascospores (3.1) after 24 h of incubation at 20ºC. Germ tubes originated predominantly from interstitial cells of *L. maculans* ascospores or terminal cells of *L. biglobosa* ascospores. Hyphae from *L. maculans* ascospores grew tortuously, whilst those from *L. biglobosa* ascospores grew in almost straight lines.

Key words: *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, ascospores, germination, water agar

Introduction

Phoma stem canker or blackleg of brassicas, caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not., is considered to be a damaging disease of oilseed rape worldwide. Disease surveys and detailed experiments show that the population of *L. maculans* can be divided into at least two main sub-groups, which are often termed A and B groups (Johnson & Lewis, 1994; Williams & Fitt, 1999) and have now been named as *Leptosphaeria maculans* and *Leptosphaeria biglobosa* (Shoemaker & Brun, 2001). *L. maculans* produces large pale leaf lesions with pycnidia in autumn and then forms basal stem lesions (stem cankers) in spring and summer, causing considerable economic losses in oilseed rape production. In the UK, *L. biglobosa* tends to produce small dark leaf spots bearing few or no pycnidia and then causes lesions on upper stems, rather than basal stem canker, in spring and summer. *L. biglobosa* is considered to be less damaging than *L. maculans* in the UK (Johnson & Lewis, 1994; West *et al*., 2001).

Epidemics of phoma stem canker are initiated by air-borne ascospores released from infected debris (McGee, 1977; Gladders & Musa, 1980; Schramm & Hoffmann, 1991). Results from detailed studies on the development of phoma leaf spot lesions on oilseed rape plants artificially inoculated with ascospores of *L. maculans* or *L. biglobosa* showed that ascospores of both species were able to infect oilseed rape leaves and produce lesions at

temperatures from 5 to 20ºC (Biddulph *et al*., 1999; Toscano-Underwood *et al*., 2001). Temperature affected the incubation period (estimated as the time from inoculation to the appearance of the first lesions) of both *L. maculans* and *L. biglobosa*. The results suggested that the incubation period (which includes germination, infection, colonisation, production of lesions) of *L. biglobosa* may be shorter than that of *L. maculans*.

However, little is known about the comparative germination of ascospores of the two groups before the infection process occurs. In comparisons of the germination of conidia and ascospores of *L. maculans* at different temperatures, ascospores germinated and produced germ tubes within the first 8 h of incubation at 4 to 28ºC, whereas conidia did not do so until after 24 h at 12 to 24ºC (Wittern & Krüger, 1985). However, the ascospores or conidia used in their studies were not identified as being of *L. maculans* or *L. biglobosa*. Petrie (1988) differentiated *L. maculans* and *L. biglobosa* on the basis of the length of germ tubes produced from conidia. Hitherto, no comparable work has been done on the germination of ascospores of *L. maculans* and *L. biglobosa*, although the germination of ascospores is normally an important component of the process of infection of oilseed rape leaves by this pathogen (West *et al*., 2001). This paper reports the effects of temperature on germination parameters of ascospores of *L. maculans* and *L. biglobosa*.

Materials and Methods

Three experiments investigated ascospore germination in temperature-regulated incubators (5, 10, 15 or 20ºC). Three agar slides were used for each temperature/incubation time tested. Prior to inoculation, all agar slides were pre-conditioned overnight at the desired temperatures. Petri dishes containing inoculated agar slides (one slide per dish) were incubated in darkness at these constant temperatures until ascospores had germinated. Petri dishes with *L. maculans* or *L. biglobosa* ascospores were arranged in completely randomised patterns in each incubator (temperature treatment). Temperature treatments were replicated in time (three experiments). Incubators were allocated at random for each experiment whenever possible, but some incubators could not be operated at the lower temperatures (5ºC and 10ºC). The temperatures in the incubators were monitored throughout the experiments and varied by ± 1ºC.

Production of ascospores

Pieces (*c*. 30 - 50 cm in length) of winter oilseed rape stem debris (cv. Lipton) showing stem canker lesions were collected after harvest from fields at Rothamsted, UK and Poznan, Poland in July/August 1999. These stem pieces from Rothamsted or Poznan were expected to produce ascospores which were predominantly of *L. maculans* or *L. biglobosa*, respectively (Jedryczka *et al.*, 1999). Stem pieces containing mature pseudothecia were stored dry at -5ºC until required. The identity of the ascospores used (*L. maculans* or *L. biglobosa*) had been previously confirmed by Toscano-Underwood *et al.* (2001). UK stem pieces from the tap root and stem base (< 10 cm above ground level) had been found to produce almost entirely *L. maculans* ascospores, but pieces from upper stems (10 - 30 cm above ground level) produced a mixture of *L. maculans* and *L. biglobosa* ascospores. Consequently, only the stem base and tap root region of UK stem debris were used for producing *L. maculans* ascospores. This Polish debris had been previously found to produce only *L. biglobosa* ascospores (Toscano-Underwood *et al.*, 2001) and was therefore used as a source of *L. biglobosa* ascospores (as it would not have been possible to obtain pure *L. biglobosa* ascospores from UK debris).

Fresh ascospore suspensions were used as inoculum in controlled environment experiments. To obtain ascospores, 3 - 4 cm lengths of stem bearing mature *L. maculans* or *L.*

biglobosa pseudothecia were attached to the underside of 9 cm diameter Petri dish lids with petroleum jelly. The pieces of stem were immersed in distilled water for 30 s to swell the asci, then the water was poured off and the lid placed over the Petri dish base. After a period of 3 h at 20ºC, large numbers of ascospores had been discharged into the dishes. Sterile distilled water (2.5 mL) was added to each Petri dish and the spores were dislodged using a sterile wooden spatula. Ascospore suspensions from several dishes were combined and the concentration of spores adjusted to 5 x 10^3 ascospores mL^{-1} using a haemocytometer.

Inoculation of agar slides

0.5 – 0.7 mL of molten distilled water agar (1.5%) (Oxoid, Basingstoke, UK) were placed in the centre of each glass slide (75 x 25 mm, previously heated to 55ºC) using a glass Pasteur pipette. The agar was spread uniformly to form a continuous layer of dimensions *c.* 4 x 1.6 cm. The agar slides were then placed in Petri dishes (diameter 9 cm) containing two layers of filter paper (Whatman no.1; Whatman International Ltd., Maidstone, UK) moistened with distilled water. Agar slides were inoculated by applying two drops (*c.* 60 µL) of fresh ascospore suspension (containing 5 x 10^3 ascospores mL^{-1}) onto each slide using a glass Pasteur pipette. After inoculation, all slides were returned to the Petri dishes lined with moistened filter paper and the insides of the dishes were sprayed with distilled water to maintain 100% relative humidity. The dishes were then placed in incubators at the desired temperatures and germination parameters were measured after incubation times of 2, 4, 6, 8, 10, 12, 14 and 24 h.

Assessment of ascospore germination

After the designated incubation time, agar slides were removed from the incubators and three drops of tryphan blue (0.1% w/v in lactophenol; BDH Microscopial Reagents, Poole, UK) were spread onto the surface of each slide to stop germination. Assessments were done using a light microscope at 250x magnification. The percentages of ascospores of *L. maculans* or *L. biglobosa* which had germinated were determined by traversing the full length of each slide and assessing the first 100 ascospores observed (except in experiment 1, when all ascospores deposited on each slide were assessed). An ascospore was considered to have germinated if the length of the germ tube exceeded the width of the ascospore (*c.* 5 µm). The lengths of the germ tubes of 20 *L. maculans* or *L. biglobosa* ascospores per slide were also assessed, for each temperature and incubation time tested. If the ascospore had produced more than one germ tube, only the length of the longest was measured.

The positions of the germ tubes (i.e. whether they originated mainly from terminal or from interstitial cells) were observed at all temperatures. As an example, the numbers of germ tubes originating from the terminal cells or interstitial cells of the *L. maculans* or *L. biglobosa* ascospores after 4, 10 and 24 h of incubation at 20ºC were counted. This temperature was used for these assessments as the percentages of both *L. maculans* and *L. biglobosa* ascospores which germinated were greatest at temperatures from 15 to 20ºC. The diameter of *L. maculans* or *L. biglobosa* germ tubes (assessed at 1000x magnification) and the total number of germ tubes per ascospore were assessed after 8 and 24 h of incubation, respectively, at 20ºC. The patterns of hyphal growth from *L. maculans* or *L. biglobosa* ascospores were observed at all temperatures and incubation times; to illustrate these patterns, they were photographed after 4, 14 and 24 h of incubation at 20ºC.

Statistical analysis

The maximum percentage of ascospores that germinated (*Go*) for each experiment and treatment was obtained from the observed data. The data were then normalised as a percentage *Gn* of *Go*. The observed time required for 50% of viable ascospores to germinate, Vo_{50}, was calculated by linear interpolation from the points just above or just below 50%. The average observed rate of germination (ro_c) over the range 5% < *Gn* < 95% was obtained

by linear regression. Analyses of variance were used to assess the effects of temperature and ascospore group on the three observed parameters, using the Genstat statistical software (Payne *et al*., 1993). To estimate the time to the germination of 50% of viable ascospores (Ve_{50}) and the rate of germination at Ve_{50} (re_{50}) for each temperature and ascospore group, a generalised linear model with a binomial distribution and a logit link function ($\log_e$ (Gn / (100 – Gn)) (logistic regression) with time as the explanatory variable was also fitted to the data.

Linear regressions of observed germ tube length on time (up to 12 h) were calculated separately for each temperature and ascospore group and analyses of position and parallelism were done to assess whether the data were best fitted by a single line or series of parallel lines or series of non-parallel lines for different temperatures and ascospore groups. The data for the total number of germ tubes per ascospore and diameter of germ tubes were analysed by analyses of variance. The data for the position of germ tubes emerging from the ascospores were analysed using a split-plot design with position as a sub-plot.

Results

Effects of temperature on ascospore germination in vitro

Ascospores of both *L. maculans* and *L. biglobosa* germinated at temperatures from 5 to 20ºC on distilled water agar by producing germ tubes. After 2 h of incubation, >5% of *L. maculans* ascospores had germinated at 10 to 20ºC and >5% of *L. biglobosa* ascospores had germinated at 5 to 20ºC (Fig. 1). At all temperatures, the percentage germination had almost reached its maximum after 14 h of incubation. The percentage germination of both *L. maculans* and *L. biglobosa* ascospores was greatest at 20ºC after 24 h of incubation (89% and 95% of the *L. maculans* and *L. biglobosa* ascospores, respectively, had germinated). The mean maximum percentage germination (*Go*) was greater for *L. biglobosa* ascospores (87.7%) than for *L. maculans* ascospores (77.4%) (Table 1, SED 3.45; $P < 0.01$; 14df).

Table 1. Maximum percentage germination (*Go*), time to 50% germination ($Vo_{50,}$ Ve_{50}) and rate of germination ($ro_{c,}$ re_{50}) of ascospores of *L. maculans* or *L. biglobosa* germinating on distilled water agar slides in darkness at 5, 10, 15 and 20ºC. Observed values (Vo_{50} , ro_c) and values estimated from regression of percentage germination on incubation time ($Ve_{50,}$ re_{50})

Temp (ºC)	Species	Germination parameter				
		Go	Vo_{50}	Ve_{50}	ro_c	re_{50}
5	*L. maculans*	65.2	8.9	8.3	7.0	8.5
	L. biglobosa	78.2	7.3	7.4	6.1	7.7
10	*L. maculans*	75.4	7.7	7.2	8.0	10.3
	L. biglobosa	85.7	5.0	5.3	7.0	11.2
15	*L. maculans*	79.8	5.7	5.7	6.6	10.0
	L. biglobosa	91.8	3.8	3.9	6.7	13.2
20	*L. maculans*	89.2	6.8	6.2	7.2	10.1
	L. biglobosa	95.3	3.3	3.4	7.7	17.5
SED		6.90	0.83	0.60[b]	1.18	1.85
(df)		(14)	(14)	(128)	(14)	(128)

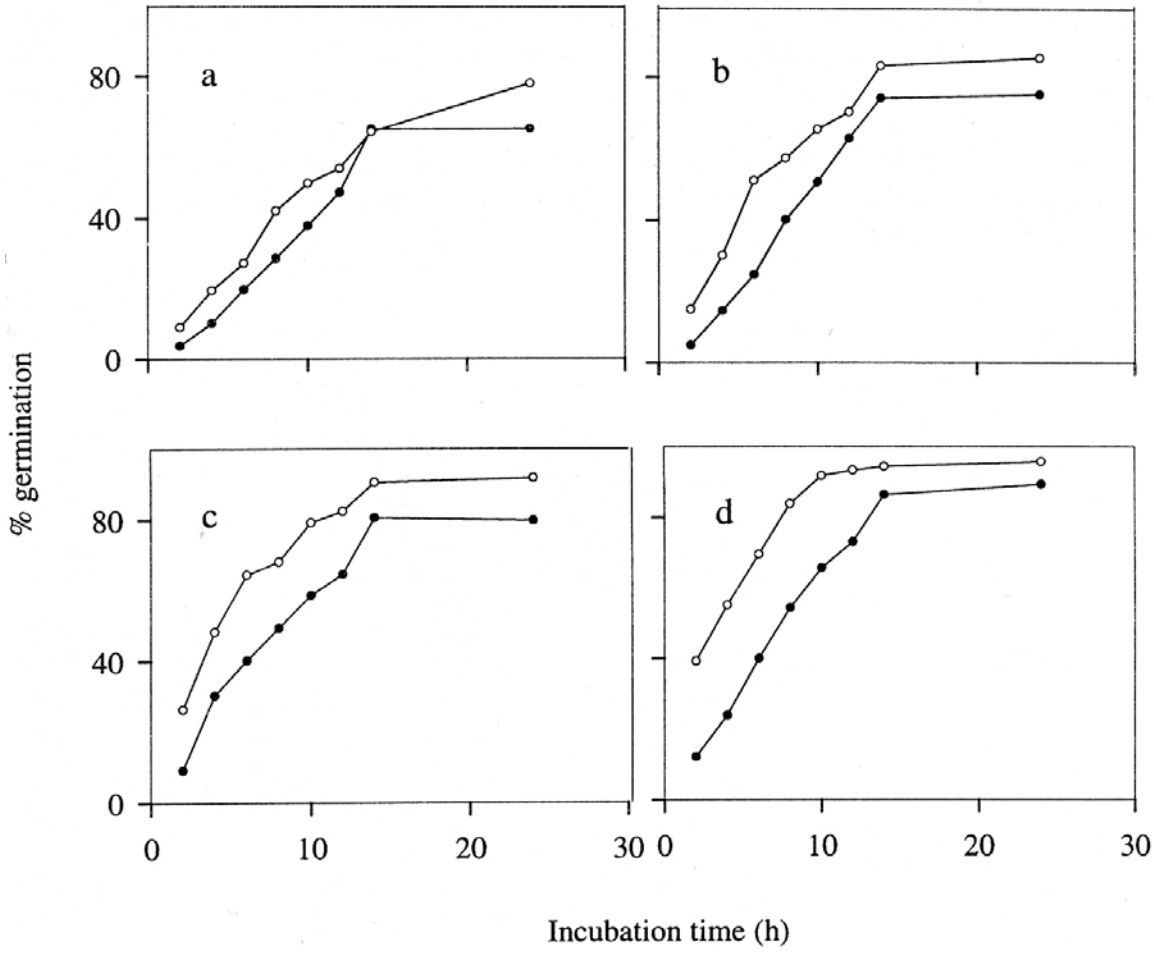


Figure 1. Changes with incubation time in the percentages of ascospores of *L. maculans* (●) or *L. biglobosa* (○) which had germinated on distilled water agar in darkness at temperatures of 5ºC (a), 10ºC (b), 15ºC (c) or 20ºC (d). Data points illustrated are averages from three experiments.

Effects of temperature on the percentage of ascospores that germinated were similar for both *L. maculans* and *L. biglobosa* ascospores. The percentages of both *L. maculans* and *L. biglobosa* ascospores that germinated after 24 h increased with increasing temperature from 65% (*L. maculans*) or 78% (*L. biglobosa*) at 5ºC to 89% (*L. maculans*) or 95% (*L. biglobosa*) at 20ºC (Fig. 1; Table 1). For both *L. maculans* and *L. biglobosa* ascospores, the observed times (Vo_{50}) which elapsed from inoculation until 50% of the spores (i.e. proportions normalised) had germinated generally decreased with increasing temperature, but Vo_{50} was shorter at 15ºC than at 20ºC for *L. maculans* ascospores. Vo_{50} was shorter for *L. biglobosa* than for *L. maculans* ascospores at all temperatures tested (the mean values of Vo_{50} for *L. maculans* and *L. biglobosa* ascospores were 7.3 and 4.8, respectively; SED 0.41; $P < 0.001$; 14df). The observed rate of germination (ro_c) did not differ significantly between temperatures or ascospore groups.

The logistic regression analyses generally confirmed the observed results. Regression curves fitted well to the normalised data for changes in the percentage germination of ascospores on agar with time (h) after inoculation with *L. maculans* or *L. biglobosa* ascospores. The curves differed significantly (deviance ratio 36.0; 47df and 128df; $P < 0.001$)

between the *L. maculans* and *L. biglobosa* and between different temperatures (Fig. 2). The estimated times from inoculation to 50% germination (Ve_{50}) generally decreased with increasing temperature for both *L. maculans* and *L. biglobosa* ascospores, but Ve_{50} was shorter at 15ºC than at 20ºC for *L. maculans* ascospores. The estimated rate of germination at 50% (re_{50}) increased with increasing temperature for *L. biglobosa* but not for *L. maculans* ascospores (Table 1). The re_{50} values for *L. biglobosa* ascospores at 15ºC and 20ºC were greater than the ro_c values.

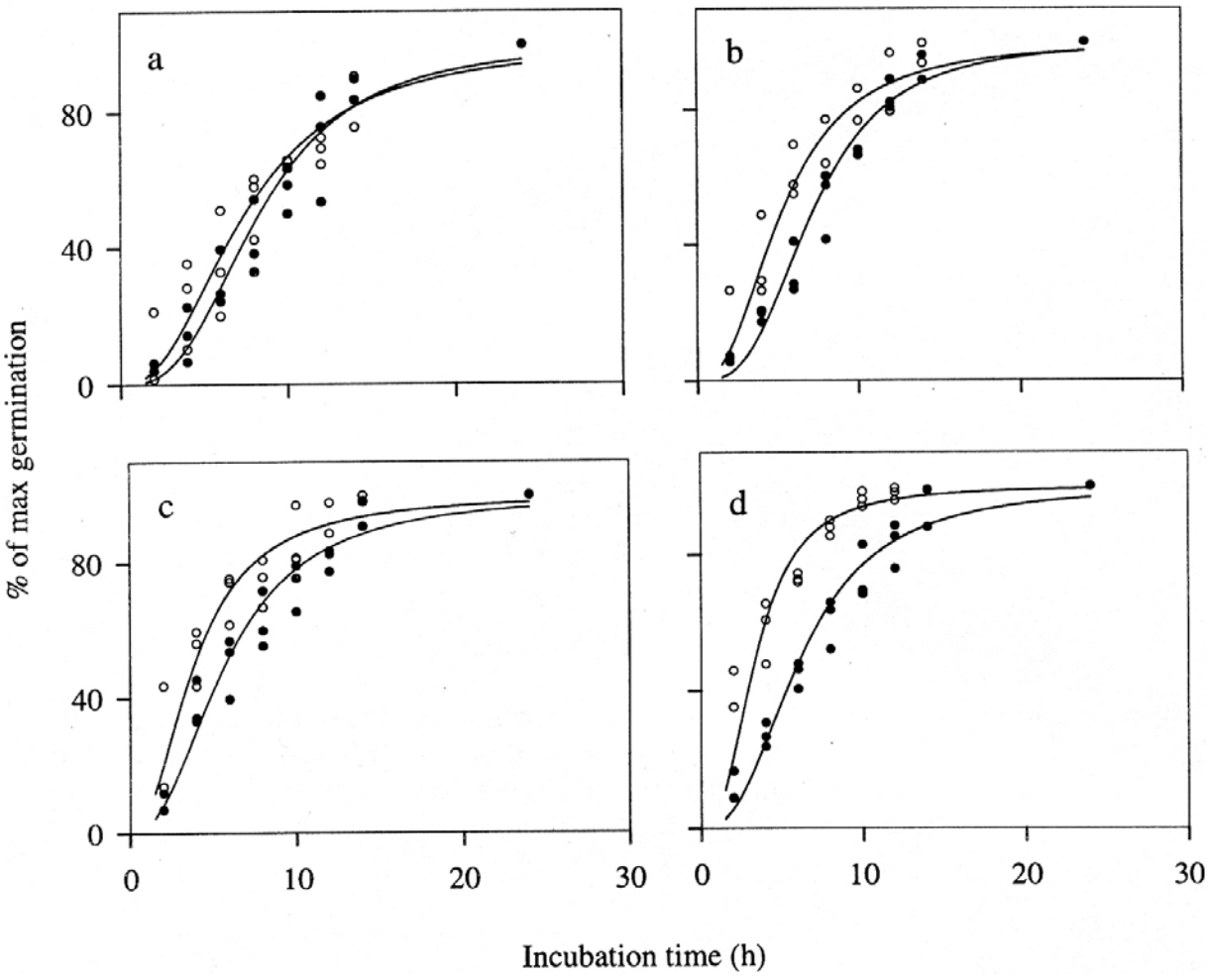


Figure 2. Changes with incubation time in the percentages of ascospores of *L. maculans* (●) or *L. biglobosa* (○) germinating on distilled water agar in darkness at temperatures of 5ºC (a), 10ºC (b), 15ºC (c) or 20ºC (d). Observed data from three experiments were normalised as a percentage (*Gn*) of the maximum number of ascospores which germinated (*Go*) for each experiment and treatment (Table 4). A generalised linear model with a binomial distribution and logit link function ($\log_e (Gn/(100 - Gn))$) was used to calculate the regression curves. Back-transformed curves are illustrated.

Effects of temperature on germ tube elongation in vitro

Germ tube length increased with increasing temperature from 5 to 20ºC for both *L. maculans* and *L. biglobosa* ascospores (Fig. 3). At 5 and 10ºC, germ tubes from *L. maculans* ascospores

grew slowly, reaching average lengths of *c.* 19 and 28 µm, respectively, after 24 h. At 15ºC, germ tubes grew rapidly after 14 h to an average length of *c.* 60 µm at 24 h. Germ tubes grew steadily at 20ºC, reaching *c.* 47 µm in length after 14 h. After 14 h of incubation, germ tubes had branched extensively, forming tortuous hyphae for which lengths were difficult to measure. Germ tubes from *L. biglobosa* ascospores grew slowly at 5ºC, reaching *c.* 21 µm in length after 24 h. At temperatures above 10ºC, elongation was rapid and after 12 h at 20ºC germ tubes had grown to an average length of 131 µm. At 15 and 20ºC, after 12 h of incubation germ tubes were too long to be measured.

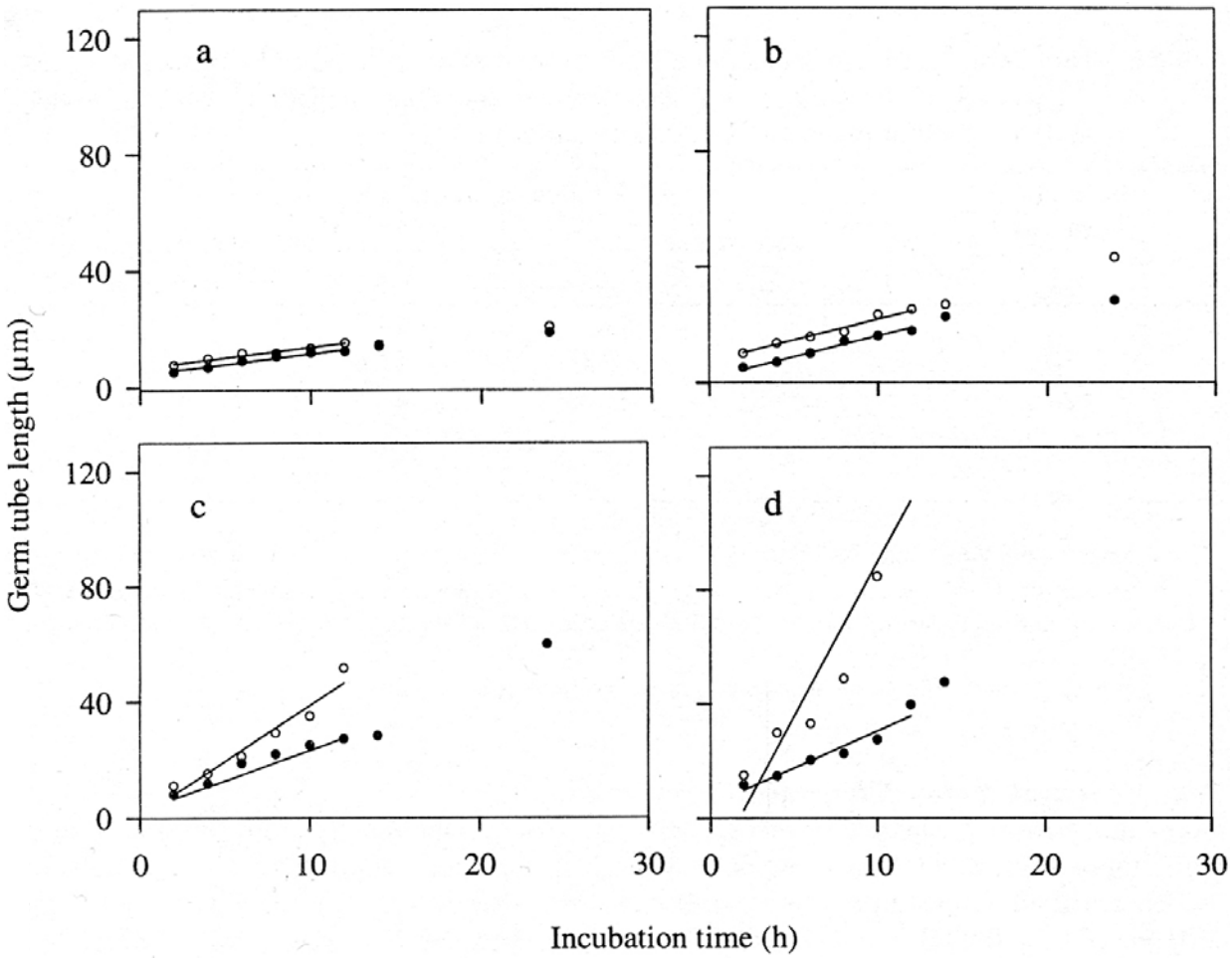


Figure 3. Changes with incubation time (*t*) in the length of germ tubes (*l*) produced from ascospores of *L. maculans* (●) or *L. biglobosa* (○) germinating on distilled water agar in darkness at temperatures of 5ºC (a), 10ºC (b), 15ºC(c) or 20ºC (d). Data points illustrated are averages from three experiments. Regression lines for the *L. maculans* and *L. biglobosa* (over the period up to 12 h) are: $l = 4.35 + 0.71t$ and $l = 6.60 + 0.71t$ at 5ºC (lines parallel); $l = 1.46 + 1.45t$ and $l = 7.26 + 1.45t$ at 10ºC (lines parallel); $l = 2.42 + 2.09t$ and $l = 0.42 + 3.83t$ at 15ºC (lines not parallel); $l = 4.72 + 2.58$ and $l = -18.71 + 10.81t$ at 20ºC (lines not parallel).

Linear regressions fitted well to the data for increase in germ tube length with time up to 12 h after inoculation of agar with ascospores (after which it was not possible to measure germ tube lengths accurately) and accounted for >80% of the variance. The combined linear

regression analyses of position and parallelism suggested that the data were fitted best by a series of different lines. Subsequent analyses were therefore done separately for each temperature. The percentages of the variance accounted for were 84%, 83%, 91% and 88% for temperatures of 5, 10, 15 and 20ºC, respectively. The data for temperatures of 5 and 10ºC were fitted best by two pairs of parallel lines for *L. maculans* and *L. biglobosa* ascospores, respectively. At temperatures of 15 and 20ºC, data were best fitted by two non-parallel lines (Fig. 3). Germ tubes from *L. biglobosa* ascospores were longer than germ tubes from *L. maculans* ascospores at all temperatures tested but the rate of increase in length was greater only at 15ºC and 20ºC ($P < 0.001$).

Table 2. Number of germ tubes emerging from interstitial or terminal cells; mean values for ascospores of *L. maculans* or *L. biglobosa* germinating on distilled water agar after 4,10 and 24 h of incubation at 20ºC in darkness

Incubation time (h)	Number of germ tubes [a]			
	Interstitial cells		Terminal cells	
	L. maculans	*L. biglobosa*	*L. maculans*	*L. biglobosa*
4	38.2	8.8	18.7	50.5
10	57.8	18.3	23.8	56.8
24	68.5	33.2	40.5	59.3
SED (df)	2.58 (59) 2.54 (30)[b]			

[a] Two experiments were done on distilled water agar. In each experiment, a total of 90 ascospores of *L. maculans* or *L. biglobosa* (30 ascospores from each of three slides) were observed for each temperature and incubation time. Mean values calculated from the data for the two experiments are presented.

[b] For comparing within the same ascospore group and time combination.

Patterns of hyphal growth from ascospores in vitro

Germ tubes from *L. maculans* ascospores originated predominantly from interstitial cells while those from *L. biglobosa* ascospores originated predominantly from terminal cells of ascospores at all temperatures observed. For example, after 4 h at 20ºC there were significant differences ($P < 0.001$) in the position of germ tubes between *L. maculans* and *L. biglobosa* ascospores (Table 2; Fig. 4a, b). There was an interaction between ascospore group, position of germ tube and incubation time. As the incubation time increased, the number of germ tubes emerging from both interstitial and terminal cells of *L. maculans* ascospores increased. For the *L. biglobosa* ascospores, the number of germ tubes emerging from interstitial cells increased with increasing incubation time while the number of germ tubes emerging from the terminal cells of ascospores did not (Table 2).

The patterns of hyphal growth differed noticeably between ascospores of *L. maculans* and *L. biglobosa* at all temperatures tested. Hyphae from *L. maculans* ascospores grew tortuously and branched extensively at all temperatures, for example after 14 h (Fig. 4c) or 24 h (Fig. 4e) of incubation at 20°C. By comparison, *L. biglobosa* ascospores produced long hyphae that grew in almost straight lines at all temperatures, for example after 14 h (Fig. 4d) or 24 h (Fig. 4f) of incubation at 20°C. The average number of germ tubes produced from *L. maculans* ascospores (3.8) was greater than the average number of germ tubes from *L.*

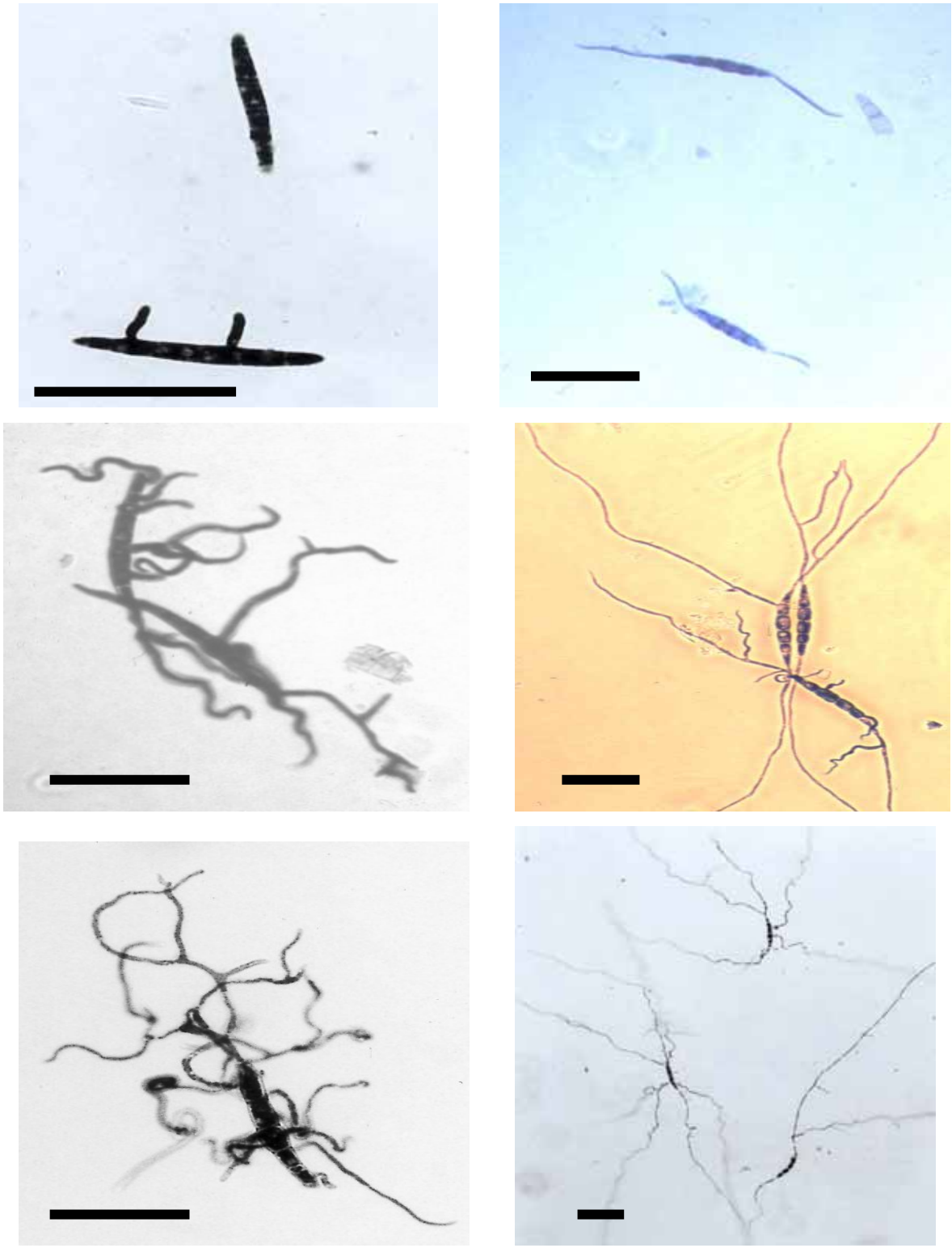


Figure 4. Patterns of germination and hyphal growth from *L. maculans* (a, c, e) (bars = 37µm) or *L. biglobosa* (b, d, f) (bars = 35µm) ascospores obtained from oilseed rape debris from field experiments at Rothmasted or Poznan, after incubated for 4h (a, b), 14h (c, d) and 24h (e, f) on distilled water agar in darkness at a temperature of 20°C

biglobosa ascospores (3.1) (SED 0.10; $P < 0.05$; 474df) after 24 h of incubation at 20ºC (Table 3). After 8 h of incubation at 15 or 20ºC, when more than 50% of both *L. maculans* and *L. biglobosa* ascospores had germinated, there were significant differences in diameter between *L. maculans* and *L. biglobosa* germ tubes. The mean diameters of germ tubes from *L. maculans* and *L. biglobosa* ascospores were 1.8 and 1.2 μm, respectively (SED 0.05; $P < 0.001$; 156df).

Table 3. Numbers of ascospores of *L. maculans* and *L. biglobosa* germinating on distilled water agar which had produced one to seven germ tubes after 24 h of incubation at 20ºC in darkness

Number of germ tubes	Number of ascospores[a]	
	L. maculans	*L. biglobosa*
1	4	0
2	32	81
3	71	84
4	62	55
5	53	12
6	15	8
7	3	0

[a] Three experiments were done on distilled water agar. In each experiment, 80 ascospores of both *L. maculans* or *L. biglobosa* were observed. The total number of germ tubes produced by each ascospore was counted.

Discussion

These controlled environment experiments suggest that ascospores of both *L. maculans* and *L. biglobosa* are able to germinate over a wide range of temperatures (5 to 20ºC) on distilled water agar. Nevertheless, there were noticeable differences between *L. maculans* and *L. biglobosa* ascospores in the position of germ tubes and pattern of hyphal growth. Previous work (Wittern & Krüger, 1985) had shown that ascospores of *L. maculans* are able to germinate in distilled water at temperatures from 4 to 28ºC. These experiments now provide detailed data on the germination of both *L. maculans* and *L. biglobosa* ascospores under controlled environment conditions and enable comparisons to be made between germination parameters for the two groups. Analyses of data from these experiments suggest that temperature is an important factor affecting the length of time from inoculation to the germination of 50% of *L. maculans* or *L. biglobosa* ascospores (Vo_{50}) and that the effects of temperature on ascospore germination are similar for both groups. Vo_{50} generally increased with increasing temperature from 5 to 20ºC for both ascospore groups.

Data from these experiments show that *L. biglobosa* ascospores germinate more rapidly than *L. maculans* ascospores on water agar. The time elapsed from inoculation of water agar to the germination of 50% of viable *L. biglobosa* ascospores (Ve_{50}) was shorter than that for *L. maculans* ascospores. The difference between the observed and the estimated rates of germination (ro_c and re_{50}) of *L. biglobosa* ascospores (at 15 and 20ºC) in these experiments indicates that the rate of germination of *L. biglobosa* ascospores at 50% germination is greater than the overall germination rate throughout most of the germination period. Results from

studies on the development of phoma leaf spot lesions on winter oilseed rape plants inoculated with *L. maculans* or *L. biglobosa* (Biddulph *et al*., 1999; Toscano-Underwood *et al*., 2001) suggest that the incubation period (time from inoculation to the appearance of leaf spot lesions) of *L. biglobosa* shorter than that of the *L. maculans*. The differences between the *L. maculans* and *L. biglobosa* in the observed times until 50% of the ascospores had germinated in these controlled environment experiments and differences between the two species in the infection process (Johnson & Lewis, 1994) may, in part, explain why the incubation period of *L. biglobosa* is shorter than that of *L. maculans* (Toscano-Underwood *et al.,* 2001).

Results from these experiments indicate that the effects of temperature on the elongation of germ tubes from ascospores were similar for *L. maculans* and *L. biglobosa*. For ascospores of both species, germs tube length increased with increasing temperature from 5 to 20ºC. Furthermore, these results suggest that higher temperatures (15 to 20ºC) appear to be particularly favourable for the elongation of germ tubes from *L. biglobosa* ascospores. These results agree with those reported by Petrie (1988), who developed a rapid method for differentiating *L. maculans* from *L. biglobosa* isolates based on differences in conidial germ tube length. Conidia of *L. biglobosa* isolates germinating on distilled water agar produced significantly longer germ tubes than did conidia of *L. maculans* isolates, after 40 – 44 h of incubation in darkness at 20ºC.

The differences between the two species in the pattern of ascospore germination may be indicative of different infection strategies. Indeed, studies on the establishment of systemic infection of leaves of oilseed rape by *L. maculans* after inoculation with conidia (Hammond *et al.*, 1985; Hammond & Lewis, 1987; Johnson & Lewis, 1994) showed that there are differences between the two groups in the early stages of infection. In their experiments, the predominant mode of infection of leaves by hyphae from *L. maculans* conidia was through stomata and hyphae grew into the substomatal cavity without forming appressoria, with penetration via wounds or intact walls of guard cells observed only occasionally. However, penetration of the leaf surface by hyphae from *L. biglobosa* conidia was primarily through wounds (Johnson & Lewis, 1994). There is a need for similar studies on the comparative mode of infection of oilseed rape leaves inoculated with ascospores of *L. maculans* or *L. biglobosa*. Differences between *L. maculans* and *L. biglobosa* in the pattern of ascospore germination could be used to help in the taxonomic revision of *L. maculans and L. biglobosa* (Shoemaker & Brun, 2001). They might also be used as a rapid method for differentiating the two groups in ascospore samples collected by Burkard samplers (West *et al.*, 1999) and thus enable more accurate predictions of the severity of stem canker epidemics.

Acknowledgements

We thank the Perry Foundation, the UK Department of Food, Environment and Rural Affairs, the Biotechnology and Biological Sciences Research Council and the China Scholarship Council for funding.

References

Biddulph, J.E, Fitt, B.D.L, Gladders, P., Jedryczka, M.& West, J.S., Welham, S. 1999: Conditions for infection of oilseed rape leaves by ascospores of UK (A group) and Polish (B group) *Leptosphaeria maculans* (stem canker). *Group Consultatif International de Recherche sur le Colza Bulletin* 16: 82-83.

Gladders, P. & Musa, T.M. 1980: Observations on the epidemiology of *Leptosphaeria maculans* stem canker in winter oilseed rape. *Plant Pathology* 29: 28-37.

Hammond, K.E, Lewis, B.G.& Musa, T.M. 1985: A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. *Plant Pathology* 34: 557-565.

Hammond, K.E. & Lewis, B.G. 1987: The establishment of systemic infection in leaves of oilseed rape by *Leptosphaeria maculans*. *Plant Pathology* 36: 135-147.

Jedryczka, M., Fitt, B.D.L., Kachlicki, P., Lewartowska, E., Balesdent, M.H.& Rouxel, T. 1999: Comparison between Polish and United Kingdom populations of *Leptosphaeria maculans*, cause of stem canker of winter oilseed rape. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 106: 608-617.

Johnson, R.D. & Lewis, B.G. 1990: DNA polymorphism in *Leptosphaeria maculans*. *Physiological and Molecular Plant Pathology* 37: 417-424.

Johnson, R.D. & Lewis, B.D. 1994: Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans*. *Plant Pathology* 43: 269-277.

McGee, D.C. 1977: Blackleg (*Leptosphaeria maculans* (Desm.) Ces. et de Not.) of rapeseed in Victoria: sources of infection and relationships between inoculum, environmental factors and disease severity. *Australian Journal of Agricultural Research* 28: 53-62.

Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunnicliffe W.G., Welham, S.J.& White, R.P. 1993: *Genstat 5 Release 3 Reference Manual.* Oxford, UK. Clarendon Press. 796 pp.

Petrie, G.A. 1988: The rapid differentiation of virulent and weakly virulent strains of *Leptosphaeria maculans* (blackleg or stem canker) and related pycnidial fungi from *Brassica* seeds and stems. *Canadian Journal of Plant Pathology* 10: 188-190.

Rouxel, T. & Séguin-Swartz, G. 1995: International blackleg of crucifers network. *Blackleg News* 5: 15-16.

Schramm, H. & Hoffmann, G.H. 1991: Biologische Grundlagen zur integrierten Bekämpfung von *Phoma lingam* (teleomorph *Leptosphaeria maculans* (Desm.) Ces. & de Not.), dem Erreger der Wurzelhals- und Stengelfäule an Winterraps. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 98: 586-596.

Shoemaker, R.A. & Brun, H. 2001: The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans. Canadian Journal of Botany* 79: 421-419.

Toscano-Underwood, C., West, J.S., Fitt, B.D.L., Todd, A.D. & Jedryczka, M. 2001: Development of phoma lesions on oilseed rape leaves inoculated with ascospores of A-group or B-group *Leptosphaeria maculans* (stem canker) at different temperatures and wetness durations. *Plant Pathology* 50: 28-41.

West, J.S., Biddulph, J.E., Fitt, B.D.L. & Gladders, P. 1999: Epidemiology of *Leptosphaeria maculans* in relation to forecasting stem canker severity on winter oilseed rape in the UK. *Annals of Applied Biology* 135: 535-546.

West, J.S., Kharbanda, P.D., Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathology* 50: 10-27.

Williams, R.H. & Fitt, B.D.L. 1999: Differentiating A and B groups of *Leptosphaeria maculans,* causal agent of stem canker (blackleg) of oilseed rape. *Plant Pathology* 48: 161-175.

Wittern, I. & Krüger, W. 1985: Sporenkeimung von *Phoma lingam* (Tode ex. Fr.) Desm. und Resistenzprüfung bei Raps im Gewächshaus. *Journal of Phytopathology* 113: 113-124.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 43 - 52

Investigations on diseases of false flax (*Camelina sativa* (L.) Crtz.) with special regard to downy mildew (*Peronospora parasitica* (Pers.) Fr.)

