IOBC / WPRS

Working Group „Integrated Control in Oilseed Crops“

OILB / SROP

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

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at / à

Prague (Czech Republic)

May 31 – June 2, 1999

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V.H. Paul, I. Föller, N. Evans & I. Williams

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Preface

The working group „Integrated control in oilseed crops“ held its 9th biannual meeting in the Czech Republic in Prague from May 31st to June 2nd 1999. We thank the local organiser Prof. Fabry, who was helped by colleagues of his faculty, especially Dr. Baranyk and Dr. Filipek for the very successful organisation.

A total of 25 participants from 6 different countries attended. On the first two days works and results were presented in lectures. This sessions included papers on:

- Monitoring Diseases – Biology of Pathogens
- Disease Resistance and Integrated Control of Diseases
- Monitoring Pest – Biology of Harmful and Beneficial Insects
- Biological and Integrated Control of Insect Pests
- Integrated Crop Protection

The third day was used for an Excursion to field trials of the Ceska Zemedelska Univerzita v Praze (Prof. Fabry) and a Field Seminar with people from the Czech advisory service for oilseed rape.

From our working group, sub-group entomology under the overall charge of Prof. Ingrid Williams, Dr. Wolfgang Büchs and Dr. David Alford, an EU-Proposal was worked out.

The next biannual meeting will be held in Germany in 2001 at the University of Paderborn, Department of Agriculture in Soest. The local organiser will be Prof. Paul.

Prof. V.H. Paul
Convenor

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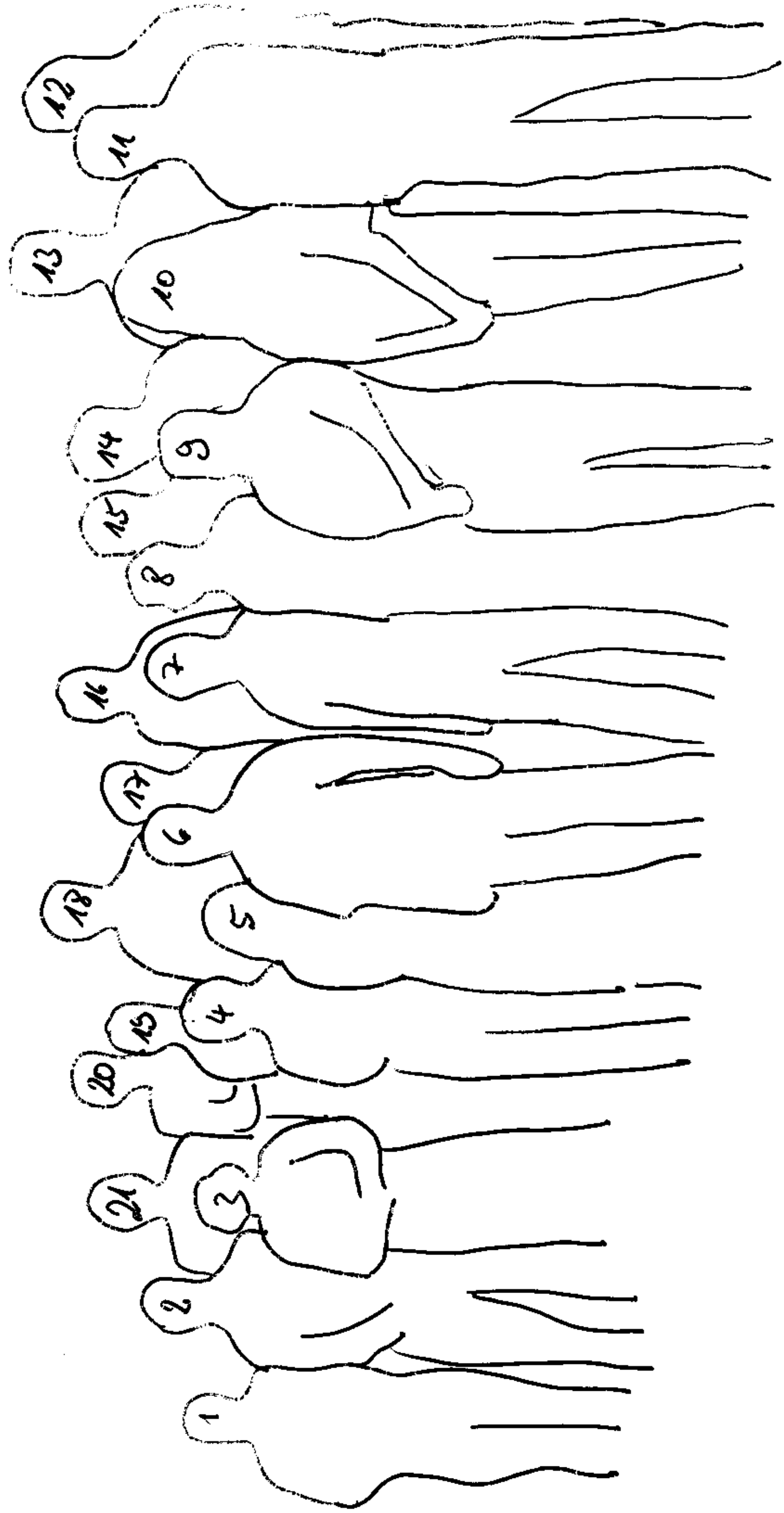

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Supplement to the IOBC Meeting from April, 10-12, 1997 in Poznan

Introduction

Integrated Control in Oilseed Crops
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Present Level of Rapeseed Production and the Development of the Crop in the Czech Republic

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Abstract: Since 1983, there have been significant increases in the development of oilseed rape as a crop in the Czech Republic. We expect ca. 350 000 ha harvest area in 1999, i.e. 11.3% share of total area of the whole republic. In some regions, where rapeseed production has increased dramatically, new diseases and pests problems have begun to arise. The ratio of domestic processing and export remaining more or less 1:1. Currently, no transgenic rapeseed is grown commercially in the Czech Republic.