Föller, I., Paul, V.H.
Universität-Gesamthochschule Paderborn, Fachbereich Agrarwirtschaft,
Labor für Biotechnologie und Qualitätssicherung, Lübecker Ring 2, D-59494 Soest

Abstract: In the study presented here, the disease susceptibility of false flax was investigated in field and laboratory trials. In these field investigations more diseases were found on false flax than the older literature suggested. These diseases were downy mildew (*Peronospora parasitica*), *g*rey mould (*Botryotinia fuckeliana*), stem rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida*), white leaf spot (*Pseudocercosporella capsellae*), stem and root rot (*Rhizoctonia solani*), powdery mildew (*Erysiphe* spec.), and bacterial blight (*Pseudomonas syringae* pv. spec.). Among the most important diseases found was downy mildew. In further laboratory studies the cultivars/lines showed clear differences in regard to their susceptibility to this disease. In these studies inoculations at three different growth stages were tested. For resistance breeding against downy mildew, the inoculation in the cotyledon stage is the most suitable. Furthermore, an easy and cost efficient storage methods for conidia of downy mildew was developed. Conidia stored on infected leaves at -25 °C survived and were viable for more than 10 months. In an additional test positive effects of the plant activator BION® were noted in regard to the susceptibility of false flax against downy mildew. With application of the plant activator BION® prior to infection, the infection with downy mildew could be severely reduced.
Keywords: False flax, *Camelina sativa*, *Peronospora parasitica*, BION®, *Botryotinia fuckeliana, Sclerotinia sclerotiorum*, *Albugo candida, Pseudocercosporella capsellae, Rhizoctonia solani, Erysiphe* spec., *Pseudomonas syringae* pv. spec

Introduction

False flax is an oilseed plant that has been cultivated in Europe since the Neolithic times and was an important plant in the iron age (400 BC – 500 AC) (Schulze-Motel, 1979). It was supposed that it established itself as a weed in flax and was then selected and cultivated separately (Knörzer, 1978). False flax belongs to the family of brassicaceae and there are now summer and winter forms available. In the discussion for regenerable resources, false flax has been one alternative for set aside lands next to hemp, oil flax, *Crambe abyssinica* and others.

Material and methods

Field investigations

Due to the unknown disease situation of the currently available cultivars and lines of false flax it was the aim of this study to investigate these cultivars/lines for their disease susceptibility. It was of special interest to assess diseases that could infect other brassicaceae (e.g. oilseed rape). For this purpose 13 cultivars/lines of false flax (6 registered cultivars, 4 German and 3 Danish breeding lines, s. Table 1) were grown in field trials at 6-7 different locations in Germany (s. Table 2). In the last year of this study only one location was available.

During the field trials all diseases that appeared were identified according to Koch´s postulates and assessed at three or four different growth stages (emergence, rosette, flowering and maturity).

Table 1. False flax cultivars and lines used for the field and laboratory experiments

Variant	Cultivar/Line	Origin
1	Lindo	DSV*
2	Bavaria	DSV
3	Soledo	DSV
4	Licalla	DSV
5	Limaga	DSV
6	Ligena	DSV
7	WIR 2/92 Genbank St. Petersburg	DSV
8	WIR 3/92 Genbank St. Petersburg	DSV
9	92/055 25/87 Genbank Gatersleben	DSV
10	92/063 28/81 Genbank Gatersleben	DSV
11	Breeding line from Denmark	Dr. Zubr**
12	Breeding line from Denmark	Dr. Zubr
13	Breeding line from Denmark	Dr. Zubr

* DSV=Deutsche Saatveredelung, Lippstadt; ** The Royal Veterinary and Agricultural University Taastrup, Denmark

Table 2. Locations of the field trials from 1995-1998, used cultivars/lines and number of repetitions

	Cultivars/lines and repetitions in year							
	1995		**1996**		**1997**		**1998**	
Location (State of Germany)	Cult.*	n	Cult.	n	Cult.	n	Cult.*	n
Merklingsen (Nordrhein-Westfalen)	1-10	5	1-10	4	1-13	4	1-13	4
Thüle (Nordrhein-Westfalen)	1-10	2	1-10	2	1-10	2	–	–
Kritzkow (Mecklenburg-Vorpommern)	1-10	1	1-10	2	–	–	–	–
Groß Gerau (Hessen)	1-10	1	1-10	1	1-10	2	–	–
Rauischholzhausen (Hessen)	1-10	1	1-10	1	1-10	1	–	–
Lübeck (Schleswig-Holstein)	–	–	1-10	2	1-10	2	–	–
Kleinmachnow (Brandenburg)	1-10	1	1-10	2	–	–	–	–
Dahnsdorf (Brandenburg)	–	–	–	-	1-10	2	–	–
Rohrbach (Thüringen)	1-10	1	–	-	–	–	–	–
Number of locations		**7**		**7**		**6**		**1**

* Cult. = cultivars/lines

Laboratory investigations

Downy mildew (*Peronospora parasitica*) on false flax had been described in older literature (Hackbarth, 1944; Becker-Dillingen, 1928; Kirchner 1906). In this study downy mildew was

observed to play an important role on false flax. This complex was further analysed in laboratory investigations.

Storing of downy mildew (*Peronospora parasitica*) of false flax

In order to carry out the different inoculation trials, a method for storing downy mildew had to be established. Since downy mildew is an obligat pathogen it is not possible to cultivate it on synthetic media. However, since it is known that different downy mildew species can be stored at –25 °C (Klodt-Bussmann, 1995; Tewari, 1993, Koch & Slusarenko, 1990), four different methods for storage at this temperature were tested.

The isolates were frozen in watery solutions with different concentrations of glycerine (5, 10, 15, and 20 %), polyethylen glycol (PEG 400) (5, 10, 15, 20, and 25 %), and dimethyl sulfoxide (DMSO) (5, 10 %) or directly on infected cotyledons. Viability of the isolates was tested fresh and after storage at –25 °C for one day, seven days, one month and three months. In an additional trial the length of survival of conidia of downy mildew on living plants was investigated.

Investigations on the susceptibility of false flax against downy mildew (*P. parasitica*) and the virulence of the different isolates

One of the aspects investigated was the susceptibility of the 13 cultivars/lines to downy mildew at different growth stages. The cultivars/lines were inoculated with downy mildew at the cotyledon, two-leaf and four-leaf stage. For this investigation six downy mildew isolates from false flax were available (s. Table 3). These isolates were additionally tested for their virulence.

Table 3. Origin of the isolates of downy mildew (*Peronospora parasitica*) from false flax

Isolate	Origin	Year
Per Rhh ´96	Rauischholzhausen, Hessen	1996
Per Rhh ´97	Rauischholzhausen, Hessen	1997
Per Me ´97	Merklingsen, Nordrhein-Westfalen	1997
Per Me 10´97	Merklingsen, Nordrhein-Westfalen	1997
Per Me ´98	Merklingsen, Nordrhein-Westfalen	1998
Per Sw ´98	Sweden	1998

Each of these isolates was used for inoculation trials in the above mentioned growth stages. For inoculation the plants of each cultivar/line were grown in a greenhouse in multi-pot-plates (51 pots per plate) until they reached the respective growth stages. The plants were then inoculated with 10 and 15 ml at the 4 leaf stages of a conidial solution with a conidial density of 10^4 conidia/ml. For incubation the plants were placed in small propagators with a fitting plastic lid and ca. 95% air humidity in a climatic chamber at 17/10 °C day/night and 14/10 h light/dark. For the assessments, a five stepped assessment scheme (Klodt-Bussmann, 1995) was used (s. Table 4).

Table 4. Assessment scheme for downy mildew (*P. parasitica*)

Assessment rate	Sporulation area on leaf surface (underside)
1	no sporulation
2	1 – 25 %
3	26 – 50 %
4	51 – 75 %
5	76 – 100 %

Influence of BION® on the susceptibility of false flax against downy mildew (*P. parasitica*)
In a further investigation the influence of BION® on the establishment of downy mildew on false flax was tested. BION® is a plant activator which is used for activating the plant defences (resistance) against different pathogens and has no direct influence on the pathogen.

For this trial false flax plants from seven cultivars/breeding lines were grown in a greenhouse in multi-pot-plates until the cotyledon stage. In this stage they were then treated with 20ml of water (control) and 20 ml of a watery solutions with 10 and 20 ppm a.i. concentrations of BION®. After 10 days these plants were then inoculated with downy mildew (s.a.) and placed in propagators in a climatic chamber at 17/10 °C day/night and 14/10 h light/dark. The assessment for disease incidence were accomplished 12 days after inoculation with the assessment scheme in Table 4.

Results and discussion

Field trials
During the investigations presented here, one bacterial and seven fungal/fungal like diseases on false flax were diagnosed. These were the bacterial blight (*Pseudomonas syringae* pv. spec.), downy mildew (*P. parasitica*), grey mould (*Botryotinia fuckeliana*), stem rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida*), white leaf spot (*Pseudocercosporella capsellae*), stem and root rot (*Rhizoctonia solani*) and powdery mildew (*Erysiphe* spec.). Of these diseases, seven had been previously described on false flax, but the bacterial blight was first found in this investigation.

Downy mildew and grey mould, followed by stem rot were the most important. Downy mildew was observed in the field trials in all years of this investigation with a marked increase in the last year. In that year 41,3 % of the plants at the rosette stage (s. Figure 1), averaged over all cultivars/lines, were infected with downy mildew, 9,5 % in the flowering stage (s. Figure 2) and 19,8 % in the maturity stage (s. Figure 3). On average over this year (all growth stages) 26,9 % of the false flax plants had been infected with downy mildew. The currently available cultivars/lines showed distinct differences with regard to their susceptibility to downy mildew. Two Danish varieties (11 and 13) and two german lines (9 and 10) showed a distinct resistance to downy mildew. The registered German cultivars (Lindo, Bavaria, Soledo, Licalla, Limaga and Ligena) as well as the lines 8 and 12 showed high infestation levels with downy mildew. Only in this year a tendentious influence of downy mildew on the yield of false flax could be observed. The varieties which were most infected showed, tendentiously, the least yield.

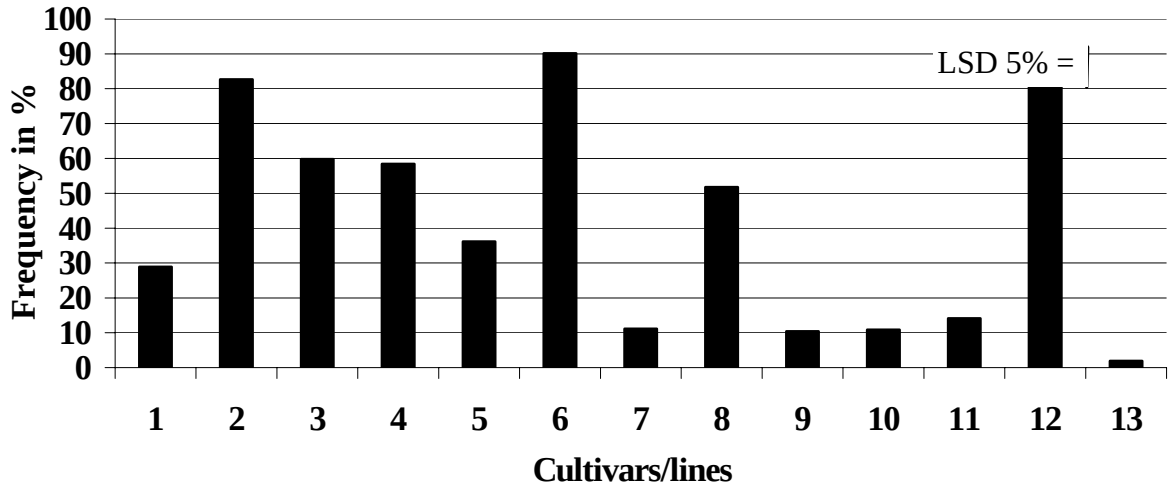


Figure 1. Downy mildew (*P. parasitica*) infestation of false flax (*C. sativa*) at the rosette stage (Merklingsen, 1998; n = 4)

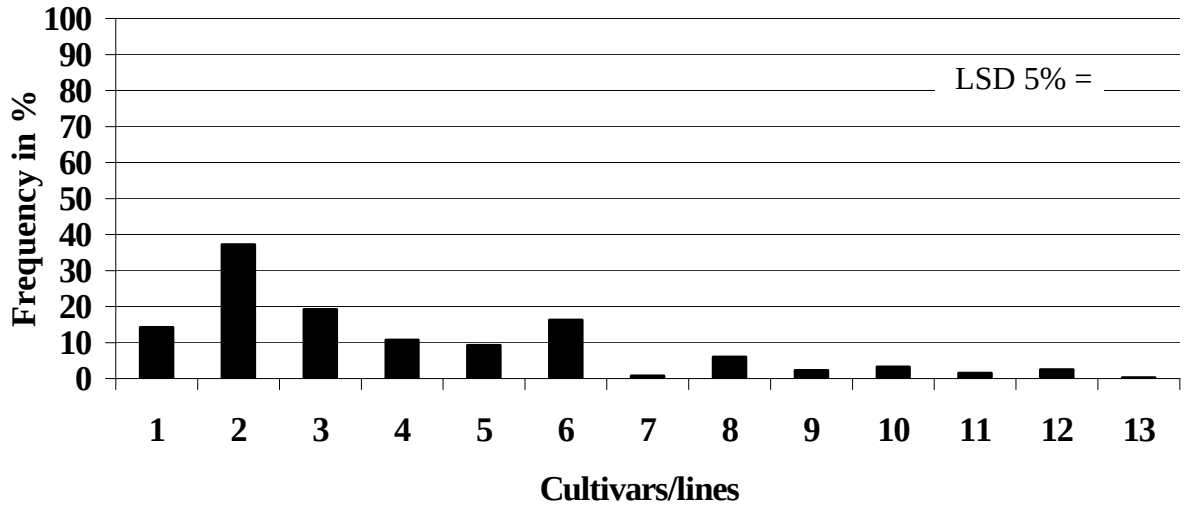


Figure 2. Downy mildew (*P. parasitica*) infestation of false flax (*C. sativa*) at the flowering stage (Merklingsen, 1998; n = 4)

Grey mould was also observed on the fields in all study years. The highest infection rate was found in 1995, where 44,4 % of the plants had been infected. Since there was no disease free control, no effect of disease on false flax yield could be measured. Nevertheless, in some locations, the cultivars/lines with the highest infestation levels had the highest yield (Thüle, 1997). Clear differences in the susceptibility of the cultivars/lines used could not be observed. On average, the assessments of the cultivars/lines at the different locations for each year varied about 14-15 % from each other. Nevertheless, in three of the four years the line 9 had the lowest infestation, which was still high in some cases e.g. 38 % infected plants in 1995. On the other hand, the cultivar 2 (Bavaria) had the highest infestation level in three of the years. In 1995 the infestation rate was 52 % of the plants averaged over all assessments and locations.

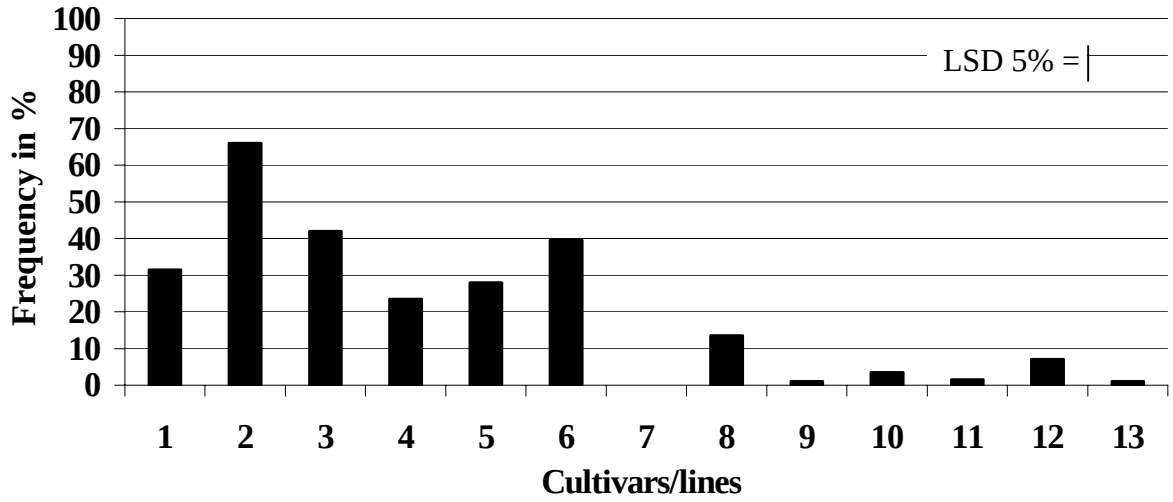


Figure 3. Downy mildew (*P. parasitica*) infestation of false flax (*C. sativa*) at maturity (Merklingsen, 1998; n = 4)

Stem rot also occurred in all study years. The highest infestation levels could be observed in 1995 and 1998. Still, averaged over all locations and assessments only <10 % of the plants had been infected. In Merklingsen (1995) up to 30 % and in Thüle (1998) up to 20 % of the plants had been infected. No cultivar/line with a clear resistance against stem rot has been observed, but tendentiously the line 8 was more susceptible than cultivar 1 (Lindo). In other studies in the EU Grey mould and stem rot have also been determined to be the most important diseases in false flax.

The occurrence of the other diseases varied also, depending on the variety of false flax, but they were not very distinct. One exception was powdery mildew. Compared with other varieties, the variety 1 (Lindo) had the least infestation of this disease.

From these results, the thesis that false flax is a very healthy plant with a low degree of diseases as stated in older literature can not be maintained. On the contrary, if the cultivation of false flax is expanded, a high increase in disease incidences has to be expected.

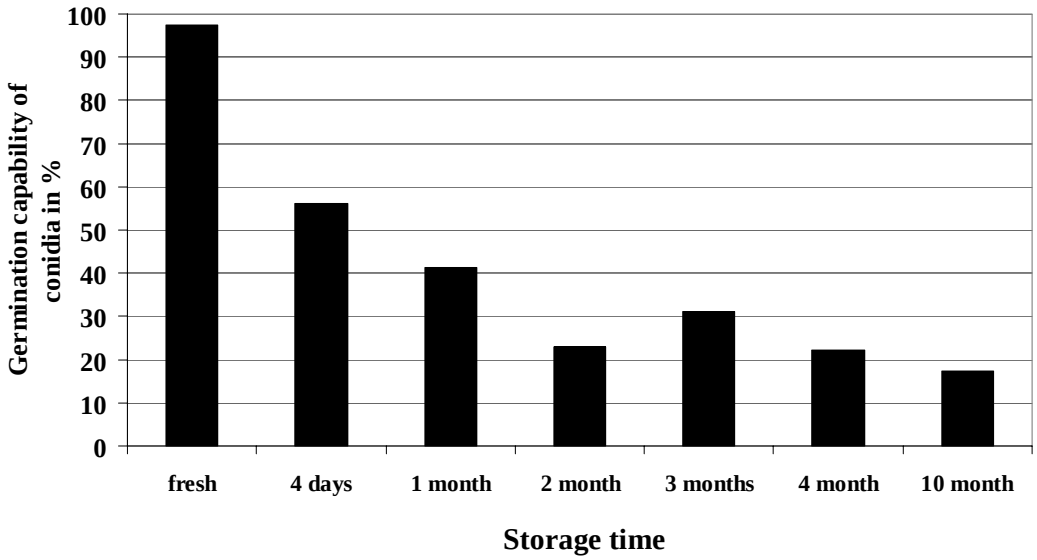


Figure 4: Survival rate of *Peronospora*-conidia on infected false flax cotyledons at -25 °C (n=4)

Laboratory experiments

Storing of downy mildew* (Peronospora parasitica) *of false flax

For our studies on downy mildew (*P. parasitica*), we had to establish a method to store the pathogen. Three different methods were tested. The most efficient method was to store downy mildew on infested leaves at –25 °C (s. Figure 4). With this method we were able to maintain the germination capacity and the infectivity of the conidia for a period of 10 months.

The next efficient method was to store downy mildew on the leaves of living plants (s. Figure 5). After four weeks on living plants, the germination capacity of the conidia was at 70 %. This experiment then had to be stopped on account of the severe damage to the plants. Almost all leaves had wilted.

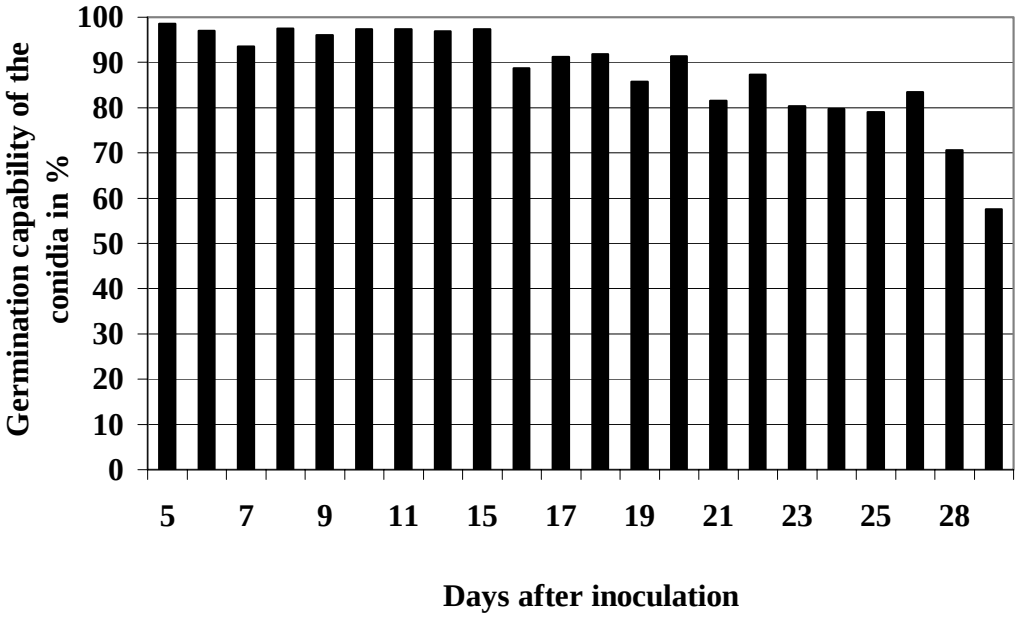


Figure 5: Survival rate of *Peronospora*-conidia on infected, living false flax plants (n=4)

The least efficient method was the storage of the conidia in watery solutions with various admixtures (s. Figure 6 and Figure 7). Here the germination capacity of the conidia was reduced to 20 % of that of the control directly after addition of 15 or 20 % of glycerine, addition of 20 or 25 % polyethylen glycol (PEG 400) reduced the germination capacity about 50 % and dimethyl sulfoxid (DMSO) showed similar results.

Investigations on the susceptibility of false flax against downy mildew (*P. parasitica*) and the virulence of the different isolates

In a comparison of different methods of inoculation, the most efficient method was inoculation at the cotyledon-leaf stage, as was described for various *Brassica* species. After inoculation in this growth stage the plants showed an average assessment rate of 3,6, whereas the average assessment rates after inoculation in the two-leaf and four-leaf stage were 1,8 and 1,6 respectively (s. Figure 8). The cultivars and lines showed clear differences and, based on this method, the available 13 cultivars/lines could be divided into 4 different degrees of susceptibility. The varieties 1 to 6 (Lindo, Bavaria, Soledo, Licalla, Limaga, Ligena) as well as 7 and 8 are susceptible (assessment rate >3,5-4,5), the varieties 9, 10 and 12 have medium

susceptibility (assessment rate >2,5-3,5). The variety 11 is resistant (assessment rate >1,5-2,5), and the variety 13 is highly resistant (assessment rate 1-1,5).

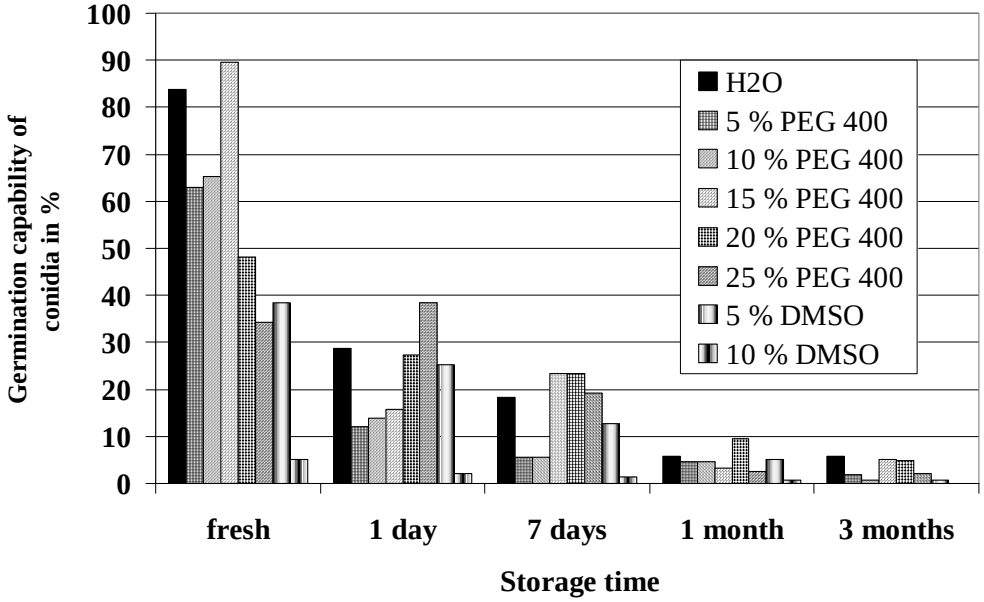


Figure 6: Survival rate of *Peronospora*-conidia in watery solutions with PEG 400 and DMSO (n=5)

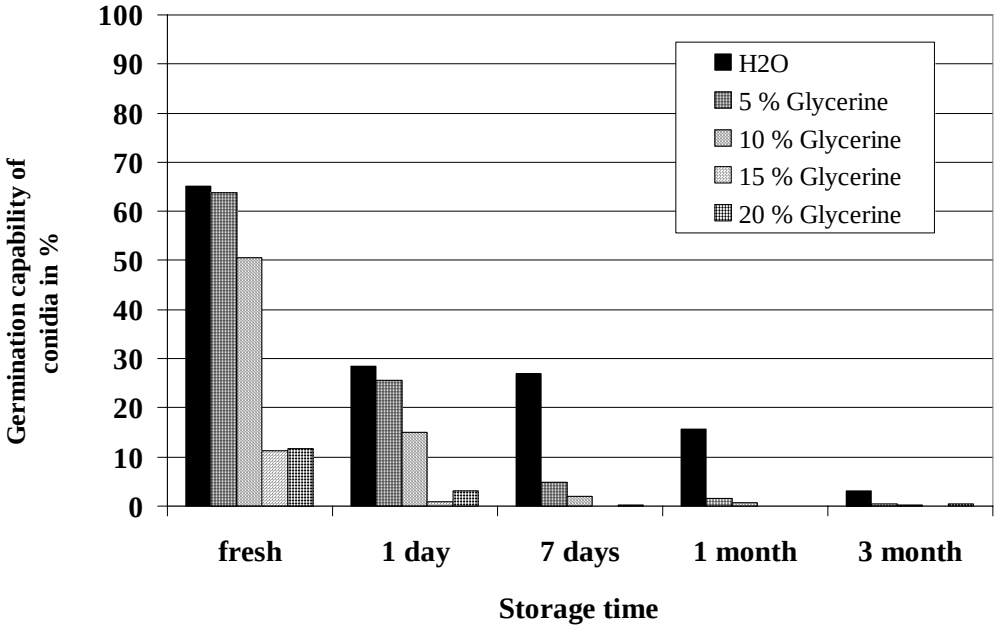


Figure 7: Survival rate of *Peronospora*-conidia in watery solutions with glycerine (n=5)

During our examination of different downy mildew-isolates of false flax, we established virulence differences between the isolates. Of six available isolates, two were intensely virulent (SW ’98 and Me ’97), and four were medium virulent (Rhh ’96, Rhh ’97, Me 10 ’97 and Me ’98). The isolate from Sweden (Sw ’98) was the most virulent (s.Table 5).

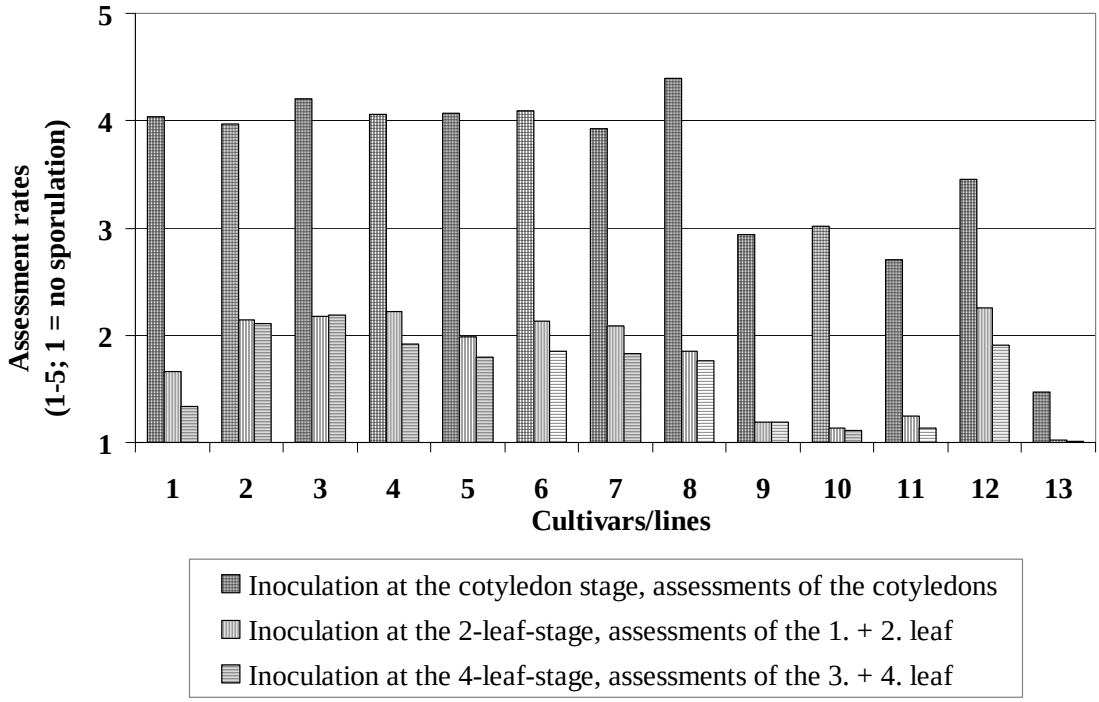


Figure 8: Differences between false flax cultivars/lines after inoculation with 6 resp. 5 *P. parasitica*-isolates at three growth stages (n=3; 17 plants each)

Table 5: Average assessment rates (assessment rates 1-5, 1=no sporulation) of 6 *P. parasitica*-isolates on the cotyledons of 13 false flax cultivars/lines (442 cotyledons [13x34], n= 3)

Isolat	Sw ´98	Rhh ´96	Rhh ´97	Me ´97	Me 10´97	Me ´98
Sw ´98	4,3					
Rhh ´96	*	3,2				
Rhh ´97	*		3,1			
Me ´97	*	*	*	3,7		
Me 10´97	*			*	3,3	
Me ´98	*	*	*			3,5

with * marked average assessment rates differ significantly (95 %)

Influence of BION® on the susceptibility of false flax against downy mildew (*P. parasitica*)

The application of BION® provoked an induced resistance in false flax. After treatment with 10 and 20 ppm respectively of a watery solution of BION® 10 days prior to inoculation with downy mildew (s.
Figure 9), three of the seven false flax-cultivars investigated showed a complete resistance. In the other five cultivars the assessment rates for downy mildew were significantly lower than in the water treated control. Due to these results it would be of interest to investigate the influence of BION® under field conditions or as a seed coating.

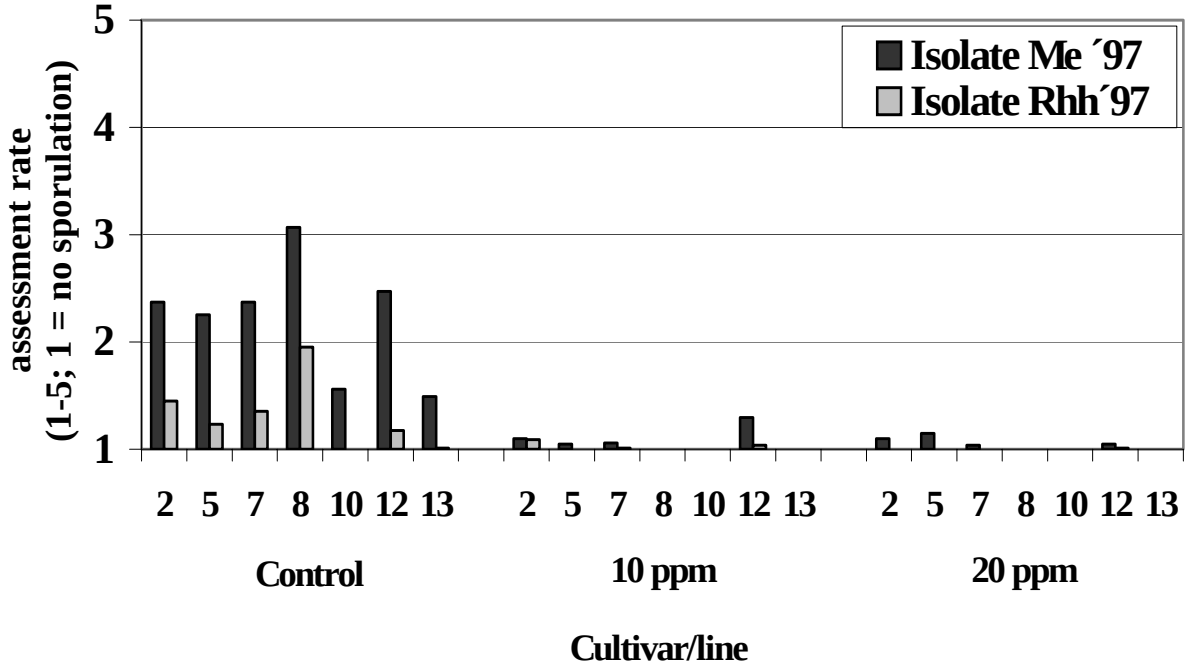


Figure 9: Sporulation intensity of *P. parasitica* on the cotyledons of false flax treated with 10 resp. 20 ppm of the plant activator BION® prior to inoculation

References

Becker-Dillingen, J., 1928: *Der Leindotter. Handbuch des Hackfruchtbaues und Handelspflanzenbaues*, Berlin, Paul Parey: 384-386.

Hackbarth, J., 1944: *Die Ölpflanzen Mitteleuropas.* Stuttgart, Wissenschaftl. Verlagsgesellschaft: 151-157.

Kirchner, O., 1906: *Die Krankheiten und Beschädigungen unserer landwirtschaftlichen Kulturpflanzen.* Stuttgart, Verlagsbuchhandlung Eugen Ulmer.

Klodt-Bussmann, E., 1995: Untersuchungen zur Epidemiologie und sortenspezifischen Pathogenität von *Peronospora parasitica* an Winterraps (*Brassica napus* L.). *Dissertation Universität Bonn*

Knörzer, K.-H., 1978: Entwicklung und Ausbreitung des Leindotters (*Camelina sativa* s.I.). *Ber. Deutsch. Bot. Ges*. 91: 187-195.

Koch, E., Slusarenko, A., 1990: *Arabidopsis* is susceptible to infection by a downy mildew fungus. *The Plant Cell* 2: 437-445.

Schulze-Motel, J., 1979: Die Anbaugeschichte des Leindotters, *Camelina sativa* (L.) Crtz.. *Archaeo-Physika* 8: 267-281.

Tewari, J.P., 1993: Cultivation, preservation, and host-range of *Peronospora parasitica* from *Capsella bursa-pastoris*. Bulletin OILB SROP 16 (9): 22-24.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 53 - 63

Factors affecting the pathogenicity of *Verticillium dahliae* to spring linseed

Dimitra Gkilpathi, Bruce D.L. Fitt, Elaine Ward and Alan Todd
IACR-Rothamsted, Harpenden, Herts. AL5 2JQ, UK

Abstract: Experiments were done to examine the pathogenicity of *V. dahliae* isolates from linseed and other hosts to linseed cultivars. No evidence of host adaptation was found as linseed isolates were not more pathogenic to linseed than isolates from other hosts. The molecular variation of *V. dahliae* isolates from different hosts was tested using RFLP analysis of ribosomal DNA; the isolates could be divided into two groups by this method; isolates from linseed were all in the same group. The effects of air temperature and soil temperature on the progress of the disease caused by *V. dahliae* were tested in controlled environment conditions. An increase in soil temperature from 16°C to 24°C increased the severity of the disease symptoms but the effect of the soil temperature also seemed to be influenced by the pathogenicity of the *V. dahliae* isolate. An increase in air temperature from 16°C to 24°C increased the severity of the disease symptoms for all isolates of *V. dahliae* used. Disease progress in a spring linseed crop was not related to the initial inoculum density or to the previous cropping sequence. At the end of flowering the plants appeared symptomless even though the fungus had already spread within them, whereas symptoms had developed by the time capsules were senescing.

Key words: RFLP, air temperature, soil temperature, soil borne

Introduction

Verticillium dahliae Kleb is a damaging pathogen causing severe wilt diseases worldwide, which is favoured by high air and soil temperatures (20-30°C, optimum 25°C) and moderate to high levels of soil moisture (Schnathorst, 1981). In the UK, the disease was observed for the first time on *Linum usitatissimum* (linseed cultivars) in 1990 at Rothamsted (Fitt *et al*., 1992). The first symptoms of the disease on the linseed crop appear in late July or early August, when the air temperature is >25°C and soil temperatures around 17-18 °C, as dark brown stripes on the stems and branches of maturing plants. This paper reports work to study the factors that might have contributed to the development of *Verticillium* on linseed in the UK (changes in pathogen populations, cropping sequences or weather patterns).

Materials and Methods

Pathogenicity experiments

Thirty isolates of *V. dahliae* (from tomato, potato, hop, strawberry, maple, quince, sunflower, cotton, olive, chrysanthemum, linseed or soil) were tested for their pathogenicity to the spring linseed cultivar Antares and four of these isolates were tested for their pathogenicity to eight other spring linseed cultivars (Jupiter, Agristar, Windermere, Master, Mikael, Omega, Barbara and Coniston). Pathogenicity experiments were done in controlled environment cabinets at a night/day temperature of 22°C, with a 16 h photoperiod and a light intensity of 600 μE m^{-2} sec^{-1}. Linseed plants were inoculated by dipping roots of seedlings at growth stage 22-23 (Freer, 1991) into a *V. dahliae* spore suspension with 1.4 x 10^7 spores ml^{-1}. Inoculated

plants in pots were arranged in a randomised block design, with nine replicates per isolate for five pathogenicity experiments with cv. Antares and three replicates per isolate for the pathogenicity experiment with the eight linseed cultivars. Plants were assessed 1, 2 and 3 weeks after inoculation for main stem height, number of tillers and % leaf area with chlorosis or necrosis on each main stem or tiller. The visual symptoms (% leaf area with chlorosis or necrosis) caused by *V. dahliae* were assessed using a disease index: 0, no visual symptoms; 1, 0-20%; 2, 20-40%; 3, 40-60%; 4, 60-80%; 5, 80-100% area affected. A plant disease index was calculated as the mean of the stem and tiller disease indices of each plant.

Molecular variation in V.dahliae isolates

DNA extractions were done with a standard phenol: chloroform extraction method (Lee and Taylor, 1990). All the *V.dahliae* isolates used for extraction of DNA were grown in a sucrose-sodium nitrate broth (SSN). For the PCR and digestion of amplified DNA, each 100µl reaction contained reaction buffer (10mM Tris HCl pH8.8, 1.5mM$MgCl_2$, 50mM KCl, 0.08% Nonidet P40, 0.1mg/ml BSA), dNTPs 0.2mM, 1 µl of each primer (100 pmol μl^{-1}) and 0.2 µl (1 unit) of *Taq* polymerase (MBI) and 80 ng fungal DNA in TE. These were incubated in a Progene Techne Thermal Cycler (Teche Ltd., UK) for 25 cycles at 94^{o}C for 1 min, 42^{o}C for 2 min and 72^{o}C for 2 min. The primers used, which anneal to consensus regions of fungal ribosomal DNA were ITS4 (5` TCCTCCGCTTATTGATATGC) and ITS5 (5` GGAAGTAAAAGTCGTAACAAGG) (White *et al*., 1990). Amplified DNA (1-2µl) was digested with restriction enzymes at 37^{o}C. Two enzymes (*Cfo* I and *Rsa* I) were chosen for the digestions. These were predicted to give different banding patterns among published *V.dahliae*, *V.albo-atrum* and *V.longisporum* isolates using the program MAP within the GCG package (Anonymous, 1994). The digestion products were separated on gels containing 2% Nusieve agarose (Flowgen) plus 1% standard agarose and the DNA stained with ethidium bromide.

Effects of soil and air temperature on disease caused by V. dahliae on linseed

Verticillium dahliae isolates VD9 (originally from strawberry) and VD60 (originally from linseed), which had proved pathogenic to linseed in previous experiments, were used to investigate the expression of the disease under different soil and air temperatures. The experiment on soil temperature was done in Wisconsin tanks. Three temperatures (16^{o}C, 20^{o}C and 24^{o}C) were tested. The air temperature during the experiment was an average of 20^{o}C, as recorded by a data logger (Tinytalk II Data Logger Gemini Data Loggers Ltd., UK). The linseed cv. Antares was used and the plants were inoculated by root dipping. The experiments on air temperature were done in controlled environment cabinets (at a night/day temperature of 22^{o}C, with a 16 h photoperiod and a light intensity of 600 µE m^{-2} sec^{-1}) and the plants were inoculated by root dipping in spore suspension. Three temperatures (15^{o}C, 20^{o}C and 24^{o}C) were examined. The plants were put in plastic pots (8.4 cm diameter) and arranged in a complete randomised design with eight replicates per isolate at each temperature. The plants in both the soil and air temperature experiments were assessed for main stem height and plant disease index 2, 3 and 4 weeks after inoculation.

Effects of previous crop species and inoculum density on progress of disease in linseed crops

A field experiment was started in 1999 to investigate the effect of thee cropping sequences on the severity of *Verticillium* on linseed and to study the effect of different cropping sequences on the population of *V.dahliae* in soil and the disease progress. There were three cropping sequences tested, in a randomized design of three blocks of nine plots (plot area 4 m x 3 m) (Table 1).

Table 1. The plan to test cropping sequences in a field experiment

Crop sequences	S1	S2	S3	S2	S3	S1	S3	S2	S1
Year 1 (1999)	Ln*	Su	P	Su	P	Ln	P	Su	Ln
Year 2 (2000)	Ln	Ln	Ln	Ln	Ln	Ln	Ln	Ln	Ln
Year 3 (2001)	Ln	Ln	Ln	Ln	Ln	Ln	Ln	Ln	Ln

*Ln: linseed, P: Potato, Su: Sunflower

After the 1999 harvest, the stubble left in each plot was incorporated into the field. In 2000, all the plots were sown with linseed. Prior to sowing in 2000, the inoculum in each plot was measured. From each plot, six soil samples were taken with a soil corer (diameter 5 cm) from a depth of 0-15 cm in a W-shaped pattern. There were then air-dried for 15 days and then milled for 10 min and passed successively through 2 mm and 1 mm mesh sieves (Termorshuizen *et al*., 1998). They were stored in paper bags at room temperature. To measure the numbers of propagules of *V.dahliae,* the modified Andersen air sampler of Butterfield and DeVay (Butterfield and DeVay, 1977) was used. From each sample, ten sub-samples (each of 100 mg) were spread onto a semi-selective medium (Harris *et al*., 1993) on agar plates. After 10 days, the soil on the agar substrate was removed by washing and the Petri dishes were checked for the presence of *V.dahliae* colonies under a stereo-microscope.

The linseed cultivar Antares was sown on 22 March 2000 (500 seeds m^{-2}). Five assessments were done in the summer of 2000. At each assessment, 10 plants per plot were taken randomly, using a W pattern of sampling in the plot. The height of plants, the disease incidence and the disease severity were recorded. For the disease severity, the areas with visual symptoms on the roots, leaves and capsules were assessed. From each sample of 10, three plants were taken randomly to be examined for the spread of fungus in the plants. From each linseed plant, parts of the roots, the lower stem, the middle stem, the upper stem, the leaves and the capsules were surfaced sterilised for 1 min in 2% sodium hypochlorite and were placed in Petri dishes containing PDA amended with antibiotics (streptomycin and penicillin) (for the first and second assessment, 23 June 2000 and 3 July 2000 respectively) and in MSEA medium (for the third and fourth assessments, 13 July 2000 and 31 July 2000 respectively) . The Petri dishes were incubated at 22^{o}C in the dark for 5-7 days. At the end of the incubation period, they were assessed for the presence of *V.dahliae* colonies.