Key words: oilseed rape, Czech Republic, pests, diseases, production, processing

The main oilseed crops grown in the Czech Republic are oilseed rape (*Brassica napus* L. var. *napus*), sunflower (*Helianthus annuus* L.), poppy (*Papaver somniferum* L.), mustard (*Sinapis alba* L.), linseed (*Linum usitatissimum* L.) and soybean (*Glycine soja* Sieb. et Zucc.). The area sown to each crop has changed rapidly in recent years, as has the importance of each crop (Table 1).

Table 1: Harvest area, yield and production of oilseeds grown in the Czech Republic in 1989 and 1998.

Crop	Harvest area (ha)			Yield (t/ha)			Production (t)		
	1989	1998	(%)	1989	1998	(%)	1989	1998	(%)
Oilseed rape	102376	264310	258	3,06	2,57	84	313253	680216	217
Mustard	7065	36136	511	1,32	1,03	78	9319	37282	400
Poppy	7611	27881	366	1,04	0,74	71	7894	20524	260
Sunflower	4453	17274	388	1,72	2,11	123	7659	36475	476
Linseed*	21186	3719	16	0,63	0,32	51	13247	1199	9
Soybean	1020	261	26	1,92	1,25	65	1956	327	17
Other	323	3762	1165	1,23	1,11	90	396	4158	1050
Total	144034	349624	243	2,46	2,19	89	353994	957927	271

**) above all fibre flax*

The largest oilseed crop in the Czech Republic is rapeseed, which is grown on ca. 75% of the total acreage sown to oilseeds (Fig. 1).

Historically, there is a very long tradition of rapeseed cultivation on Czech land and the industry was revived in a latter half of the 19th century. Yields at that time were so high that Czech crushing mills were not able to process the total crop. As a consequence, rapeseed was exported to Germany, crushed and the rape oil was then re-imported back into Czechoslovakia.

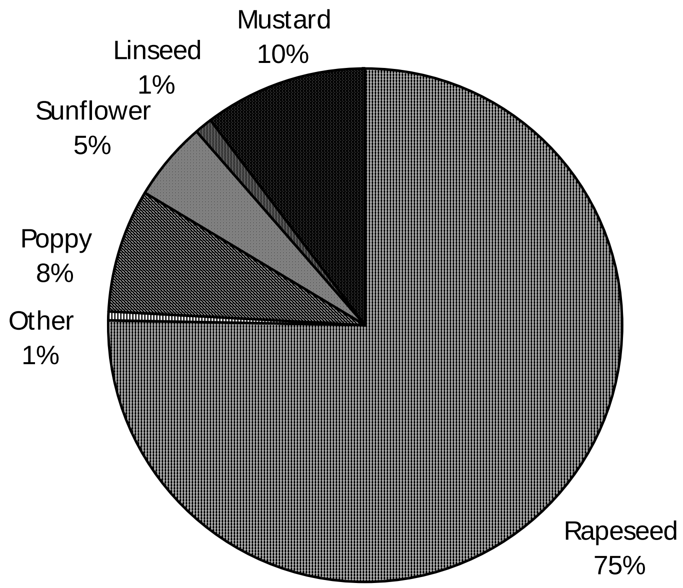

Figure 1. Harvest area of oilseed crops, Czech Republic 1998.

After World War II, Czechoslovakia was importing large amounts of the raw materials used by the edible oil industry and animal fodder industry on an annual basis. This untenable situation stimulated the formation of a complex rapeseed growing system named the **System of Rapeseed Production** (SRP).

SRP was established in 1983 and consisted of the use of current scientific and technical knowledge under field conditions through increased co-operation between the Czech University of Agriculture, oilseeds processors, seed producers and agricultural businesses.

Since 1983, there have been significant increases in the development of oilseed rape as a crop in the Czech Republic, due to the four following improvements:

1. Change over to the new double low varieties, which produce better quality oil (low erucic acid/low glucosinolate content). The success of the breeders in the development of the new varieties has enabled rapeseed oil to become one of the best-valued edible oil worldwide and to compete in the market place with sunflower, soybean and palm oil. The decreased glucosinolate content facilitates better exploitation of rapeseed meal and cake for fodder production.
2. In addition to improvements in oil quality, there has been a significant increase in yield potential from the new varieties, which can reach for 8 t/ha under trial conditions. Following improvements to yield, we may expect further improvements through the use of heterosis in hybrid varieties.
3. The increase in rapeseed cropping in the Czech Republic (Fig. 2) was also positively influenced both by increased domestic processing capacity and by the increased oilseed demand worldwide.

4. Active operation by the SRP through consultancy (variety choice, cropping technology, crop protection considerations, etc.) currently accounts for yield differences of ca. 250 kg/ha between members of SRP and non-members.

Due to the good price for rapeseed in recent years (1997; ca. 7000 CZK/t, 1DEM = ca. 19 CZK) the area sown to rapeseed in the Czech Republic increased rapidly during the 1998/99 season to ca. 350 000 ha (winter rapeseed - ca. 92%; spring rapeseed - ca 8%). This constitutes an 11.3% share of the total area of the whole republic and more than 20% of the rotation in some regions and farms (in extreme cases, 40-50% of arable land may be sown to rapeseed!). In regions such as these, where rapeseed production has increased dramatically, new diseases and pests problems have begun to arise and many farmers have been forced to treat crops with pesticides. However, treatment is expensive and decreases the profitability of the crop. Although it is only used when necessary, in some areas it is not profitable to grow rapeseed without treatment.