Results and discussion

Pathogenicity experiments

All thirty *V. dahliae* isolates tested were pathogenic to the spring linseed cultivar Antares. Infection by *V. dahliae* generally decreased the number of tillers and the height of the linseed main stems to cause severe stunting (Fig. 1). Isolates from linseed did not produce more severe disease symptoms than isolates from other hosts. The plant disease index ranged from 4.6 for a highly pathogenic isolate to 0.2 for the least pathogenic isolate. The four *V. dahliae* isolates tested on eight linseed cultivars were pathogenic to all of them (Fig. 2). The plant disease index ranged from 2.5 to 4.3 (Fig. 2). The cultivars showed differences in their susceptibility to *V. dahliae*; cvs. Barbara and Agristar had the lowest disease indices.

Molecular variation of V.dahliae isolates

Restriction analysis of PCR amplified ribosomal DNA

The primers ITS4 and ITS5 amplify a region of DNA that stretches from the 3` end of the small ribosomal subunit to the 5` end of the large ribosomal subunit and includes the 5.8S gene and the two internal transcribed spacer (ITS) regions. The size of the fragment amplified by ITS4/ITS5 was 560bp. From the restriction analysis of the fragments using the enzymes *Rsa* I and *Cfo* I, the *V.dahliae* isolates tested could be divided in two groups (AC or BD). All the linseed isolates were in the same group (BD), which included 91% of the *V.dahliae* isolates tested (Table 5). The AC pattern was observed for three haploid *V.dahliae* isolates and the diploid *V.longisporum* isolates. One of the three haploid *V.dahliae* isolates which gave the AC pattern, isolate V8 (original code 1928) was also reported to have an atypical RFLP pattern by using RFLP analysis with random genomic probes; the other two isolates with pattern AC, VD1 (original code 1893) and VD37 (original code 1807) had similar RFLP patterns to other *V.dahliae* isolates in this earlier study (Ocoli *et al*., 1993).

Effects of soil and air temperature on disease caused by V. dahliae on linseed

Soil temperature

Results from these experiments are shown in Fig. 3. At 2, 3 and 4 weeks after inoculation, increasing the soil temperature from 16^{o}C to 24^{o}C decreased the height of stems of the linseed plants infected with isolate VD9. The disease index at 2 and 3 weeks post inoculation also increased with increasing soil temperature from 16^{o}C to 24^{o}C for VD9, whereas the linseed plants infected with VD60 showed the highest disease index at 16^{o}C. At 4 weeks post inoculation, the disease index was significantly higher at 24^{o}C for both VD9 and VD60 isolates.

Air temperature

Increasing air temperature in the cabinets from 15^{o}C to 20^{o}C and 24^{o}C increased the severity of the symptoms (plant disease index) caused by the isolate VD9, even from 2 weeks after inoculation (Fig. 4). The linseed plants showed severe stunting at 24^{o}C at the first assessment (2 weeks after inoculation). For isolate VD60, the symptoms (plant disease index) increased in severity as air temperature increased, but the height did not follow the same pattern. The height of the linseed plants decreased significantly as the temperature increased from 15^{o}C to 20^{o}C and 24^{o}C; however, the plants were taller at 20^{o}C than at 15^{o}C, although the symptoms as measured by the disease index (% area of the plant with necrosis/chlorosis) were more severe at 20^{o}C.

Effects of previous crop species and inoculum density on progress of disease in linseed crops

The inoculum density in the field plots varied from 145 cfu g^{-1} soil to 26 cfu g^{-1} soil (Table 3). Differences between plots with different cropping sequences were observed but these were not related to the previous crop cultivated in the plot. In all the assessments, the previous crop did not affect the disease incidence or the disease severity (Fig. 5). The plants were symptomless at the first assessment (96 days after sowing) but *V.dahliae* could be isolated from the leaves and the middle stem of the linseed plants. The disease severity increased with time and reached 38 % by the end of the growing season as plants were approaching maturity. Then the fungus was easily isolated from all the parts of the plant (root, lower stem, middle stem and capsules) and at a low percentage from the seed. No correlation could be found between the initial inoculum density and the final disease incidence or severity (data not shown) or the height of the plant.

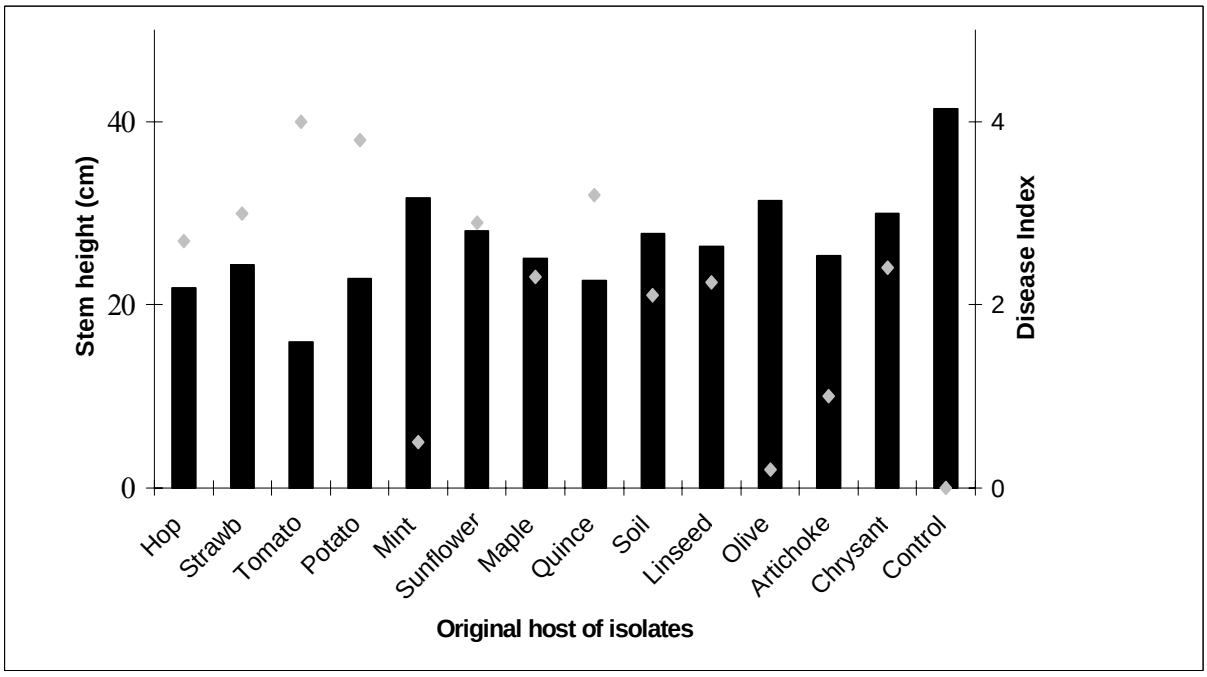


Figure 1. Effects *of V.dahliae* isolates from different original hosts on the mean height (ν) and the mean disease index (υ) of lineseed cv. Antares 3 weeks after inoculation (data are means of five experiments).

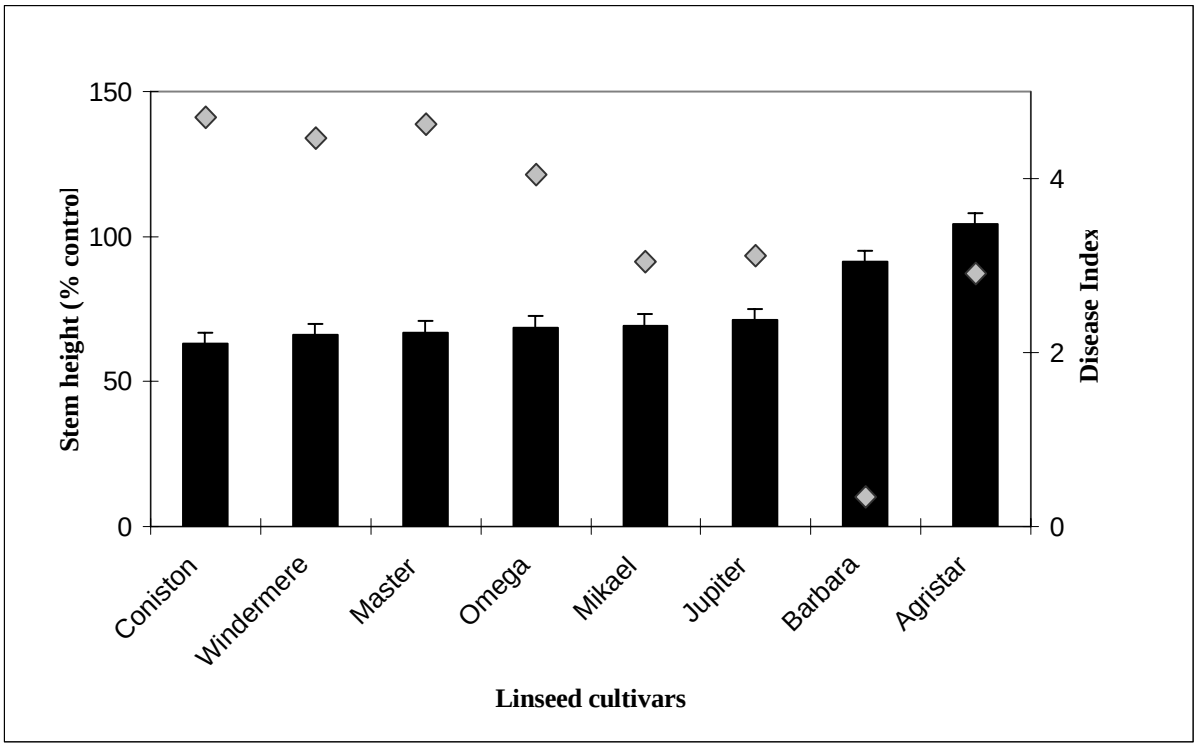


Figure 2. Effects of four *V.dahliae* isolates on the height (expressed as % of the uninoculated control) (ν) and disease index (expressed as mean score on stems and tillers minus control score) (υ) on linseed cultivars Coniston, Windermere, Master, Omega, Master, Mikael, Jupiter, Barbara and Agristar (Error bar = SED, P = 0.05).

Table 2. RFLP analysis of *V.dahliae* isolates using their ribosomal DNA, amplified by the primers ITS4/ITS5 and digested with the restriction enzymes *Cfo* I and *Rsa* I.

Isolate	**Original Host/ Country**	**Pattern of isolates**	
		ITS4/5 Rsa *I*	**ITS4/5** Cfo *I*
VD1	Hop, UK	A[+]	C
VD2	Hop, UK	B	D
VD3	Hop, UK	B	D
VD4	Hop, UK	B	D
VD5	Hop, UK	B	D
VD8	Tomato, UK	A	C
VD9	Strawberry, UK	B	D
VD10	Strawberry, UK	N/A	N/A
VD11	Strawberry, UK	B	D
VD12	Strawberry, UK	B	D
VD17	Potato, Canada	B	D
VD20	Mint, UK	B	D
VD21	Linseed, UK	B	D
VD23	Sunflower, UK	B	D
VD33	Sunflower, UK	B	D
VD34*	Oilseed rape, Poland	A	C
VD35*	Oilseed rape, Poland	A	C
VD37	Quince, UK	A	C
VD40	Soil, UK	B	D
VD41	Soil, UK	B	D
VD42	Soil, UK	B	D
VD44	Soil, UK	B	D
VD45	Cotton, Greece	B	D
VD46	Olive, Greece	B	D
VD51	Chrysanthemum,	B	D
VD53	Artichoke, Greece	B	D
VD56	Linseed, UK	B	D
VD57	Potato, UK	B	D
VD58	Linseed, UK	B	D
VD59	Linseed, UK	B	D
VD60	Linseed, UK	B	D
VD62	Linseed, UK	B	D

[+] The band sizes corresponding to RFLP types A-D are (in bp) A, 425, 115; B, 540; C, 298, 252; D, 295, 176, 71

***** *V. longisporum,* previously classified *as V.dahliae* var. *longisporum*

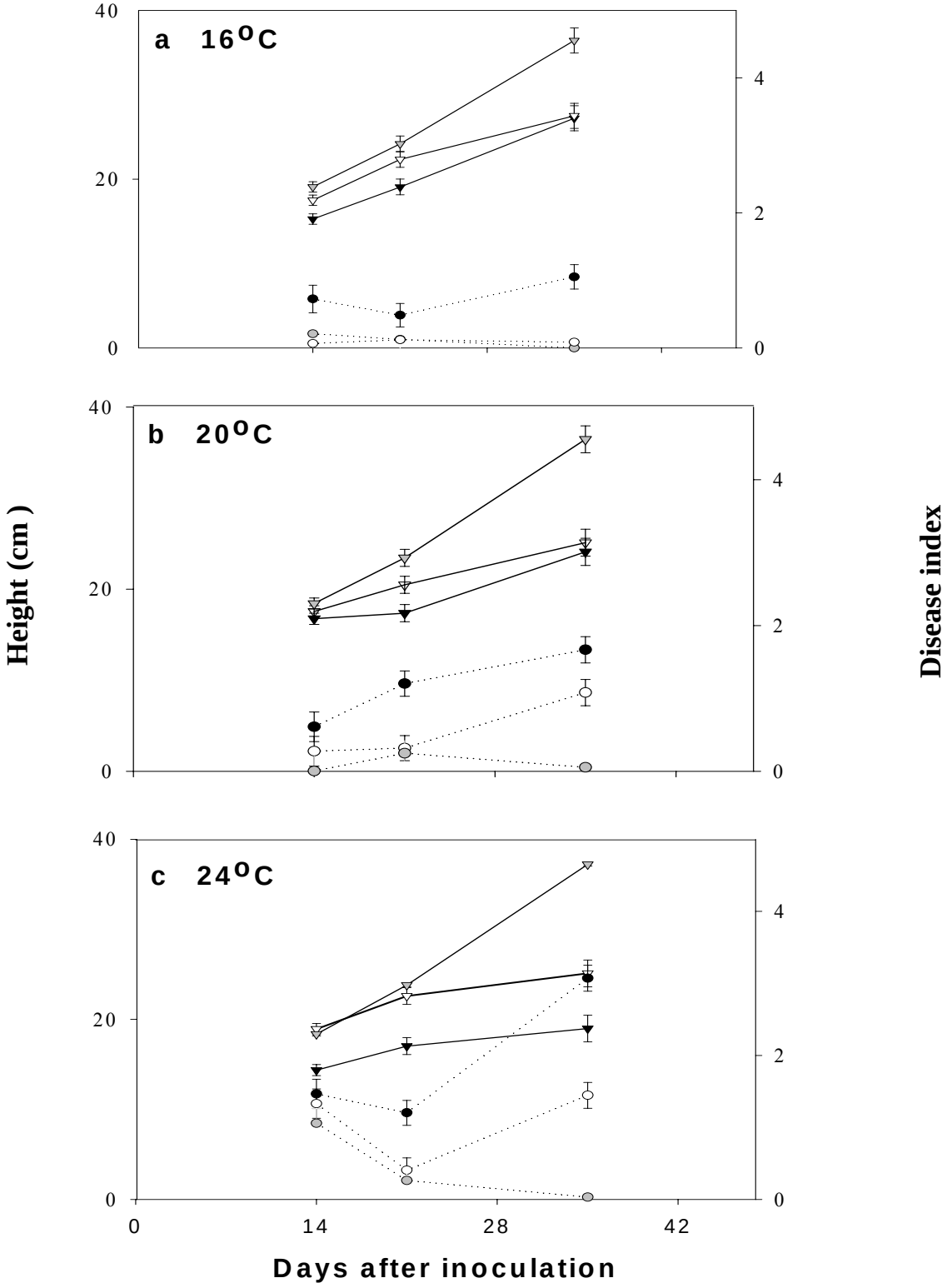


Figure 3. Effects of *V. dahliae* isolates VD9 and VD60 on the height (uninoculated ▼, VD9 ▼, VD60 ▽) and plant disease index of linseed plants (uninoculated ●, VD 9 ●, VD60 ○) at soil temperatures of 16°C (a), 20°C (b) and 24°C (c) 2, 3 and 5 weeks after inoculation (Error bar = SED, P = 0. 05)

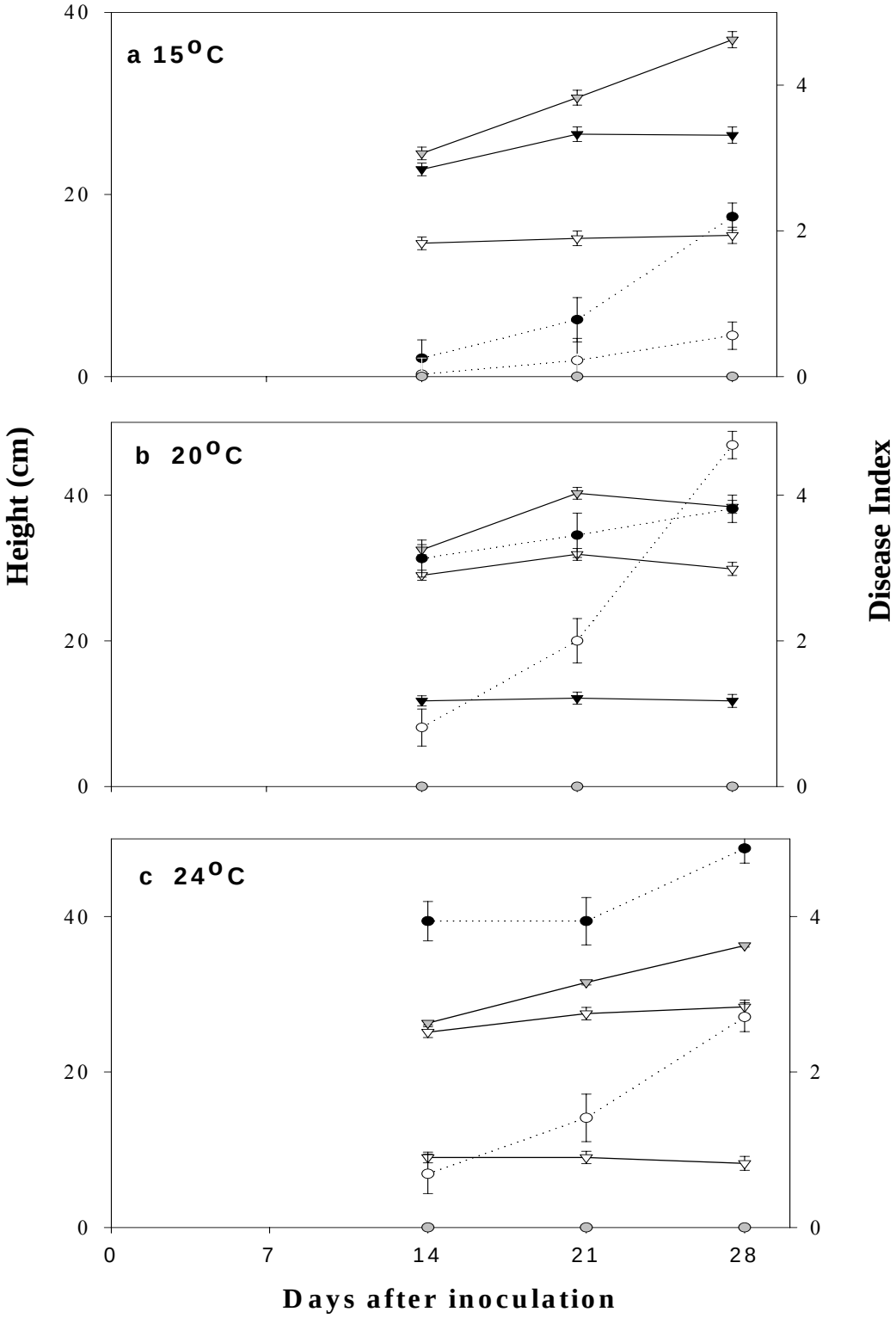


Figure 4. Effects of *V. dahliae* isolates VD9 and VD60 on the height (uninoculated ▼, VD9 ▼, VD60 ▽) and plant disease index (uninoculated ●, VD 9 ●, VD60 ○) of linseed plants at air temperatures 15°C (a), 20°C (b) and 24°C (c) 2,3 and 4 weeks after inoculation (Error bar = SED, P = 0.05)

Table 3. Inoculum levels of *V.dahliae* as cfu g^{-1} soil in the field experiment (harvest year 2000), where different crops were cultivated the previous harvest year (1999)*

Crop sequence*	Previous crop (1999)	cfu g^{-1} soil ln-transformed	(untransformed data)
S2	Sunflower	4.8	(145.0)
S1	Linseed	4.7	(121.7)
S3	Potato	4.4	(108.7)
S1	Linseed	4.2	(72.7)
S2	Sunflower	3.9	(48.7)
S1	Linseed	3.7	(40.7)
S3	Potato	3.4	(32.7)
S2	Sunflower	3.3	(30.7)
S3	Potato	3.2	(26.0)
SED (8 df)		0.49	

* See Table 1

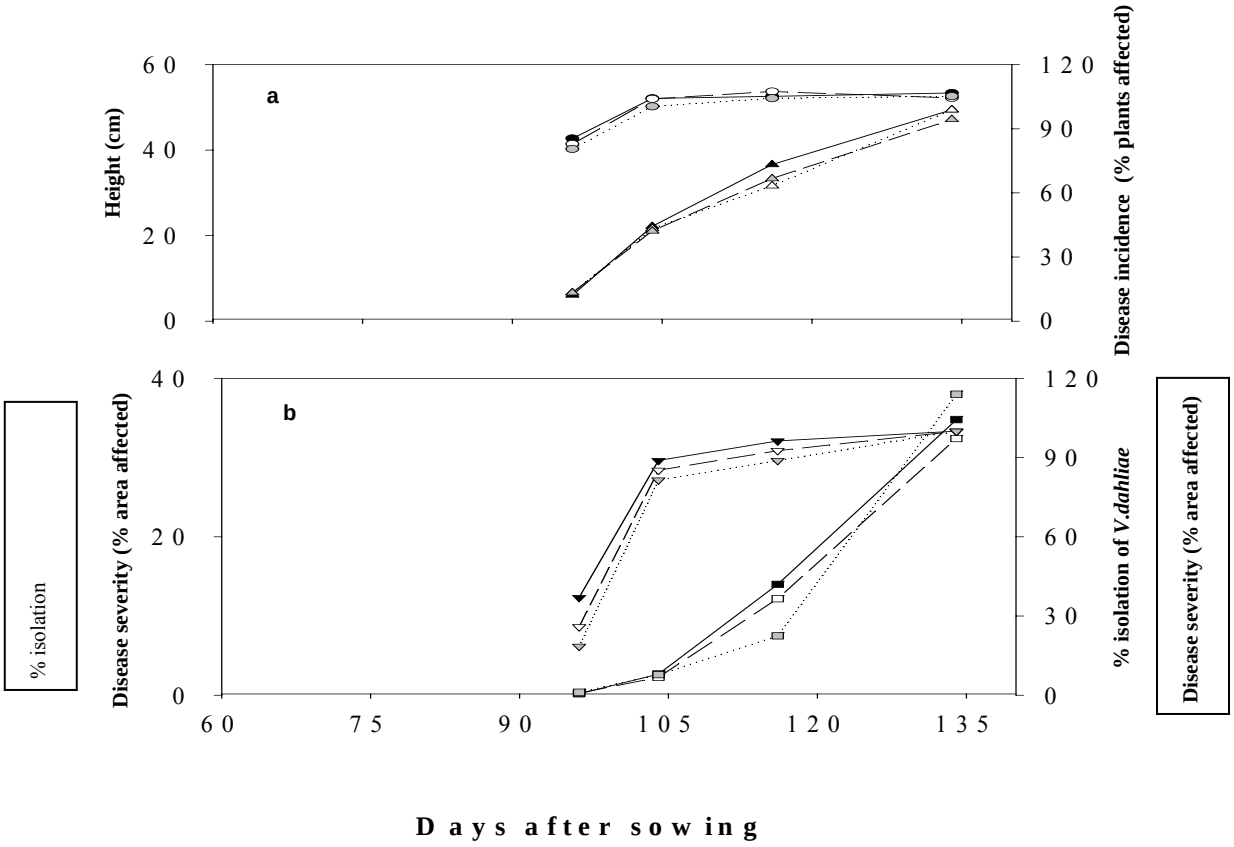


Figure 5. Effects of the previous crop species (linseed (L), sunflower (SU) and potato (P)) on the height (L ●, SU ●, P ○), disease incidence (L ▲, SU▲, P △) (a) and severity of disease (L ■, SU ■, P □) caused by *V.dahliae,* and isolation of *V.dahliae* (ISVD) (L ▼, SU ▼, P ▽) from the linseed plants 96, 104, 116 and 134 days after sowing (b) of a field experiment in spring 2000.

The pathogenicity experiments suggested that no host adaptation is present in the *V.dahliae* isolates from linseed plants. In the experiments, it was observed that some isolates from hosts other than linseed were more pathogenic to the linseed than isolates from linseed. The lack of host specificity in these *V.dahliae* isolates towards their host of origin is not unusual (Bhat and Subbarao, 1999). It has been reported that the *V.dahliae* isolates reflect the previous cropping history of the field rather than the host they have been isolated from (Tjamos, 1981), and as the isolates from linseed in these experiments were from a field in which various crops had been cultivated in the past, it might explain the lack of host adaptation of the isolates.

In the RFLP analysis of the *V.dahliae* isolates, the *V.dahliae* isolates from linseed did not show any distinctive pattern compared with other *V.dahliae* isolates used; they were in the same group as most of the other isolates which were analysed. The differences observed between isolates tested could not be related to host or to geographic origin. rDNA has been useful for distinguishing different *Verticillium* species (Carder and Barbara, 1991) but often has not produced enough information for differentiating isolates in the same species (Morton *et al*., 1995).

The effects of soil temperature on the expression of disease caused by *V.dahliae* were not clear-cut. The effect on the height of the plant was influenced by the isolate used and only with highly pathogenic isolates were the effects evident. When the effects of the soil temperature have been investigated, the maximum temperature at which *V.dahliae* can affect the crop was usually studied (Bell 1992; Schnathorst 1981), rather than the minimum temperatures at which *V.dahliae* could cause problems. However, inoculating linseed plants using spore suspensions may have reduced the effect of the soil temperature on the expression of the disease; in the field the fungus survives as microsclerotia, which germinate and infect the roots, a process which is affected by temperature. The effects of the air temperature were clearer, with the increase in temperature accelerating and enhancing the disease symptoms. At the lowest temperature tested (15^{o}C), not uncommon in UK during the summer, the fungus produced moderate symptoms, even though *V.dahliae* is favoured by higher temperatures.

In the field experiment, the previous crop that had been cultivated did not seem affect the amount of the inoculum, the disease incidence or severity. It has been reported that the previous crop can influence inoculum density; crops may decrease the inoculum density by stimulating the microsclerotia to germinate and die or increase the inoculum density though release of further microsclerotia into the soil. All the plant species used in the field experiment are hosts of *V.dahliae.* The increase in the *V.dahliae* inoculum density in soil did not differ significantly between plots where hosts other than linseed were grown and those where linseed was grown. This suggests that, although *V.dahliae* produces many microsclerotia on linseed plants, linseed does not increase the inoculum in the soil more than other hosts do.

Acknowledgements

We thank Dr D. Harris for providing the East Malling isolates, Dr K Elena from Benaki Phytopathological Institute for the Greek isolates, A. Barrow (Semundo Ltd.) for providing linseed seed. This work was funded by the EU (Project FAIR-BM-971336) under a Marie Curie Fellowship and by the UK Department of Environment, Food and Rural Affairs.

References

Anonymous 1994: *Genetics Computer Group (GCG),* Madison, Wisconsin.

Bell, A.A. 1992: *Verticillium* Wilt Cotton Diseases, R. J. Hillocks, ed., CAB International, Oxon, UK.

Bhat, R.G. and Subbarao, K.V. 1999: Host range specificity in *Verticillium dahliae*. *Phytopath*. 89: 1218-1225.

Butterfield, E.J. and DeVay, J.E. 1977: Reassessment of soil assays for *Verticillium dahliae*. *Phytopath.* 67: 1073-1078.

Carder, J.H. and Barbara, D.J. 199: Molecular variation and Restriction-Fragment-Length-Polymorphisms (RFLPs) within and between 6 species of *Verticillium*. *Mycol. Res.* 95: 935-942.

Freer, J.B.S. 1991: A development stage key for linseed (*Linum usitatissimum*). *Asp. Appl. Biol.* 28: 33-40.

Fitt, B.D. L., Bauers, F., Burhenne, S. and Paul, V.H. 1992: Occurence of *Verticillium dahliae* on linseed (*Linum usitatissimum*) in the UK and Germany. *Plant. Pathol.* 41: 86-90.

Harris, D.C., Yang, J.R. and Ridout, M.Z. 1993: The detection and estimation of *Verticillium dahliae* in naturally infested soil. *Plant. Pathol.* 42: 238-250.

Lee, S.B. and Taylor, J.W. 1990: Isolation of DNA from fungal mycelia and single spores. PCR protocols, a guide to methods and applications, M.A. Innis, ed., Academic Press, San Diego: 282-287.

Morton, A., Carder, J.H. and Barbara, D.J. 1995: Sequences of the Internal Transcribed Spacers of the Ribosomal RNA genes and relationships between isolates of *Verticillium albo-atrum* and *V. dahliae*. *Plant. Pathol.* 44: 183-190.

Okoli, C.A.N., Carder, J.H. and Barbara, D.J. 1993: Molecular variation and sub-specific groupings within *Verticillium dahliae*. *Mycol. Res.* 97: 233-239.

Schnathorst, W.C. 1981: *Life cycle and epidemiology of Verticillium. Fungal Wilt Diseases of Plants*, Academic Press Inc., New York, USA.

Termorshuizen, A.J. et al. 1998: Interlaboratory comparizon of methods to quantify microsclerotia of *Verticillium dahliae* in soil. *Appl. and Environ. Microb.* 64: 3846-3853.

Tjamos, E.C. 1981: Virulence of *Verticillium dahliae* and *V. albo-atrum* in tomato seedlings in relation to their host origin and applied cropping system. *Phytopath* 71: 98-100.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols. A guide for methods and applications,* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds., Academic Press, San Diego, CA, USA: 315-322.

Biological and Integrated Control of Diseases

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 67 - 75

First results of a three year field trial on the influence of Metconazol on plant morphology and yield development of oilseed rape (*Brassica napus*)

Dapprich, P.D.[1], Liu, Y.[1,2], Henneken, M.[1], Paul, V.H.[1], Föller, I.[1]

[1] *Universität-GH Paderborn, Abteilung Soest, Fachbereich Agrarwirtschaft, Labor für Biotechnologie und Qualitätssicherung, Lübecker Ring 2, D-59494 Soest*

[2] *Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, 610066 Chengdu, Sichuan, P.R. China*

Abstract: The triazole fungicide, tebuconazole, has been used in winter oilseed rape for about seven years. This group of triazoles is known not only for its fungicidal but also for its growth regulatory effects. In 1999 a new triazole fungicide with the active ingredient metconazole was registered as a growth regulator with fungicidal effects. This paper compares the growth regulatory characteristics of both triazoles, measuring typical parameters of plant development and yield formation.

In a three year field study, established in 1998 at the experimental farm of the Department of Agriculture in Merklingsen, metconazole was applied at three different concentrations (100 %, 75 % and 50 % of the standard application rate of 1,5 l/ha) at 10 different application times (early autumn, late autumn, early spring, late spring) and application numbers (one / two applications). At the growth stages 1.0, 1.5 (before winter), 1.10 (early spring), 5.0, 6.3, 6.7 (maturity), plant response (plant height, leaf position and colour, diameter of hypocotyl, fresh/dry matter, root length and tendency to lodge) was recorded.

A good correlation was found between product concentration and application time. Applications also had a significant effect on plant height, leaf position and colour and lodging. For example, the effect of the chemical on lodging was very evident after an unexpected snowfall during flowering in mid-April 1999 (5 cm of snow !).

Best results were seen with the early autumn treatment with an assessment rate of 3.0 – 3.8 versus 6.8 of the control. Also a combination of late autumn and early spring application tended to stand better than the control and other applications. For hypocotyl diameter, larger diameters were observed with increased concentration, but the effects were not significant and not reproducible over the years. However, yield was higher than the untreated control regardless of the treatment concentration.

Key words: Triazole, metconazole, winter oilseed rape, growth regulatory effects

Introduction

With regard to market prices and quality standards of winter oilseed rape it is necessary to optimise yield and production efficiency to ensure reliable profits for the producer/farmer. In order to increase yield of winter oilseed rape, in addition to changes in agromomic practises such as optimising drilling date or optimising cultivar selection and plant protection measures, control of plant growth is a predominant method to ensure yield and harvest efficiency.

With the use of triazole-fungicides it is now possible to take two measures like plant protection and growth regulation in one. The triazoles regulate growth height, resistance to lodging and thus lodging itself and they also increase winter hardiness and stress tolerance. Usually the strongest effects are to be expected when the triazoles are applied in autumn and in spring whilst the plant is undergoing active growth.

This study focuses on plant height, leaf position and colour, diameter hypocotyl, fresh and dry matter, root length and the tendency to lodge at different growth stages. This three year field study which began in 1998 used three different concentrations of metconazole as an active triazole ingredient. The results from 1998/99 and 1999/00 are presented. Fungicidal effect was also estimated, but will be dealt with in another article (see Henneken et al., 2001).

Materials and method

The experiment was sown in August 1998 at the experimental station of the Department of Agriculture in Merklingsen. The experiment consisted of four replicates (cv Lirajet) of 14 treatments of the growth regulating fungicides Caramba (a.i. metconazole) and Folicur (a.i. tebuconazole), applied in three concentrations and at different application times (Table 1). Each plot was 6 m long and 2,25 m wide. The central 5 x 2 m strip was harvested for yield estimation.

Table 1. Application times and concentrations of the growth regulating fungicides Caramba (metconazole) and Folicur (tebuconazole)

Variant	Product	l/ha	Application time
1	Caramba	1,5	autumn 1
2	Caramba	1,0	autumn 1
3	Caramba	0,7	autumn 1
4	Caramba	1,5 / 1,5	autumn 2/ spring 1
5	Caramba	1,0 / 1,0	autumn 2/ spring 1
6	Caramba	0,7 / 0,7	autumn 2/ spring 1
7	Caramba	1,5 / 1,5	autumn 2/ spring 2
8	Caramba	1,0 / 1,0	autumn 2 + spring 2
9	Caramba	0,7 / 0,7 / 1,0	autumn 2/ spring 1/ spring 2
10	Folicur	1,0	autumn 1
11	Folicur	1,0 / 1,0	autumn 2/ spring 1
12	Folicur	1,0 / 1,0	autumn 2/ spring 2
13	Folicur	0,5 / 0,5 / 0,7	autumn 2/ spring 1/ spring 2
14	control	–	–

autumn 1 = EC 18-19 (3-4-leaf stage); autumn 2 = EC 23-25 (6-8-leaf stage); spring 1 = EC 39-59 (prior flowering); spring 2 = EC 65 (main flowering)

Table 2. Assessment parameters and frequencies used to determine the effects of the products to plant growth. The parameters were assessed in the field and in the laboratory (e.g. root mass etc.).

no	assessment	unit	frequency	growth stage (EC)
1.	Root length	cm	7	24-25,26,26,27-31,64,83,87-89
2.	Fresh-/ dry-matter of the roots	g	7	24-25,26,26,27-31,64,83,87-89
3.	Hypocotyl diameter	cm	7	24-25,26,26,27-31,64,83,87-89
4.	Fresh-/ dry-matter of the plants	g	7	24-25,26,26,27-31,64,83,87-89
5.	Leaf position and colour		7	24-25,26,26,27-31,64,83,87-89
6.	Plant height	cm	7	24-25,26,26,27-31,64,83,87-89
7.	Tendency to lodging		7	24-25,26,26,27-31,64,83,87-89
8.	Yield	dt/ha	1	89

Results

1. Root length

Root length was measured because in theory a good root growth is believed to ensure good plant growth and optimal supply of the plant with nutrients and water. As winter oilseed rape forms a strong taproot that branches readily, the length of the taproot was estimated during the vegetative period of plant growth.

The results obtained show significant differences only in winter 1998/99 for treatment 5 with 14.4 cm versus 11.5 cm root length (see fig. 1).

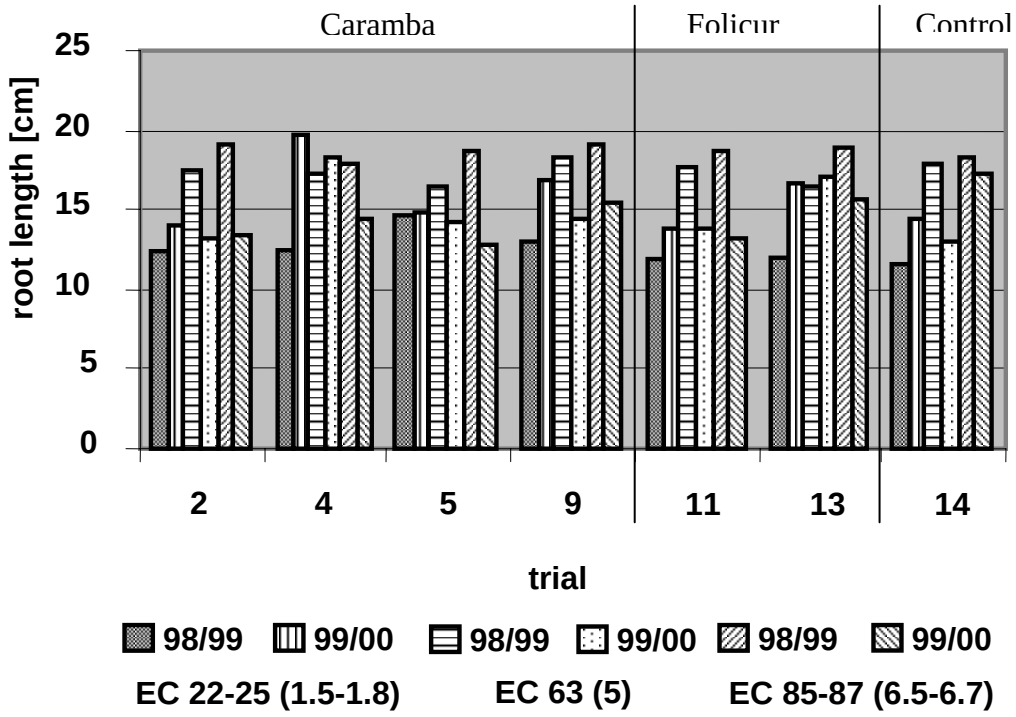


Figure 1. Effect of triazoles on root length of winter oilseed rape applied at different development stages in 1998/99 and 1999/00

2. Fresh-/dry-matter of the roots

The fresh and dry weights of the roots show clear differences in 1999 and 2000. Even within years, there are differences between treatments with a tendency for increased fresh weight of root matter when applied twice, with one application in autumn and one in spring (see fig. 2, applications 8 and 13) in comparison to the control. The difference between 1999 and 2000 and the control for treatment 1 (1.5 l Caramba early in autumn) is not easy to explain and must be regarded as an unreliable result.

Statistical analysis for differences in fresh and dry weight of roots was not possible due to too the lack of replication with regard to application date.

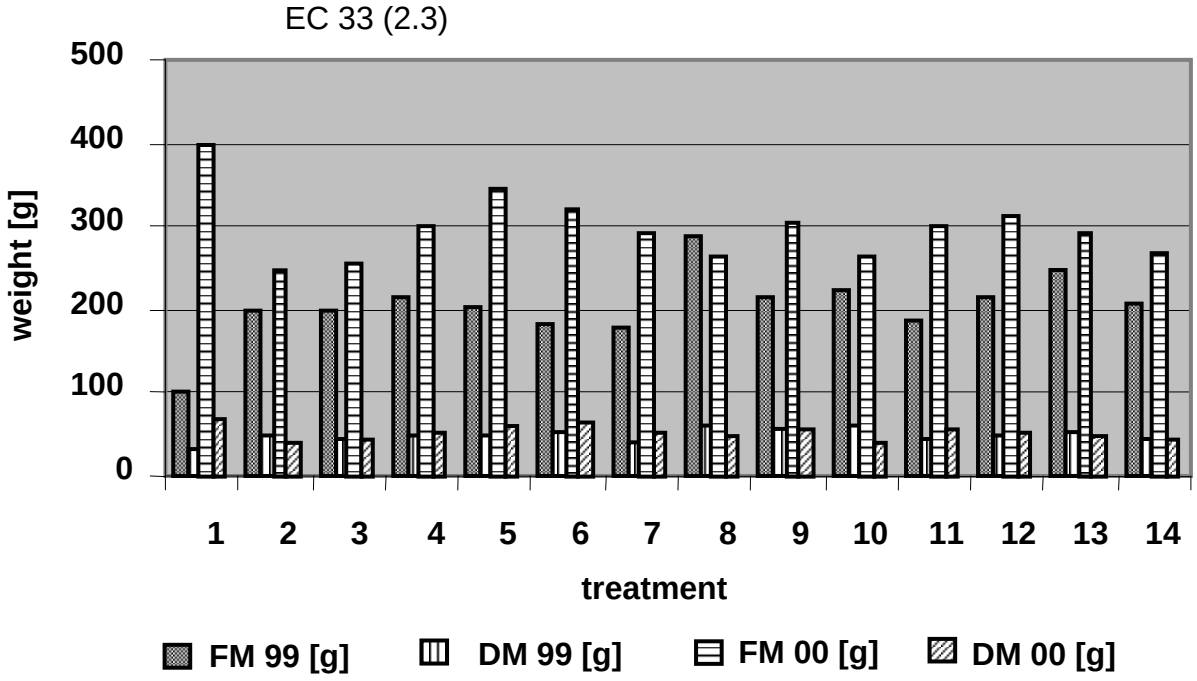


Figure 2. Fresh and dry weight of root biomass in spring 1999 and 2000

3. Hypocotyl diameter

There was a clear difference in hypocotyl diameter between the two experimental periods (1998/99 and 1999/00) with larger hypocotyl diameters in 1999/00. The expected increase in hypocotyl diameters during the vegetative period is very evident with only few significant differences within the treatments (figure 3).

In winter 1998/99 (EC 22-25) treatments 9 and 12 showed significantly smaller hypocotyl diameters in comparison to the control (fig. 3). However, this effect was outgrown by early spring. At EC 63 only treatment 6 had significantly larger hypocotyl diameters than the control. By harvest, only treatments 1 and 2 outperformed the control significantly.

A somewhat different picture was obtained in 1999/00. For all treatments apart from treatments 2 and 8, significant larger hypocotyl diameters were found in comparison to the control (data not shown). However, by harvest these differences were outgrown and the treatments only tended to induce increased hypocotyl diameters but differences were not significant (see fig. 3).

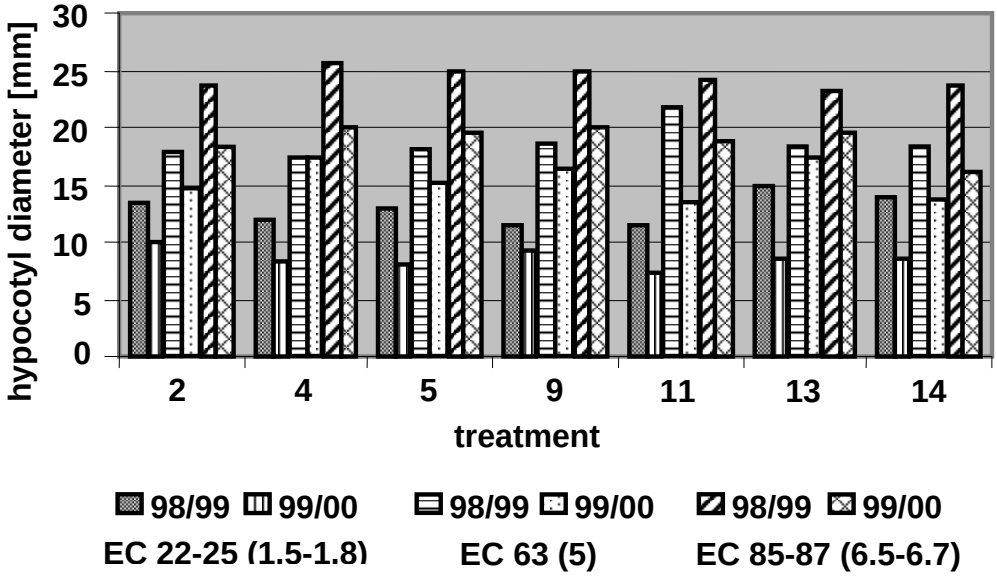


Figure 3. Effect of triazoles on hypocotyl diameter of winter oilseed rape applied at different developing stages in 1998/99 and 1999/00

4. Fresh-/drymatter of winter oilseed rape plants (without roots)

With regard to plant fresh matter there was a tendency for increased fresh matter with reduced triazole application as well as with split applications in late autumn and spring (fig. 4). These effects are evident despite the fact that the amount of fresh matter varied between the two seasons. Caramba most evidently produced this effect in 1999 (see fig. 4 variant 1, 2 and 3) and provides equal control with an application of 50% of the advised concentration.

The results for the dry matter mirror the results from the fresh matter for both fungicides and all treatments. Again it should be kept in mind that differences could not be reliably verified by statistic methods due to the lack of replication.

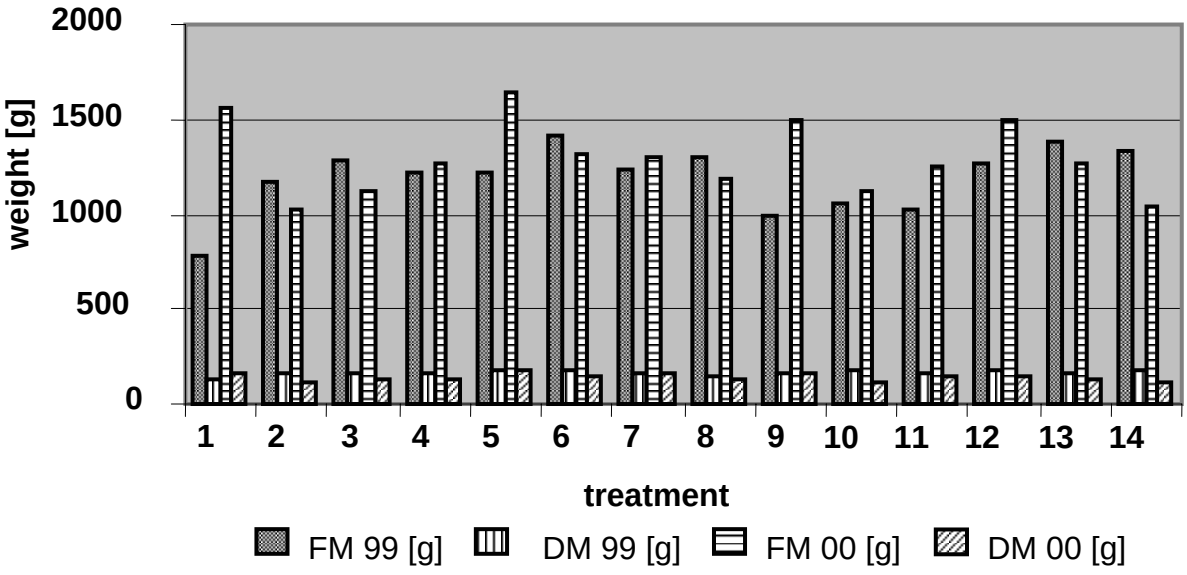


Figure 4. Fresh-/drymatter of winter oilseed rape plants harvested in spring (EC 39) in 1999 and 2000

5. Leaf position and colour of winter oilseed rape

Both triazole fungicides seem to influence the overall appearance of the plants in a positive way compared to the control. The effect seemed more evident for Caramba then for Folicur (fig. 5). In 1998/99, all three full-rate Caramba and the full-rate Folicur applications (see fig. 5 treatments 1, 2, 3 and 10) resulted in plants having a significantly better overall appearance. Treatments 1, 2 and 10 were significantly (p>0.1 %) better than the control (data not shown).

This effect was not significant, but was still evident, in 1999 (see fig. 5). Prior to harvest other treatments showed more effect such as treatments 5, 8 and 9. Even the low concentration, split application of Folicur was adjudged to be highly significantly (p>0.1 %) better in overall appearance in comparison to the control. It would appear that there was a tendency that even at reduced rates of product application, there was still a good positive effect if applied early in autumn. A split application in late autumn, in combination with a second application in spring, did not result in the same positive effect. A triple split application even equals the control (with both products) and thus showed no effect on leaf position and colour at all (see application 9 and 13 fig. 5).

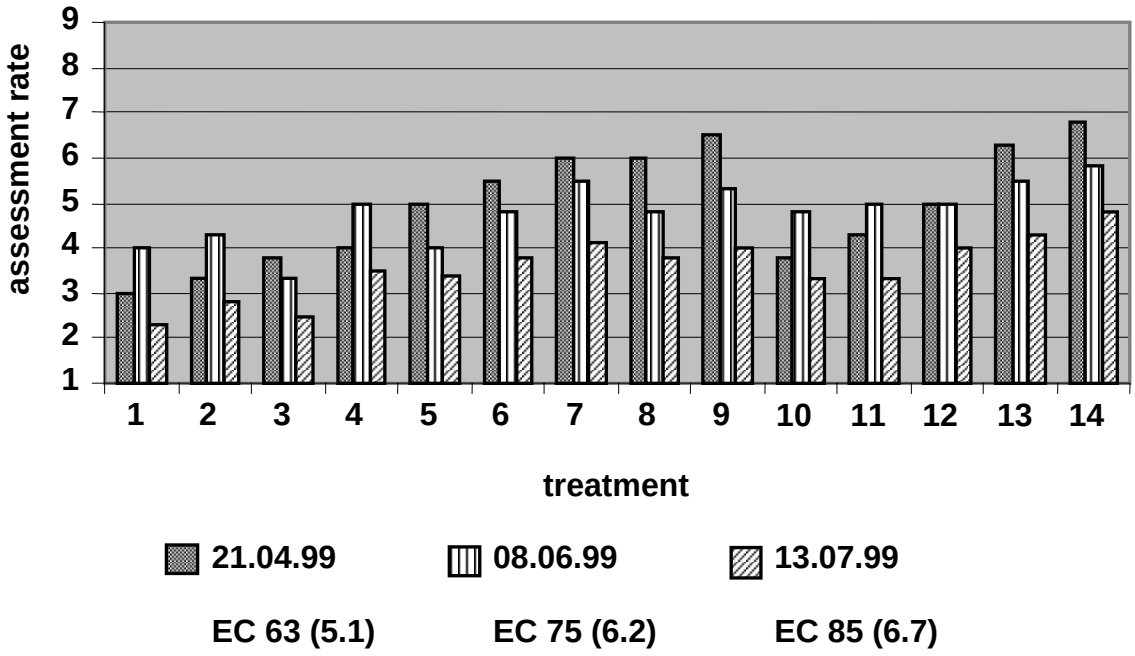


Figure 5. Leaf position and colour of winter oilseed rape after different treatments with triazoles in 1999 (1=good overall appearance of the plant, 9=bad overall appearance of the plant)

6. Plant height

With regard to plant height, best results were obtained with split application of each product (fig. 6). In late winter, at EC 23-24 significantly reduced plant height was found for treatment 4, 5, 6, 7, 9, 10, 11 and 12 with highest growth reduction in treatment 12 with 5.9 cm versus 6.9 cm in the control. In early spring, treatments 5, 6 and 7 showed significantly longer shoots than the control with 12.1 versus 10.6 cm. No significant difference was found during flowering (EC 64). At the early ripening stage (EC 75) and prior to harvest (EC 87), significant reduction in plant height was found for treatments 3, 4, 5, 6, 7, 9, 11, 13 and 4, 5,

6, 7, 9, 11,13 in comparison to the control. The highest reduction compared to the control was found for treatment 5 with 126 cm versus 154.8 at EC 75 and for treatment 7 with 134.6 versus 152.2 at EC 87 (see fig. 6)

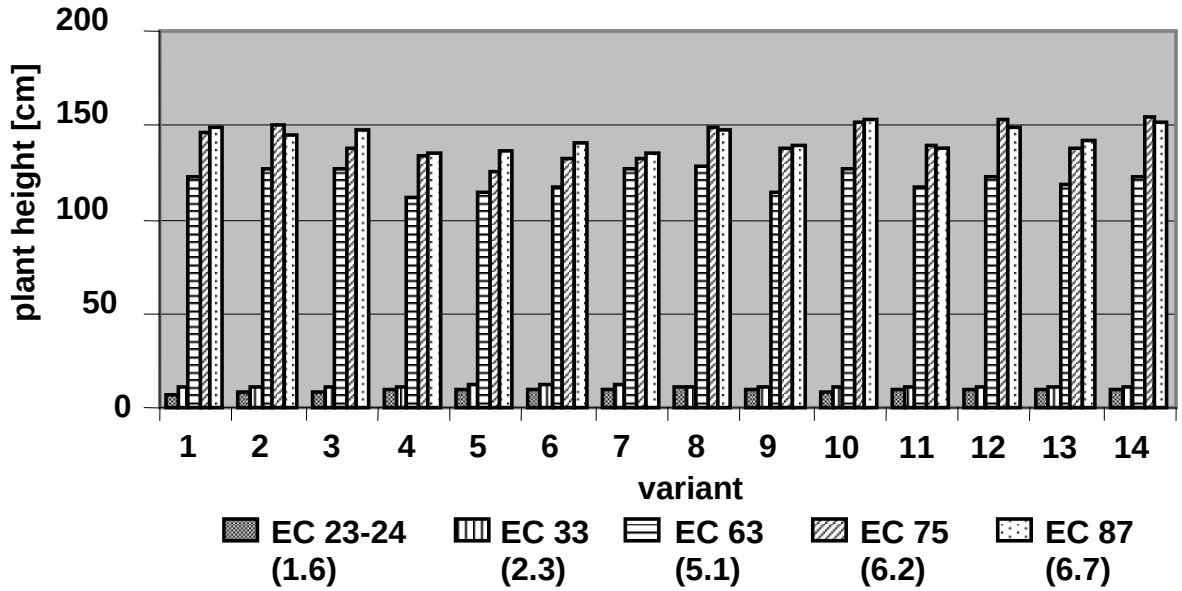
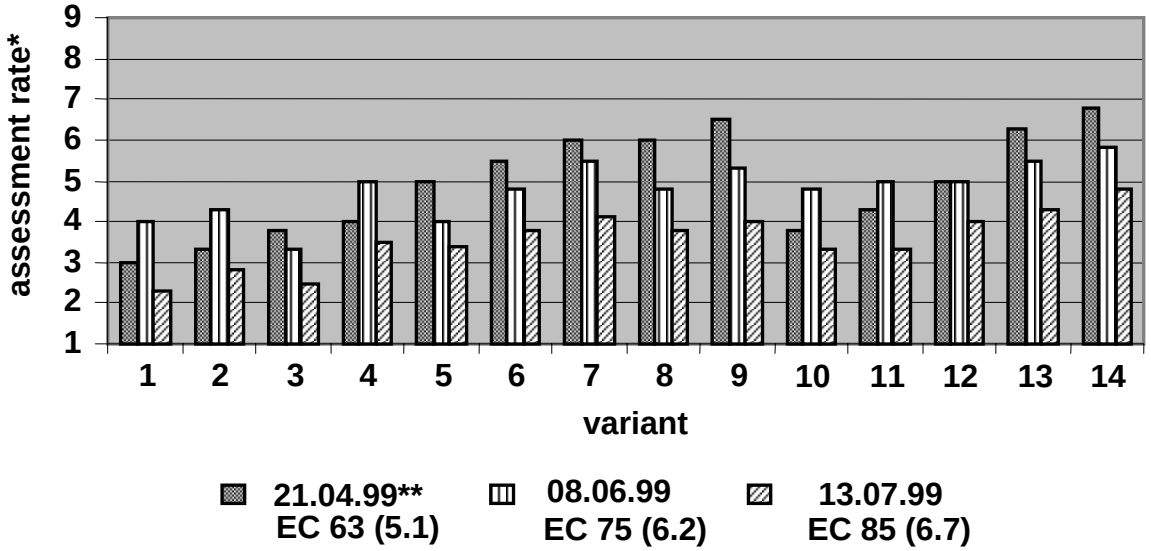


Figure 6. Plant height of winter oilseed rape after different treatments with triazoles in 1999/00



*assessment rate 1 = no lodging, 9 = no triazole effect; **assessed two days after heavy snowfall

Figure 7. Tendency to lodging of winter oilseed rape after different treatments with triazoles in 1999

7. Tendency to lodging

In 1998 no significant differences in tendency to lodging were observed. In 1999 the tendency to lodging seemed to be controlled well by full and even reduced doses of Caramba when applied early in spring (see treatment 1, 2, 3 fig. 7). Split applications and the application of Folicur also performed well (treatments 4, 5, 6 10, 11, 12 fig. 7) but these effects were only highly significant (p>0.1 %) for split applications of both triazoles prior to harvest (treatments 4, 5, 6, 9, 11, 13 fig. 7).

8. Yield

With regard to yield in 1998/99 only one treatment (treatment 3 fig. 8) performed significantly better than the control and the rest of the treatments with 42.8 dt/ha in comparison to 33 dt/ha. In 1999/00 all treatments except the early autumn Caramba applications (treatments 1, 2 and 3, fig. 8) performed significantly better than the control. With the exception of treatment 5, all other applications yielded significantly better than the control (p>0.1). The best yield was obtained from treatent 12 (split application Folicur late autumn and late spring) with 42.05 dt/ha compared to 32.35 dt/ha for the control (see fig. 8).

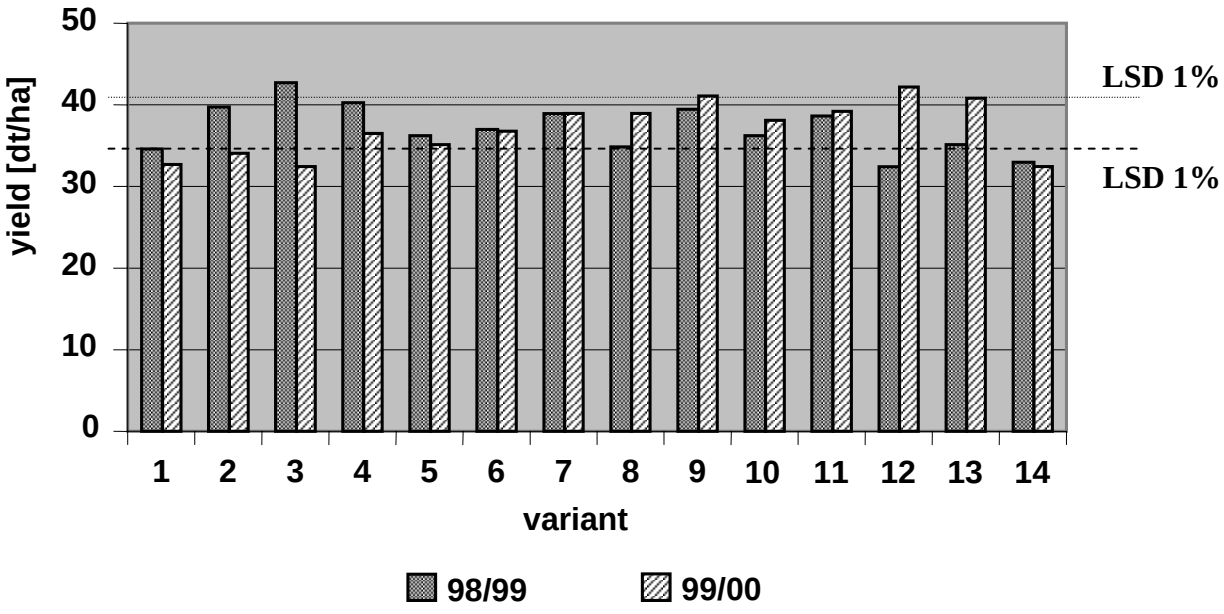


Figure 8. Yield of winter oilseed rape after different treatments with triazoles in 1998/99 and 1999/00

Discussion

The results of this study indicate that triazoles do effect plant growth in various ways. All major parameters such as root length, fresh and dry weight matter of the roots, hypocotyl diameter, fresh and dry weight of the plant parts above ground, overall appearance, plant height, tendency to lodge and yield are affected. Yet the results show that all these effects are strongly dependent on weather conditions as can be seen e.g. in the effects on root length,

hypocotyl diameter and yield. A potential cultivar influence can be assumed, but this can not be verified by the results of this study.

The most predominant positive effect of triazoles was observed with regard to the tendency to lodge. Though not always significant, use of the chemicals produced a clear tendency to the production of better standing plants. The effect was strongest with full concentration application early in autumn of both triazoles tested with a slightly better performance of Caramba over Folicur. This better standing ability probably leads to better yield as both triazoles increased yield to a comparable extent. Yet one major effect of the triazoles that definately occurred during the study, but which has not been included in the results is the fungicidal effects associated with these compounds. These plant-protecting effects probably outperform the effect on plant growth and thus are predominantly responsible for the yield increase.

Summarising all the results of this study, it is possible to conclude that positive effects on plant growth like increased root length, increased hypocotyl diameter and reduced plant height can be expected when triazoles are applied. This is true for both triazoles tested and for singular and split applications. If these effects turn out to be significant and yield affecting in the end is strongly dependent from weather influence, overall management and probably cultivar effects.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 77 - 85

Investigations on the effects of Metconazol on selected oilseed rape diseases under controlled conditions

Liu, Y. [1,2]; Föller, I. [1]; Henneken, M. [1]; Paul, V.H. [1]
[1] *Labor für Biotechnologie und Qualitätssicherung, Universität-GH Paderborn, Fachbereich Agrarwirtschaft, Lübecker Ring 2, D-59494 Soest, Germany*
[2] *Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, 610066 Chengdu, Sichuan, P.R. China*

Abstract: First results of the effects of Metconazol (C) and a comparative product (VGM) on stem rot, powdery mildew and *Rhizoctonia*-Damping off on *Brassica napus* have been obtained in climatic chamber experiments. Through different inoculation methods the protective and curative effects of Metconazol in 3 different concentrations (100%, 75% und 50% of the registered doses of 1,5 l/ha) were investigated.

Application date and application concentration showed a high correlation with the effects of the fungicide. The earlier the application and the higher the concentration the better the effects of the fungicide against the pathogens tested.

Keywords: *Brassica napus*, Metconazole, Tebuconazole, *Sclerotinia sclerotiorum*, *Erysiphe cruciferarum*, *Rhizoctonia solani*

Introduction

Triazole fungicides have been used in plant production for more than 20 years and are known to stabilize or raise the yields even without the presence of fungal diseases (Sampson,1992; Bolton, 1992). There are a multitude of reasons for this and they are not easy to differentiate. Generally the fungicide induces a better fitness against stress combined with reduced aging. Beside the triazole fungicides which are already established in oilseed rape production, a new fungicide with the active ingredient Metconazol is now on the market(Terence, 1995; Cyanamid Agrar 1995). It was the goal of this study two compare these to fungicides under different conditions. Beside the field trials (see Dapprich et al.; Paul 2001), extensive laboratory studies were carried out to assess the effects of the two fungicides on Stem rot (*Sclerotinia sclerotiorum*), Powdery mildew (*Erysiphe cruciferarum*), and *Rhizoctonia*-Damping off (*Rhizoctonia solani*).

Material and methods

Inoculation with stem rot

For inoculation with the pathogen of stem rot *Sclerotinia sclerotiorum* (s. Table 1) true leaves of *Brassica napus* were cut of thew plants and then contact-inoculated with 25 µl drops of a mycelial suspension and placed in a humid chamber (approximately 100 % rel. humidity).

To study the curative effects the leafs were treated with Metconazol and the comparative product 24, 48, 96 and 144 hours after inoculation.

For the study of protective effects the leafs were treated with Metconazol and the comparative product 24, 48, 96 and 120 hours prior to inoculation.

The assessment (measurement of the diameter of the lesions) was accomplished 8 days after inoculation.

Table 1. Inoculation of *Brassica napus* true leaves with the pathogen of stem rot *Sclerotinia sclerotiorum* and application of Metconazol/VGM and the comparative product

Plant material	2nd to 4th true leaf of *Brassica napus*
Inoculation material	Mycelia suspension of *S. sclerotiorum*
Inoculation method	25 µl of the mycelia suspension placed on the surface of true leafs
Treatment	Protective: application 1, 24, 48, 96, and 144 h before inoculation Curative: application 24, 48, 96 and 120 h after inoculation
Incubation	At 14/10 h day/night and 18/12 °C at approximately 100 % relative humidity in an humid chamber
Assessment	Diameter of lesions/spots on the leafs

Inoculation with powdery mildew

The inoculation with the pathogen of powdery mildew *Erysiphe cruciferarum* was accomplished using a spray inoculation (conidia conzentration 10^4 conidia/ml).

To study of the curative effects the plants were treated with the full conzentartion (100 %) of Metconazol and the comparative product 120 and 240 hours after inoculation.

To study of protective effects plants were treated with the fullconzentration (100 %) and reduced conzentrations (75 % and 50 %) of Metconazol and the comparative product 120 hours prior to inoculation.

Table 2. Inoculation of *Brassica napus* with the pathogen of powdery mildew *Erysiphe cruciferarum* and application of Metconazole/VGM

Plant material	*B. napus* in the 2nd to 3rd true leaf stage
Inoculation material	Conidia suspension of *Erysiphe cruciferarum* from infected plants
Inoculation method	Spray inoculation with a conidia density of 10^4 conidia/ml
Treatment	Protective: application 120 h prior to inoculation Curative: application 120 and 240 h after inoculation
Incubation	At 14/10 h day/night and 18/12 °C at 6000 Lux 60 - 85 % relative humidity
Assessment	Sporulation area (in %) on the leaves

The assessments for the curative and protective effects were carried out 33 days after inoculation according to the assessment scheme in Table 3.

Table 3. Assessment scheme for powdery mildew (*Erysiphe cruciferarum*)

Assessment rate	Sporulation area on the leaf surface in %
no sporulation	1
>0-5	2
>5-10	3
>10-15	4
>15-20	5
>20-25	6
>25-50	7
>50-75	8
>75	9

Inoculation with Rhizoctonia-Damping off

To study the effect of Metconazol on *Rhizoctonia* damping off, this pathogen was mixed (in petri dishes (PDA, Merck)) with substrate (20g Agar/kg soil). Oilseed rape seeds were sown into this substrate after an incubation period of 48 h.

For the study of the curative effects each seed was treated with 25 µl of full conzentration (100 %) and reduced conzentrations (75 % and 50 %) of Metconazol and the comparative product (seed coating) 96 and 168 h after sowing. The two fungitides were placed on the seeds in form of drops.

For the study of protective effects each seed was treated with 25 µl of the above mentioned solutions of Metconazol and the comparative product (seed coating) directly on the seeds.

Table 4. Substrate inoculation of *Brassica napus* with the pathogen of *Rhizoctonia*-Damping off *Rhizoctonia solani* and application of Metconazole/VGM

Plant material	Seeds of *B. napus*
Inoculation material	Different isolates of *R. solani*
Inoculation method	Mixing of agar plates (PDA) with *R. solani* in substrate (20 g agar plates/kg substrate) and sowing of *B. napus* 48 h after substrate incubation
Treatment	Protective: application of 25µl drops of the respective solutions directly on each seed after sowing Curative: application of 25µl on each seed 96 and 168 h after sowing
Incubation	At 14/10 h day/night and 18/12 °C at 6000 Lux 60 - 85 % relative humidity
Assessment	see assessment schema

The assessments for the curative effects were carried out 15 days after substrate inoculation (13 days after sowing) and for the protective effects 19 days after substrate inoculation (17 days after sowing) according to the assessment scheme in Table 5.

Table 5. Assessment scheme for *Rhizoctonia*-Damping off *(Rhizoctonia solani*)

Assessment rate	Description of the symptoms
1	Plant without symptoms
2	Plant without unfolded cotyledons, stem or stem base partly grey
3	Stem base grey to brown, not rotten
4	Stem base partly rotten
5	Stem base white, rotten, girdled, not fallen over
6	Stem base brown, destroyed and fallen over
7	Stem base brown to black, and nearly dry
8	Plant died off
9	Plant not emerged

Results and discussion

Stem rot

A protective application of Metconazol (C) generally reduced the extent of lesions of stem rot (*Sclerotinia sclerotiorum*). A treatment with Metconazole reduced the lesions from between 6,9 and 8,1 cm in the control (Ko) to between 0,2 and 2,8 cm (s. Figure 1). Best results were obtained when the time between application of Metconazol and inoculation was short. When the inoculation was carried out 96 to 144 h after the protective application of Metconazol the positive effects were reduced. Similar results were obtained with the comparative product (VGM). Here the lesions were reduced to between 0,5 and 0,9 cm. Additionally, the positive reductive effect lasted longer than with the Metconazole fungicide.

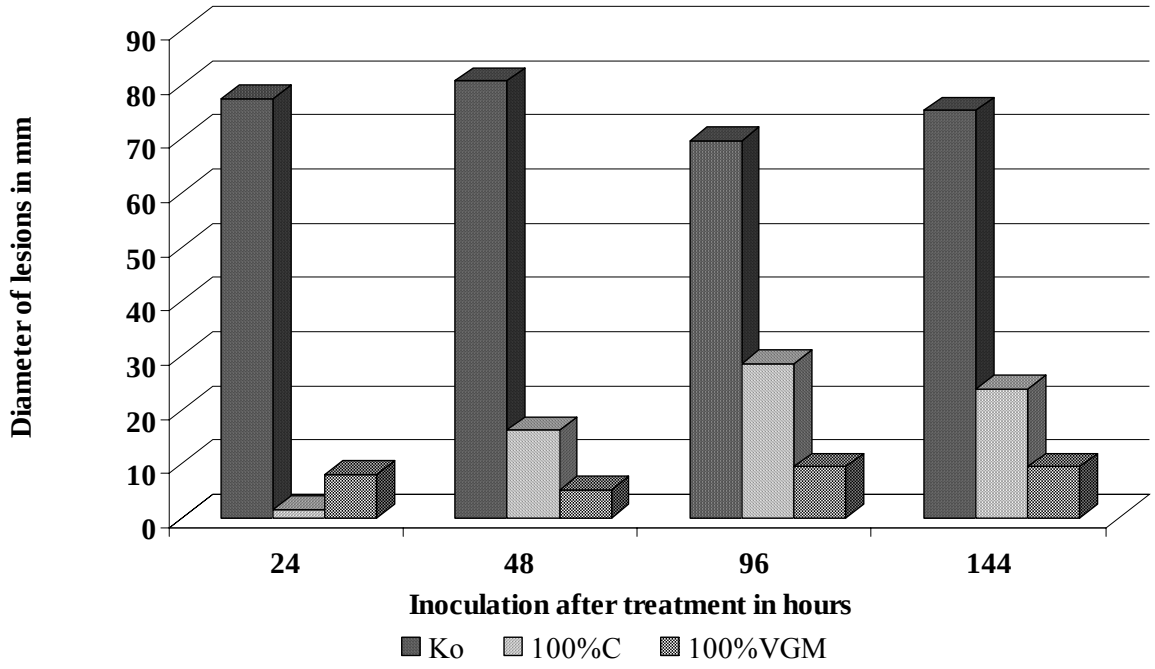


Figure 1. Protective effects of Metconazol/VGM on stem rot (*Sclerotinia sclerotiorum*); True leaf testA curative application of Metconazole (C) and the comparative product

(VGM) also reduced the extend of stem rot lesions. A treatment with 100 % Metconazole reduced the lesions from 10 cm in the control to between 0,1 and 5,6 cm (s. Figure 2). Best results were obtained when application of Metconazol was shortly after the inoculation with stem rot. Similar results were obtained with the comparative product (VGM). Here the lesions were reduced to between 0,2 and 5,7 cm. In the curative treatment no differences in the two products with regard to the duration of the positive effects could be observed. The best positive effects were obtained when the treatment with each product were carried out 24 to 48 h after inoculation with stem rot.

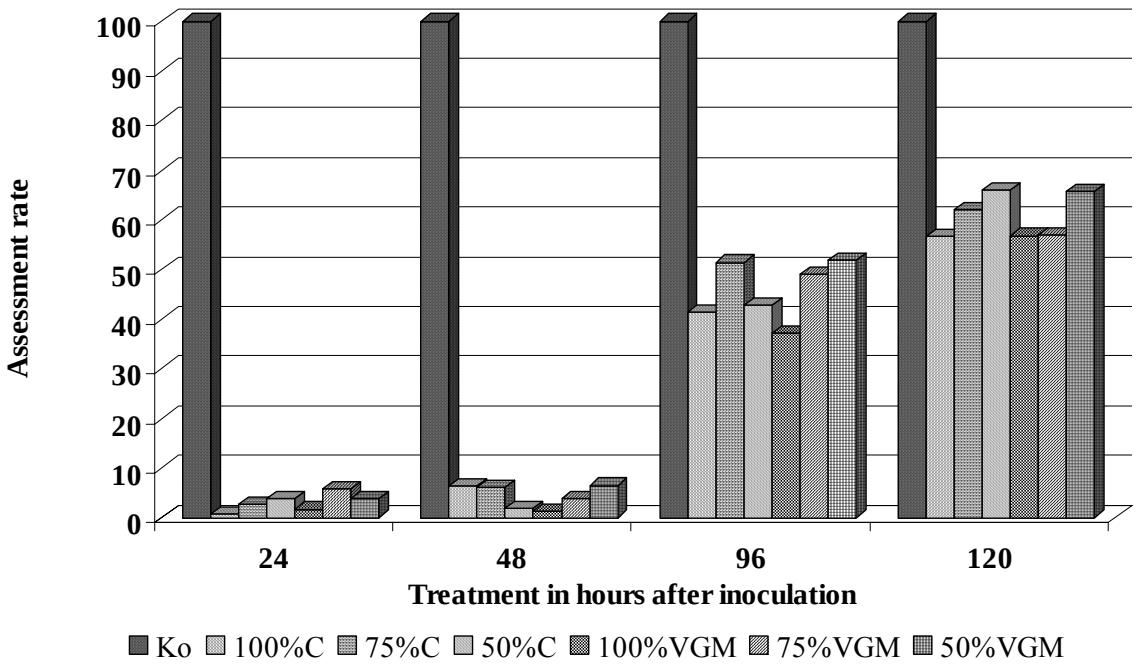


Figure 2. Curative effects of Metconazol/VGM on stem rot (*Sclerotinia sclerotiorum*); True leaf test

Powdery mildew

Both protective and curative applications of Metconazol (C) and the comparative product (VGM) reduced powdery mildew (*Erysiphe cruciferarum*). In the protective treatments the assessment rates were reduced from 8,7 in the control to between 1,4 and 2,6 in the Metconazole and between 1,7 and 2,5 in the VGM treated variants (s. Figure 3).

In the curative treatments a positive effect was noted as well. Here the assessment rates were reduced from between 7,8 and 8,1 in the control to between 1,2 to 1,5 for both products (s. Figure 4). In addition the infection frequency 33 days after inoculation was reduced. Approximately 70 % of the treated and 100 % of the control plants were infected. Furthermore it was observed that the production of conidia started 20 days later than in the control.

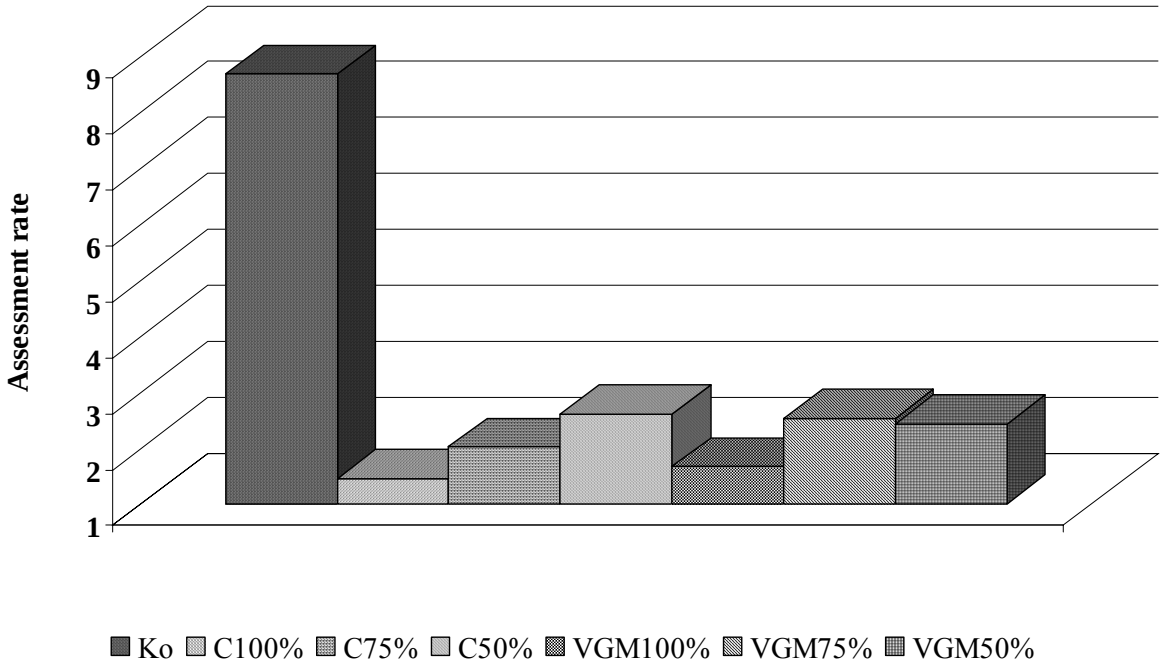


Figure 3. Protective effects of Metconazol/VGM on powdery mildew (*Erysiphe cruciferarum*), treatment 5 days before inoculation, first symptoms appeared after 12 days; assessment 33 days after inoculation

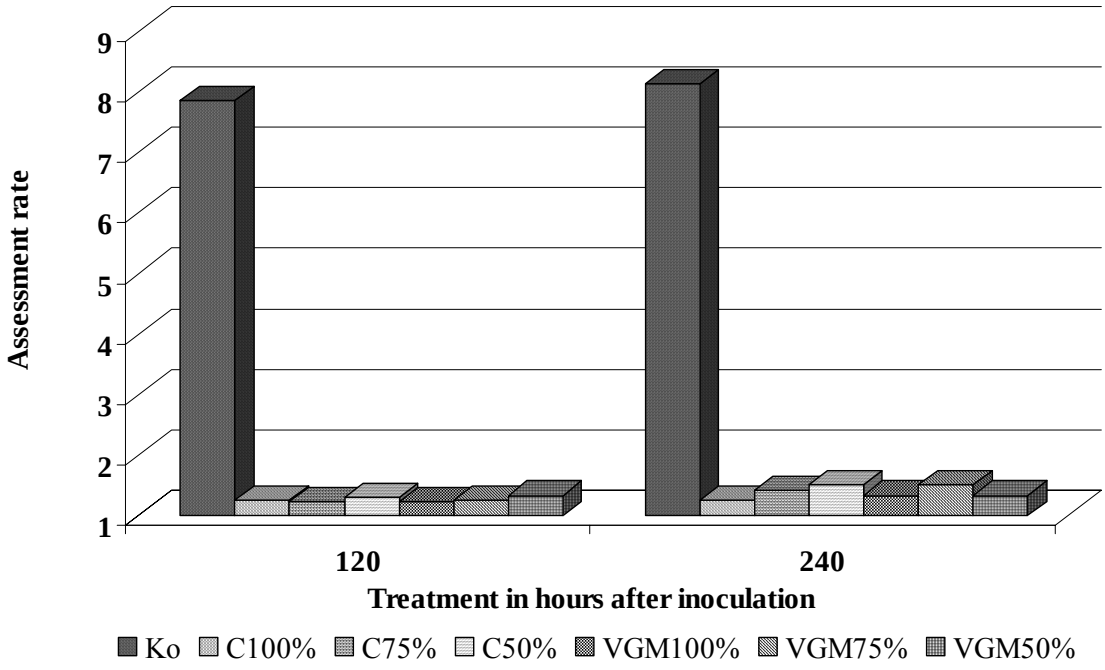


Figure 4. Curative effects of Metconazol/VGM on powdery mildew (*Erysiphe cruciferarum*); assessment 33 days after inoculation

Rhizoctonia damping off

Metconazol (C) and the comparative product (VGM) showed positive effects against *Rhizoctonia* damping off (*Rhizoctonia solani*) as well.

With a protective application the infection intensity was reduced from assessment rate 6,7 in the control to between 1,4 to 2,2 in the Metconazole and between 1,7 and 2,2 in the VGM treated variants (s. Figure 5).

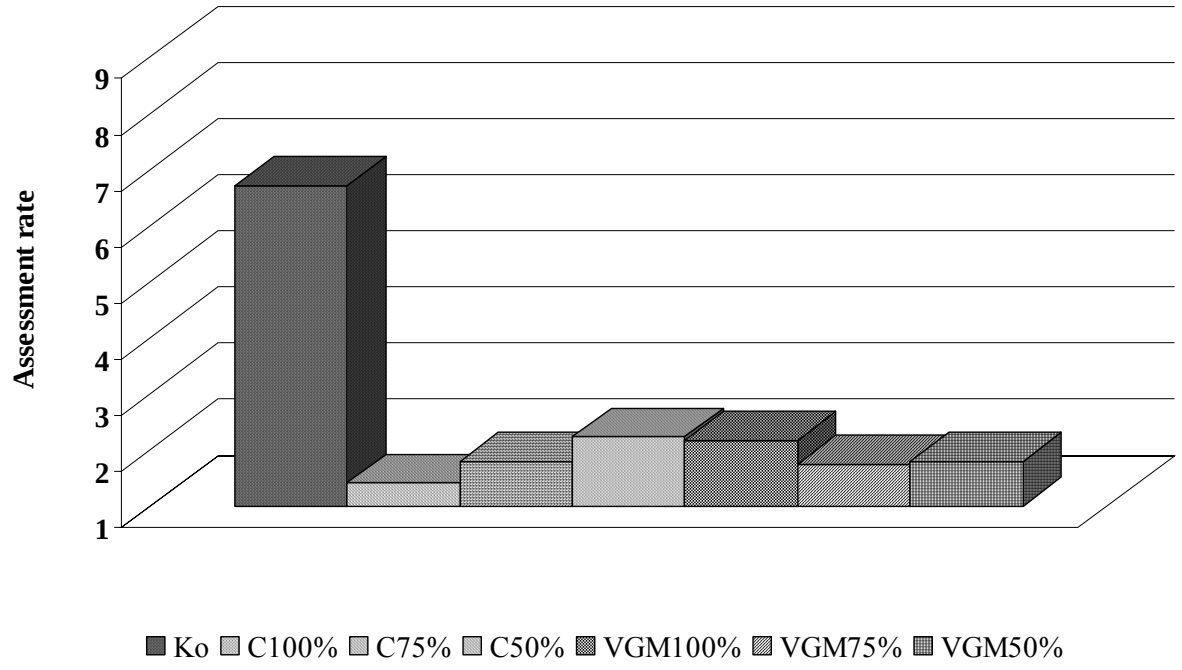


Figure 5. Protective effects of Metconazol/VGM on *Rhizoctonia*-Damping off (*Rhizoctonia solani*); treatment as seed coating; assessment 18 days after substrate inoculation

The effects of the curative applications depended on the application date (s. Figure 6). The application of Metconazol (C) and the comparative product (VGM) 96 h after inoculation lead to a higher reduction of disease symptoms than a later application date. The assessment rate of the Metconazol treated variants were reduced from 4,4 in the control to between 2,4 and 2,6 96 h after inoculation and to between 3,1 and 3,7 168 h after inoculation. The VGM treated variants were reduced from 4,4 in the control to between 2,0 and 2,8 96 h after inoculation and to between 2,8 and 3,4 168 h after inoculation. Furthermore, a reduction of effectiveness at both application dates could be observed in the VGM when lower concentration rates (75 and 50 % of registered doses) were used. When the VGM was applied 96 h after inoculation the effectiveness was reduced from an assessment rate of 2,0 at 100 % registered doses to 2,7 (75 %) and 2,8 (50 %), and in the treatment 168 h after inoculation from 2,8 (100 %) to 3,3 (75%) and 3,4 (50 %).

In the Metconazol treatments a similar reduction with decreasing concentrations was not observed. A reduction of effectiveness was only observed in the combination of the later application date with lower concentration rates (3,1 at 100% and 3,7 and 3,3 at 75 and 50 % registered doses respectively).

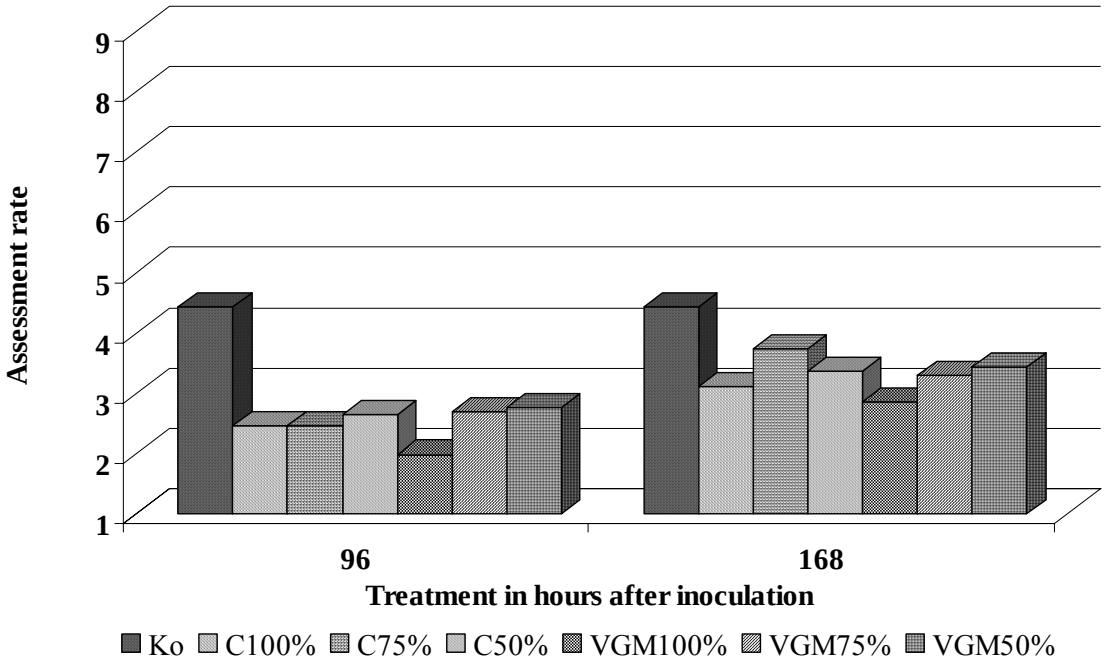


Figure 6. Curative effects of Metconazol/VGM on *Rhizoctonia*-Damping off (*Rhizoctonia solani*)

Table 6. Summary and overview on the effects of Metconazol on diseases/pathogens of oilseed rape (*Brassica napus*)

Diseases/ Pathogen	Inoculation method	Effects		Duration of effects	
		protective	curative	protective	curative
Stem rot *Sclerotinia sclerotiorum*	Drop inoculation of true leafs	good	good	up to 8 days good	up to 7 days good
Powdery mildew *Erysiphe cruciferarum*	Spray inoculation of complete plants	good	good	up to 34 days good	up to 38 days good
Rhizoctonia-Damping off *Rhizoctonia solani*	Substrate inoculation	good	some	up to 17 days good	up to 21 days some
Stem canker *Phoma lingam*	Drop inoculation of the cotyledon	good	good	up to 25 days good	up to 26 days good
Grey mould *Botrytis cinerea*	Inoculation with agar pieces	good	good	up to 8 days good	up to 6 days good
Dark leaf and pod spot *Alternaria brassicae*	Spray inoculation of true leafs	good	n.k.	up to 20 days good	n.k.
Verticillium Wilt *Verticillium dahliae*	Dip inoculation	some	n.k.	up to 28 days medium	n.k.

n.k. = not known; some ≈ 50 % reduction of symptoms compared to control; good ≈ 75 % reduction of symptoms compared to control**Summary**

Good effects against stem root, powdery mildew and *Rhizoctonia*-Damping have been obtained applying Metconazole protectively (s. Table 6). Similar results have been obtained applying the comparative product. There was a marked difference in the duration of the effects of Metconazole, as well as the comparative product, against different pathogens. The best protective results were obtained against powdery mildew where the effect of Metconazole lasted for 34 days. The protective effect against stem rot was only 8 days, but during this time the fungus was reduced by 75 % compared to the control.