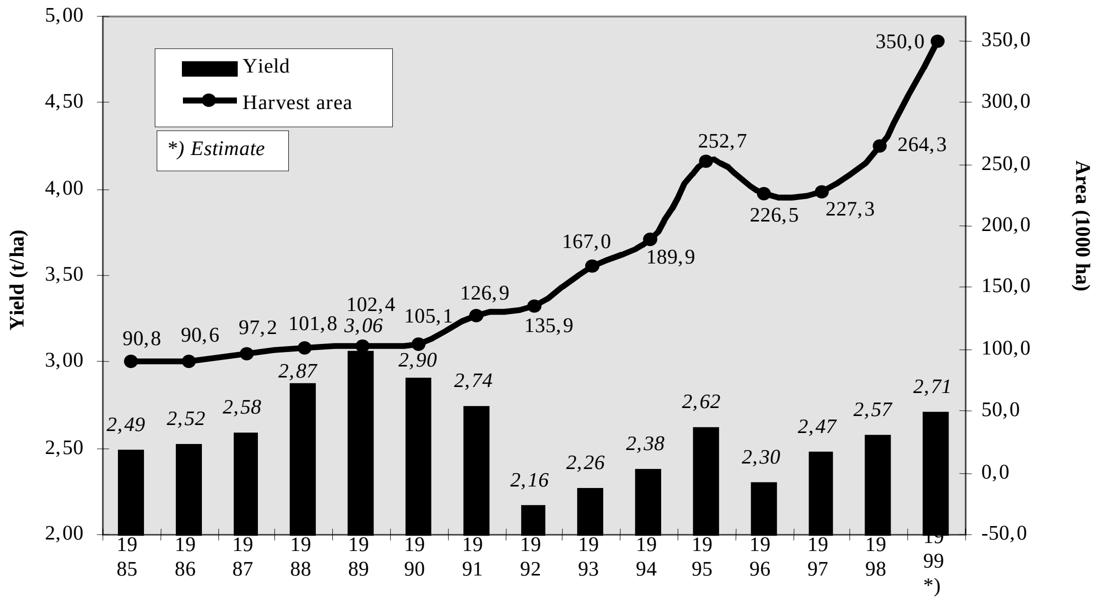

Figure 2. Yield and harvest area of rapeseed, Czech Republic 1985-1999.

This season (1998/99), based on the large area sown to the crop, relatively good overwintering and positive yield predictions, we expect the biggest rapeseed production in Czech history at harvest (Table 2).

Table 2. Rapeseed production in Czech Republic during last 10 years (1000 t)

1990	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999*)**
304,5	348,3	293,0	377,2	451,6	662,2	520,6	560,5	680,2	949,0

*) *Prediction*

In contrast to increases in production, Czech domestic processing potential has not increased in proportionally. The industry can only process ca. 50% of domestic production and the nonsensical use of export licenses for the remaining 50 % is still common place. We assume that this will continue not only this year, but also next years with the ratio of domestic processing and export remaining more or less 1:1. Another problem is that on the Czech edible oil market, pressure from foreign competitors continues to grow stronger as a consequence of the expansion of exterior market chains (e.g. Delvita, Penny Market, Plus, Norma, etc in other countries).

Due to absence of any subsidy payment, Czech farmers have to produce rapeseed with lower than optimal inputs. Czech growers are also at the disadvantage that rapeseed produce can only be sold at a level below the world market price, either domestically or abroad (at the world price with transport costs subtracted). Currently, no transgenic rapeseed is grown commercially in the Czech republic, although some varieties (Liberty Link, Roundup Ready, Seed Link) are being tested under trial conditions. It is not expected that any GM-rapeseed will undergo registration before 2002, although the grower's acceptance of the benefits of the introduction of transgenic varieties appears to be higher than that of the consumer.

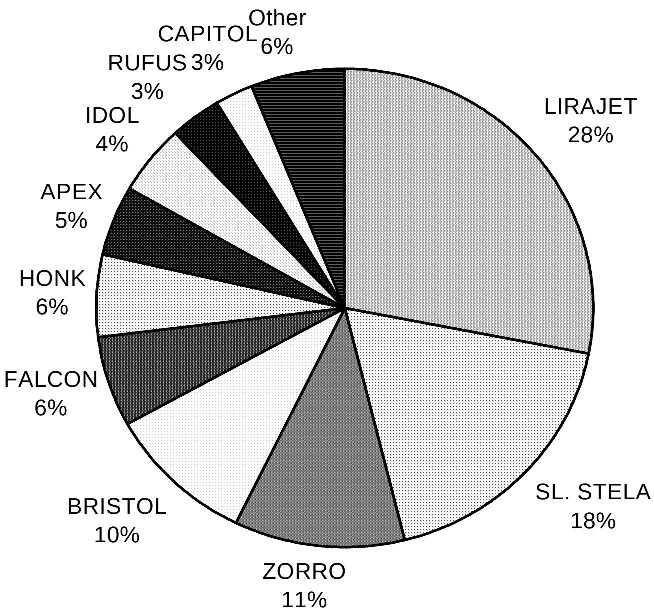

Figure 3.Varieties Market Share, Czech Republic 1998/99.