When the products were applied curatively good results were obtained against stem root, powdery mildew and some results against *Rhizoctonia*-Damping off. Here, similar to the results of the protective application, marked differences in the duration of the positive effects were noted. The best curative results were obtained against powdery mildew where the effect of Metconazole lasted for 38 days (4 days more than the protective effects). The curative effect against stem rot was only 7 days (one day less than the protective effects), but during this time the fungus was reduced as good as in the protective application.

References

Sampson, A.J. 1992: Metconazole, an advance in disease control in cereals and other crops. *Brighton Protection Conference – Pests and Diseases* 5-4: 419-426.

Bolton, B.J.G. 1992: The use of Tebuconazole for disease control and subsequent effects on lodging in oilseed rape. *Brighton Protection Conference – Pests and Diseases* 60-24: 675-680.

Terence, G.B., Boyd, S.L., Sampson, A.J. 1995: Effect of adjuvants on the performance of the new Fungizide metconazole. *Pestic. Sci.* 45: 153-160.

Cyanamid Agrar 1998: Informationsbroschüre: Metconazol – der innovative Wirkstoff für Getreide und Raps.

Paul, V.H., Dapprich, P.D., Liu, Y., Schulze-Wietis, F. 2001: Einsatz von Wachstums-regulatoren. *Raps* 19 (4): 184-189.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 87 - 92

Optimising control of phoma stem canker in winter oilseed rape in the UK

J. M. Steed[1], B.D.L. Fitt[1], P. Gladders[2], A. Naik[2]
[1] *IACR Rothamsted, Harpenden, Herts. AL5 2JQ, UK*
[2] *ADAS Boxworth, Boxworth, Cambs, CB3 8NN, UK*

Abstract As part of a project to improve strategies for phoma stem canker control, field experiments were done in two seasons at two sites (Rothamsted and Boxworth) in south east England. Four spray timings, determined by epidemic progress, were used in a full factorial design to investigate the effect of the number and timing of fungicide applications on phoma leaf spot and stem canker, other diseases and on yield. While phoma leaf spot / stem canker was the only major disease present at Boxworth, moderate light leaf spot epidemics also occurred at Rothamsted. In 1998/99, phoma leaf spot epidemics started in November and resulted in only moderate stem canker epidemics at both sites. In this season, economic yield responses were only achieved at Rothamsted where yield benefits were associated with light leaf spot control. In 1999/2000, early and prolonged phoma leaf spot epidemics caused severe stem canker epidemics at both sites and untreated yields were *c.* 1t/ha lower than in the previous season. In 1999/2000, multiple spray programmes gave good control of canker and yield increases of up to 0.7 t/ha. Single fungicide applications gave economic but variable yield responses at Boxworth and a minimum of two applications were needed at Rothamsted where light leaf spot was also present. Management of early phoma epidemics was shown to be cost effective with well timed sprays of fungicide.

Key words *Leptosphaeria maculans*, stem canker, winter oilseed rape

Introduction

Leptosphaeria maculans is considered a monocyclic fungal pathogen in the UK. Ascospores are released in autumn causing leaf lesions (phoma leaf spot) from which the fungus grows systemically down the petiole to infect the stem and cause stem canker in the spring and summer. Losses from stem canker in the UK were estimated to average £38M per annum between 1993 and 1995 (Fitt et al., 1997). However losses vary considerably from season to season (e.g. £ 11M in 1991 and £ 42M in 1993). Data from disease surveys (Gladders et al., 1997) show there is also variation in severity of stem canker between regions of the UK, with epidemics being generally more severe in the south and east, where more than 70% of winter oilseed rape is grown. It is important to ensure that fungicides are applied only when necessary and when they will be most effective. This paper reports results of field experiments to identify the optimum timing and number of fungicide applications for stem canker control and economic benefit.

Materials and methods

Field experiments were done in 1998/99 and 1999/2000 at two sites in the south east of England (ADAS Boxworth, Cambridgeshire and IACR Rothamsted, Hertfordshire). All four experiments used cv. Apex and a randomised block design with 16 different fungicide treatments with three (Rothamsted) or four (Boxworth) replicates. The two experiments at

Rothamsted were inoculated in the autumn with crop debris from the previous season to ensure that epidemics of stem canker and light leaf spot occurred. Treatments comprised a tank mix of difenoconazole (Plover) at 0.25 l/ha product + carbendazim (Bavistin DF) at 0.25 l/ha product. The first fungicide application was made at the onset of phoma leaf spotting in the autumn, with three subsequent fungicide applications at 4 to 6 week intervals thereafter. Actual application dates are shown in Table 1. These four treatments were applied as a full factorial programme to provide varying degrees of fungicide control from disease onset in autumn until the early stem extension stage in spring. Assessments of disease were made *c.* monthly on a sample of ten plants/plot. Stem canker severity was assessed on a 0 – 4 scale (0 = no stem canker, 1 = <50% stem girdled, 2 = >50% stem girdled, 3 = stem girdled and weakened, 4 = plant dead).

Table 1. Scheduled and actual dates of fungicide application at Rothamsted and Boxworth in 1998/99 and 1999/2000

Treatment code	Scheduled timing	Actual timing			
		1998/99		1999/2000	
		Boxworth	Rothamsted	Boxworth	Rothamsted
UN	Untreated				
1	Onset of phoma leaf spot	20 Oct	6 Nov	6 Oct	5 Oct
2	4 to 6 weeks later	3 Dec	16 Dec	15 Nov	3 Nov
3	4 to 6 weeks later	21 Jan	21 Jan	10 Jan	13 Dec
4	4 to 6 weeks later	9 March	25 Feb	22 Feb	20 Jan

Results and discussion

Phoma leaf spot appeared earlier and increased more rapidly in autumn 1999 than in autumn 1998 (Fig. 1). In consequence, fungicide treatments were started 14-29 days earlier in autumn 1999 and were completed earlier than in 1998/99 (Table 1). Stem canker developed earlier and became more severe by harvest in 2000 than in 1999 (Fig. 2). These seasonal differences were reflected in the yield responses to fungicide treatments. In 1998/99, control of slight stem canker at Boxworth produced no significant yield responses and responses at Rothamsted were associated with control of light leaf spot.

In all four experiments, fungicide application generally decreased severity of phoma leaf spot for *c.* 2 months after application (Table 2). The two earlier sprays generally had a greater effect, causing a greater decrease in phoma leaf spot severity and for a longer period of time than did the later sprays. The first spray timing, at disease onset, was rather less effective than the second spray timing, 1 month after disease onset, which reduced the maximum incidence of phoma leaf spot (Jan/Feb. 1999 and Dec. 1999 to March 2000). This coincides with the main period when phoma leaf spots produce cankers (Sun et al., 2000) and may explain the greater control of stem canker achieved by the second spray timing, particularly in 1999/2000. In this second season, additional applications reduced stem canker severity further, but spray programmes that did not include the second spray timing were generally less effective than

those that did (Fig. 2). Light leaf spot was also present on the experiments at Rothamsted, reaching a maximum of 10% leaf area affected in March of both seasons with 16% pod area affected in 1999 and 23% in 2000. Most fungicide treatments reduced light leaf spot incidence on leaves and pods by *c*. 50% in 1998/99, but had less effect in 1999/2000.

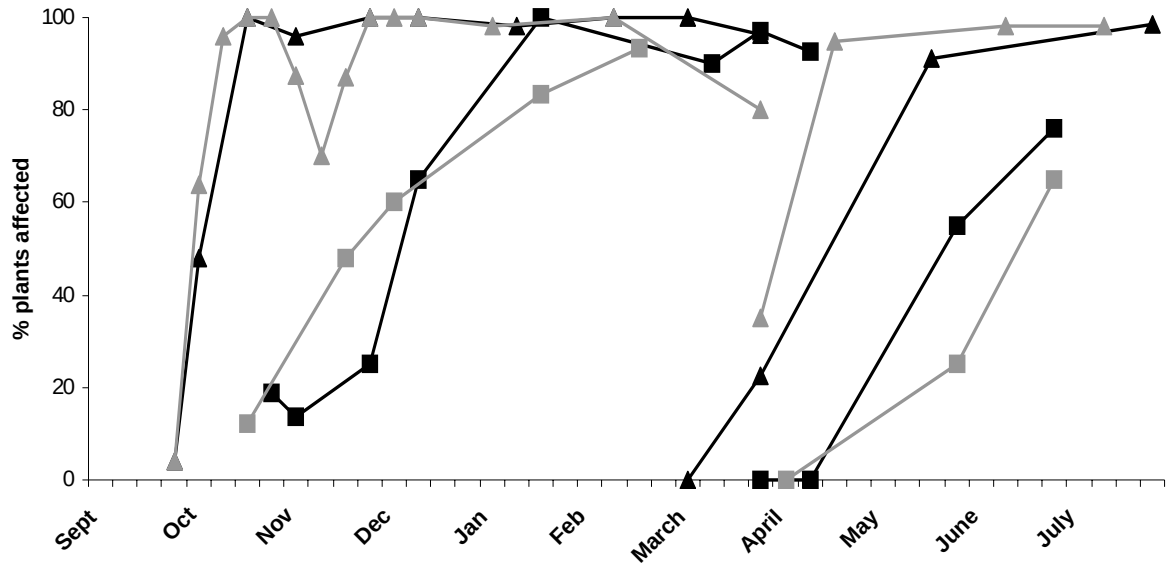


Figure 1. Development of phoma leaf spot from October to March and stem canker from March to July at Boxworth (black) and Rothamsted (grey) in 1998/99 ( ) and 1999/2000 ( )

Table 2. Effects of single sprays at disease onset (treatment 1) or at 4 to 6 week intervals thereafter (treatments 2, 3 and 4) on severity of phoma leaf spot (% leaf area affected) compared to untreated (UN) in 1998/99 and 1999/00 at Boxworth and Rothamsted

Date of disease assessment	% leaf area affected									
	Boxworth					Rothamsted				
	1998/99									
	UN	1(Oct)	2 (Dec)	3 (Jan)	4 (Mar)	UN	1 (Nov)	2 (Dec)	3 (Jan)	4 (Feb)
Oct	0.04					0.04				
Nov						0.05				
Dec	0.21	0.02[b]	0.16			0.41	0.21			
Jan	0.96	0.97	0.38[c]			0.95	0.86	0.63		
Feb						2.39	1.55	1.37[a]	1.7	
Mar	0.39	0.34	0.24	0.31						
Apr	0.84	0.53	0.48	0.42	0.05[b]	0.23	0.1	0.13	0.14	0.1
	1999/2000									
	UN	1 (Oct)	2 (Nov)	3 (Jan)	4 (Feb)	UN	1 (Oct)	2 (Nov)	3 (Dec)	4 (Jan)
Oct	0.09					1.14	0.29[a]			
Nov	1.85	1.16	1.77			0.65	0.19[a]	0.65		
Dec						0.96	0.34[c]	0.54[b]		
Jan	0.77	0.75	0.22[b]			2.49	2.71	0.5[c]	2.51	
Feb						1.18	1.0	1.03	1.07	1.10
Mar	0.98	1.56c	0.97	0.39[c]	0.96	0.49	0.26[a]	0.17[b]	0.21[a]	0.30
Apr	0.48	0.55	0.53	0.37	0.14[c]					

Significant difference compared to untreated control: a P=5%, b P=1%, c P=0.1%

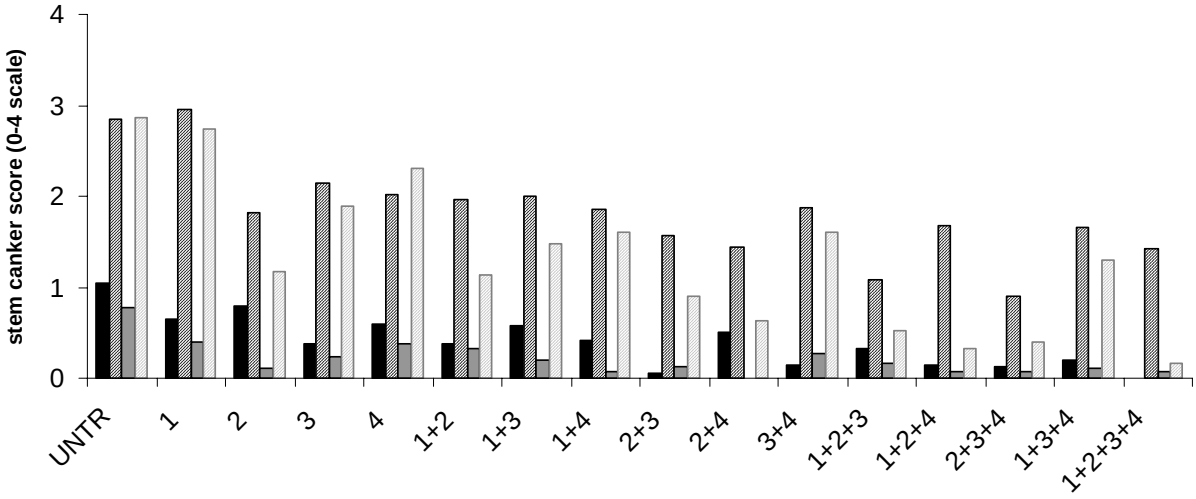


Figure 2. Effects of fungicide timing (1 = disease onset; 2, 3 and 4 at 4 - 6 week intervals thereafter) and number of sprays (all combinations of four spray timings) on severity of stem canker at harvest at Boxworth (black columns) and Rothamsted (grey columns) in 1998/99 (solid columns) and 1999/2000 (broken columns)

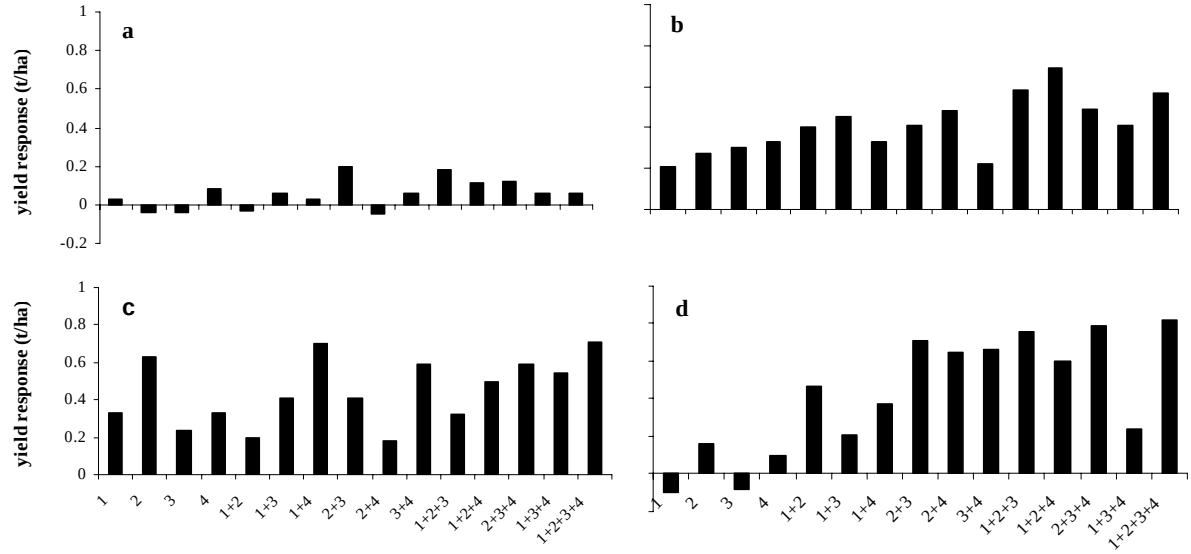


Figure 3. Effect of fungicide timing (1 = disease onset; 2, 3 and 4 at 4 – 6 week intervals thereafter) and number of sprays (all combinations of four spray timings) on yield response (tonnes/ha difference in yield compared to untreated) in 1998/99 (a and c) and 1999/2000 (b and d) at Boxworth (a and b) and at Rothamsted (c and d)

Yields from untreated plots in 1998/99 were 4.34 t/ha at Boxworth and 4.64 t/ha at Rothamsted. In 1999/2000 they were *c.* 1 t/ha lower (3.07 t/ha at Boxworth and 3.75 t/ha at Rothamsted). The seasonal differences are largely explained by the high disease severity in 1999/2000. In both seasons, the larger yield responses at Rothamsted (Fig. 3) were attributed to light leaf spot control, as stem canker severity was similar at both sites.

Growers require a yield response of *c.* 0.2 t/ha to recoup the cost of a single fungicide application. In 1999, when stem canker severity was low, yield responses were very low at

Boxworth and there was no economic return from any spray programme. However, at Rothamsted, where both light leaf spot and stem canker were present, there was a yield response of at least 0.2 t/ha from all single sprays and a response of 0.6 t/ha (significant at 5% level) from the second spray timing. Additional sprays gave little or no additional yield benefit.

In 2000, when stem canker epidemics were severe, all single sprays gave a yield response of at least 0.2 t/ha (significant at 5% level) at Boxworth, despite untreated yields being >1 t/ha lower than in the previous season. At Rothamsted, a single spray was not sufficient to give a yield response (0.4t/ha yield response was significant at 5% level). A second spray was needed, and those programmes that included the second spray timing gave higher yield responses than those that did not. There was little additional benefit from a third spray.

Results from these experiments show that early and severe stem canker epidemics are an important cause of yield loss in the UK, even on moderately resistant cvs such as Apex. Results also suggest that where a phoma leaf spot epidemic develops after late November on robust plants, stem canker severity is likely to be low and yield responses from autumn/winter fungicides are unlikely to be cost effective, unless other diseases, such as light leaf spot, are also present. When stem canker is severe, a single, well timed fungicide spray can give significant yield responses, but a second spray may be required to maximise the economic benefits. The first spray should be applied when 10-20% plants are affected with phoma leaf spot and a second application made 4-6 weeks later.

Acknowledgements

Funding for this work was provided by the UK Home-Grown Cereals Authority and the Department of Environment, Food and Rural Affairs.

References

Fitt, B.D.L., Gladders, P., Turner, J.A., Sutherland, K.G., Welham, S.J. & Davies, J.M.L 1997: Prospects for developing a forecasting scheme to optimise use of fungicides for disease control in winter oilseed rape in the UK. *Aspects of Applied Biol.* 48: 135-142.

Gladders, P. & Symonds, B.V. 1995: Occurrence of canker (*Leptosphaeria maculans*) in winter oilseed rape in eastern England, 1977-1993. *IOBC/WPRS Bulletin* 18(4): 1-11.

Gladders, P., Turner, J.A., Walters, K.F.A. & Wright, D.M. 1997: Winter oilseed rape: survey of pests and diseases, 1995/96. *ADAS/CSL Research and Development*. 42 pp.

Hammond, K.E. & Lewis, B.G. 1986: The timing and sequence of events leading to stem canker disease in populations of *Brassica napus* var. *oleifera* in the field. *Plant Pathology* 35: 551-564.

Penaud, A. 1995. Studies on the optimum timing to control rapeseed blackleg caused by *Leptosphaeria maculans* with fungicides. IOBC/WPRS Bulletin 18(4): 56-61.

Sansford, C.E., Fitt, B.D.L., Gladders, P., Lockley, K.D. & Sutherland, K.G. 1996. Oilseed rape: disease development, forecasting and yield loss relationships. *HGCA Project Report* OS17: 185 pp.

Sun, P., Fitt, B.D.L., Gladders, P. & Welham, S.J. 2000. Relationships between phoma leaf spot and development of stem canker (*Leptosphaeria maculans*) on winter oilseed rape (*Brassica napus*) in southern England. *Annals of Applied Biology* 137, 113-125.

West, J.S., Biddulph, J.E., Fitt, B.D.L., & Gladders, P. 1999: Epidemiology of *Leptosphaeria maculans* in relation to forecasting stem canker severity on winter oilseed rape in the UK. *Annals of Applied Biology* 135: 535-546.

Zhou, Y. Fitt, B.D.L., Welham, S.J., Gladders, P., Sansford, C.E. & West, J.S. 1999: Effects of severity and timing of stem canker (*Leptosphaeria maculans*) symptoms on yield of winter oilseed rape (*Brassica napus*) in the UK. *European Journal of Plant Pathology* 105: 715-728.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 93 - 102

Health status of spring rape plants as affected by the sowing date and fertilisation with sulphur, boron and magnesium

Sadowski Czeslaw [1], Jankowski Kazimierz[2], Lukanowski Aleksander [1], Trzcinski Jacek
[1] *Dept. od Phytopathology, University of Technology and Agriculture, Kordeckiego 20, 85-225 Bydgoszcz, Poland*
[2] *University of Warmia and Mazury, Oczapowskiego 2, 10-719 Olsztyn, Poland*

Abstract: The experiment was carried out in two sowing dates of oilseed rape, early one (at the time of spring cereals sowing) and three weeks later. The cultivation field was fertilised with sulphur (0 or 35 kg S per ha) before sowing, while the plant leaves were sprayed with nitrogen and magnesium (0 or 6 kg N per ha + 1 kg Mg per ha) or boron (0,5 kg of boron per ha). Healthiness of plants was evaluated in the flowering phase and maturation phase using the relevant scales of infection. Infection index was also calculated.

Downy mildew (*Peronospora parasitica*), dark leaf and pod spot (*Alternaria* spp.) and powdery mildew (*Erysiphe cruciferarum*) were detected most often on the plants, while *Phoma lingam, Sclerotinia sclerotiorum* and *Botrytis cinerea* occurred by far less intensively.

The date of sowing and fertilisation systems did not have any effect of statistical significance on the infection with downy mildew. Mean infection index fluctuated between 12.4% and 14.2%. The occurrence of *Alternaria* spp. was relatively high. This genus was present on leaves, shoots and pods. Significant differences in the infection degree occurred in respect of the date of sowing. The plants of the later sowing time were more strongly infected. Fertilisation with sulphur decreased pathogenic effects on siliques, while its impact on the infection of shoots and leaves was less pronounced. Fertilisation with magnesium decreased significantly the occurrence of the pathogen on leaves and shoots in case of earlier sowing, while the effect of fertilisation with boron was negligible. The positive effect of fertilisation with all three elements ($S_1Mg_1B_1$) was observed in the case of earlier date of sowing in two years of investigations. Disease occurrence on leaves was significantly lower. Powdery mildew occurred over two years at a high level. It this case the plants of the earlier sowing date were infected by far more intensely. The use of boron limited this effect to some extent.

Key words: spring oilseed rape, fertilisation, sulphur, magnesium, boron, diseases. healthiness, Poland

Introduction

The basic oleic crop in Poland is winter oilseed rape. However, in some years it may be necessary to cultivate the spring form of it. It may happen after severe winters and freezing of winter oilseed rape or when there are difficulties with sowing of winter form in autumn caused by the late cereal harvest (Budzynski 1998).

The oilseed rape like the other oleic crops has high demand for sulphur and it is very sensitive to its deficiency. The yield of 10 dt of seeds consumes about 16 kg of sulphur, in the comparison with the cereals where this amount is about 2-3 kg (Zhao et al. 1999). When deficiency is very high there is low number of pods with the low number of seeds, which will be small and their quality will be very poor (Schnug and Haneklaus 1995, Walkowski 2000). According to Schnug et al. (1995) the symptoms of deficiency of sulphur in the flowers are sometimes mistaken with genetic instability of variety.

Sulphur deficiency negatively influences on nitrogen assimilate ability from soil solution and on biosynthesis of proteins by oilseed rape. Not proper nitrogen metabolism may be the direct cause of abnormal development of flowers and pods (Bilsborrow et al. 1995, Mc Grath and Zhao 1996).

In the past sulphur fertilisation was ignored because of sufficient amount of this element in the atmosphere. In the last two decades the emission of sulphur to the atmosphere was reduced about 40%. The use of fertilizers containing sulphur was also reduced and it was the other cause of increasing sulphur deficiency in the soil. In Poland sulphur emission was up to 80 kg S/ha. The research from 1995 showed its serious deficiency on the large areas in north-west regions of Poland (Grzebisz and Fotyma 1996).

There is quite a lot of data concerning the reaction on differentiated fertilisation with sulphur and its influence on yield and quality. There is some data about plant health status after usage differentiated fertilisation with nitrogen (Lemanczyk et al. 1997) but very few concerning fertilisation with other elements.

The higher fertilisation with sulphur the more sulphur in leaves. It may cause the increase of glucosinolate content both in vegetative parts and in seeds, what in the case of double improved varieties is negative feature. However, the increase of these compounds content may be the reason of the lower susceptibility of the plants on fungal pathogens (Schnug and Ceynowa 1990, Schnug and Haneklaus 1995, Schnug et al. 1995, Drozdowska et al. in press).

Oilseed rape has also high demand for boron, sometimes even five times higher then in the case of cereals. Many plantations of oilseed rape in Poland shows its deficiency in soil. The results of boron deficiency are: flower bud necrosis, lower number of pods and seeds in them, decrease of disease resistance and the lower yield (Grzebisz and Gaj 2000).

Demand of oilseed rape for magnesium is quite high. Its deficiency limits growth and development of the plants which makes the yield and oil content lower and its quality.

Materials and methods

The experiment carried out at Bałcyny near Ostróda by the Department of Crop Production of University of Warmia and Mazury in Olsztyn.

It was established in system of partial replication k^{n-1} with four factors (n=4) and two levels (k=2) and it covered:

– two terms of sowing (early – the term of spring cereal sowing, late – 3 weeks later)
– pre-sowing sulphur fertilisation (0 or 36 kg S/ha)
– foliar fertilisation with magnesium and nitrogen (0 or 8 kg N + 1 kg Mg)
– foliar fertilisation with boron (0 or 0,5 kg B/ha).

There were 8 fertilisation combinations: ($S_1Mg_0B_0$, S_1Mg_1 B_0 , $S_1Mg_1B_1$, S_1Mg_0 B_1, $S_1Mg_1B_1$, S_1Mg_1 B_0 , $S_1Mg_1B_1$, S_1Mg_0 B_0, both in early and late term of sowing. Variety "Star" was cultivated every year.

The health status of the plants in 1997-1999 was determined twice: during flowering and ripening and in 2000 only during ripening. Every time 25 randomly chosen plants from each plot were analysed. The incidence (per cent) of infected plants, leaves, stems and pods and their severity of infection in per cent area or a specific scale was determined.

Peronospora parasitica severity was determined on 4 lower leaves with the use of a six-degree scale (Sadowski 1987), where 0 – no disease symptoms on leaves; 5° – symptoms cover over 75% of the leaf area. *Alternaria* pod spot severity was estimated during flowering phase on four lower leaves (0-4°), and during ripening the per cent of pod and stem area with disease symptoms was noted. For estimation of *Alternaria* spp. incidence on leaves five-degree scale was used (Evens and Gladders 1981), where 0 – no spots on leaf; 4° – the spots

cover over 50% of leaf area. Determination of *Alternaria* spp occurrence on stems and pods was carried out with the use of five-degree scale (Babadoost and Gabrielson 1979), where 0 – no disease symptoms; 4° – symptoms cover over 60% stem/pod area. For the evaluation of leaf infection with *Erysiphe cruciferarum*, the scale for estimation of *P. parasitica* was used (0-5°). During the ripening phase, a six-degree scale was used to describe *P. parasitica* infestation on the whole plant (Penaud 1999), where 0 – no visible mycelium on the plant; 5° – stems brown or black. The results were converted into disease index (DI, max = 100%) using Townsend-Heuberger formula.

The other pathogens occurred very rarely and their intensity was estimated using per cent of infected plants or leaves and it was not analysed statistically.

Table 1. Infestation of leaves with *Alternaria* spp. depending on fertilisation, Balcyny 1997 – 1999.

Combination		1997		1998		1999		1997-1999	
		Early	Late	Early	Late	Early	Late	Early	Late
1	S_0	14,6 a*	10,4 a	30,0 a	13,0 a	34,1 a	16,2 a	26,2 a	13,2 a
	S_1	16,4 a	10,7 a	28,4 a	12,2 a	30,6 b	15,7 a	25,1 a	12,9 a
2	Mg_0	15,8 a	10,5 a	33,5 a	14,2 a	34,1 a	16,2 a	27,3 a	13,7 a
	Mg_1	15,3 a	10,9 a	25,0 b	11,0 b	30,6 a	15,7 a	23,0 b	12,5 a
3	B_0	17,2 a	12,2 a	28,7 a	12,7 a	31,6 a	16,9 a	25,9 a	13,9 a
	B_1	13,9 b	9,3 b	29,7 a	12,6 a	29,7 a	15,0 a	24,5 a	12,3 a
4	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_1Mg_1B_1$	16,3 a	8,8 a	25,0 b	10,0 a	27,2 b	15,2 a	22,8 b	11,3 a
5	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_1Mg_0B_0$	17,5 a	11,3 a	31,3 b	13,7 a	33,0 a	16,2 a	27,3 a	13,7 a
6	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_0Mg_1B_0$	15,3 a	15,0 a	25,0 b	12,5 a	33,7 a	16,2 a	24,7 a	14,6 a
7	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_0Mg_0B_1$	13,0 b	10,3 a	33,8 a	14,0 a	35,5 a	15,0 a	27,4 a	13,1 a
8	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_1Mg_1B_0$	17,5 a	12,5 a	23,8 b	10,2 a	30,2 b	16,3 a	23,8 b	13,0 a
9	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_1Mg_0B_1$	14,3 b	10,5 a	33,8 a	15,0 a	32,2 b	15,0 a	26,8 a	13,5 a
10	$S_0Mg_0B_0$	18,5 a	10,0 a	35,0 a	14,2 a	36,1 a	18,8 a	29,9 a	14,3 a
	$S_0Mg_1B_1$	12,0 b	7,5 a	26,3 b	11,3 a	31,2 b	15,0 b	23,2 b	11,3 a

*/ values in the same column (for every combination separately) followed by different letters are significantly different Results

The highest incidence of fungal pathogens occurring on plants concerned black pod spot (*Alternaria* spp.), downy mildew (*Peronospora parasitica*) and powdery mildew (*Erysiphe cruciferarum*).

When the effect of sulphur, magnesium and boron was analysed – all combinations, where the element was present, were taken into consideration.

Downy mildew (*P. parasitica*) occurred in every year of observations but in relatively low intensity. Disease index (DI) in 1997 was from 10,2 to 13,4%, in 1998 from 15,2 to 20,2% and in 1999 from 8,4 to 11,2%, and the mean value for three years was 13,3% (early sowing) and 12,8% (late sowing). There was no significant differentiation in infestation depending on the experimental factors.

Alternaria black spot (*Alternaria* spp.) was observed every year in high intensity depending on the sowing date. Both per cent of infested leaves and their infection degree was distinctly higher for the plants sown earlier. DI for 1997, 1998 and 1999 for early sowing date was 15,5%, 29,2% and 32,3% respectively and for late sowing 10,5%, 12,6% and 15,9%. In all years the differences were statistically significant. Mean DI calculated for 3 years was 25,6% (early sowing) and only 13,0% (late sowing).

The influence of the other factors was very differentiated. Comparison of leaf health status on all plots without sulphur fertilisation and fertilised with it, independently from the other ingredients used [Tab. 1.1], showed significant influence of sulphur only in one year of investigations, when the seeds were sown on earlier date (1998, DI for S_0 = 34,1; for S_1 = 30,6%). In the other years the intensity of disease was on the same level.

After magnesium application [1.2], only in 1998, less disease symptoms were visible in both terms of sowing and in the case of mean DI value calculated for three years after earlier sowing. Boron [1.3] influenced significantly on decrease of intensity of *Alternaria* pod spot symptoms only in one year – 1997.

The positive effect of fertilisation with all three elements ($S_1Mg_1B_1$) was observed in the case of earlier date of sowing in two years of investigations. Disease occurrence on leaves was significantly lower [1.4].

The observations of *Alternaria* black spot occurrence on the plots fertilised only with sulphur (without magnesium and boron – $S_1Mg_0B_0$) and without sulphur ($S_0Mg_0B_0$) showed significant decrease of disease intensity only in 1998 for earlier date of sowing. In the other years there was no significant differences [1.5]. The situation was similar in the case of fertilisation with magnesium [1.6] and boron [1.7], where lower values of DI was noted after application of these elements but significant differentiation was observed only in one year in earlier term of sowing. Sulphur and magnesium usage on the earlier date of sowing resulted in significant decrease of disease occurrence. [1.8]. Decrease of disease intensity was also observed after sulphur and boron usage [1.9]. Application of magnesium and boron significantly influenced on lower disease intensity after earlier sowing in every year of investigations [1.10].

Second observation, in phase of ripening, showed quite high incidence of pod spot symptoms on main and lateral stems. The relationship between disease intensity and date of sowing was very clear. In every year of experiments significantly higher infestation of stems was noted on the plants sown on the earlier date. DI was 15,1 and 4,5%, 24,3 and 6,0%, 27,0 and 5,3%, 29,7 and 8,6% respectively for 1997, 1998, 1999 and 2000 and for both dates of sowing. The mean DI was 24,1 and 6,2%.

The usage of sulphur (for every combination where sulphur was applied) did not resulted in decrease of disease intensity on stems. Statistically lower infestation was noted only in 1999 on the plots fertilised with this element after earlier date of sowing. In the case of the late date of sowing there were no differences noted [Tab. 2.1]. When the plants were fertilised

only with sulphur alone [2.5], some symptoms of pod spot was noted, however significant differences was recorded in 1999 and 2000 (early date of sowing).

Table 2. Infestation of stems with *Alternaria* spp. depending on fertilisation, Balcyny 1997 – 2000.

Combination		1997		1998		1999		2000		1997-2000	
		Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
1	S_0	16,0 a	4,8 a	25,2 a	6,7 a	29,4 a	5,3 a	30,9 a	8,2 a	25,4 a	6,2 a
	S_1	14,3 a	4,3 a	23,5 a	5,3 a	24,7 b	5,4 a	28,5 a	9,1 a	22,7 a	6,0 a
2	Mg_0	16,3 a	4,1 a	25,9 a	7,0 a	30,9 a	5,5 a	30,1 a	8,7 a	25,8 a	6,3 a
	Mg_1	14,1 a	5,0 a	23,1 b	5,1 b	23,3 b	5,4 a	29,2 a	8,6 a	22,4 b	6,0 a
3	B_0	15,1 a	4,8 a	25,8 a	5,8 a	28,9 a	5,7 a	29,1 a	8,7 a	24,7 a	6,2 a
	B_1	15,2 a	4,3 a	23,1 a	6,2 a	25,3 a	5,1 a	30,2 a	8,6 a	23,4 a	6,1 a
4	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_1Mg_1B_1$	12,3 b	4,2 a	20,0 b	5,5 a	17,5 b	5,0 a	30,0 a	8,0 a	19,9 b	5,7 a
5	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_1Mg_0B_0$	15,7 a	4,2 a	26,0 a	6,5 a	30,2 b	5,0 a	28,0 b	9,0 a	25,0 a	6,2 a
6	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_0Mg_1B_0$	15,2 a	6,4 a	25,0 a	5,5 a	29,0 b	5,0 a	30,0 a	7,0 a	24,8 a	6,0 a
7	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_0Mg_0B_1$	17,1 a	4,2 a	26,0 a	8,5 a	30,4 b	5,0 a	30,0 a	6,5 a	25,9 a	6,0 a
8	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_1Mg_1B_0$	13,2 a	4,8 a	24,2 a	4,0 a	22,3 b	6,5 a	26,0 b	7,5 a	21,4 b	5,7 a
9	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_1Mg_0B_1$	16,1 a	4,0 a	24,0 a	5,5 a	29,0 b	5,5 a	30,0 a	12,0 a	24,8 a	6,7 a
10	$S_0Mg_0B_0$	16,4 a	4,0 a	27,5 a	7,5 a	34,0 a	6,5 a	32,5 a	7,5 a	27,6 a	6,4 a
	$S_0Mg_1B_1$	15,5 a	4,6 a	22,5 b	5,5 a	24,3 b	5,0 a	31,0 a	8,0 a	23,3 b	5,8 a

The number of symptoms of stem infestation was lower after magnesium application in the case of earlier sowing. Significant differences were noted in 1998 and 1999 and in mean value of DI calculated for earlier sowing date. In the case of late sowing, statistically proven differences was noted only in 1998 [2.2]. Analyses of disease intensity in combinations $S_0Mg_1B_0$ and $S_0Mg_0B_0$ showed differences significance only in1999, early sowing [2.6].

Application of boron with the connection with the other elements has no significant importance [2.3]. Also in combination $S_0Mg_0B_1$, except one early sowing in 1999, such an influence was not noted [2.7]. Fertilisation with sulphur and magnesium influenced positively

on stem healthiness**.** In two years, 1999 and 2000, in the case of early sowing and for mean value calculated for all years of investigations, differences were significant [2.8].

The positive influence of fertilisation with magnesium and boron was observed in two years and for all period of experiment in the case of earlier date of sowing [2.10].

It may be stated that single ingredients (S, Mg, and B) applied alone had low influence on stem healthiness, but when they were used together on early date of sowing – disease intensity was significantly lower [2.4].

The highest threat for oilseed rape was black pod spot on pods. Disease occurred in very high intensity in three years of investigations – 1998, 1999 and 2000. In every year there was significantly more disease symptoms observed on the plants sown earlier in comparison to late date of sowing. Differences in pod infection were very high and they were respectively: 13,9 and 7,0%, 57,0 and 19,0%, 58,6 and 17,0% 68,0 and 11,5%.

Table 3. Infestation of pods with *Alternaria* spp. depending on fertilisation, Balcyny 1997 – 2000.

Combination		1997		1998		1999		2000		1997-2000	
		Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
1	S_0	15,1 a	7,9 a	57,4 a	17,7 a	62,1 a	16,4 a	72,6 a	10,3 a	51,8 a	13,1 a
	S_1	12,8 b	6,2 b	56,6 a	20,4 a	55,2 b	17,6 a	63,3 b	10,9 a	47,0 b	13,8 a
2	Mg_0	13,7 a	7,1 a	56,0 a	18,5 a	58,5 a	16,6 a	69,1 a	10,4 a	49,3 a	13,1 a
	Mg_1	14,3 a	7,1 a	58,1 a	19,7 a	58,9 a	17,5 a	66,7 a	10,7 a	49,5 a	13,7 a
3	B_0	14,2 a	7,2 a	57,4 a	20,9 a	60,6 a	18,9 a	70,0 a	11,4 a	50,5 a	14,6 a
	B_1	13,7 a	7,0 a	56,8 a	17,4 a	56,7 a	15,2 b	65,9 a	9,7 a	48,3 a	12,3 a
4	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_1Mg_1B_1$	12,3 b	5,7 a	55,0 a	21,5 a	50,5 b	14,0 a	59,5 b	7,5 a	44,3 b	12,2 a
5	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_1Mg_0B_0$	12,9 a	6,0 a	56,5 a	22,5 a	57,0 b	19,0 a	67,5 b	10,0 a	48,5 a	14,4 a
6	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_0Mg_1B_0$	15,9 a	8,3 a	58,5 a	19,5 a	62,5 a	18,5 a	75,0 a	13,5 a	53,0 a	15,0 a
7	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_0Mg_0B_1$	14,3 a	7,6 a	56,0 a	18,0 a	57,5 b	15,0 a	70,5 a	7,5 a	49,6 a	12,0 a
8	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_1Mg_1B_0$	13,4 a	6,6 a	59,0 a	23,0 a	58,5 b	21,5 a	62,5 b	12,0 a	48,3 a	15,8 a
9	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_1Mg_0B_1$	12,7 a	6,9 a	56,2 a	15,0 a	55,0 b	16,0 a	63,0 b	14,0 a	46,7 b	13,0 a
10	$S_0Mg_0B_0$	14,7 a	8,0 a	55,5 a	18,5 a	64,5 a	16,5 a	75,0 a	10,0 a	52,4 a	13,2 a
	$S_0Mg_1B_1$	15,5 a	7,9 a	60,0 a	15,0 a	64,0 a	16,0 a	70,0 b	10,0 a	52,4 a	12,2 a

The plants fertilised with sulphur were characterised with lower pod infection index, but in 1997 for both dates of sowing, and in 1999 and 2000 for earlier sowing – differences were significant [Tab. 3.1] Application of sulphur alone [3.5] significantly affected disease intensity in two years (early sowing).

The usage of magnesium, independently form the other elements [3.2], and in combination $S_0Mg_1B_0$ [3.6], had no significant importance. The situation with boron was similar [3.3 and 3.7].

Table 4. Infestation of plants with *Erysiphe cruciferarum* depending on fertilisation, Balcyny 1997 – 2000.

Combination		1997		1998		1999		2000		1998-2000	
		Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
1	S_0	Trace	Trace	33,2 a	37,2 a	32,2 a	36,7 a	50,6 a	56,2 a	38,7 a	43,4 a
	S_1	Trace	Trace	33,0 a	36,5 a	33,8 a	40,7 a	53,1 a	55,7 a	40,0 a	44,3 a
2	Mg_0	Trace	Trace	33,7 a	35,7 a	32,8 a	38,0 a	51,8 a	57,5 a	39,4 a	43,7 a
	Mg_1	Trace	Trace	32,5 a	38,0 a	33,2 a	39,5 a	51,5 a	54,5 a	39,1 a	44,0 a
3	B_0	Trace	Trace	34,0 a	39,0 a	34,5 a	41,0 a	52,5 a	55,6 a	40,3 a	45,2 a
	B_1	Trace	Trace	32,2 a	34,7 b	31,5 a	36,5 b	51,2 a	53,4 a	38,3 a	41,5 b
4	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_1Mg_1B_1$	Trace	Trace	31,0 a	38,0 a	32,0 a	41,0 a	52,5 a	55,5 a	38,5 a	44,8 a
5	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_1Mg_0B_0$	Trace	Trace	36,0 a	36,0 a	32,2 a	44,0 a	55,0 a	60,0 a	41,1 a	46,7 a
6	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_0Mg_1B_0$	Trace	Trace	33,0 a	43,0 a	33,0 a	42,0 a	47,5 a	52,5 a	37,8 a	45,8 a
7	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_0Mg_0B_1$	Trace	Trace	35,0 a	38,0 a	31,0 b	32,0 b	50,0 a	57,5 a	38,7 a	42,5 a
8	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_1Mg_1B_0$	Trace	Trace	34,0 a	40,0 a	38,0 a	40,0 a	55,0 a	52,5 a	42,3 a	44,2 a
9	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_1Mg_0B_1$	Trace	Trace	31,0 a	32,0 b	33,0 a	38,0 a	50,0 a	55,0 a	38,0 a	41,7 a
10	$S_0Mg_0B_0$	Trace	Trace	33,0 a	37,0 a	35,0 a	38,0 a	52,5 a	57,5 a	40,2 a	44,2 a
	$S_0Mg_1B_1$	Trace	Trace	32,0 a	31,0 b	30,0 a	35,0 a	52,5 a	57,5 a	38,2 a	41,2 a

Fertilisation with all three elements ($S_1Mg_1B_1$) positively influenced on healthiness of pods after earlier sowing. Significantly lower infestation was noted in 1997, 1999 and 2000 and analysing all period of the investigations. When the plants were sown later there was no

impact of sulphur, magnesium and boron on health status of pods [3.4]. The usage of sulphur and magnesium made the occurrence of pod spot lower significantly in two years after early sowing. Positive effect was observed also after application of sulphur and boron [3.9].

In three years of observations there was powdery mildew (*Erysiphe cruciferarum*) noted in its high intensity. Its symptoms were visible on leaves, stems and pods. The occurrence was estimated on the whole plant in the ripening phase. DI in 1998 for the plants sown earlier was 33,1%, and for those sown later – 36,9. In 1999 33,0 and 38,7% respectively, and in 2000 – 51,8 and 56,0%. Statistical analysis showed that fertilisation had no significant influence on powdery mildew occurrence. There was no significant effect of sulphur in the whole period of researches [Tab. 4.1 and 4.4]. The situation with magnesium was the same. Only in 1998, after late sowing and application of sulphur alone, higher intensity of disease was noted (DI = 43,0%) in comparison with the plants not fertilised wit it (DI = 37,0%) – tables 4.2 and 4.6.

The influence of boron was more distinct. It affected significantly on the lower disease intensity when it was applied after late sowing in 1998 and 1999 and in combination $S_0Mg_0B_1$ (1999 – both dates of sowing) [Table 4.3 and 4.7]. Combination of sulphur with magnesium had no effect on powdery mildew. Slightly better influence was observed after fertilisation with sulphur +boron and magnesium+boron, one year - late sowing [4.9 and 4.10].

Discussion

Sulphur fertilisation is very important practice in oilseed rape cultivation. There is very few data concerning its indirect influence on plant healthiness. Sulphur metabolism is the cause of various defence mechanisms against biotic and abiotic factors. Schnug et al. (1995) claim that sulphur increases disease plant resistance. In the other investigations Schnug and Ceynowa (1990) showed higher intensity of cylindrosporiosis (*Cylidrocarpon concentricum*) when there was sulphur deficiency. Walker and Booth (1994) also reported positive effect of sulphur on plant healthiness. According to Johnston et al. (1999) sulphur is the third microelement, following nitrogen and phosphorus. Sulphur content is especially important from the beginning of vegetation. According to McGrath and Zhao (1996), in northern England and Scotland, the dose of 40 kg S/ha increased the yield about 0,7-1,6 t/ha. Haneklaus et al. (1999) claim that fertilisation with sulphur on the soils with the high absence of sulphur may increase the yield very distinctly.

Sulphur fertilisation, in our experiment, had an effect on plant healthiness. Lower incidence of black pod spot was observed. Development of *Alternaria brassicae* induces defence reactions in the plants by producing phytoalexins. They have some fungistatic properties. It may be the cause of lower plant infection.

Lower infection also could be a result of increase of glucosinolate content in the plants after sulphur fertilisation. The role of glucosinolates in the plant disease resistance is not well known. Wallsgrove et al. (1999) suppose that they are the defence factor, together with enzyme – myrosinase, which is a part of oilseed rape defence system. Authors noted significant increase of these compounds in leaves of resistant lines of oilseed rape after infection with *Sclerotinia* sclerotiorum.

Szulc et al. (2000) showed that fertilisation with sulphur not always leads to increase of total glucosinolate content. There is a suppose that not the total glucosinolate content has the importance, but their qualitative composition (Booth et al. 1995, Drozdowska et al. – in press.). If, in our investigations, the content of glucosinolates after fertilisation with sulphur increased – our results are compatible with the theory of Giamoustaris and Mithen (1995) that infestation with *Alternaria brassicae* depends on glucosinolates.

Foliar fertilisation with boron resulted in lower incidence of powdery mildew (*E. cruciferarum*). This effect was not so clear in the case of black pod spot (*Alternaria* spp.). So far, there is no sufficient data concerning directly the influence of rape fertilisation with boron on fungal pathogen occurrence. Boron is essential for proper growth and development of oilseed rape, and its deficiency may negatively affect on general plant condition. Boron deficiency may cause the increased susceptibility for diseases (Grzebisz and Gaj 2000). High demand for this element is observed in the phase of budding and flowering (Sienkiewicz-Cholewa and Gembarzewski 2000). In order to make the increase of oilseed rape cultivation profitability – fertilisation with this microelement is necessary.

The result of magnesium fertilisation was lower intensity of black spot on leaves and stems of the plants sown earlier. According to Wielebski (2000) and Fabry et al. (2000) for the good yielding of oilseed rape the sufficient amounts of boron and magnesium are required.

According to Grzebisz and Gaj (2000) foliar fertilisation may be used in the phases of critical demand for this element: from phase of rosette to flowering. Barlog and Potarzycki (2000) claim that foliar fertilization of winter form of oilseed rape with $MgSO_4$ significantly increased seed and oil yield. Wielbski (2000) indicates that for good yielding the oilseed rape demands sufficient amounts of boron and magnesium.

The other pathogens, such as grey mould (*Botrytis cinerea*), blackleg (*Phoma lingam*), sclerotinia stem rot (*Sclerotinia sclerotiorum*) and *Verticillium dahliae* were observed rarely or they were not present at all. There were a very few plants named as “early ripening”, which is often noted on the winter form of oilseed rape.

Conclusions

1. Sulphur fertilisation resulted in decreasing of *Alternaria* black spot, especially on stems and pods. Boron had low effect on that disease, but it influenced significantly on lower powdery mildew incidence.
2. In phytopathological aspect, much more advantageous was usage of sulphur, magnesium and boron together. In such combination the positive influence on plant healthiness was much higher than in the case of fertilisation with single elements.
3. The date of sowing had a serious influence on *Alternaria* black spot intensity. There was less disease symptoms on the plants sown later. This won’t be useful in Polish cultivation conditions because late sowing causes lower yielding.

References

Bilsborrow P.E., Evans E.J., Zhao F.J. 1995: Changes in the individual glucosinolate profile of double low oilseed rape as influenced by spring nitrogen application. *9th Intern. Rapeseed Congress, Cambridge.* Vol. 2: 553-555.

Booth E.J., Walker K.C., Taylor B.R, Griffiths D.,W. 1995: The influence of foliar glucosinolates in varietal resistance to light leaf spot *(Pyrenopeziza brassicae*). *9th Intern. Rapeseed Congress, Cambridge.* Vol.4: 1272-1274.

Budzynski W. 1998: Reaction of rapeseed on date of sowing and the method of weed controlling (in Polish). *Rosliny Oleiste* 19(1): 125-133.

Drozdowska L., Szulc P., Lukanowski A., Sadowski Cz. (in press): Glucosinolate content and pathogenic fungi occurrence in seeds of spring oilseed rape fertilised with sulphur. *Plant Breeding and Seed Science*.

Fabry A., Volf M., Skerik J. 2000: Actual conditions of growing oilseed rape and rapeseed market in Czech Republic (in Polish). *Rosliny Oleiste* 21(2): 419-427.

Giamoustaris A., Mithen R. 1995: Modifying leaf glucosinolates in oilseed rape and its effects upon pest and pathogen interactions. *9th International Rapeseed Congress „Rapeseed today and tomorrow" Cambridge.* Vol. 4: 1220-1222.

Grzebisz W., Fotyma E. 1996: Assessment of the nutritional sulphur status of winter oilseed rape grown in the north-west Poland (in Polish). *Rosliny Oleiste* 17(1):275-280.

Grzebisz W., Gaj R. 2000: Balanced fertilisation of winter oilseed rape. *In: „Zbilansowane nawozenie rzepaku", praca zbiorowa pod redakcja W. Grzebisza, Poznan* 2000.

Haneklaus S., Paulsen H.M., Gupta A.K., Bloem E., Schnug E. 1999: Influence of sulphur fertilization on yield and quality of oilseed rape and mustard. *10th Internnational Rapeseed Congress, Canberra. New Horizons for Old Crop Australia*. CD-ROM, Doc. No. 13: 1-5.

Johnston A.M., Grant C.A., Clayton G.W. 1999: Sulphur management of canola. *10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra.* CD-ROM, Doc. No. 353: 1-9.

Lemanczyk G., Jankowski K., Sadowski Cz., Klepin J., Muchalski T. 1997: Effect of differentiated nitrogen fertilisation and desistance form pest control on the health status of rapeseed. *Rosliny Oleiste* 18(2): 371-379.

McGrath S.P., Zhao F.J. 1996: Sulphur uptake, yield responses and the interactions between nitrogen and sulphur on winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science, Cambridge*. 126: 53-62.

Schnug E., Ceynowa J. 1990: Phytopathological aspects of glucosinolates in oilseed rape. *J. Agron. & Crop Science* 165: 319-328.

Schnug E., Haneklaus S., Booth E., Walker K.C., 1995: Sulphur supply and stress resistance in oilseed rape. *Proc. of the 9th Intern. Rape Congress, Cambridge*. Vol. 1: 229-231.

Schnug E., S. Haneklaus. 1995: Sulphur deficiency in oilseed rape flowers - biochemistry and ecological impact. *9th International Rapeseed Congress, Cambridge*. Vol. 1: 296-298.

Sienkiewicz-Cholewa U., Gembarzewski H. 2000: Effect of winter oilseed rape fertilisation with boron and molybdenum on yield. (in Polish). *In: „Zbilansowane nawozenie rzepaku" pod redakcja W. Grzebisza*: 175-180.

Szulc P., Piotrowski R., Drozdowska L., Skinder Z. 2000: Effect of sulphur fertilisation on yield and sulphur compound accumulation in seeds of spring oilseed rape "Star" (in Polish). *Folia Univ. Agric. Stetin. 204 Agricultura* (81): 157-163.

Walker K., Booth E.J. 1994: Sulphur deficiency in Scotland and the effects of sulphur supplementation on yield and quality of oilseed rape. *Norwegian J. Agric. Sci. Suppl*. 15: 98-104.

Walkowski T. 2000: *Spring oilseed rape* (in Polish). IHAR Poznań: 1-68.

Wallsgrove R., Bennett R., Kiddle G., Bartlet E., Ludwig-Mueller J. 1999. Glucosinolate biosynthesis and pest/disease interaction. *10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra, Australia*. CD-ROM, Doc. No. 393: 1-5.

Wielebski F. 2000: Actual problems of rapeseed fertilisation in Poland (in Polish). *In: „Zbilansowane nawozenie rzepaku"pod red. W. Grzebisza*: 261-276.

Zhao F.J., Blake-Kalff M.M.K., Riley N., Hawkesford M.J., McGrath S.P. 1999: Sulphur Utilisation Efficiency In Oilseed Rape. *10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra, Australia.* CD-ROM, Doc. No. 301: 1-5.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 103 - 107

Interactive forecasting on the Internet of light leaf spot (*Pyrenopeziza brassicae*) risk for winter oilseed rape

Neal Evans, Julie M Steed, Sue J Welham, John F Antoniw, Judith A Turner*, Peter Gladders[+] and Bruce D L Fitt
IACR – Rothamsted, Harpenden, Herts., AL5 2JQ, U.K.
** Central Science Laboratory, DEFRA, Sand Hutton, York, YO41 1LZ, U.K.*
[+] ADAS Boxworth, Cambridge, CB3 8NN, U.K.

Abstract: Detailed disease survey and weather data from different regions of the UK were used to produce a model that predicts the risk of severe light leaf spot for winter oilseed rape crops in that region. At the start of the season (in October), a prediction is made for each region, using the deviation of summer temperature from the 30 year mean and the incidence of light leaf spot on the pods of the previous crop immediately before harvest (previous July). The forecast is then updated periodically to take account of observed deviations from the average winter rainfall. Recently, the Internet version of the model that is hosted on the web-site at www3.res.bbsrc.ac.uk/leafspot/ has been updated to make it more crop-specific. Oilseed rape growers are required to input two pieces of information specific for their crop; cultivar (to take account of the light leaf spot resistance rating of the cultivar) and sowing date (as the model indicates that early sowing increases the risk from the disease). A crop-specific prediction of risk from light leaf spot, with or without the effect of an autumn fungicide application targeted at light leaf spot, is then delivered to the grower. The interactive light leaf spot model will be incorporated into a two-way interactive oilseed rape pest and disease Decision Support System (DSS) for winter oilseed rape (PASSWORD).

Key words: Forecast, interactive, Internet, light leaf spot, *Pyrenopeziza brassicae*.

Introduction

Oilseed rape is the most important arable crop in the UK after cereals (>400,000 ha *per annum*). It is estimated that diseases can cause up to £ 80M of losses per season in winter oilseed rape, athough losses differ greatly from season to season (Fitt *et al.*, 1997). Light leaf spot (*Pyrenopeziza brassicae*) and stem canker (*Leptosphaeria maculans*) are the two diseases that consistently cause the greatest losses. However, there are regional differences in the severity of the two diseases and light leaf spot causes the greatest losses in the north of England and in Scotland (Sutherland *et al.*, 1995).

Fungicide timing for the control of light leaf spot has not been optimal (Hardwick & Turner, 1994). For environmental and economic reasons, fungicide timing needs to be optimised so that only crops which require treatment are treated. Recommendations on spray timing depend on an understanding of the epidemiology of light leaf spot and an ability to forecast the risk of severe epidemics. A forecasting scheme, based on empirical relationships between disease incidence and weather factors (e.g. temperature, rainfall) has been developed over a number of years at IACR – Rothamsted. The model is continually being improved by the incorporation of new epidemiological information on *P. brassicae*. This paper describes the recent development of an Internet-based version of this system which provides information to help growers to optimise fungicide use for control of light leaf spot on winter oilseed rape.

Materials and methods

Development of initial regional model

The scheme for forecasting the severity of light leaf spot epidemics involves regional risk and crop risk forecasts at the beginning of the growing season in October, combined with a protocol for sampling crops to confirm the presence of light leaf spot (Fitt *et al*., 1998; Welham *et al*., 1999). Seasonal, regional risk indices, predicting the % crops in a region with light leaf spot in the following March, have now been issued in October since 1996. Spring disease survey data (i.e. March 1997, 1998, 1999 and 2000) were used to validate predictions made the previous autumn (i.e. October 1996, 1997, 1998 and 1999). Observed light leaf spot incidence in spring was never greater than that predicted for a region but was sometimes considerably smaller, most probably because many crops at risk had been sprayed with fungicide.

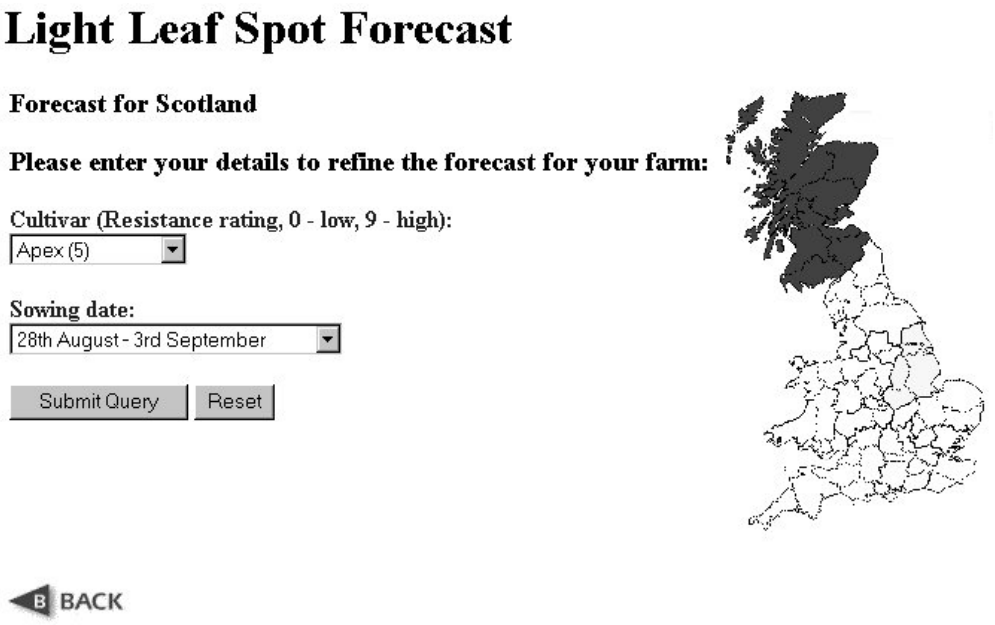


Figure 1. Light leaf spot forecast input page for a farm in Scotland. Cultivar to be grown and week of sowing can be selected by clicking on the arrows and selecting from the pull-down menus. The model is then run by clicking on the "Submit Query" button.

Development of an interactive, Internet-based model

The light leaf spot web pages were first produced in 1998. During this first year, the forecast was issued as a map showing the light leaf spot risk for different regions of the UK. Recently, the use of active server page (ASP) technology has allowed the development of an interactive crop-specific model. The ASP's were produced using Delphi software (Borland Software Corp., California, USA) and the pages are hosted on an interactive internet server. The pages (www.iacr.bbsrc.ac.uk/leafspot) now contain two input fields; cultivar (chosen from the current list of recommended cultivars) and sowing date (Fig. 1). Growers input information about their situation, press "Submit query" and are presented with two risk predictions for their specific area of the country under the cultural practices used on their farm (Fig. 2). The first risk prediction provides information for an unsprayed crop, whilst the second risk prediction takes into account the effect of a fungicide spray targeted against light leaf spot. A

recent addition to the website is a registration/comments form. Growers or their advisors are encouraged to register their email address or mobile telephone number and will be alerted to forecast model updates either by email to their computer or an SMS text message to their mobile phone (Fig. 3).

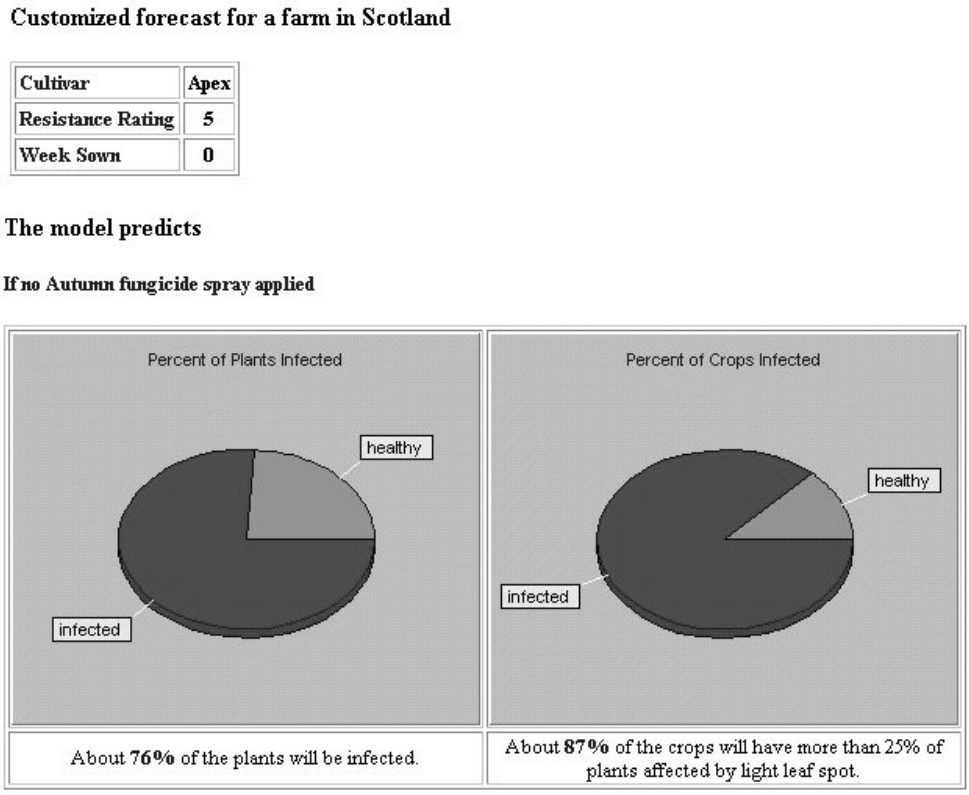


Figure 2. Light leaf spot forecast output page showing the high light leaf spot risk for a farm in Scotland growing winter oilseed rape cv. Apex (resistance rating 5) sown during the week 28 August to 3 September. Two similar charts on the same page provide the grower with information on the effect of an autumn fungicide application.

Discussion

The recent advances provided by the interactive crop-specific model have increased the potential of the model to provide the grower with valuable information before fungicide application decisions are made. The model is now more crop-specific than before, with cultivar resistance ratings and sowing date being taken into account. Both of these factors provide a further refinement to the original regional forecast information available to the grower. Ultimately, there is a need for crop risk indices that can be updated by using information about local weather (e.g. occurrence of infection conditions) and fungicide use throughout the season. Furthermore, predictive models need to be derived for situations where a combination of diseases occurs together.

The interactive model also provides growers with a useful tool which can be used before sowing the crop. Because resistance information on commonly grown cultivars has been included in the interactive web site, the grower has the opportunity to assess the relative merits of using cultivars with different resistance ratings on a farm in a specific region. From

this, it is possible to ascertain the potential risk from light leaf spot for a particular cultivar in a given region of the UK in a particular season and to evaluate the benefits of growing a more resistant cultivar.

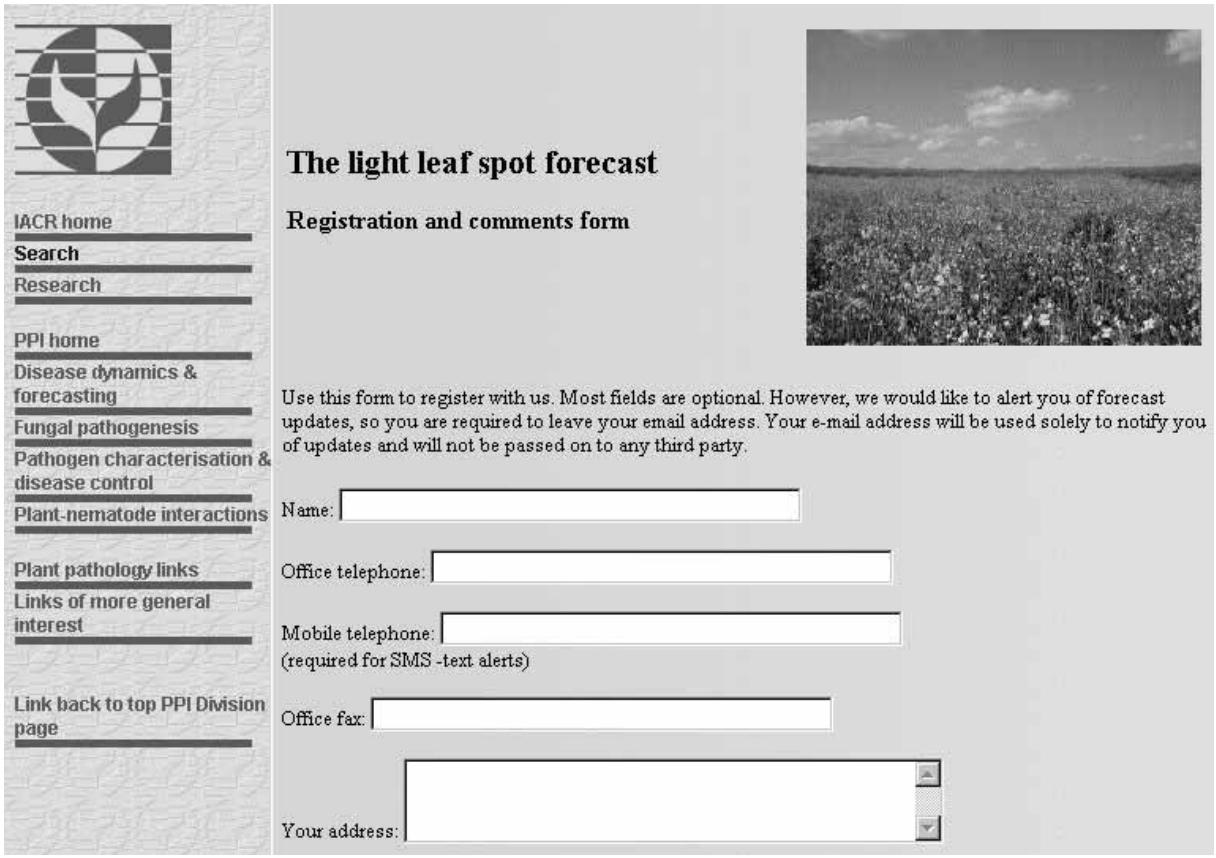


Figure 3. Registration / comments form on the light leaf forecast website. The form allows growers / advisers to register their email address / phone number for automatic notification of updates to the model.

The recent addition of a registration/comments form (Fig. 3) allows the forecast to become two-way interactive for the first time. Growers or advisers who register can choose whether they would like automatic notification of forecast updates, either by email or by SMS text message to their mobile telephone. It is envisaged that the further development of mobile telecommunications technology will allow real-time interaction with the forecast website, for example, to take into account a recent disease assessment or to incorporate on-farm meteorological data. For example, a study in Finland utilised SMS text messaging not only to alert growers to developing outbreaks of common agricultural pests, but allowed the development of a dynamic real-time database of actual pest levels at the field level through farmer response SMS text messages sent to the study centre (Markkula *et al*., 2000).

A new project (PASSWORD, Pest and Disease mAnagement System Supporting Winter Oilseed Rape Decisions) has just begun. Ultimately, PASSWORD aims to develop a decision support system for integrated management for stem canker, light leaf spot and the major pests of winter oilseed rape in the UK. However, such a decision support system can be reliable and robust only if it is based on accurate understanding and accurate models of the epidemiology

of the important diseases. The pest module of PASSWORD (DORIS: developed by colleagues at the Central Science Laboratory, York) is already being tested under field conditions. The priority now must be to obtain accurate biological data about the development of stem canker, the other important disease of winter oilseed rape in the UK. This will allow the construction of accurate models to describe development of stem canker epidemics and will facilitate the development of a combined regional risk and crop risk forecast system for light leaf spot and stem canker on winter oilseed rape.

Acknowledgements

This work was funded by the UK Department for Environment, Food and Rural Affairs, the Biotechnology and Biological Sciences Research Council and the Home-Grown Cereals Authority. We thank Stewart Souter for initial development work on the web pages.

References

Fitt, B.D.L., Doughty, K.J., Gilles, T., Gladders, P., Steed, J.M., Su, H. & Sutherland, K.G. (1998): Methods for assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. *Annals of Applied Biology* 133: 329-341.

Fitt, B.D.L., Gladders, P., Turner, J.A., Sutherland, K.G., Welham, S.J. & Davies, J.M.L. (1997): Prospects for developing a forecasting scheme to optimise use of fungicides for disease control on winter oilseed rape in the UK. *Aspects of Applied Biology* 48: 135-142.

Hardwick, N.V. & Turner, J.A. (1994): Fungicide use on winter oilseed rape in England and Wales, 1986-1993. *4th International Conference on Plant Diseases, Bordeaux*. ANPP: 1163-1170.

Markkula, I., Hannukkala, A., Lehtinen, A., Mattila, I., Ojanen, H., Raiskio, S., Reijonen, P. & Tiilikkala, K. 2000: Pest warnings and forecasts sent as SMS messages from models into "farmer's pocket". *The BCPC Conference Proceedings: Pests and diseases*, Vol. 1: 285-290.

Sutherland, K.G., Wale, S.J., Sansford, C.E. (1995): Effect of different epidemics of *Pyrenopeziza brassicae* on yield loss in winter oilseed rape. *Proceedings 9th International Rapeseed Congress, Cambridge*: 1004-1006.

Welham, S.J., Fitt, B.D.L., Turner, J.A., Gladders, P. & Sutherland, K. (1999): Relationships between regional weather and incidence of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in England and Wales. *Aspects of Applied Biology* 56: 51-59.

Biological and Integrated Control

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 111 - 115

The influence of temperature on the production of Camalexin and Methoxycamalexin in *Camelina sativa* (L) Crtz

Henneken, M. [1]; Föller, I. [1]; Paul, V.H. [1] and Krohn, K. [2]
[1] *Labor für Biotechnologie und Qualitätssicherung, Universität-GH Paderborn, Fachbereich Agrarwirtschaft, Lübecker Ring 2, D-59494 Soest*
[2] *Universität-GH Paderborn, Fachbereich Chemie und Chemietechnik, Warburger Straße 100, D- Paderborn*

Abstract: A method to extract the indolic *C. sativa* phytoalexins Camalexin and Methoxycamalexin with C-18 sorbent and simultaneous determination with HPLC was optimised. With this method the influence of temperature on the production of phytoalexins was investigated in *Camelina sativa*. After activation of the production of phytoalexins in *C. sativa* with the plant activator BION® the optimised method was used to measure the amount of phytoalexins. For this investigation four different *C. sativa* cultivars were used. These were cultivated under climatic chamber conditions at 18/11 °C and 14/10 day/night rhythm to rosette stage. At this stage the plants were treated with BION®. The plants of the control were treated with tap water. For incubation three different temperatures were used. A cold regime with 11/6 °C, a middle regime with 18/11 °C and a warm regime with 24/16 °C. After fourteen days the plants were harvested and immediately stored at –21 °C. For the extraction and analyses of the phytoalexins with the optimised method it was not required to dry the plant material, so frozen fresh material was used.
The amount of the phytoalexins Camalexin and Methoxycamalexin produced show a high variation with regard to the temperature regimes and the four cultivars concerned. The control (only one cultivar was taken representatively) shows the lowest amount of phytoalexins.
Of the used cultivars, one produced a high amount of Camalexin and a low amount of Methoxycamalexin. In another cultivar the amounts of Camalexin and Methoxycamalexin produced were approximately equal. While in the other two cultivars the amount of Methoxycamalexin was higher than the amount of Camalexin, and one of them produced a lower quantity of phytoalexins compared with the other three cultivars.
The total amounts of Camalexin and Methoxycamalexin decreases with warmer temperatures.
When the amounts of Camalexin and Methoxycamalexin were added up for the total quantity of phytoalexins produced the concentration of phytoalexins in three of the four cultivars at the cold regime were between 800 and 1100 µg/kg fresh matter (FM). One cultivar showed a quantity of 200 µg/kg FM. At the middle temperature regime the quantity of phytoalexins decreases (200 to 600 µg/kg FM) and there were further differences between the cultivars noted. The total quantity of pytoalexin was similar in all cultivars at the warm temperature regime and ranged between 100 and 180 µg/kg FM.

Key words: *Camelina sativa*. anti-microbial compounds, phytoalexins, plant activator, BION®

Introduction

In the beginning of the 21st century it is an important task of the human race to find some alternative raw materials to the fossil resources. One possibility is the use of regenerable resources, especially oilseed crops as a source of raw material. One old oilseed crop described first around 2000 BC is *Camelina sativa* (Grigson, 1960; Schultze-Motel, 1979; Mansfeld,

1986). In the discussion about regenerable crops *C. sativa* was rediscovered. To use *C. sativa* effectively as a regenerable oilseed crop it is required to start a breeding program with regard to yield and simultaneously to resistance to pathogens, especially fungal pathogens. *C. sativa* produces the pytoalexins Camalexin and Methoxycamalexin. These phytoalexins are discussed in regard to the resistance of *C. sativa* to *Alternaria brassicae* (Conn et al., 1991). It is furthermore of interest that *Arabidopsis thaliana* produces the pytoalexin Camalexin. In some investigations it was noted that mutants of *A. thaliana* unable to produce Camalexin were infested with pathogens whereas the Camalexin producing plants were resistance (Rogers, et al. 1996). Therefore it is probable that the phytoalexins Camalexin and very probably Methoxycamalexin plays an important part in the defence against pathogens in *C. sativa*. In this paper the influence of temperature on production of phytoalexins in *C. sativa* cultivars were investigated.

Material and method

Plant cultivation and elicitation of the phytoalexins

Four cultivars of *C. sativa*, labelled 2, 7, 12 and 13, were cultivated in a climatic chamber at 18/11 °C, 14/10 h day/night rhythm to rosette stage. At this stage they were treated with the plant activator BION® dissolved in tap water (0.9 mg/20 ml/0.2 m^2) and than cultivated at three different temperatures (11/6, 18/11, 24/16 °C 14/10 h day/night rhythm). The upper parts of the plants were harvested and stored at –21 °C immediately 17 days after treatment.

Sample clean up

3 g plant material was mixed with 40 ml methyl alcohol, treated with microwaves (1000 W) for 1 min and filtered. The extract was evaporated near to dryness under reduced pressure at 40 °C. The residue was taken up in 1 ml acetonitrile, diluted with phosphate buffer pH 8 (3.5 mM H_3PO_4, 0.3 mM NaCl) (25% v/v) and passed through a C-18 cartridge, solvated with phosphate buffer pH 8. The cartridge was washed with phosphate buffer pH 8. Than the cartridge was eluted with 1.5 ml acetonitrile / phosphate buffer ph 4 (80% v/v). The obtained coloured eluate was directly measured with HPLC.

Quantitative analysis of phytolaexins with HPLC

Analysis were performed on a HPLC system from Merck Hitachi (pump L6200A, fluorescence detector F 1080F, dioden array detector (DAD) L4500, auto sampler AS 4000A). To separate the phytoalexins a LiChrosorb C-18, 250-4 mm, 5µm column was used. The excitation and emission wavelengths were set at 318 and 385 nm (Turk et al., 1998). The phytoalexins were eluated from the C-18 column with 3.5 mmol Phosphatpuffer, MeCN, THF (60%, 35%, 5% v/v/v) and a flow rate of 1ml/min (Jamieson et al., 1988; Browne et al., 1989, Henneken, 2001). Quantification of Camalexin and Methoxycamalexin was carried out with Camalexin standard. The pure standard was obtained by Prof. Dr. Ayer (University of Alberta, Edmonton, Alberta Canada T6G 2E1).

Results and Discussion

In previous investigations it was shown that *C. sativa* in the rosette and cotyledon stage produced the highest amounts of phytoalexins after treatment with BION®. That is the reason why plants in rosette stage were used in this investigation. Fig. 1 shows the concentration of the phytoalexins Camalexin and Methoxycamalexin after treatment with BION® and incubation at three different temperatures. After treatment with BION® the plants of all cultivars showed depressions in growth compared to the untreated control. Concerning the production of phytoalexins the investigated cultivars showed differences regarding

concentration of Camalexin and Methoxycamalexin and furthermore a negative correlation between the temperature and the concentration of phytoalexin was found. The colder temperatures caused a higher amount of phytoalexin.

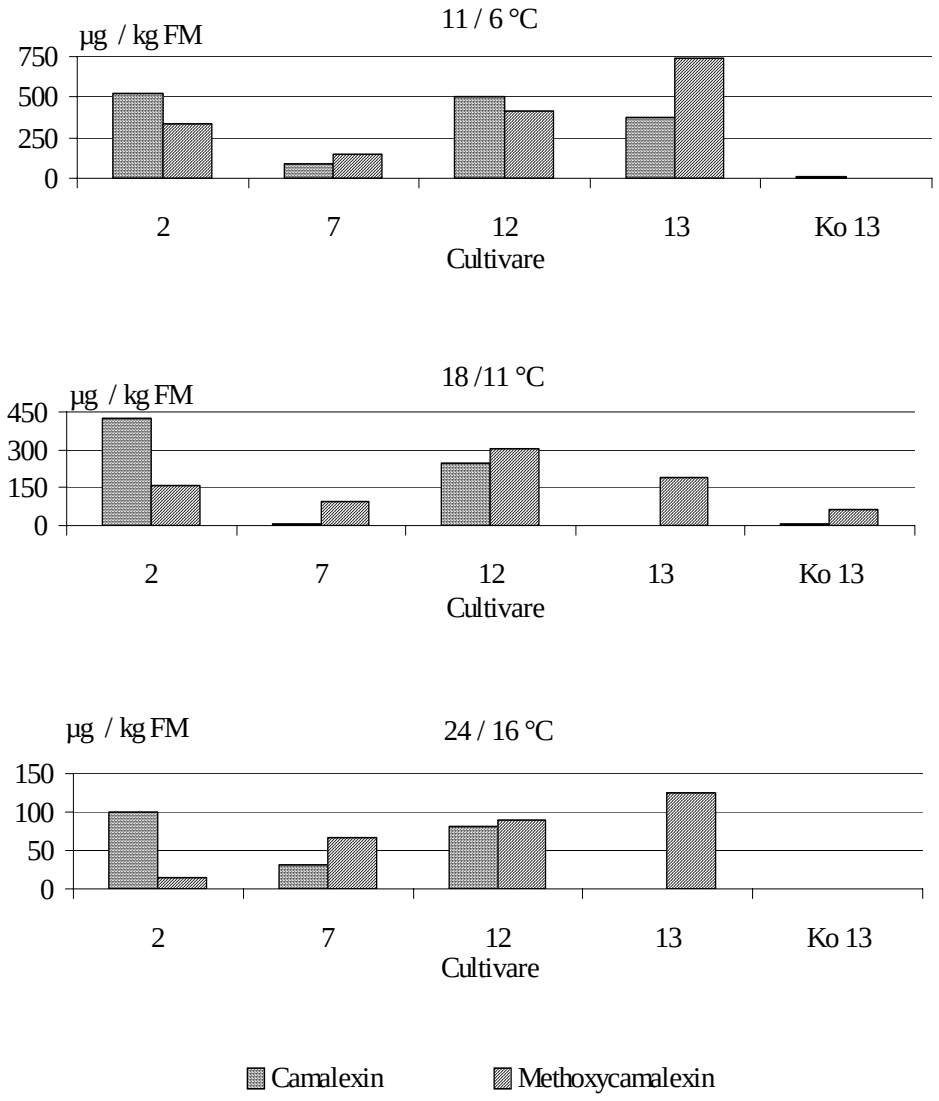


Fig. 1. Production of Camalexin and Methoxycamalexin in the upper parts of *C. sativa* plants incubation at three different temperature regimes after elicitation of the phytoalexin production with BION® and a control treated with tap water.

That *C. sativa* produces higher amounts of phytoalexin at low temperature is opposite to that what would be expected. If we transfer the results to the situation in field the behaviour concerning production of phytoalexins becomes more clear. Because *C. sativa* has a good agreeableness to late frosts the sowing date can de placed very early. At this temperature the growth of plants are low. In this situation infestations are potentially most harmful. *C. sativa*, like many other plants, can fall back on an inducible defence system. At high temperatures the growing rate is fast and a potential infestation has less harmful effects on plants and it is not necessary to activate an additional defence system. Since the activation of the inducible system is combined with the use of energy it should only be activated in case of needs e.g.

infestation with pathogens, abiotic stress. At higher temperatures and later growth stages there is no longer needs to activate the additional defence system in case of an infestation. Therefore it appears that a regulatory mechanism dose exist to suppress the activation of the inducible defence system and to save the energy to invest it in other areas.

In this investigation the used cultivars of *C. sativa* showed differences concerning the amount of produced phytoalexins. The cultivars 2, 12 and 13 produced high amounts, whereas the cultivar 7 produced low amounts of phytoalexins.

Furthermore there exists differences concerning the produced amounts of Camalexin and Methoxycamalexin (Fig. 1). The cultivar 2 produced more Camalexin than Methoxy-camalexin whereas the cultivar 13 produced more Methoxycamalexin than Camalexin. Concerning the cultivars 7 and 12 the produced amount of Camalexin and Methoxycamalexin are more or les the same.

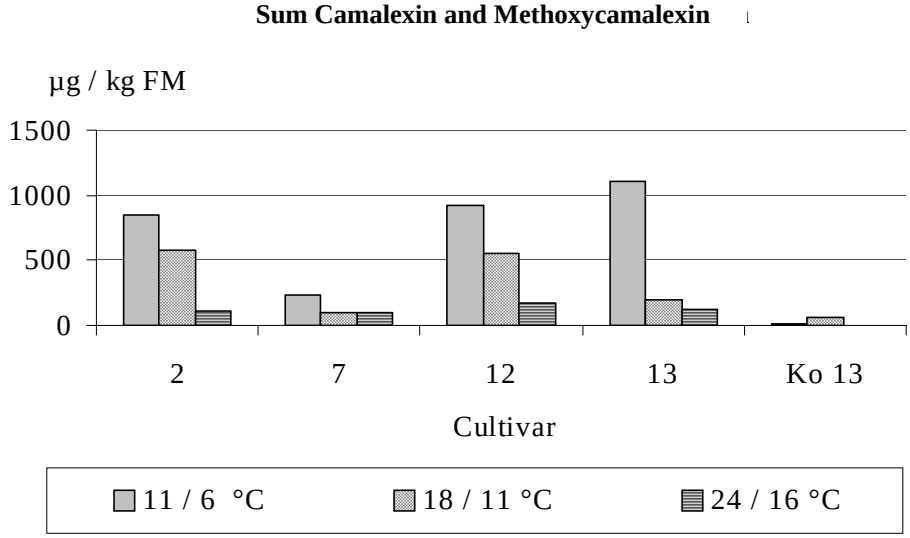


Fig. 2. Production of phytoalexins (sum of Camalexin and Methoxycamalexin) in the upper parts of *C. sativa* after treatment with BION® and cultivation at three difference temperatures compared to a control.

Fig. 2 depicts the total amount of phytoalexins (Camalexin and Methoxycamalexin). Here the interdependence of temperature and amount of phytoalexins becomes more distinct. Furthermore the investigated cultivars showed differences concerning the amount of Phytoalexin in the different temperature regimes. The amount of phytoalexin of cultivars 2 and 12 decreased about one third from the temperature regime 11/6 °C to 18/11 °C. From 18/11 °C to 24//16 °C the value decrease from about 500 to 50 µg/kg FM. The cultivar 13 showed a high decrease from 11/6 °C to 18 /11 °C. The concentration of phytoalexin was in the temperature regime 18/11 °C and 24/16° C more or less equal. The concentration of phytoalexin in the cultivar 7 was at 11/6° C about 100 µg/kg FM, the other temperature regimes the concentration was about 50 µg/kg FM.

It is very interesting to note that in every cultivar at the temperature regime 24/16° C the concentration of phytoalexin is more or less equal. This shows clearly that it was very important to take into consideration the temperature in realising such investigations with the goal to differentiate *C. sativa* cultivars in regard to their phytoalexin production. Concerning

the obtained concentration on Camalexin and Methoxycamalexin it is possible to differentiate the investigated cultivars. Now it is necessary to test more *C. sativa* cultivars.

Acknowledgement

We would like to thank Prof. Dr. Ayer (University Alberta, Edmonton Canada) to make a sample of Camalexin available to us.

References

Browne, L.M.; Conn, K.L.; Ayer, W.A. & Tewari, J.P. 1991: The Camalexins: New Phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). *Tetrahedron* 47(24): 3909-3914.

Grigson, G. 1960: Gold of Pleasure. In: *The Englishman's Flora*, Phoenix House LTD, London: 64.

Henneken, M. 2001: HPLC-Analyse von Phytoalexinen zur Differenzierung von *Camelina sativa* (L.) Crtz.-Sorten. *Dissertation*.

Jamieson, J. & Dunkan, H. J. 1989: Determination of thiabendazole residues in potatoes using high pressure liquid chromatography. *Potato Research* 32: 123-126.

Mansfeld, R. 1986: *Camelina*. In: *Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen),* Schultze-Motel, J. (ed.), Springer Verlag Berlin: 281-284.

Rogers, E.E.; Glazebrook, J. & Ausubel, F.M. 1996: Mode of action of the *Arabidopsis thaliana* phytoalexin camalexin and its role in *Arabidopsis*-pathogen interactions. *Molecular-Plant-Microbe-Interactions* 9(8): 748-757.

Schultze-Motel, J. 1979: Die Anbaugeschichte des Leindotters, *Camelina sativa* (L.) Crantz. In: Körber-Grohne, U. (Ed.), *Festschrift Maria Hoff, Archeo-Physika 8*. Habelt, Bonn: 267-281.

Turk, F.M.; Bennett, M.H.; Glazebrook, J.; Mansfield, J.W. & Holub E.B. 1998: Biotic and abiotic elicitation of Camalexin in phytoalexin-deficient (PAD) mutants and several wild-type accession of *Arabidopsis*. *7th International Congress of Plant Pathology, Edinburgh, Scotland August 1998*.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 117 - 122

Parasitization Rates of the Oil Seed Rape Pests *Ceutorhynchus napi, Ceutorhynchus pallidactylus* (Coleoptera, Curculionidae) and *Meligethes aeneus* (Coleoptera, Nitidulidae) by Ichneumonids in Several Localities of Eastern Austria

Petra Kraus and Bernhard Kromp
Ludwig Boltzmann Institute for Biological Agriculture and Applied Ecology, Rinnböckstraße 15, A-1110 Vienna, Austria

Abstract: In winter rape fields of Eastern Austria investigations were carried out to evaluate the parasitization rates of the rape stem weevil (*Ceutorhynchus napi* Gyll.), the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh., both Coleoptera, Curculionidae) in 1996, 1999 and 2000 and the pollen beetle (*Meligethes aeneus* F., Coleoptera, Nitidulidae) in 1999 and 2000 by their larval parasitoids *Tersilochus fulvipes* Grav., *Tersilochus obscurator* Aub. and *Tersilochus heterocerus* Thoms. (all Hymenoptera, Ichneumonidae), respectively.

For the weevils, larvae were sampled from unsprayed field plots (1996: 144m^2 each; 1999 and 2000: 1000m^2 each) and reared in the laboratory to evaluate their parasitization rates. The adult parasitoids in pre-emergence state were removed from their cocoons and identified. For the pollen beetles, rape flowers were sampled from the unsprayed plots and handsorted for *Meligethes aeneus* larvae in the laboratory. The parasitization rate was evaluated by counting the infested larvae.

In 1996, the parasitization rates ranged from 60% to 81% for *C. napi* and from 49% to 81% for *C. pallidactylus*. In 1999, the parasitization rates were between 2% and 26% for *C. napi*, between 40% and 76% for *C. pallidactylus* and between 16% and 29% for *M. aeneus*. In 2000, the parasitization rates ranged from 7% to 28% for *C. napi*, from 49% to 53% for *C. pallidactylus* and from 22% to 83% for *Meligethes aeneus*.

Key words: oil seed rape, pests, *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus*, *Meligethes aeneus*, larval parasitoids, Tersilochinae, parasitization rates

Introduction

In Austria, organic oilseed rape production is limited mainly because of the pest pressure of the rape stem weevil (*Ceutorhynchus napi* Gyll.), the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh., both Coleoptera, Curculionidae) and the pollen beetle (*Meligethes aeneus* F., Coleoptera, Nitidulidae).