Czech Republic growers have been sowing some hybrid rapeseed varieties since 1998 and four varieties are currently available (PRONTO, SYNERGY, ARTUS, BETTY). The proportion of each of the main varieties grown is shown in Fig. 3.

Rapeseed is not only used for edible oil production, but also in the production of biodiesel (rape methyl ester - RME) which can be used in most modern diesel engines. In the Czech Republic there is also the „oleoprogramme", one aim of which is to modify rapeseed oil through a re-esterification process. Between 1992-1995, seventeen RME production facilities were built. The annual capacity of the facilities is 63 000 t of RME (equivalent to 70 000 ha of rapeseed). However, only 20 000 t RME/year is produced, mainly because RME can be cheaply imported from Germany and Austria. Because of the higher price of RME in comparison to mineral diesel, almost all RME is used in the production of „Second generation biodiesel". This contains a mixture of min. 30% RME to which biodegradable mineral oil fractions are added. This mixture constitutes 4-5% of the Czech market of fuel for diesel engines (170 000 t in 1997).

Even though growers can expect a significantly worse price for rapeseed this year (forecast price = ca. 5000 CZK/t) and the crop suffers from a lack of alternative markets, the authors believe that the substantial recent increase in area sown to rapeseed will be maintained during the 1999/2000 season, as the crop acts as a good breakcrop in the cereal rotation.

Monitoring Diseases – Biology of Pathogens

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Characterisation of the strains of *Leptosphaeria maculans* isolated from oilseed rape in the Czech Republic and Slovakia

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Abstract: Stems of winter oilseed rape with symptoms of the fungal infection caused by *L. maculans* were collected in 1997, from commercial fields in the central and eastern part of the Czech Republic (Moravia and Silesia) and from western Slovakia. The aim of the study was to characterise the populations of *L. maculans* from the different geographical regions. Pure, single spore isolates of the fungus were classified using morphological (growth rate, sporulation intensity, pigment production) and biochemical tools (the production of secondary metabolites from the sirodesmin group). On the basis of the complex of these characters, isolates were classified as A (Siro^{+}) or B (Siro^{0}) group. It was found that the majority (87%) of isolates belonged to the B group and only 4 isolates out of 30 studied (13%, 2 per country) belonged to the A group, isolates of which are regarded to be very aggressive.

This is the first report on the composition of the *L. maculans* population on winter oilseed rape in the Czech Republic and Slovakia. This study will be supplemented with more isolates from the remaining geographical regions of both countries in the near future. On the basis of the results obtained so far, the prevalence of B group over A group isolates has been found.

Key words: *Leptosphaeria maculans*, stem canker, oilseed rape, sirodesmins, A (Siro^{+}), B (Siro^{0})

Introduction

The cultivation of oilseed plants, mainly winter rape (*Brassica napus* L.), plays an important role in the agricultural production of the Czech Republic and Slovakia (Havel 1996). The climatic conditions of these regions are good for the growth and development of oilseed rape, which results in a high yield from the crop. One of the best regions for the intensive cultivation of this crop in the Czech Republic are the Czech-Moravian Highlands located near Jihlava (Moravia), but oilseed rape is also grown in other regions (Bohemia, Silesia). In Slovakia, oilseed rape is mainly grown in the western and southern part of the country. The area of oilseed rape cultivation is smaller than in the Czech Republic, as large parts of Slovakia are hilly or mountainous and are unsuitable for oilseed rape cultivation. Due to a high intensity of oilseed rape production, a constant increase in disease incidence and severity has been observed (Vasak *et al*. 1997). One of the most damaging diseases is blackleg or stem canker (Flanderkova and Hampejs 1988, Vitasek 1994). The disease, regarded as one of the most damaging to oilseed rape worldwide, is caused by the ascomycete fungus *Leptosphaeria maculans* (Desm.) Ces. et De Not. It has been shown that strains of the fungus differ in regard to pathogenicity to different crucifers (Petrie *et al*. 1995), colony morphology (Koch *et al*. 1989), sporulation (Petrie 1988), secondary metabolite production (Badawy and Hoppe 1989) and isozymes (Balesdent et al. 1992). Substantial differences between strains can also be observed at the molecular level (Johnson and Lewis 1990, Schäfer and Wöstemeyer 1992, Morales et al. 1993). On the basis of several characters, the isolates of *L. maculans* have been divided into two groups: isolates pathogenic to oilseed rape and those specific for several cruciferous weeds. These groups should be regarded to be different species (Rouxel et al. 1995). The most important, from the economic point of view, are isolates pathogenic to oilseed rape. These isolates can be further sub-divided into two groups that differ according to many charcteristics, with the aggressivity level being one of the most important factors. Isolates from the A group are very pathogenic / aggressive, whereas the pathogenicity / aggressivity of the B group isolates is regarded as negligible (Gugel and Petrie, 1992). Symptoms caused on oilseed rape plants by both types of isolates are similar to identical. Although some differences between the symptoms produced on leaves exist (Biddulph *et al*. 1999), macroscopic differentiation between these two groups may be difficult and misleading (Brun *et al*. 1997). In the case of stems, isolate identification in the field is not possible at all.

There are several methods for the differentiation of *L. maculans* isolates, of which, molecular methods are one of the more recent advances (Goodwin and Annis 1991, Meyer *et al*. 1992, Plummer *et al*. 1994, Balesdent *et al*. 1998). However, the most common and easiest method to differentiate isolates is based on their ability to produce *in vitro* yellowish-brown pigments, or phytotoxins called sirodesmins. Pathogenic strains do not secrete pigments, but they do produce secondary metabolites from the sirodesmin group and this gave rise to the term Tox^+ (Balesdent *et al*. 1992) or $Siro^+$ (as proposed by Pedras). The other group comprises isolates that do produce pigments, but do not produce sirodesmins (Tox^0 or $Siro^0$). It was found that various regions of Europe differ in regard to the ratio of the isolate types. Isolates from the A group apparently dominate the population in the UK (Jedryczka *et al*. 1999) and Germany (Kuswinanti *et al*. 1995) and they constitute the only *L. maculans* isolates present in the southern part of France (Balesdent *et al*. 1997). In contrast, isolates from the B group dominate the population in Poland (Jedryczka *et al*. 1994, 1997). Varieties of oilseed rape differ with regard to resistance to different isolates of *L. maculans*, hence local proportions between these groups may play an important role for the selection of most suitable varieties for a particular region of cultivation.

Materials and methods

Collection of infected plants

The expedition to collect infected oilseed rape plants was carried out in a number of regions of the Czech Republic and Slovakia between the 18 to 23 June 1997. Symptoms of stem canker were diagnosed macroscopically in the field. A few to several infected oilseed rape stems were collected from each field and samples were packed separately and labelled. Plants were collected from fields along the following routes:

* in the Czech Republic

18 June – the region of the Czech-Moravian Highlands (Ceskomoravská Vrchovina) and southern Moravia (Jieni Morava);

19 June – central and north-eastern Moravia including the region of Valašsko

20 June – Silesia (Slezsko)

* in Slovakia

21 June – the region around Bratislava and the route along the western border of Slovakia with the Czech Republic

22 June – the route along the southern border of Slovakia with Hungary

23 June – the region of central and north-western Slovakia

At each field, a visual observation of disease incidence was noted, according to a scale from 0 to 3, where:
0 – no plants with stem canker symptoms
1 – a few plants with stem canker symptoms (1 % - 5 %)
2 – about 10 %-20 % of plants with stem canker symptoms
3 - many plants with stem canker symptoms (>20 %)

Isolation of fungal strains
Small fragments of infected stems were surface sterilised with sodium hypochlorite, washed three times in sterile distilled water and cultured on Potato Dextrose Agar (PDA) medium. The growing fungal colonies were subcultured onto PDA and V8 juice agar and observed under a light microscope. Single spore isolates were produced from fungal colonies identified as *Phoma lingam* [Tode ex Fr.] Desm. (teleomorph: *Leptosphaeria maculans* Desm. [Ces. et De Not.]) and isolates were characterised further using morphological and biochemical tools. One representative isolate was produced per field.

Characterisation of colony morphology
The morphology of fungal colonies was characterised using several methods:

- **Growth rate –** Agar discs overgrown with the mycelium of an isolate were subcultured onto PDA (3 replicates) and were incubated at 20^0C; colony diameter was measured daily.
- **Sporulation intensity –** Evaluated after 3 weeks of growth on PDA, according to a visual scale with three grades: little, medium, profound.
- **Pigment production –** Evaluated after 2 and 4 weeks of subculture in liquid Czapek-Dox medium supplemented with yeast extract (2g/L) (CDY); the colour of medium was described as: colourless, straw coloured, yellow, beige, brown or dark brown.

Characterisation of secondary metabolites
High Performance Liquid Chromatography (HPLC) was used to characterise secondary metabolites produced *in vitro*. Isolates of *L. maculans* were subcultured into 150 mL of CDY medium. Culture filtrate was collected from 3 replicate flasks, after 3 weeks of static growth at 20ºC. Culture filtrate was extracted twice with an equal volume of ethyl acetate. The combined organic phases were evaporated to dryness *in vacuo* and redissolved in 2 mL of methanol. HPLC analyses were done using a Beckman Gold instrument with a Diode Array Detector. Methanol solution containing metabolites (20 µL) was injected onto a Lichrosorb C_{18} column (250×4 mm). Separation was carried out using two solvents: water (A) and acetonitrile (B) at 1 mL/min flow rate with the following gradient: 30% of B for 3 min; linear gradient to 50% of B over 18 min; to 95% of B over 2 min; 95% of B for 6 min; before returning to the starting conditions. Chromatograms were recorded at 225 nm and 270 nm and the UV spectra of individual compounds were also recorded (in the wavelength range 200-450 nm). Compounds were identified by comparison of their retention time and UV spectra with those of standards.

Results

Stem canker symptoms were found on the stems of plants in all oilseed rape fields studied, although lesions were sometimes small and infrequent. However, in the majority of fields, symptoms were large, profound, and easy to identify. Lesions were located at the bottom or the middle of the stem. No *L. maculans* symptoms were found on leaves.