For organic production there are no direct control measures available due to a ban on chemical synthetic insecticides according to the EU-regulative 2092/91. So far, in Austria no information is available on natural enemies of OSR-pests as a basis for the development of biological control strategies. To obtain information on parasitoid antagonists, investigations were carried out in winter rape fields in several localities of Eastern Austria.

The objective of the study was the evaluation of parasitization rates of *C. napi*, *C. pallidactylus* and *M. aeneus* by larval parasitoids (Ichneumonidae, Tersilochinae), in 1996, 1999 and 2000.

Material and methods

Study sites

All studies were carried out in unsprayed winter rape fields or field plots. In 1996, sampling was performed in 5 fields (size of the unsprayed field plots: 144m^2) on the northern and southern outskirts of Vienna. In 1999 and 2000, the studies were carried out in 4 fields (size of the unsprayed plots: 1000m^2) of the Agricultural Schools of Gießhübl, Pyhra, Tulln and Mistelbach in Lower Austria and 3 fields (size of the unsprayed field plots: 144m^2) in the north of Vienna (Raasdorf, Mühlleiten, Vienna). The distance from Vienna to the farthest locality Gießhübl (G) is about 116 km (Figure 1)

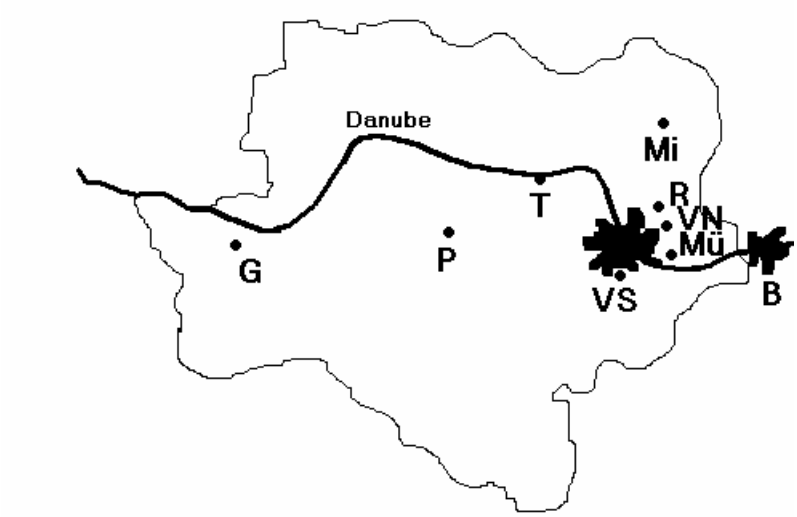


Fig. 1. Localities of the research fields in Lower Austria (G-Gießhübl, P-Pyhra, T-Tulln, Mi-Mistelbach) and the outskirts of Vienna (R-Raasdorf, Mü-Mühlleiten, VN-Vienna North, VS-Vienna South) in 1999 and 2000. B indicates the city of Bratislava nearby the Austrian border.

Evaluation of the parasitization rates of the weevils

The parasitization rates were evaluated by rearing the larvae in the laboratory according to the method of Klingenberg & Ulber (1994) and Ulber (personal communication). The 3rd-instar larvae were dissected from 100-200 rape stems per field plot and put into plastic boxes with soil (sterilized and moistened) for pupation. Adult beetles of *C. pallidactylus* were removed and counted continuously. After 8 weeks cocoons of *C. napi* were counted. Parasitoids in pre-emergence state were removed from their cocoons and identified (Horstmann, 1971 & 1981). The parasitization rates were calculated by using the following formula:

$$\frac{\text{Number of parasitoid cocoons x 100}}{\text{Number of rediscovered cocoons}}$$

Using the number of rediscovered cocoons instead of the total number of reared larvae allowed us to exclude the mortality rate under laboratory conditions.

Evaluation of the parasitization rates of the pollen beetles

Fifty rape inflorescences were sampled per field plot and handsorted for 3rd-instar pollen beetle larvae. The parasitization rates were calculated by counting the larvae which were infested by black eggs of the ichneumonid *Tersilochus heterocerus*.

Results

Parasitization rates of weevil larvae in 1996

The larvae of *C. napi* were parasitised by the ichneumonid wasp *T. fulvipes* and the larvae of *C. pallidactylus* by *T. obscurator*.

In southern Vienna the parasitization rates ranged between 62% and 81% for *C. napi* and between 53% and 81% for *C. pallidactylus* (Figure 2). In northern Vienna the pest incidence was very low, so only a small number of larvae could be sampled. Accordingly, the number of rediscovered larvae was very low. For the larvae of both weevil species, the mortality rates under laboratory rearing conditions were about 30% for Vienna South and about 50% for Vienna North.

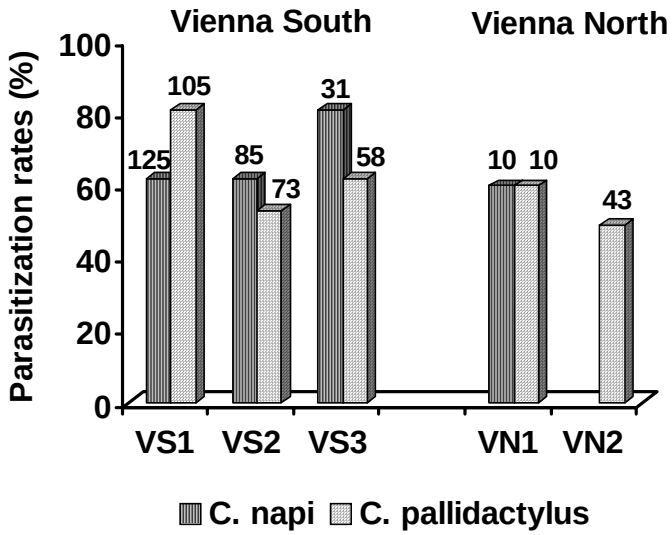


Fig. 2. Parasitization rates (in %) of *Ceutorhynchus napi* and *C. pallidactylus* in unsprayed field plots in the south and north of Vienna in 1996. The numbers on top of the bars indicate the numbers of rediscovered cocoons in the laboratory.

Parasitization rates of weevil larvae in 1999/2000

In the study period of 1999 and 2000 the same parasitoids of *C. napi* and *C. pallidactylus* larvae were discovered as in 1996.

In 1999, *C. napi* could only be sampled in 4 sites. At these localities the parasitization rates were between 0% and 26%. *C. pallidactylus* occurred at all sites and its parasitization rates ranged from 40% to 76%. In 2000, the weevil larvae could be sampled only in 2 sites due to low pest incidences. The parasitization rates for *C. napi* ranged between 7% and 28% and were as low as in the year before, the rates for *C. pallidactylus* ranged from 49% to 53% and were in average as high as in 1996 and 1999 (Figure 3). In 1999 and 2000, for both species the mortality rates under laboratory rearing conditions ranged between 10% and 40%.

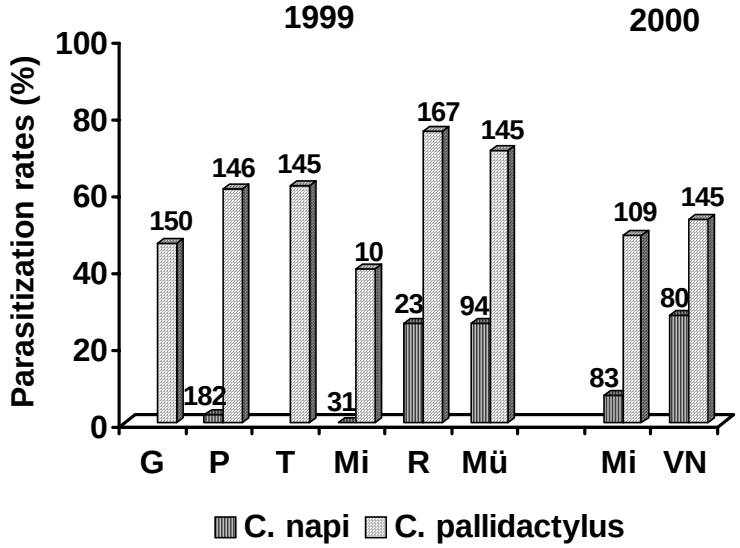


Fig. 3. Parasitization rates (in %) of *Ceutorhynchus napi* and *C. pallidactylus* from unsprayed field plots in 6 fields in Lower Austria and the outskirts of Vienna in 1999 and 2000. The numbers on top of the bars indicate the numbers of rediscovered cocoons in the laboratory (for abbreviations of localities see legend Figure 1).

Parasitization rates of pollen beetle larvae in 1999 and 2000

Concerning the pollen beetle larvae we only evaluated the parasitization rates by *Tersilochus heterocerus*. According to various studies from other European countries, *T. heterocerus* is the predominant parasitoid in *M. aeneus* larvae although a few other parasitoid species also occur occasionally (Nilsson & Andreasson, 1987; Büchi, 1991; Nissen, 1999; Alford et al., 2000).

In 1999, parasitization rates were evaluated at 3 localities of Lower Austria. They ranged from 16% to 29% (Figure 4). In the following year, the parasitization rates for Gießhübl (G) and Pyhra (P) were as high as in 1999, the rates for Tulln (T) were nearly 2,5 times higher. The parasitization rates for the sites Mistelbach (Mi), Raasdorf (R) and Vienna North (VN) ranged from 38% up to 83%.

Concerning *Meligethes aeneus*, our results showed parasitization rates by *T. heterocerus* ranging from 16% to 83% in Lower Austria. In Sweden, Nilsson & Andreasson (1987) found *M. aeneus* to be parasitised by *T. heterocerus* between 3% and 57%. In 1992 and 1993, Nissen (1999) evaluated parasitization rates by *T. heterocerus* between 34% and 48% in Germany. Büchi (1991) evaluated in 1990 parasitization rates between 0% and 87% in several regions of northern Switzerland. The latter author found 4 species of parasitoids: *Tersilochus heterocerus, Phradis morionellus, Diospilus capito* and one unknown species. *T. heterocerus* was the predominant parasitoid species with a rate of parasitization between 0% and 26%. In the valley of Rhine between Bad Ragaz and Domat-Enns the parasitization rate of *M. aeneus* by *T. heterocerus* ranged from 0% to 87% (Büchi, 1991).

The results from the literature and our own study show varying degrees of naturally occurring parasitization of the above mentioned rape pests. However, due to the fact that the parasitoids kill the host larvae not before they have migrated into the soil the feeding damage by the larvae cannot be reduced in the current season. Parasitization might have an effect on the damage level only in the following year. The actual contribution of the parasitoids to pest reduction, however, can only be estimated, as the impact of the parasitoids can not be seen

isolated from other natural mortality factors which are especially effective while the pest larvae migrate into the soil and overwinter (Klingenberg & Ulber 1994). According to Klingenberg & Ulber (1994) parasitization rates of up to 50% are likely to have an influence on pest abundance in the long run. Hokkanen et al. (1988) reported that in some areas of Finland where parasitization rates of *Meligethes aeneus* were as high as 60% to 80%, the need for chemical control had been by far less important than in areas with low parasitzation rates. As a consequence, Hokkanen (1989) developed a biological control strategy against the rape blossom beetle, which is based on protection of the parasitoids by avoiding soil cultivation after rape harvest until the next mid-summer. This allows the larvae to overwinter in the soil of the field unaffected by mechanical destruction. Additionally, the rape is undersown with white clover. By following that strategy, small-scale organic production of oilseed rape for producing cold-pressed oil has been well-established. However, this strategy is applicable only in Finland where only summer rape is grown and the rape blossom beetle is the only main pest species. Similar or even more complex strategies have still to be developed for other European countries which grow predominantly winter rape and have to face a wider spectrum of pest species.

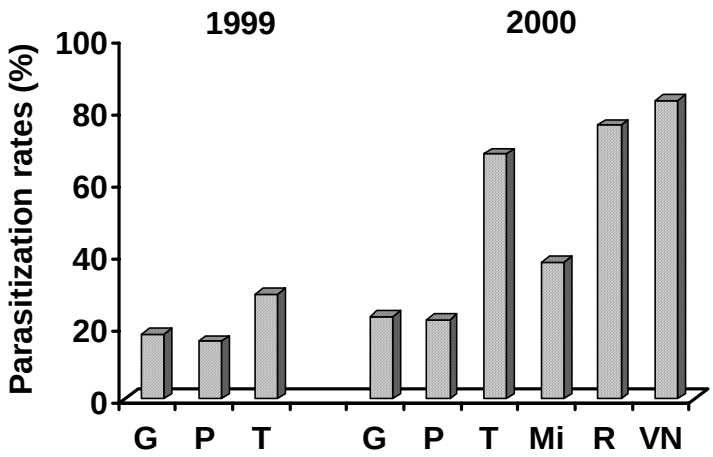


Fig. 4. Parasitization rates (in %) of *Meligethes aeneus* in unsprayed field plots in 6 fields in Lower Austria in 1999 and 2000 (for abbreviations of localities see legend Figure 1).

Acknowledgements

We want to thank the personnel of the Agricultural Schools of Lower Austria, especially their research coordinator J. Rosner as well as the farm managers F. Kaindl (City of Vienna Farm Wallhof) and K. Refenner (Experimental Farm of the Agricultural University of Vienna).

Special thanks are due to B. Ulber, University of Göttingen, Germany for providing information on the rearing methods of weevil larvae.

References

Alford, D.V., Ballanger, Y., Büchi, R., Büchs, W., Ekbom, B., Hansen, L.M., Hokkanen, H.M.T., Kromp, B., Nilsson, C., Ulber, B., Walters, K.F.A., Williams, I. & Young, J.E.B. 2000: Minimizing pesticide use and environmental impact by the developement

and promotion of bio-control strategies for oilseed rape pests. *BORIS – The Final Report. FAIR – CT*: 96-1314.

Büchi, R. 1991: Machen Nützlinge Spritzungen gegen Rapsschädlinge überflüssig?. *Raps* 9: 200-202.

Hokkanen, H. 1989: Biological and agrotechnical control of the rape blossom beetle *Meligethes aeneus* (Coleoptera, Nitidulidae). *Acta Entomologica Fennica* 53: 25-29.

Hokkanen, H., Husberg, G.-B. & Söderblom, M. 1988: Natural Enemy Conservation for the Integrated Control of the Rape Blossom Beetle *Meligethes aeneus* F. *Annales Agriculturae Fenniae*. 27: 281-294.

Horstmann, K. 1971: Revision der europäischen Tersilochinen I. (Hym. Ichneumonidae). *Veröff. Zool. Staatssamml. München* 15: 47-138.

Horstmann, K. 1981: Revision der europäischen Tersilochinen II. (Hym. Ichneumonidae). *Spixiana Suppl*. 4: 1-76.

Jordheuil, P. 1960: Influence de quelques facteurs écologiques sur les fluctuations de population d'une biocénose parasitaire: etude relative a quelques hymenopteres (Ophioninae, Diospilinae, Euphorinae) parasites de divers coleopteres infeodes aux crudiferes. *Ann. Épiphyt*. 11: 445-658.

Klingenberg, A. & Ulber, B. 1994: Untersuchungen zum Auftreten der Tersilochinae (Hym. Ichneumonidae) als Larvalparasitoide einiger Rapsschädlinge im Raum Göttingen 1990 und 1991 und zu deren Schlupfabundanz nach unterschiedlicher Bodenbearbeitung. *J. Appl. Ent.* 117: 287-299.

Nilsson, Ch. & Andreasson, B. 1987: Parasitoids and predators attacking pollen beetles (*Meligethes aeneus* F.) in spring and winter rape in Southern Sweden. IOBC/WPRS Bulletin 10(4): 64-73.

Nissen, U. 1995: Zeitliches Auftreten von Parasitoiden im Rapsbestand. In: *Rapssymposium zu Fragen der Phytopathologie und des Pflanzenschutzes 13./14. Oktober 1995*. Universität Rostock: 25-28.

Nissen, U. 1999: Natürliche Gegenspieler von Rapsschädlingen. *Raps* 17(2): 76-82.

Sedivy, J. 1983: Tersilochinae as parasitoids of insect pests of winter rape (Hymenoptera: Ichneumonidae). *Contrib. Americ. Ent. Inst.* 20: 266-276.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 123 - 131

Aphids in oil seed rape in autumn, possibilities to reduce virus transmission

Udo Heimbach, Christa Eggers and Thomas Thieme [1]
BBA, Institute for Plant Protection in Arable Crops and Grassland, Messeweg 11/12, D-38104 Braunschweig, Germany
[1] BTL-PE, Sagerheide, Birkenallee 19, D-18184 Sagerheide, Germany

Abstract: Aphids, mainly *Brevicoryne brassicae* and *Myzus persicae*, can colonise oil seed rape in autumn. They can transmit Turnip yellows virus (TuYV) disease to rape, which can affect the yield in certain situations. Therefore field experiments were carried out for several years, in which different methods were used to reduce aphid numbers. Aphid numbers and TuYV infection rates in autumn were reduced by straw mulch or seed treatment with Poncho (β-cyfluthrin 2 g a.i. + imidacloprid 10 g a.i./kg seed) to a similar degree, but the effect of Poncho lasted longer. Seed treatment with isofenphos had no effect on aphid number and virus infection, whereas seed treatment with Chinook (2 g a.i. β-cyfluthrin plus 2 g a.i. imidacloprid/kg seed) reduced aphid numbers and virus infection only slightly. The reason for lower aphid numbers in mulched plots seems to be an altered settling behaviour of aphids as the number of trapped alate aphids indicate. The degree of virus infection was correlated to a certain degree with the number of aphids counted in the plots. Yield was not influenced in the trials though different aphid / virus infections were reached.

In one year aphid and TuYV infection were analysed in small plots (approx. 50 m² per plot) parallel to larger plots (approx. 1000 m² per plot). Results show that control and other untreated areas neighbouring the experimental area influence the degree of virus infection in treated plots for several meters. Thus virus infection rates in e.g. Poncho treated plots near to untreated areas were about 80 % whereas in 20 m distance they were only about 40 %.

Key words: aphid settlement behaviour, oil seed rape, Turnip yellows virus (TuYV), mulch, imidacloprid seed treatment, plot size

Introduction

Aphids are known to transmit Turnip yellows virus (TuYV) to oil seed rape and virus infection can reduce the yield (Graichen & Schliephake 1996). Mainly *Brevicoryne brassicae* and *Myzus persicae* settle in autumn in newly germinated rape plants and both species are capable of transmitting the Turnip yellows virus (Schliephake et al. 2000). To control aphid numbers and so virus infection insecticides (e.g. seed treatment with imidacloprid) or alternative control measures can be used. First results indicate that less aphids (Heimbach et al. 2001 in press) and more epigeic predators were found when mulching was used compared to non mulched plots (Heimbach et al. 1997). Alate aphids are able to use optical and/or olfactorial stimulants for orientation to find host plants during flight. The reaction patterns are species specific for different species. The differences of colour (e.g. Moericke 1950) as well as contrast between crop and background (Neitzel & Müller 1959, Müller 1957, 1964) and the plant odour (Nottingham & Hardie 1993, Nottingham et al. 1991) are important for the settlement of alate aphids. Therefore effects influencing settling behaviour could be used to reduce aphid numbers in some crops.

Material and methods

Field experiments were carried out in the area around Braunschweig in Germany between 1998 and 2000 in oil seed rape (variety Mohican) with 4 replicated plots per variant. The plot size was about 50 m² (in 2000 additionally 1000 m², 3 replicates only). Plots were mulched by applying a thin layer of straw (100 – 200 g per m²) after sowing but before emergence and were compared to plots without mulch. The insecticides were applied as seed dressings or sprays (see Tab. 1). All other treatments were kept the same for all plots including insecticidal treatments in the springs of the three years.

Sowing was carried out with a small experimental seeder except the large plots in 2000 for which usual equipment was used one day after sowing the small plots. The result was a distinct lower emergence rate and later emergence of the crop compared to the small plots on the same field.

Table 1. Type of treatments in autumn rape 1998 – 2000.

Variant	seed treatments	g a.i./kg seed	other treatments	years
Thiram	thiram (fungicide)	4.4	-	1998-1999
Pyrethroid	isofenphos + thiram	16 + 4.4	λ-cyhalothrin, 7.5 g a.i./ha, 27.9.00	2000
Isofenphos	isofenphos + thiram	16 + 4.4	-	1998–2000
Straw	isofenphos + thiram	16 + 4.4	straw, 100 - 200 g/m²	1998–2000
Chinook	imidacloprid + β-cyfluthrin + thiram	10 + 2 + 4.4	-	1998–2000
Poncho	imidacloprid + β-cyfluthrin + thiram	2 + 2 + 4.4	-	1998-2000

The number of alate aphids flying into the crop was analysed by using one sticky net of 0.5 m² per plot (mesh size about 8 x 4 mm) which was kept 2 - 10 cm above the crop canopy in a horizontal position. Aphids were collected from the sticky nets every 2 – 4 days except in rainy periods. Additionally the number of aphids colonising the crop plants was counted mostly in weekly intervals. Depending on the degree of infection between 10 and 50 plants per plot were assessed.

The degree of infection with Turnip yellows virus was determined by ELISA on 25 - 30 plants taken from the central area of each small plot usually in autumn and spring. In autumn 2000 in each of the large plots 12 plants were sampled in seeding direction 3 m away from the two plot borderlines and in the centre, altogether 36 plants per plot. Samples of 5 plants were taken in spring 2001 every 2 m from one side of each plot to the other side (altogether 70 plants per plot) to check for border effects.

Results

Aphid numbers were quite low in 1998 reaching less than 1 aphid per plant at maximum in variants without aphicides whereas in 1999 and 2000 much higher numbers were found. Therefore in Tab. 2 the sum of all aphids collected is given whereas for the other years average values per plant and assessment are given.

Isofenphos did not have any effects on aphid number on plants compared to the thiram variant (only fungicidal treatment) in the 2 years tested (Tab. 2, 3), whereas seed dressing containing imidacloprid showed an effect in relation to the dose as expected with higher efficacy of Poncho compared to Chinook (Tab. 2 - 4). Spraying of a pyrethroid in 2000 did not have any effects on aphid numbers (Tab. 4) though it was very efficient on flea beetle population in this variant. Mulching had similar effect on aphid numbers in early autumn 1999 and 2000 as the high rate of imidacloprid though in later autumn aphid number increased in mulched plots (Tab. 3, 4). This effect is not visible in 1998, but aphid numbers were very low that year (Tab. 2). In 2000 the larger plots (only about 11 plants per m row developed) were much more attractive for aphids than the small plots (about 16 plants per m row developed), with more aphids found on the rape (Fig. 1, Tab. 4) and more aphids caught in sticky nets in the large plots (Tab. 5). Differences in aphid numbers between mulched and isofenphos plots are more pronounced in large plots compared to small ones. In 1999 and 2000 less alate aphids were detected using sticky nets in straw mulched plots compared to not mulched ones (Tab. 5). The differences in numbers of alate aphids settling were diminished when the crop cover increased (Tab. 5).

Table 2. Total number of aphids collected per variant and TuYV infection in autumn and spring and yield using different treatments in autumn 1998.

Variant	sum of all assessments, 1998		TuYV infection in %		yield
	all aphids	alate only	autumn 98	spring 99	in t/ha
Thiram	329	52	10.8	8.6	4.08
Isofenphos	371	61	10.0	8.5	3.87
Straw	403	59	0.0	0.9	4.16
Poncho	122	49	2.5	1.6	4.13
Chinook	218	49	5.0	7.3	3.96

Table 3. Average number of aphids per plant and TuYV infection in autumn and spring and yield using different treatments in autumn 1999.

Variant	no. aphids per plant, 1999			TuYV infection in %		yield
	total, 14.9.–12.10.	alate only	total, 30.11.	autumn 99	spring 00	in t/ha
Thiram	3.83	0.11	3.95	85	91	3.73
Isofenphos	2.93	0.22	5.68	77	88	3.80
Straw	0.84	0.08	5.95	54	87	3.97
Poncho	0.28	0.10	0.23	45	66	3.78
Chinook	1.54	0.19	3.50	67	92	3.89

Table 4. Average number of aphids per plant in small (approx. 50 m²) and large plots (approx. 1000 m²) on the same field using different treatments in autumn 2000.

Variant	small plots, 2000			large plots, 2000		
	11.-29.9.	23.10.	20./29.11.	11.-29.9.	23.10.	20./29.11.
Isofenphos	0.57	0.61	3.28	1.39	5.35	1.97
Straw	0.16	1.23	4.98	0.32	0.88	3.63
Poncho	0.09	0.00	0.623	0.28	0.63	0.33
Chinook	0.16	1.50	1.10	-	-	-
Pyrethroid	0.52	0.66	3.15	-	-	-

Table 5. Number of alate aphids per sticky trap (0.5 m²) in isofenphos treated plots with and without straw mulch.

Year	1998		1999		2000, small plots		2000, large plots	
Period	15.9.-14.10.	4.10.-18.11.	3.9.-20.9.	20.9-15.10	4.9.-28.9.	28.9.-26.10.	4.9.-28.9.	28.9.-26.10.
No. assess.	7	7	6	5	8	8	8	8
Straw	0.61	0.18	1.21	6.50	1.22	3.78	0.75	3.79
No straw	0.36	0.18	3.04	8.65	1.66	4.78	3.42	7.42

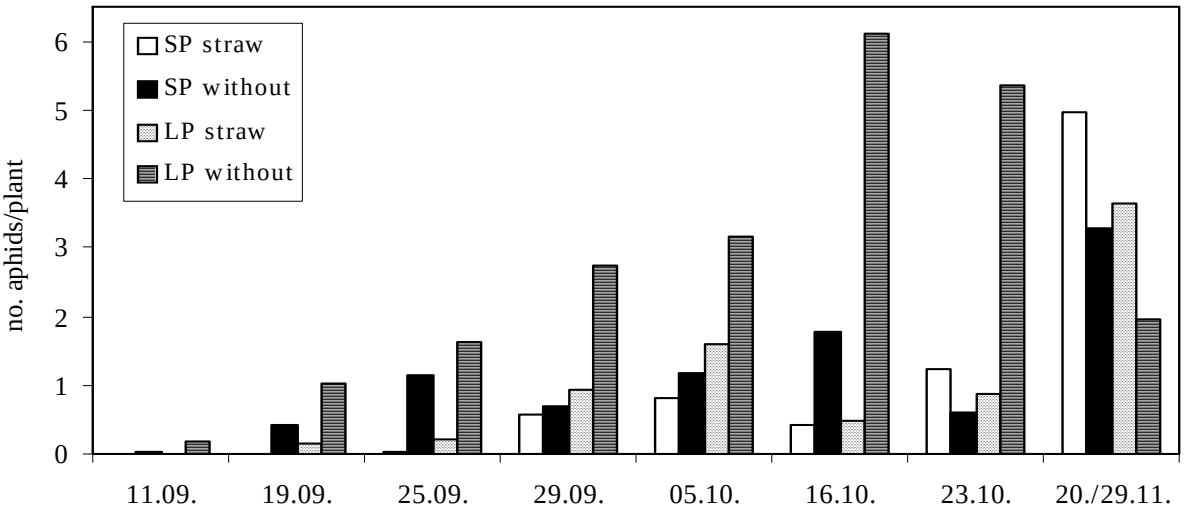


Fig. 1. Number of aphids per rape plant in small (SP) and large plots (LP) on the same field with or without straw in 2000.

Aphid species developing in the crops were mainly *Myzus persicae* and *Brevicoryne brassicae*. *M. persicae* dominated over *B. brassicae* in the two years for which a species composition was determined (Tab. 6, 7). Species composition differed slightly between variants within each year. In those cases when higher numbers were collected, usually *M. persicae* was reduced to a much higher extent than *B. brassicae* in mulched plots compared to not mulched ones (Tab. 6, 7).

Aphid numbers and TuYV infection seem to be correlated. Thus insecticidal treatments reduced aphid densities more efficiently and had also a higher effect on the virus infection rate (Tab. 2, 3, 8). This is also visible in the virus infection rates of mulched plots which are in the same range as for Poncho when infection rates were tested in autumn 1999 and 2000 (large plots), but they were higher when samples were collected in spring 2000 and 2001 again. In large plots virus infection rates are reduced in mulched plots to higher extent than in small plots (Tab. 8). The yield of the different variants differed only slightly in the 2 years for which results are available yet (Tab. 2, 3).

TuYV infection of plants collected in autumn 2000 in large plots with Poncho or straw mulch showed that sampling near (3 m) to areas that were not treated with any aphicide - as isofenphos plots and the surroundings of the experimental site - had higher virus infection values than plot areas being at least 15 m away from these “untreated” areas (Tab. 9). In spring 2001 plants for TuYV analysis were taken every 2 m across the large plots. The

achieved values were grouped between the three replicates of the variants Poncho and Straw and arranged in relation to the distance from directly neighbouring rape areas without aphicidal treatments. A significant negative effect of the distance from this borderlines on the virus infection was calculated for Poncho ($y = 84 – 1.84\ x$, $r = 0.818$). For straw also a negative relationship was found ($y = 60.7 – 0.92\ x$, $r = 0.444$) (Fig. 2), which was not significant because of high variability of the data.

Table 6. Number of alate and apterous *Brevicoryne brassicae* and *Myzus persicae* sampled from 10 plants per plot during 12.10.99 and 30.11.99 using different treatments in autumn 1999.

Variant	12.10.1999				30.11.1999			
	B. brassicae		*M. persicae*		*B. brassicae*		*M. persicae*	
	alate	apterous	alate	apterous	alate	apterous	alate	apterous
Thiram	0	30	1	34	0	0	10	10
Isofenphos	0	13	2	33	0	2	3	22
Straw	0	20	1	6	0	1	2	19
Poncho	0	0	2	5	0	0	0	2
Chinook	1	16	0	16	0	0	4	16

Table 7. Number of *Brevicoryne brassicae*, *Myzus persicae* and other aphid species sampled from 25 plants per plot (only alate aphids found) during 4 sampling dates (11.9. - 29.9.00) in small (approx. 50 m²) and large plots (approx. 1000 m²) on the same field using different treatments in autumn 2000.

Variant	small plots, 2000			large plots, 2000		
	B. brassicae	*M. persicae*	others	*B. brassicae*	*M. persicae*	others
Isofenphos	2	6	1	8	43	9
Straw	2	6	2	7	11	10
Poncho	0	9	0	5	23	6
Chinook	2	7	2	-	-	-
Pyrethroid	3	8	0	-	-	-

Table 8. Rates of TuYV infection and efficacy calculated with isofenphos as control at 2 sampling dates in small (approx. 50 m²) and large plots (approx. 1000 m²) on the same field using different treatments in autumn 2000.

Variant	small plots		large plots			
	December 2000		December 2000		April 2001	
	TuYV	efficacy	TuYV	efficacy	TuYV	efficacy
Isofenphos	55.8 % a	-	85.2 % a	-	91.9 % a	-
Straw	36.0 % b	-35.5%	41.7 % c	-51.1 %	49.5 % b	-46.1 %
Poncho	36.4 % b	-33.8%	63.0 % b	-26.1 %	60.0 % b	-34.7 %
Chinook	46.0 % ab	-17.6%	-	-	-	-
Pyrethroid	49.9 % ab	-10.6%	-	-	-	-

Different letters indicate significant differences ($p < 0.05$) using t-test (small plots) and Tuckey (large plots autumn 2000) and Kruskal-Wallis (large plots spring 2001).

Table 9. Rates of TuYV infection (%) sampled in December 2000 in lines 3 m away from plot border areas with no aphicidal treatments (neighbouring) or at least 15 m away (not neighbouring) in large plots (approx. 1000 m²) using different treatments in autumn 2000.

Variant	site of sampling	TuYV in %	
Straw	no. = 5, not neighbouring (≥15m) no. = 4, neighbouring (3m)	33.3 52.1	not sign.
Poncho	no. = 5, not neighbouring (≥15m) no. = 4, neighbouring (3m)	55.0 72.9	not sign.

Grouping of the variants straw and Poncho shows significant differences between neighbouring/not neighbouring sites (ANOVA followed by Bonferroni adjustment, $p < 0.05$)

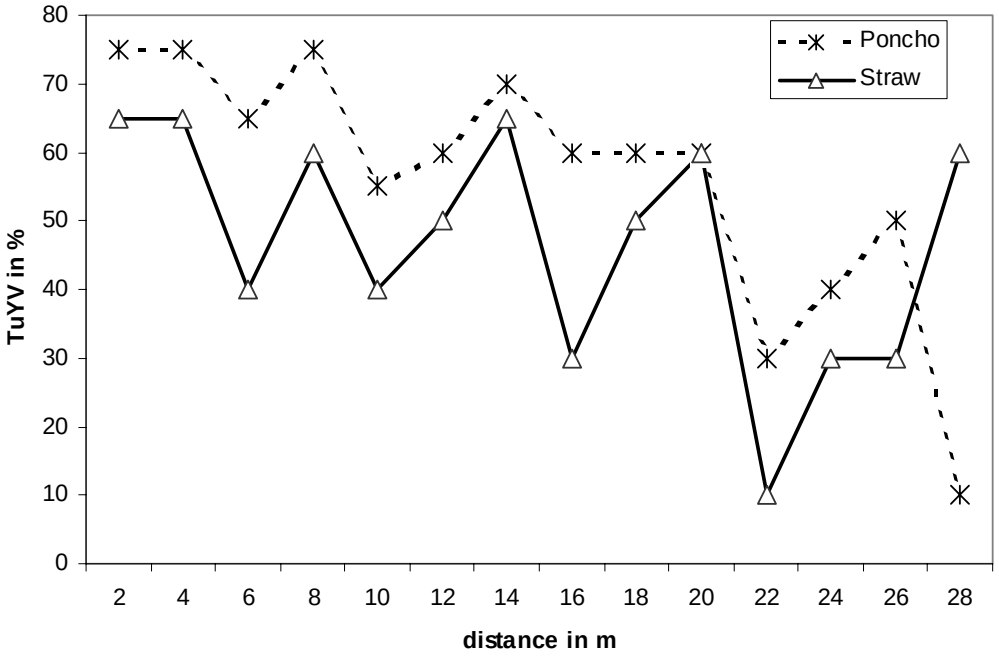


Fig. 2. Percent TuYV infected rape plants in relation to the distance from bordering areas treated with isofenphos (having no effect on aphids) grouped for all plots treated with Poncho or mulched with straw.

Discussion

Aphid numbers were reduced by seed treatment with imidacloprid, which is known to be an efficient seed treatment in several crops (Leicht 1996). Both tested rates are quite low compared to rates used in other crops (sugar beet up to 100 g a.i./ha according to the registered rate in Germany) which may explain that even at the tested high rate still aphids are present in the crop and are able to reproduce. In contrast seed treatment with isofenphos was not effective on aphids. This can be explained by the non systemic action of this insecticide in upper plant parts (Perkow & Ploss 1999). The pyrethroid was interestingly not effective on the aphids though flee beetles (*Psylliodes chrysocephala*) were very well controlled (Heimbach unpublished) in the same experiment. This might be due to the fact that in autumn aphids suck mostly on the underside of the rape leafs and therefore they are not in direct contact with the pyrethroid.

A reason for the lower infestation with aphids which was detected in the mulch variants could be the different fitness of the host plants (Nozon 1990) influencing aphid development and optical effects of the mulch on alate aphids settling. Colours (Moericke 1952) are important in addition to olfactory effects (Nottingham & Hardie 1993) in settling of aphids on the host plant. Also the contrast between plant and soil seems to play an important role in the orientation (Müller 1964, Smith 1976) and it is known that inhomogeneous crops, as the large plots in 2000, will usually be much more infested than homogeneously distributed crops (Müller 1957). As in this study also from other crops a reduction of settlement by alate aphids and/or a reduction of aphid-transmitted virus infections is known (Eggers et al. 2001 in press, Finch & Edmonds 1994, Heimbach et al. 1997 & 2001 in press, Jones 1994, Kendall et al. 1991, Wyman et al. 1979). The results presented here confirm the reducing effect of mulch on aphid numbers and virus transmitting in rape. The settlement behaviour of the alate aphids caused by mulch can be taken as an important reason for lower aphid numbers developing in the crop. In most cases both, approaching aphids and the density of aphids on the plants were reduced. Reduced numbers of settling aphids were found as long as the culture did not cover the soil surface completely and the contrast between culture and soil surface was visible. This seems to be the reason for a longer and more intensive reduction of settling aphids in rape in 2000 in mulched large plots with inhomogeneous and lower crop cover compared to the homogenous crop in small ones. The higher reduction of *Myzus persicae* due to mulching with straw compared to *Brevicoryne brassicae* might be due to the fact that aphids can perceive volatiles of the host plant (Nottingham & Hardie 1993, Nottingham et al. 1991), thus optical effects may be less important for some species. It is known that some polyphagous predators like Staphylinidae and Araneae may occur in higher numbers in mulched plots (Heimbach et al. 1997), which may reduce aphid numbers additionally. Consequently, the way of the cultivation of a crop (use of mulch, avoidance of inhomogeneity of the crop) seems to offer a good possibility to reduce the number of aphids settling and thus virus infection rates and should be considered for integrated and biological crop protection.

TuYV infection rates varied in relation to aphid number which is expected for a persistent virus. Higher infection rates in spring compared to autumn were found in two years, which were characterized by having a mild winter enabling long periods of activity for the aphids. Thus autumn samples often did not show final infection rates. No effect of virus infection rates on yield was found for the two years for which yield is available. Other pest insects did not occur in such numbers that they could have influenced yield in the two year (Heimbach unpublished). Thus it seems that yield reduction as described by Graichen & Schliephake (1996) occurs only in certain circumstances.

In 2000 the plot size had an important influence on the virus infection values obtained. Small plot experiments do not always seem to be able to represent realistic efficacy data for pesticides on virus transmission, because neighbouring effects of other plots with no or little efficacy have to be expected. The importance of the plot size will increase with the period of activity of the pest organisms and the period until the final measurements on virus infection are taken. Therefore field experiments have to be planned very carefully in regard to sufficient plot size and neighbouring effects.

Acknowledgements

We would like to thank K. Graichen (BAZ Aschersleben) for carrying out the ELISA for Turnip yellows virus in rape and Mrs. Busch, Mrs. Ionascu and Mr. Heinke and other colleagues of the BBA for aphid sampling from plants and sticky nets.

References

Eggers, C. & Heimbach, U. 2001: Reduzierung des Blattlausbefalls durch Mulchsaatverfahren in Ackerbohne. *Mitt. dtsch. Ges. allg. Entomologie* 13: 253-256.

Finch, S. & Edmonds, G.H. 1994: Undersowing cabbage crops with clover - the effects on pest insects, ground beetles and crop yield. *IOBC/WPRS Bull.* 17(8): 159-167.

Graichen, K. & Schliephake, E. 1996: Auftreten, Symptome und Vektoren des Wasserrübenvergilbungsvirus (Syn. Westliches Rübenvergilbungsvirus) am Winterraps. *Nachrichtenbl. Deut. Pflanzenschutzd.* 48: 186-191.

Heimbach, U., Knolle, B., Sokolowski, A. & Garbe, V. 1997: Einfluß von Direktsaat-/Mulchverfahren auf räuberische Arthropoden in und auf dem Boden. *Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin Dahlem* (232): 145-154.

Heimbach, U., Eggers, C. & Thieme, T. 2001 (in press): Effect of mulch on settlement and population of aphids in some arable crops. *Proceedings of the 6th International Symposium on Aphids, September 2001*.

Jones, R.A.C. 1994: Effect of mulching with cereal straw and row spacing on spread of bean yellow mosaic potyvirus into narrowleafed lupins (*Lupinus angustifolius*). *Ann. Appl. Biol.* 124: 45-58.

Kendall, DA., Chinn, N.E., Smith, B.D., Tidboald, C., Winstone, L. & Western, N.M. 1991: Effects of straw disposal and tillage on spread of barley yellow dwarf virus in winter barley. *Ann. appl. Biol*. 119: 359-364.

Leicht, W. 1996: Imidacloprid – ein Chloronicotinyl-Insektizid: Biologische Aktivität und landwirtschaftliche Bedeutung. *Pflanzenschutz-Nachrichten Bayer* 49: 71-86.

Moericke, V. 1950: Über das Farbensehen der Pfirsichblattlaus (*Myzodes persicae* SULZ.). *Z. Tierpsych.* 7: 265-274.

Moericke, V. 1952: Farben als Landereize für geflügelte Blattläuse (Aphidoidea). *Z. Naturforsch.* 7b: 304-309.

Müller, H.J. 1957: Über die Entwicklung erhöhten Randbefalls von Ackerbohnen-Beständen durch *Aphis fabae* Scop. *Z. für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz* 64: 7/10, 593-599.

Müller, H.J. 1964: Über die Anflugdichte von Aphiden auf farbige Salatpflanzen. *Ent. exp. & appl.* 7: 85-104.

Neitzel, K. & Müller, H.J. 1959: Erhöhter Virusbefall in den Randreihen von Kartoffelbeständen als Folge des Flugverhaltens der Vektoren. *Ent. exp. & appl.* 2: 27-37.

Nottingham, S.F. & Hardie, J. 1993: Flight behaviour of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and non-host plant odour. *Physiol. Entomol.* 18: 389-394.

Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. 1991: Behavioural and electrophysiological response of aphids to host and non-host plant volatiles. *Journal of Chemical Ecology* 17: 1231-1242.

Nozon, S. 1990: Untersuchungen zur Konkurrenz von *Aphis fabae* Scopoli und *Acyrthosiphon pisum* (Harris) und zur Beeinflußung ihrer Wirtspflanze *Vicia faba* L. (Homoptera: Aphididae). *Diplomarbeit Universität Rostock*, 36 pp.

Perkow, W. & Ploss, H. 1999: Isofenphos. In: *Wirksubstanzen der Pflanzenschutz- und Schädlingsbekämpfungsmittel.* Parey Buchverlag, 3. Ergänzungslieferung zur 3. Auflage.

Schliephake, E., Graichen, K. & Rabenstein, F. 2000: Investigations on the vector transmission of the Beet mild yellowing virus (BMYV) and the Turnip yellows virus (TuYV). *Journal of Plant Diseases and Protection* 107: 81-87.

Smith, J.G. 1976: Influence of crop background on aphids and other phytophagous insects on Brussels sprouts. *Ann. Appl. Biol.* 83: 1-13.

Wyman, J.R., Toscano, N.C., Kido, K., Johnson, H. & Mayberry, K.S. 1979: Effects of mulching on the spread of aphid-transmitted watermelon mosaic virus to summer splash. *J. Econ. Entomol.* 72: 139-143.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 133 - 139

Strategies for the control of cabbage stem flea beetle on winter rape in Sweden

Christer Nilsson
SLU, Department of Crop Science, P.O. Box 44, SE-230 53 Alnarp, Sweden

Abstract. Cabbage stem flea beetle (CSFB) is a winter active pest of oilseed rape distributed over most regions of northern Europe with a maritime climate. In Sweden, it is confined to the coastal areas of the southern part. It can be controlled with seed treatments against adults or sprays against adults and/or larvae. Control thresholds have been established and prognoses have been used for the past 30 years. The different strategies and their effects on economic damage and the population dynamics of CSFB are discussed.

Key words: chemical control, cabbage stem flea beetle, winter rape, seed dressing, spraying, prognoses

Introduction

Cabbage stem flea beetle (CSFB) is a winter active pest of oilseed rape distributed over most regions of northern Europe with a maritime climate. In Sweden, it is usually confined to the coastal areas of the southern part, but with several consecutive mild winters it can extend over the whole of the winter rape growing area. After a short summer rest, the beetles migrate into fields of newly emerged rape plants. The beetles eat the rape leaves, and after a couple of weeks, ovary maturation is completed and oviposition starts. Larvae hatch in the autumn and mine leaf petioles during winter and will also often move into the stems the following spring. With many larvae per stem, plants can show wilting symptoms and flowering can be retarded or decreased. If winter temperatures are relatively high, most larvae and beetles will survive and oviposition can continue during warmer periods in winter and during spring. There are three larval stages and pupation takes place during late spring. New adults appear in the middle of the summer. The larvae are considered responsible for most of the economic losses, through the tunnelling of petioles and stems and due to Phoma infecting these tunnels. Yield losses can also come from a lower plant stand produced by adult leaf feeding, often already before plant emerge. The control threshold for seed dressing has been shown to be as low as 1 larva/plant, but this varies with the year (Nilsson 1990). In this paper different control strategies are described and their effect on economic losses and population dynamics of the CSFB be discussed.

Material and methods

Chemical control

CSFB numbers fluctuate from year to year and field trials are only worthwhile in outbreak years when numbers are higher. In this paper, trials from 3 outbreak periods (1982/84, 1991/93 and 2000) are reported.