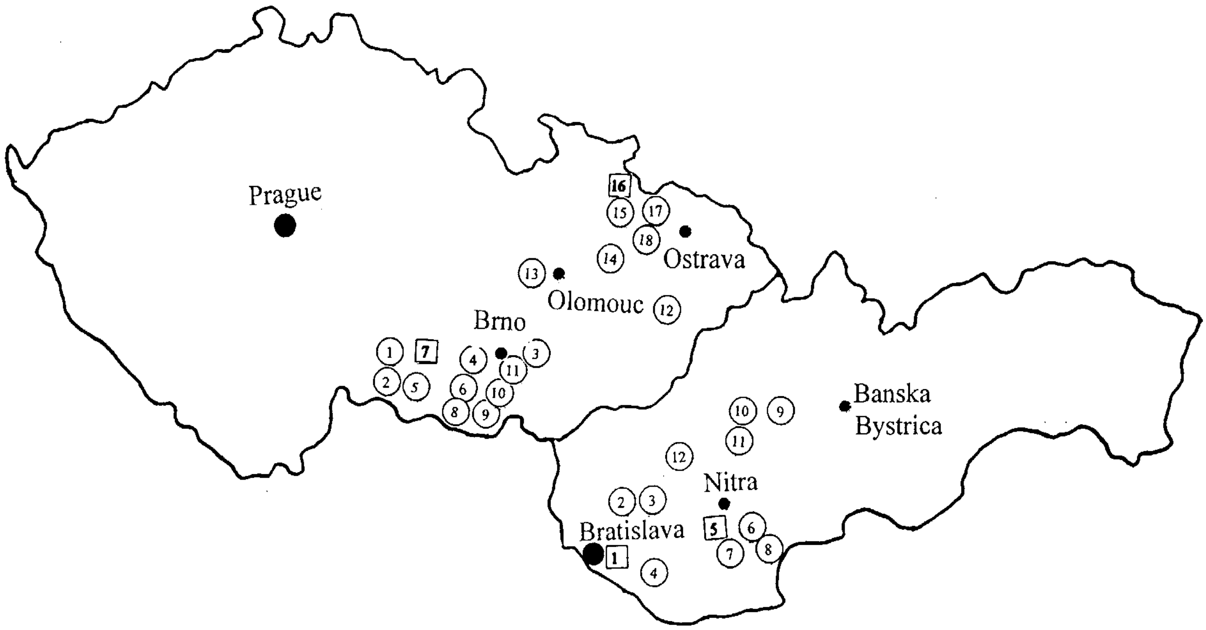

Figure 1. Origin of *Leptosphaeria maculans* isolates from the Czech Republic and Slovakia.

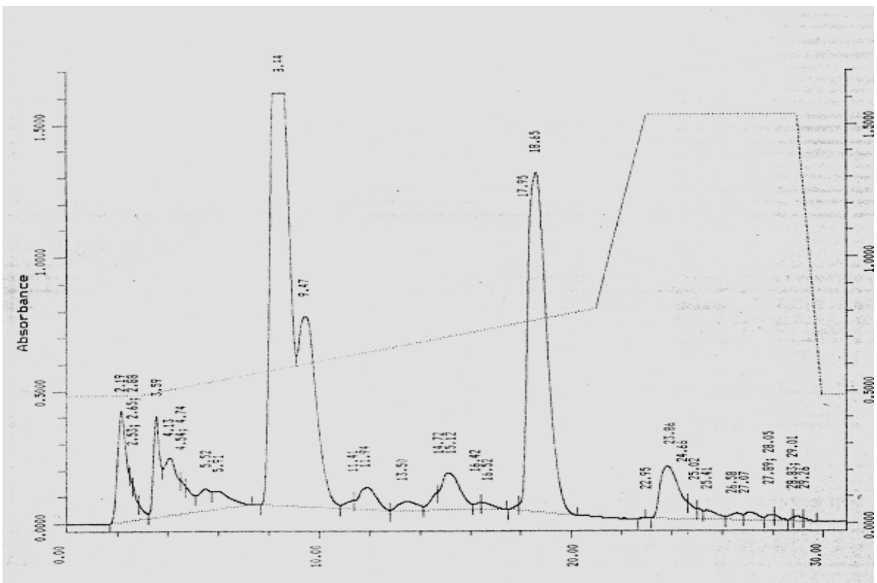

Figure 2. HPLC of secondary metabolites produced by Tox^+ isolate CZ7.

Out of 30 fields studied, 30 single-spore isolates of the fungus *Phoma lingam* were obtained: 18 from the Czech Republic (isolate symbols CZ1-CZ18) and 12 from Slovakia (SK1-SK12). The origin of the isolates is given in Table 1 and shown on Figure 1. Most of isolates were characterised by a fast growth rate and the presence of pigment produced *in vitro* on both PDA and CDY media. The intensity of pigmentation was very variable and it varied from straw coloured to dark brown. However, in most cases the CDY medium became brown or dark brown 3-4 weeks following subculture. Only four isolates (CZ7, CZ16, SK1 and SK5) did not produce any pigment either on PDA and CDY media. The intensity of sporulation differed from little to profound. Detailed data concerning pigment production and sporulation intensity are presented elsewhere (Jedryczka *et al*. 1998).

Due to the extraction of the filtrates with ethyl acetate, most of the inorganic components of the medium, as well as highly polar organic components were discarded. High performance liquid chromatography of secondary metabolites revealed noticeable differences in secondary metabolite patterns among the isolates. Four fungal strains (CZ7, CZ16, SK1 and SK5) produced sirodesmin PL and it's deacetyl derivative as quantitatively dominating compounds (Figure 2).

Table 1. Characterisation of *Phoma lingam* (*Leptosphaeria maculans*) isolates collected from infected oilseed rape plants in the Czech Republic and Slovakia.

No	**Location**	**Isolate symbol**	**Disease incidence**	**Sirodesmin PL**	**Wasabidienone B**	**Group**
			The Czech Republic			
1	Zverkovice	CZ1	1	-	++	B (Siro^0)
2	Jevisovka	CZ2	3	-	+	B (Siro^0)
3	Ujezd	CZ3	3	-	?	B (Siro^0)
4	Dukovany	CZ4	3	-	++	B (Siro^0)
5	Visnove	CZ5	3	-	+++	B (Siro^0)
6	Visnove-Skalice	CZ6	1	-	+++	B (Siro^0)
7	Skalice	CZ7	3	+	-	A (Siro$^+$)
8	Chlupice	CZ8	1	-	+	B (Siro^0)
9	Domnice	CZ9	1	-	+++	B (Siro^0)
10	Branisovice	CZ10	1	-	?	B (Siro^0)
11	Pohorelice	CZ11	1	-	+	B (Siro^0)
12	Zubri-Zasova	CZ12	3	-	++	B (Siro^0)
13	Lastany	CZ13	1	-	+++	B (Siro^0)
14	Mladecko	CZ14	1	-	?	B (Siro^0)
15	Brumovice	CZ15	2	-	++	B (Siro^0)
16	Opava-Jaktar	CZ16	1	+	-	A (Siro$^+$)
17	Trebom	CZ17	1	-	+	B (Siro^0)
18	Opava-Kylesovice	CZ18	1	-	++	B (Siro^0)
			Slovakia			
19	Cerna Voda	SK1	3	+	-	A (Siro$^+$)
20	Senec	SK2	3	-	?	B (Siro^0)
21	Seneckie Jazera	SK3	3	-	-	B (Siro^0)
22	Velke Ulany	SK4	3	-	-	B (Siro^0)
23	Vrable 1	SK5	2	+	-	A (Siro$^+$)
24	Vrable 2	SK6	2	-	+++	B (Siro^0)
25	Telnice	SK7	2	-	+++	B (Siro^0)
26	Velky Dur	SK8	1	-	+++	B (Siro^0)
27	Oslany	SK9	1	-	+	B (Siro^0)
28	Brodzany	SK10	3	-	+	B (Siro^0)
29	Oponice	SK11	2	-	++	B (Siro^0)
30	Bocany	SK12	3	-	+	B (Siro^0)

Twenty-six isolates belonged to the Tox^0 group. Chromatograms of the majority of them (18 isolates) revealed very similar patterns of secondary metabolites. The remaining 8 isolates produced a range of various compounds produced in different amounts. Isolate CZ13 produced large amounts of wasabidienone B. In addition to this compound and it's known derivatives, the *P. lingam* Tox^0 isolates studied produced several additional compounds; the UV spectra of some of them have been previously published (Kachlicki and Jedryczka 1999).

On the basis of several characters studied, the isolates of *P lingam* were classified as A (Tox$^+$, Siro$^+$) or B (Tox^0, Siro^0) group. Most of the isolates (87%) were identified as B group. Four isolates (CZ7, CZ16, SK1 and SK5) were attributed to the A group. All of these isolates were characterised by slower growth rate, medium to profound sporulation, non-production of pigment and the production of sirodesmin PL and it's deacetyl derivative. A detailed characterisation of all of the isolates tested is presented in Table 1.

Discussion

The results of extensive studies on the composition of the *L. maculans* (*P. lingam*) population that have been carried out in Canada and western European countries are closely connected with everyday agricultural practice. As most strains isolated from infected oilseed rape plants were identified to be Tox$^+$ subgroup PG3 isolates (Balesdent *et al*. 1997), there was a change to the cultivation of varieties resistant to these isolates, *eg.* Capitol. Growers from Canada and Germany were also made aware of the serious risk from stem canker or blackleg, after reports of dramatic changes in *L. maculans* populations from less aggressive to very aggressive isolates (Petrie *et al*. 1985, Kuswinanti *et al*. 1995). The aim of this study was to investigate the *L. maculans* population structure in two countries located in central Europe: the Czech Republic and Slovakia. From published reports, it can be concluded that stem canker is a great threat to winter oilseed rape production in both countries (Svoren 1997, Kohout *et al*. 1998). Plachka (1996) reported that autumn infections of oilseed rape are, to a great extent, caused mainly by the fungus *L. maculans*.

The results of our study showed that the majority of *L. maculans* isolates (87%) belonged to the less aggressive B group. However, the fact that many of them (40%) were collected from fields with severe symptoms of stem canker suggests that the B group isolates may be more aggressive than previously thought. Only 4 isolates out of 30 studied (2 per country) represented the A (Tox$^+$/Siro$^+$) group. A similar situation was observed in a neighbouring country of Poland, where the majority of *L. maculans* strains isolated from winter oilseed rape in different regions of the country, were also identified as representatives of the B (Tox^0/Siro^0) group (Jedryczka *et al*. 1994, 1997, Kachlicki and Jedryczka 1994). As is the case in the Czech Republic and Slovakia, stem canker is also regarded to be a serious fungal disease of oilseed rape in Poland, where epidemics are characterised by plants with profound symptoms and high disease incidence (Lewartowski and Orlikowska 1986, Frencel *et al*. 1991, Starzycki 1998).

Some of the isolates from the B group, often described as "non aggressive", caused severe symptoms of blackleg, when introduced into soil used as a substrate for the growth of oilseed rape in the glasshouse (Jedryczka *et al*. 1994). Moreover, it was found that although these strains were unable to produce sirodesmins, some other phytotoxic metabolites could be detected in culture filtrate in which they had been grown (Kachlicki *et al*. 1996, Zhang *et al*. 1998). The majority of Czech and Slovakian isolates also produced comparable amounts of the same secondary metabolites *in vitro*. However, in some cases big differences between the composition of metabolites were found. A similar case was observed for some Polish B group isolates, which also produced different sets of compounds (Jedryczka *et al*. 1999). These results support the theory that the *L. maculans* species, and especially the B group, represent a highly diverse species complex (Balesdent *et al*. 1992). The results presented in this study are quite preliminary, as they represent the population of *L. maculans* on winter oilseed rape in only one season. The expedition of 1997 covered a few, but not all regions of the Czech Republic (Moravia and Silesia) and Slovakia (western part of the country). The study is a result of the ongoing collaboration between plant pathologists from Poland, the Czech Republic and Slovakia.