Chemical control trials reported here are all field trials with a randomised block design, usually with 4 replicates, and located in commercial crops. Except for the treatments, the trials were managed using standard field practice. Larval density was assessed by taking 10

plants at random from each plot in early spring when the plants just had resumed growth. Plants were dissected under the microscope by cutting all petioles and stems in mm-thick slices. Plant number was determined on 2 m row in each plot. The same rows were examined both in the autumn and spring. Damaged leaf area was estimated on 25 plants per plot on several occasions during early plant development.

Normally 25 sqm. of the plots were harvested with a trial combine and seed samples analysed for cleanness, water, chlorophyll and crude fat content.

Analysis of isofenphos content of emerging plant leaves was done by a commercial laboratory. Seed pelleted with isofenphos by two companies using different methods, was sown in a glasshouse and plants harvested weekly after emergence. Growth stages of harvested plants were recorded simultaneously.

Prognoses

Each year, farmers were asked to take 50 plants at random, walking diagonally across their fields. The number of fields sampled varied from about 40 to 110 per year. The farmers were also questioned about their control methods and other data. Plant samples were sent to a central laboratory where they were dissected to estimate larval density. These data are not directly comparable with those from field trials, which were more accurate and indicated higher larval numbers. Because of large number of samples in a prognosis study it was not possible to dissect plants under a microscope.

Strategies for control

Seed treatment

Seed treatments with insecticides, often in combination with fungicides, have been used for more than 25 years during outbreak periods. Until about 1980 the treatment was applied in the form of a dry powder formulation. However, a substantial part of the powder was lost in the seed drill and during packing and distribution, and the real dose was much lower than assumed, sometimes too low to give a god protection of the seedlings. Moreover a few farmers were poisoned when calibrating their seed drills, and consequently powder formulations were banned and the insecticide was applied in coatings (pelleting). Gamma BHC (lindane) was used during the first part of this period, but was later exchanged for isofenphos (Oftanol) and during the late 1990s, carbosulfan (Marshal MUP, Promet) was also used. Other compounds, like imidacloprid, were tested in field trials and shown to be as effective as the standards, but were not introduced commercially.

Coating was slightly more effective than the powder formulation (Table 1) both in terms of yield increase and reduction of the number of larvae per plant. However, seed treatment, irrespective of application method, only reduced the final larval population by about 50% (Table 1, 3 and 4).

Table 1. Seed dressing against adults, 15 trials 1982-84 (Oftanol T = isofenphos, 400 g/kg + thiram 100 g/kg).

		g/kg seed	larvae per plant	rel.	crude fat kg/hectare	rel.
Untreated			2.5 a	100	1020 a	100
Oftanol T	Dry powder	40	1.6 b	64	1090 b	107
Oftanol T	pelleting	40	1.3 b	52	1120 b	110

In a glasshouse experiment, the insecticide concentration in cotyledons and leaves of winter rape grown from seed coated with isofenphose was measured (figure 1.). The concentration decreased rapidly when the first true leaf emerged. Seed dressing will kill those beetles that arrive first to the crop and its main effect is to secure an adequate plant stand, which is gaining increasing importance as winter rape all over Europe is sown at lower and lower seeding rates. However, plant losses from early attacks of CSFB is not very usual and in the trials of 1991-1993, an increase in plant stand from seed dressing was observed in only 2 of 11 trials.

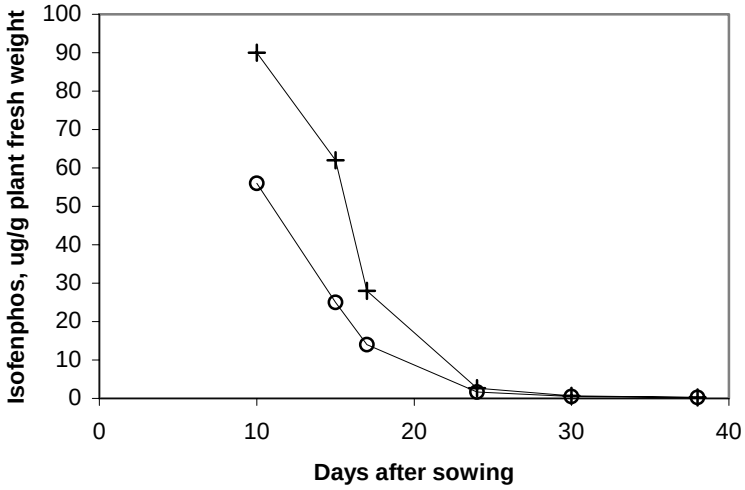


Fig. 1. Isofenphos in cotyledons and leaves after emergence (µg a. i. /plant) in a greenhouse trial. Seed coated in two different ways with 40 g/kg Oftanol T.

Insecticide sprays against adults

During September farmers are very busy harvesting cereals and preparing their fields for the sowing of winter cereals. Spray applications are difficult to fit into this work period and the often rainy weather also results in heavy wheeling damage. OP compounds were not effective for more than a few days, and had to be sprayed repeatedly to have an effect better than seed dressing. The introduction of pyrethroids in the 1980s offered the possibility to spray during early September, as soon as the plant stand is established, as pyrethroids, at least in higher doses, have a marked long-term effect. Conditions for spraying at this time are generally quite favourable compared to later in autumn and the herbicide treatment can be delayed to coincide with the first part of the optimal period for CSFB control. This control method has been tested in field trials during recent population peaks (Table 2, 3 and 4).

The different pyrethroids tested were equally effective at appropriate doses. Early treatments were, in general, more effective than later ones in terms of yield, leaf damage and reduction of the larval population. Differences between cotyledon, 2 and 4 leaf stage treatments are however small. In some years there can be a late migration of beetles when the plants are in the 2 leaf stage or sometimes even later. This is probably a result of soil preparation for winter wheat, which usually follows rape, and the destruction of volunteer rape plants on the former winter rape fields. It is consequently safer to spray at the 2 leaf stage instead of at cotyledon stage.

Table 2. Sprays against adult beetles, 3 + 3 trials 1983-84

		l or kg/ha	larvae per plant	rel.	crude fat, kg/hectare	rel.
Untreated			9.4 a	100	910 a	100
Decis	2 - 4 leaves	0,4	1.2 b	13	1120 b	126
Untreated			2.1 a	100	790 a	100
Decis	2 - 4 leaves	0,3	0.5 b	24	950 b	121
Cymbush	1 month later	0,5	0.4 b	19	880 b	112

Table 3. Sprays and seed dressing against adults. 12 trials in 1991-1993 (Seed dressing: Oftanol T, 20 g/kg; Spray: Decis 25 EC, 0,3 l/ha and no seed dressing)

	plants per m^2, in autumn	% damaged leaf area	rel.	larvae per plant	rel.	crude fat, kg/hectare	rel.
Untreated	83 a	3.9 a	100	4.6 a	100	1270 a	100
Seed dressing	86 a	1.7 b d	42	3.1 a	67	1300a	102
Spray at cotyledons	85 a	0.1 b c	3	0.5 b	11	1360 a	107
Spray at 2 leaves	82 a	1.5 b d	38	0.4 b	9	1340 a	106
Spray at 4 leaves	84 a	1.8 b d	45	1.0 b	21	1340 a	106

Spraying with pyrethroids will decrease the larval population with 80-90%. decrease leaf damage considerably, at least as much as seed dressing and increase yields substantially some years. Moreover, winter survival is a important factor. There is a good relationship between larval population density and the reduction in plant stand during winter (Figure 2).

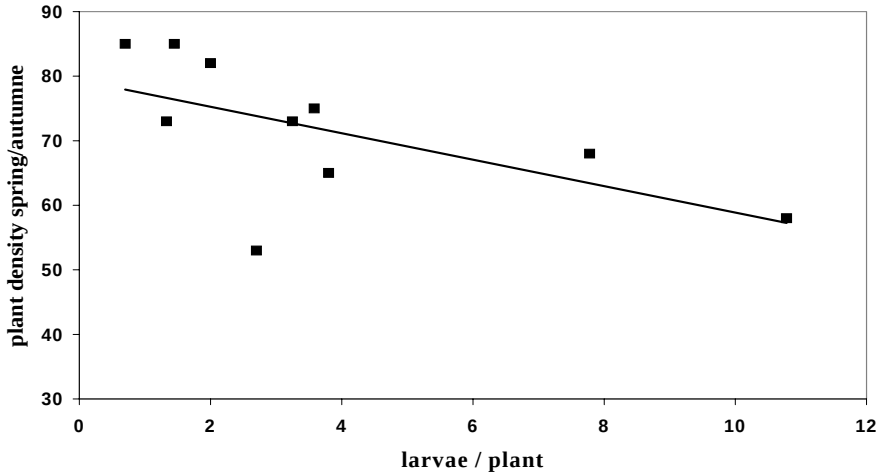


Fig. 2. Plant winter survival. Data from 1991-1993

Trials with sprayed and unsprayed plots also show that beetles, once they have settled, are less mobile than has been assumed. There is almost no increase in the larval populations in plots close to untreated plots, even at high beetle densities.

Insecticide sprays against larva

Spraying against CSFB larva has only been tested in a few field trials in early spring, but not in the autumn. The conditions for spraying are not favourable late in the autumn and farmers are reluctant to accept late spray operations. Effects on larval populations were not found, neither from pyrothroids nor from a systemic compound like Dipterex (trichlorfon, 800 g/kg; 0,75 kg/ha).

Table 4. Average results from 5 trials in 2000.

	larvae per plant	rel.	crude fat kg/hectare	rel.
Untreated	3.2 a	100	1943 a	100
Seed dressing (Marshal, Promet, Chinock)	3.2 a	98	1913 a	98
Sprayed (alfa fenvalerate, 0,5 l/hectare)	0.4 b	13	1948 a	100

Control thresholds

Yields in winter rape vary considerably, mainly due to winter conditions. If yields are plotted against larval population densities no pattern is usually detectable. Data emanate, however, often from untreated plots and plots treated to control CSFB, which means that the data points can be treated pairwise. As all data intervals don't cover the whole range it is not possible to relate all yields to each other. Instead data points have been connected and the mean coefficient of elevation has been calculated for all lines passing a certain interval of larval density. In this way, a series of coefficients can be used to construct a yield-loss-larval density curve starting at the mean yield for plots without attack or with very low abundance of larva. From this relationship, the yield loss equivalent to the treatment cost can be estimated, e. g. the control threshold of spraying or seed dressing.

Table 5. Yield losses, kg crude fat / larva and hectare. Cost of a seed dressing and one spraying with pyrethroid correspond to about 20-40 and 90 kg seed/ha respectively.

1982	35
1983	23
1984	114
1991-1993	174

This has been done for single years or a couple of years depending on the volume of data available (Table 5). The variation between years in yield loss is great, greater than with most other pests. The reasons for this are not known in detail, but winter stress to plants also varies considerably, as does stand thinning and *Phoma* infections following CSFB attacks.

Prognoses

CSFB has been monitored in the southern part of Sweden for more than 30 years, which has revealed a very cyclic abundance pattern with peaks about every 7th year (Figure 3). From the northern part of Germany, Erichsen (1993) has published comparable data from Schwerin for

a ten year period. It is interesting to note that the same pattern of abundance appears to exist in both regions. Godan (1947) showed the larval abundance during the years 1936 to 1947 for northern Germany and also found a 7 year time span between two peaks. This seemingly regular abundance pattern can only be created by biological factors and should give a good opportunity for prediction and prognoses. The observed pattern seem to be little effected by seed dressing, but without control, peaks may well have been much higher. During the last outbreak that begun in 2000, farmers sprayed to a much higher extent than previously, sometimes in combination with seed dressing, especially with hybrid varieties. This change in control technique may alter the abundance pattern in the future.

In some locations beetles caught in yellow water traps and the leaf damage produced during the first month, were measured. There is no apparent correlation between these two factors and the larval population density the following spring.

In Sweden, seed producers also have the facilities for seed dressing. Prognoses based on larval counts in spring and on population abundance pattern have made it possible to convince farmers, advisers, seed retailers and seed producers to refrain from seed dressing in years when less than approximately 1 larva per 2 plants were found (Figure 3). Practically no treated seed has been sown in these periods as no treated seed has been available, unless farmers made a special order well before delivery.

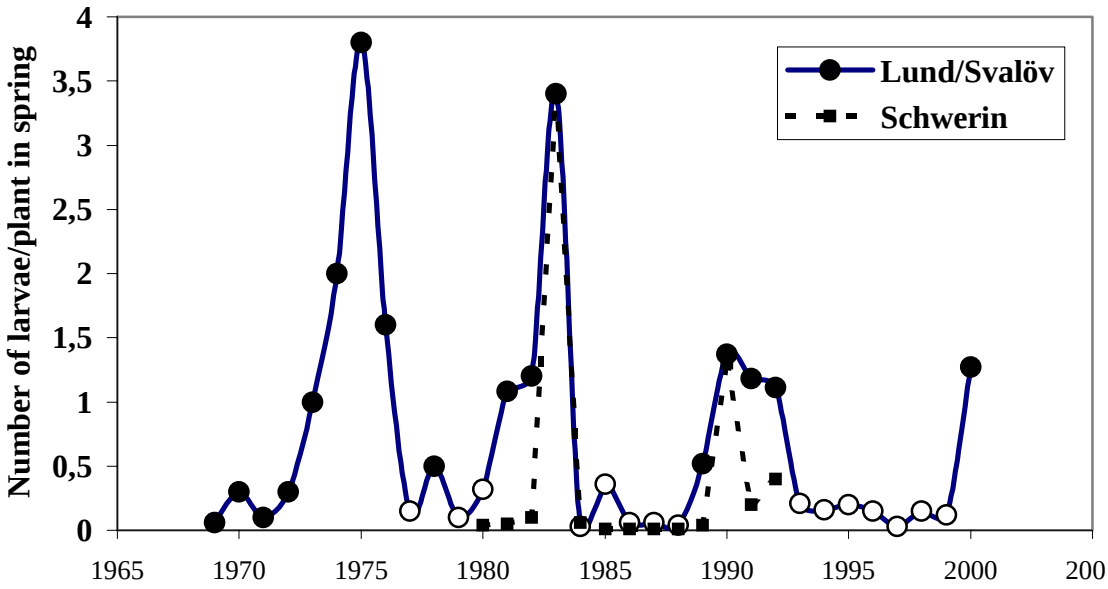


Fig. 3. CSFB larval abundance in southern Sweden and in Schwerin, Germany. Open circles denote years when no seed dressing have been used in the region (after Berg 2001 and Erichsen 1993).

Discussion

Routine use of insecticides is obviously not to be recommended and a strategy is needed for what control to use and when. Such a strategy can only be operational if population densities are monitored. Monitoring larval populations seems to be far more practical than counting adults in yellow traps in the autumn, when farmers also are occupied with harvesting and soil

preparation for next year's crops. Monitoring larval populations in the spring can be done over at least a month. However, some samples have to be taken quite late, to see if any females have survived winter and produced a second batch of larvae.

About 0.3-1 larva per plant is equivalent to a seed dressing. The increasing use of hybrid varieties and precision seeding during recent years have increased the importance of plant losses due to adult damage and it is probably that in future all seed every year will be treated with a combination of an insecticide and a fungicide. New field trials are needed to show if this is justified.

In Sweden, seed dressing has, up to now, been used at larval densities between 0.5 and 1 larva per plant and spraying at densities higher than 1 larva. It could be argued that spraying is relatively cheap and very effective and could be used already at lower larval densities. However, spraying has a great impact on the general field fauna including those parasitoids and predators that spend the winter in the field. Spraying should be restricted to situations where an economic benefit is clearly evident. One major obstacle to this strategy is the great variation in yield response between years. In some years even a very low population density seems to justify control. A better understanding of the mechanisms behind yield losses and the factors that produce them would greatly enhance our ability to restrict chemical control to ensure a yield benefit.

One way of avoiding some of the environmental effects is to use band spraying and combine herbicide and insecticide in a crop sown to large row width. Mechanical weeding can be used in between rows. This restricts impact of the chemical control to half of the acreage or less. We have tested this method for a couple of years and it works fairly well, even if the weed control is not complete.

The apparent cyclic pattern of the CSFB populations could merit an investigation on its dynamics. Until now it has not been affected very much by the control measures used, but this could be altered substantially if spraying is practised more frequently.

Acknowledgements

I wish to thank B. Åhman and B. Christensson for skilful technical assistance with laboratory and field work, Prof. Ingrid Williams for reviewing the manuscript and the Swedish Oilseed Growers' Association for permission to use data.

References

Berg, G. 2001. Rapsjordloppa – fortsatt starka angrepp. *Svensk Frötidning* 70: 14-15.

Erichsen, E. 1993. Auftreten und Ûberwachung von Rapsschädlingen in Mecklenburg Vorpommern. *Gesunde Pflanzen* 45: 12-15

Godan, D. 1947. Der Einfluss der Witterung auf den Massenwechsel des Rapserdflohs (*Psylliodes chrysocephala* L.). *Nachrichtenblatt Dtsch Pflzschutzd*. NF 1 : 101-104

Nilsson, C. 1990. Yield losses in winter rape caused by cabbage stem flea beetle larvae (*Psylliodes chrysocephala* (L.)). Bull IOBC/WPRS 13(4): 53-56.

Nilsson, C. 1988. Kemisk bekämpning av rapsjordloppa. (Insecticides against Cabbage Stem Flea Beetle) *Växtskyddsnotiser* 52(6): 153-157.

Supplement to the IOBC Meeting from May 31st to June 2nd, 1999 in Prague

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 25 (2) 2002
pp. 143 - 151

Within-field distributions of the seed weevil, *Ceutorhynchus assimilis* (Paykull) and its parasitoid, *Trichomalus perfectus* (Walker), on winter oilseed rape

Ingrid H. Williams[1], Archie K. Murchie[1,2], Andrew W. Ferguson[1], Zdzislaw Klukowski[1,3], Joe N. Perry[1], Barbara Walczak[1,3]

[1] *IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK*

[2] *present address: Department of Agriculture for Northern Ireland and Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, UK*

[3] *present address: Katedra Entomologii Rolniczej, Akademia Rolnicza, Ul. Cybulskiego 32, 50-205 Wroclaw, Poland*

Abstract: *Ceutorhynchus assimilis* (Paykull), the cabbage seed weevil, is a major pest of winter oilseed rape in Europe. It is attacked by *Trichomalus perfectus* (Walker), a pteromalid larval ectoparasitoid, and parasitism rates can exceed 70%. The spatio-temporal distributions of *C. assimilis* and of *T. perfectus* within a crop of winter rape were investigated over two years. Insects were sampled from the points of intersections of a grid on the crop, and their distributions were mapped. Spatial Analysis by Distance IndicEs (SADIE) and a randomisation procedure were used to describe and compare patterns of distribution across time and between species. During immigration, adult *C. assimilis* were aggregated at the edges of the crop, but later were more widespread. Adult *T. perfectus* migrated to the crop later than *C. assimilis* and were not aggregated at the crop edge except briefly during the early phase of immigration. Adult female and larval *C. assimilis* were spatially associated, as were densities of *C. assimilis* larvae and those parasitised by *T. perfectus.* The implications of the observed distributions of *C. assimilis* and *T. perfectus* for a) integrated pest management strategies for winter rape that seek to employ insecticides, targeted in time and space, together with parasitoids for biological control, b) accurate sampling for pest and parasitoid, and c) push-pull strategies incorporating semiochemicals, are discussed.

Key words: pest, parasitoid, *Ceutorhynchus assimilis*, *Trichomalus perfectus*, insecticide targeting

Introduction

Ceutorhynchus assimilis (Paykull) (syn. *obstrictus*), the cabbage seed weevil, is a major pest of oilseed rape in Europe. It lays its eggs in the pods and the larvae cause economic damage by eating developing seeds. The pteromalid wasp, *Trichomalus perfectus* (Walker), is an important and widespread ectoparasitoid that attacks *C. assimilis* larvae in the pods, often killing more than 70% (Alford *et al.*, 1995; Murchie & Williams, 1998). The effectiveness of a parasitoid as a biological control agent depends partly on good co-incidence, in both time and space, between the adult parasitoid and its host. Information about within-field temporal and spatial distribution of *T. perfectus* in relation to that of *C. assimilis* could lead to ways of enhancing the effectiveness of the parasitoid as a biocontrol agent in an integrated pest management (IPM) strategy for winter rape. Any dissociations between their temporal and spatial distributions could also provide opportunities for targeting insecticide against the pest without harming the parasitoid.

Both *C. assimilis* and *T. perfectus* migrate to winter rape in the spring from overwintering sites in perennial vegetation or litter in woodland and field boundaries. The pest migrates two to four weeks earlier than the parasitoid. A recently proposed IPM strategy for control of *C.*

assimilis populations on winter rape in the UK (Alford *et al.*, 1996), takes advantage of this temporal dissociation between the immigration flights of *C. assimilis* and *T. perfectus*. It recommends the temporal targeting of insecticide treatments during the main immigration flight of *C. assimilis* and before that of *T. perfectus*, thus aiming to kill the pest without harming the parasitoid. The strategy is based on work by Murchie *et al.*, (1997b), who showed that a pyrethroid insecticide targeted against adult *C. assimilis* and applied during flowering had little effect on parasitism rates, whereas an organophosphate insecticide, targeted against the larvae of *C. assimilis* and applied post-flowering, reduced parasitism rates substantially. In commercial crops in the UK, the recent decline in the use of post-flowering treatments appears to have resulted in substantially increased rates of parasitism of *C. assimilis* by *T. perfectus* (Alford *et al.*, 1996).

Precision targeting of insecticide treatments to those areas of crop where *C. assimilis* is most dense, could offer further potential for more effective pest control, conservation of *T. perfectus* and the reduction of insecticide; development of such a strategy, however, requires more knowledge of the spatial distribution of *C. assimilis* and of *T. perfectus* relative to its host. Previous studies of the distribution of *C. assimilis* on winter rape, sampling along transects and from discrete areas, have shown that, during immigration, crop edges are more heavily infested than crop centres (eg. Free & Williams, 1979) and this has led to the suggestion that application of insecticide to crop borders alone could effectively reduce *C. assimilis* numbers. However, there have been no previous studies of the spatial distribution of *T. perfectus* on winter rape.

In this paper, we report on two studies into the spatio-temporal distributions of *C. assimilis* and *T. perfectus* on winter rape, using sampling points for the insects arranged in a grid pattern within the crop. Pest and parasitoid distributions were mapped, and Spatial Analysis by Distance IndicEs (SADIE) and a randomisation procedure were used to describe and compare the patterns of distribution across time and between species. We discuss the implications of our findings for IPM strategies in winter rape that might incorporate spatial as well as temporal targeting of insecticide treatments and for the accurate sampling of the pest and its parasitoid on the crop.

Materials and methods

Insect sampling

Adult insects were sampled from late April to mid-July using traps positioned on selected intersections of a grid (10 m in 1992; 43.5 m in 1995) across crops of winter rape (1.1 ha in 1992; 6.6 ha in 1995), in Hertfordshire and Bedfordshire, UK, respectively. In 1992, water traps (n=23) were used to sample both adult *C. assimilis* and adult *T. perfectus.* In 1995, flight traps (n=36), baited with the host plant volatiles 2-propenyl isothiocyanate and 2-phenylethyl isothiocyanate (Murchie *et al*., 1997a), were used to sample adult *C. assimilis.* Traps were emptied weekly. In early July 1995, numbers of *C. assimilis* larvae and their parasitism by *T. perfectus* were assessed in a sample of 400 pods from each of 19 of the trap sites.

Mapping and spatial analyses

To visualise the spatial distributions of sampled *C. assimilis* and *T. perfectus*, counts were mapped using Unimap 2000 software (Uniras Ltd., Slough, UK). In 1992, frequency distributions of species were investigated by fitting Taylors' Power Law (Taylor, 1961) to sample variances and means derived from weekly trap counts. To determine the strength of edge effects, a randomisation test of permuted rearrangements of the counts was used (Perry, 1995; Murchie, 1996). In 1995, the spatial patterns were described using Spatial Analysis by Distance IndicEs (SADIE; Perry & Klukowski, 1997; Perry 1998a); this describes the spatial pattern of a single set of counts using three indices, I_a, and K_a, for which values greater than unity indicate aggregated

arrangements of the counts and J_a, for which values equal or less than unity indicate the presence of more than one cluster. Another index, I_m (Perry, 1998b), was used to compare two sets of counts; values greater than zero indicating positive spatial association between them.

Results

1992

Distribution of C. assimilis *adults*

Ceutorhynchus assimilis adults first invaded the crop in late April, reached maximum numbers in early/mid June and declined from early July, with few caught after mid-July; the numbers caught from 8 May to 10 July are given in Table 1. Regression of variance against mean indicated heterogeneity of catches (a = 0.420; b = 1.556). Numbers on the edge were greater than at the centre in early/mid-May, but less so during the second half of May when densities stabilised. In early June, there was a similar edge effect. This declined in late June and early July when densities became greatest in two longitudinal regions parallel with the northern and southern edges of the crop.

Table 1. Mean numbers of *C. assimilis* adults and *T. perfectus* females caught weekly in water traps in 1992; * indicates a significant (P < 0.05) edge effect.

Date trap emptied	Mean no. of *C. assimilis* per trap	Mean no. of *T. perfectus* per trap
8 May	19.5*	0.5
15 May	10.9*	2.3*
22 May	12.2	0.9
29 May	15.0	0.6
5 June	13.3*	3.3
12 June	50.1	5.1
19 June	36.7	4.5
26 June	37.4	9.0
3 July	39.7	8.3
10 July	7.1	13.4

Distribution of T. perfectus *females*

Only female *T. perfectus* were identified from trap samples because of the difficulty in identifying Pteromalid males. The first females were caught during early May; numbers remained small until early June and then increased steadily until mid-July (Table 1). Regression of variance against mean indicated strong heterogeneity of catches (a = 0.229, b = 1.824). There was a marked edge effect in mid-May only and thereafter more were caught from the centre than from the edge of the crop.

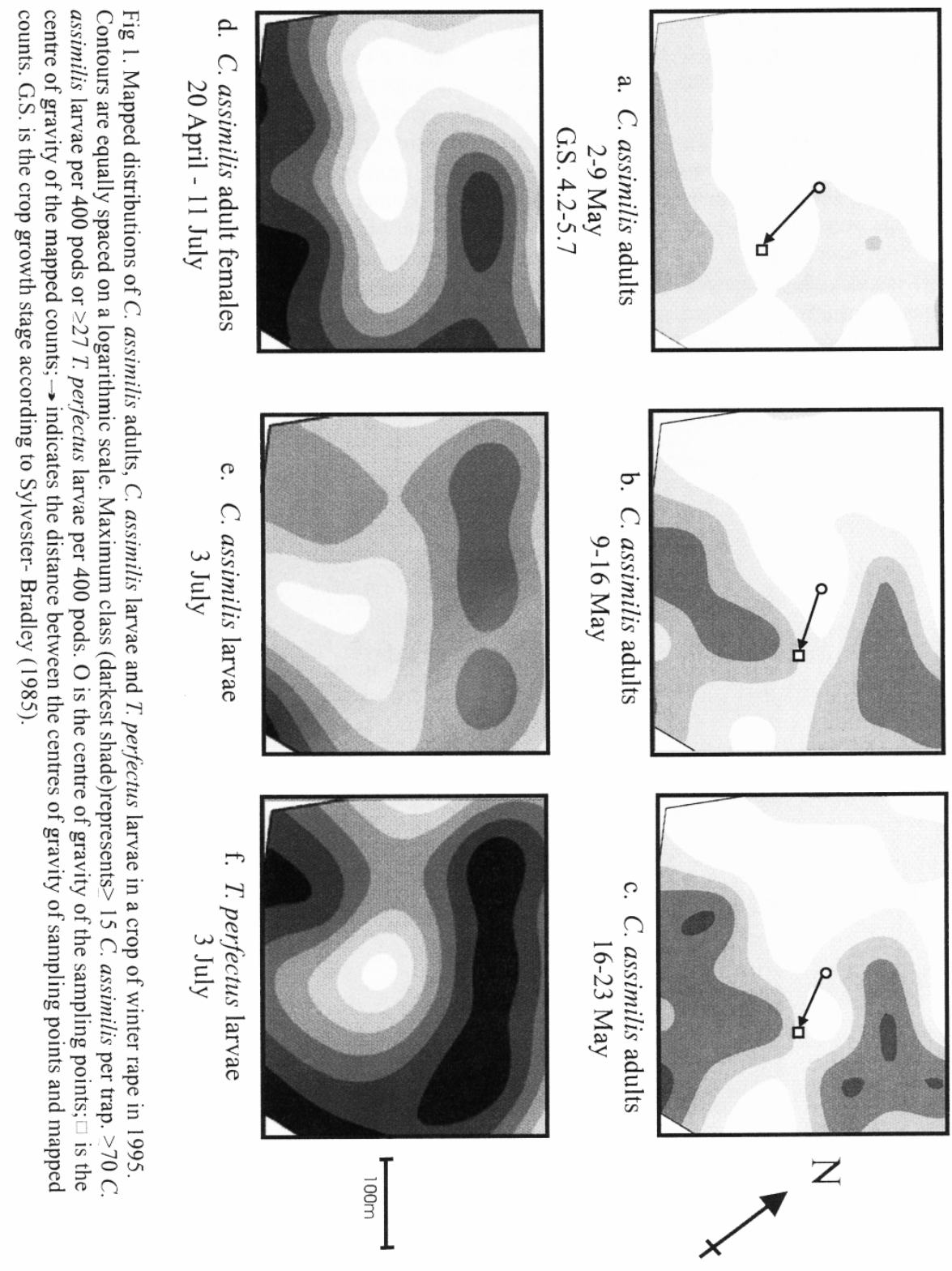


Fig 1. Mapped distributions of *C. assimilis* adults, *C. assimilis* larvae and *T. perfectus* larvae in a crop of winter rape in 1995. Contours are equally spaced on a logarithmic scale. Maximum class (darkest shade)represents> 15 *C. assimilis* per trap, >70 *C. assimilis* larvae per 400 pods or ≥27 *T. perfectus* larvae per 400 pods. O is the centre of gravity of the sampling points; □ is the centre of gravity of the mapped counts; → indicates the distance between the centres of gravity of sampling points and mapped counts. G.S. is the crop growth stage according to Sylvester- Bradley (1985).

Comparison of distributions of* C. assimilis *adults and* T. perfectus *females

The numbers of *T. perfectus* caught on most occasions were negatively correlated with those of *C. assimilis* except during mid-May and mid-July. They were also negatively correlated with those of *C. assimilis* females three weeks earlier, when the host larvae attacked by *T. perfectus* would have been at the egg stage (*T. perfectus* on 3 July *v. C. assimilis* on 12 June $r = -0.4855$, $P = 0.02$).

1995

Distribution of* C. assimilis *adults

Mapped counts of *C. assimilis* adults suggested two main phases of crop colonisation, namely crop invasion followed by population decline. Invasion began at the south-east and south-west field boundaries during 20-25 April. During the following four weeks, it spread from these areas to other parts of the crop, the two foci almost merging to give a single cluster covering most of the south and, less densely, parts of the north of the crop at the peak of colonisation during 16-23 May (Fig 1a,b,c). Thereafter numbers caught from all parts of the crop declined, with those parts most heavily infested being the last to retain a population. SADIE analyses of these distributions (Table 2) indicated that they were strongly and significantly aggregated on all dates (1_a >1). The invasion on two fronts was confirmed by the index J_a , which was not significantly greater than unity (Perry, 1998) except near the peak of abundance (9-16 May) and at the end of colonisation (13-20 June). There was also a noticeable smaller scale pattern of aggregation within the south of the field where most *C. assimilis* occurred, as shown by values of index K_a which were substantially greater than unity especially during May (Perry & Klukowski, 1997).

Table 2. Analyses of the spatio-temporal distribution of *C. assimilis* adults caught weekly in flight traps in 1995. * indicates a significant (P < 0.05) degree of aggregation of counts (I_a, K_a) or the presence of a single cluster (J_a).

Date trap emptied	Mean no. per trap	SADIE index		
		I_a	J_a	K_a
25 April	0.44	1.62*	1.22	0.99
2 May	0.88	1.49*	0.91	1.03
9 May	1.06	1.94*	1.08	1.08*
16 May	3.50	1.71*	1.14*	1.03
23 May	4.92	1.63*	1.09	1.05
30 May	2.92	1.57*	1.02	1.09*
6 June	1.31	1.40*	1.06	1.01
13 June	0.19	1.52*	1.47	1.03
20 June	0.14	1.51*	3.05*	1.00

Comparison of distributions of* C. assimilis *adults,* C. assimilis *larvae and* T. perfectus *larvae

The distributions of the cumulative totals of *C. assimilis* adults (Fig. 1d) and of their larvae (Fig. 1e) showed some inconsistencies especially in the northern quarter of the crop where traps caught few adults but plants contained many larvae. Although the correlation coefficient between the numbers of adult female and of larval *C. assimilis* was only 0.30, they were spatially associated (I_m = 3.48, P = 0.003). The distributions of both healthy *C. assimilis* larvae (Fig. 1e) and of parasitised *C. assimilis* larvae (Fig. 1f) appeared to be aggregated into regions *c*. 0-80m from crop edges and their densities were strongly associated (I_m = 4.82, P< 0.003*)*. The mean percentage parasitism was 57% and this did not vary with host density.

Discussion and conclusions

Grid sampling, mapping of the distributions over time and novel analyses of spatial distributions, have revealed for the first time, the complexity of the pattern of crop colonisation by *C. assimilis*, with invasion on multiple fronts, significant aggregation throughout colonisation, on different scales, and a simultaneous decline in infestation from all areas of the crop towards the end of

flowering. The pattern of colonisation undoubtedly reflects the interplay of environmental factors, such as a suitable temperature for flight, the location of overwintering sites and windbreaks relative to the position of the crop and the direction of the wind, and the behavioural responses of the pest, particularly those involved in crop location and host plant selection. Greater understanding of the ways in which these factors determine the distribution patterns of *C. assimilis* could lead to the prediction of areas of a crop most at risk of infestation.

Although the distributions of adult female and of larval *C. assimilis* were spatially associated, they were not coincident in all parts of the crop, possibly because flight traps sampled flying rather than ovipositing females. Despite negative correlations between the numbers of *C. assimilis* adults and *T. perfectus* females caught, there was a close spatial association between healthy and parasitised larvae, with no areas where larvae were not attacked. The latter indicates that presence of the host was the main factor limiting the distribution of the parasitoid; any disparity would have indicated that other factors restricted parasitoid distribution. The uniform and large proportion of larvae parasitised, over the crop area occupied by the host, shows that *T. perfectus* was effective both in its dispersal over the crop and in host location.

The spatio-temporal distributions of *C. assimilis* and *T. perfectus* have implications for IPM strategies that aim to control the pest below damaging levels with the minimum use of insecticide, while conserving the parasitoid. In the UK, most crops of winter rape are treated with insecticides, applied to the entire area of the crop, to kill *C. assimilis*, either prophylactically or when the threshold number of two per plant during flowering is reached (Alford *et al*., 1991; Walters & Lane, 1994). The aggregated nature of the distribution of *C. assimilis* adults, in the two crops studied, suggests a potential for targeting insecticide treatment only to those crop areas where pests are densest, thereby maximising control of the pest while minimising pesticide use. The recommended time for the application of a pyrethroid to kill adult *C. assimilis* on winter rape (Whitehead, 1998) is during flowering, between 20 pod set and 80% petal fall on the main raceme (Sylvester-Bradley, 1985: Growth Stage 4.7-5.8); this timing avoids the main immigration flight of *T. perfectus* (Alford *et al.*, 1996). In this study, both crops were between these stages during early to mid May. At this time, *C. assimilis* adults infested only part of the crop area and their density varied considerably within the infested areas (Fig. 1a,b,c). However, on both crops, edge effects were strong, suggesting that application of insecticide to crop borders alone, during early flowering, could be an effective way of spatially targeting the densest part of the population of *C. assimilis*. If insecticide had been applied to the whole crop during this time much would have missed its target pest. Furthermore, the 1992 experiment showed that, although both species displayed an edge effect at the same time, *T. perfectus* did so early during its immigration only, when its density was low, so that application of insecticide at the recommended time, and to the crop borders only, would have caused it little harm. The close spatial associations between the distributions of *C. assimilis* larvae and those parasitised by *T. perfectus*, further confirm that application of insecticide post-flowering would be as likely to kill the larvae of the parasitoid as those of the pest.

To assess the value of this approach further, more work is needed to quantify the edge distributions of pest and parasitoid, particularly their width, on a range of crops of different sizes and infestation levels. Any IPM strategy that incorporates spatial as well as temporal targeting of insecticide to kill *C. assimilis* should be compatible with the need to conserve the parasitoids of other pests of winter rape that might be active at the time of insecticide application eg. those of *Meligethes* spp.. It must also take into account the proposed introduction of unsprayed buffer zones at crop borders, designed to avoid spray drift to field margins which can provide reservoirs for beneficial insects.

The aggregated nature of the distribution of *C. assimilis* adults in the crop, has implications for the accurate sampling of this pest to estimate populations for monitoring and to provide a

basis for decisions for insecticide application. Sampling only 20 plants, along a single transect into the crop, as currently recommended, could lead to severe inaccuracies and contribute to the reported unreliability of the method (Walters & Lane, 1994). This study supports the acknowledged need for the improvement of population assessment methods for *C. assimilis* on oilseed rape and the need for more research into and information on its spatial distribution.

Advances in our knowledge of the environmental factors and behavioural responses determining the spatio-temporal distributions of *C. assimilis* and *T. perfectus*, may lead to the development of more sophisticated integrated pest management strategies for oilseed rape, for example, push-pull or stimulo-deterrent diversion strategies. These would incorporate not only spatially targeted insecticides, but also spatially targeted semiochemicals; for example, the combined use of pest resistant cultivars (Bartlet *et al.* 1999), trap crops, host plant volatiles (Bartlet *et al.*, 1993; 1997) or pheromones, such as the oviposition-deterring pheromone of *C. assimilis* (Ferguson & Williams, 1993; Ferguson *et al.*, 1999a & b) to manipulate the movement and distribution of pest and parasitoid on the crop. Little is known about how *T. perfectus* locates the oilseed rape crop. It has not been caught in traps baited with rape plant volatiles, such as isothiocyanates, which attract other parasitoids of rape pests (Murchie *et al.*, 1997a) but has been reported to use the frass produced by the last instar of *C. assimilis* to locate its host within the crop (Dmoch & Rutkowska-Ostrowska, 1978). More information about its responses to host plant and host semiochemicals could lead to ways of manipulating its distribution and behaviour on the crop.

Acknowledgements

This study was supported financially by the Ministry of Agriculture, Fisheries and Food (Arable Crops and Horticulture), by the Home-Grown Cereals Association (Oilseeds) and the Ministry of Agriculture, Fisheries and Food LINK Programme: Technologies for Sustainable Farming Systems. IACR - Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council. Fellowships granted to Z. Klukowski by Rothamsted International and to Z. Klukowski and B. Walczak by the British Council enabled them to work at IACR-Rothamsted on this research. We thank Suzanne Clark and Moira Mugglestone for discussion during the course of this study.

References

Alford, D.V., Cooper, D.A. & Williams, I.H. 1991: Insect Pests of Oilseed Rape. *HGCA Oilseeds Research Review no. 0S1. Home-grown Cereals Authority, Research and Development*: 130 pp.

Alford, D.V., Williams, I.H., Murchie, A.K. & Walters, K.F.A. 1995: The status and potential of the parasitoids of the seed weevil and pod midge on winter oilseed rape. *HGCA Oilseeds Research Project no. OS 14 (Project no. OS15/1/91), August 1995*: 89pp.

Alford, D.V., Walters, K.F.A. , Williams, I.H. & Murchie, A.K. 1996: A commercially viable low-cost strategy for the management of seed weevil populations on winter oilseed rape in the UK. *Proceedings of the British Crop Protection Conference, Pests & Diseases, Brighton pp. 609-614.*

Bartlet, E., Blight, M.M. Hick, A.J. & Williams, I.H. 1993: The responses of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) to the odour of oilseed rape (*Brassica napus*) and to some volatile isothiocyanates. *Entomologia experimentalis et applicata* 68: 295-302.

Bartlet, E., Blight, M.M., Lane, P. & Williams, I.H. 1997: The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from oilseed rape in a linear track olfactometer. *Entomologia Experimentalis et Applicata* 85: 257-262.

Bartlet, E., Williams, I.H. & Pickett, J.A. 1999: The ideal glucosinolate profile for pest resistance in oilseed rape. *IOBC/WPRS Bulletin (Breeding for Resistance to Insects and Mites)* 22(10): 13-17.

Dmoch, J. & Rutkowska-Ostrowska, Z. 1978: Host-finding and host-acceptance mechanism in *Trichomalus perfectus* Walker (Hymenoptera, Pteromalidae). *Bulletin de l'Academie Polonaise des Sciences* 26: 317-323.

Ferguson, A.W. & Williams, I.H. 1993: Studies of the oviposition deterring pheromone of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.). Behavioural bioassays and oviposition by weevils wintered in the laboratory. *IOBC/WPRS Bulletin* (*Integrated Control in Oilseed Crops)* 16(9): 193-201.

Free, J.B. & Williams, I.H. 1979: The distribution of insect pests on crops of oilseed rape (*Brassica napus* L.) and the damage they cause. *Journal of agricultural Science, Cambridge* 93: 139-149.

Ferguson, A.W., Solinas, M., Ziesmann, J., Isidoro, N., Williams, I.H., Scubla, P. & Mudd, A. 1999a: Identification of the gland that secretes oviposition-deterring pheromone in the cabbage seed weevil, *Ceutorhynchus assimilis* Payk. (Coleoptera: Curculionidae). *Journal of Insect Physiology* (in press).

Ferguson, A.W., Ziesmann, J., Blight, M.M., Williams, I.H., Wadhams, L.J., Clark., Woodcock, C.M., Mudd, A. 1999b: Perception of oviposition-deterring pheromone by the cabbage seed weevil (*Ceutorhynchus assimilis). Journal of Chemical Ecology* (in press).

Murchie, A.K. 1996: Parasitoids of cabbage seed weevil and brassica pod midge in oilseed rape. *Unpublished Ph.D. thesis. University of Keele, UK*.

Murchie, A.K., Smart, L.E. & Williams, I.H. 1997a: Responses of *Dasineura brassicae* and its parasitoids *Platygaster subuliformis* and *Omphale clypealis* to field traps baited with organic isothiocyanates. *Journal of Chemical Ecology* 23: 917-926.

Murchie, A.K. Williams, I.H. & Alford, D.V. 1997b: Effects of commercial insecticide treatments to winter oilseed rape on parasitism of *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae) by *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae). *Crop Protection* 16: 199-202.

Murchie, A.K. & Williams, I.H. 1998: A bibliography of the parasitoids of the cabbage seed weevil (*Ceutorhynchus assimilis* Paykull). *Bulletin IOBC/WPRS, Integrated Control in Oilseed Crops* 21(5): 177-181.

Perry, J.N. 1995: Spatial analysis by distance indices. *Journal of Animal Ecology* 64: 303-314.

Perry, J.N. 1998a: Measures of Spatial Pattern for Counts. *Ecology* 79(3): 1008-1017.

Perry, J.N. 1998b: Measures of spatial pattern and spatial association for insect counts. In: *Population and Community Ecology for Insect Management and Conservation.* (eds. J. Baumgartner, P. Brandmayr & B.F.J. Manly). Balkema, Rotterdam. *Proceedings of the Ecology and Population Dynamics Section of the 20th International Congress of Entomology, Florence, Italy, 25-31 August 1996.* ISBN 90 5410 9300.

Perry, J.N., Klukowski, Z. 1997: Spatial distributions of counts at the edges of sample areas. *Proceedings of the VI Conference of the Biometric Society, Cordoba 21-24 September 1997*: 103-108.

Sylvester-Bradley, R: 1985. Revision of a code for stages of development in oilseed rape (*Brassica napus* L.). *Aspects of Applied Biology* 10: 395-400.

Taylor, L.R. 1961: Aggregation, variance and the mean. *Nature* 189: 732-735.

Walters, K.F.A. & Lane, A. 1994: The development of an improved management strategy for cabbage seed weevil on oilseed rape. In: *Individuals, Populations and Patterns in Ecology*. Eds. S.R. Leather, N. Mills, A.D. Watt, K.F.A. Walters. Andover, UK: 187-197.

Whitehead, R. (ed.) 1999: *The UK Pesticide Guide*. CAB International and British Crop Protection Council, U.K., 683 pp.