A shift in composition of the *L. maculans* population towards a higher ratio of A group isolates has recently been observed in Poland (Jedryczka unpublished data). Although the situation in the Czech Republic and Slovakia seems to be quite optimistic if the pathogen population structure remains as it apparently is at present, there is a high probability that the shift observed in Poland may happen in these countries in the near future.

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Secondary metabolites of Tox^0 *Phoma lingam* strains and their phytotoxic activity

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Abstract: Our previous studies of the fungus *Phoma lingam* (generative stage: *Leptosphaeria maculans*) population show that strains belonging to the Tox^0 (type B, "weakly virulent", "non-aggressive") subspecies dominate in Poland. Despite that these strains do not produce phytotoxins from the sirodesmin group, some phytotoxic activity is observed in their culture filtrates. Partially purified phytotoxic fractions were obtained from culture filtrates of several isolates using column chromatographic procedure. Some compounds such as benzoic acid, phenylacetic acid or succinic acid were identified in these fractions.

Microspore-derived embryo and protoplast cultures of winter oilseed rape (*Brassica napus* L.) were used for the studies of phytotoxic activity of *P. lingam* metabolites. The cultures were treated with sodium benzoate, partially purified fraction of PL68 isolate metabolites with the highest phytotoxic activity and with succinic acid present in this fraction. Phytotoxic activity of sodium benzoate and of the studied fraction of PL68 metabolites was observed both in embryo and protoplast cultures. In spite of structural similarity of succinate to oxalate (the known phytotoxin of the fungus *Sclerotinia sclerotiorum*) no influence of this compound was observed in the studied cultures.

Single leaves of four week old oilseed rape plants cvs Apex, Bolko and Leo were treated with sodium benzoate, sodium phenylacetate, crude mixture of metabolites of isolates RAW4 and PL27 and partially purified phytotoxic fraction of PL27 metabolites. The treated and untreated leaves were taken for analyses after 1, 3 and 7 days. The indolyl glucosinolate contents were measured using HPLC of desulphoglucosinolates and compared to that observed for water treated control plants.

Considerable induction of indolyl glucosinolate content was observed 24 hours after treatment with all metabolites studied. The total indolyl glucosinolate contents remained on the increased level until the seventh day but some changes in the composition of this group was observed then. The role of plant and fungal metabolites in the process of infection will be discussed.

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Predicting leaf infection by *Leptosphaeria maculans* on winter oilseed rape

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Abstract: Ascospores of *Leptosphaeria maculans* infect leaves of oilseed rape to cause phoma leaf spots, from which the fungus can grow to infect the stem. Yield losses due to early senescence and lodging result if the stem infections reach a threshold severity prior to harvest. Host resistance alone has not been sufficient to control the disease in Europe, where two forms of the fungus (A (Tox^+) and B (Tox^0) occur, each with different pathogenicity groups. Currently fungicides do not eradicate mycelium once it is inside the stem so management relies on protection of the leaves. One or two well-timed fungicide applications can give good economic control of the disease. The first releases of ascospores often cause leaf infections which lead to the most damaging stem cankers. These first releases of spores can be predicted by monitoring weather conditions and maturation of *L. maculans* pseudothecia. Accurate forecasting of severe epidemics can improve disease control and also decrease fungicide use when the risk of crop damage is low.

Key words: *Leptosphaeria maculans*, disease forecasting, pseudothecial maturation

Introduction

The fungus *Leptosphaeria maculans* (Desm.) Ces & De Not. causes stem canker (blackleg) of winter oilseed rape. Pseudothecia are produced on stubble and release ascospores from the autumn onwards. The spores infect leaves to cause leaf spots, from which the fungus can grow to infect the stem (Hammond *et al*., 1985). Hammond and Lewis (1986) found that the most damaging cankers are formed at the stem base from leaf infections occurring before the onset of rapid stem extension. Additionally, the first six leaves appear to be more susceptible to infections than those produced later (McGee and Petrie, 1979). Therefore, any control strategies should focus on this vulnerable period. Two forms of the fungus occur; A (Tox^+) which causes damaging cankers on the stem and B (Tox^0) which infects the pith (Johnson and Lewis, 1994).

It has been suggested that fungicides do not control *L. maculans* once it has reached the stem so it is important to protect the leaves (Gladders, 1988). However, due to the small size of young leaves and the relatively warm temperatures of early autumn, the pathogen can rapidly spread to the stem, so it is important to apply fungicides very promptly if infections occur. Biddulph *et al.* (1998) showed that lesions start to appear after only 3 days at 20ºC, 5 days at 16ºC, and 13 days at 8ºC. Additionally, some reports indicate that fungicides applied to control leaf infections have not always prevented the development of cankers (Rawlinson and Muthyalu, 1979). Hammond (1985) speculated that the fungus within the leaf might escape the effects of fungicides. If this is so, as some fungicides promote leaf retention rather than abscission, late fungicide applications may even be counterproductive by allowing the fungus to reach the stem. Applications of fungicide, in response to the first observation of leaf spots (commonly once a threshold of 10% of plants affected is reached) may be too late to prevent visible infections from spreading to the stem but should protect the majority of uninfected leaves. Very frequent observations may be required to detect these first leaf infections before the incidence of leaf spotting becomes too high. Furthermore, application of the control spray can be delayed by bad weather. A method for advanced prediction of leaf infection would improve disease control. This paper describes how the number of spores in the air can be predicted and monitored, as part of a disease forecasting scheme to enhance disease control and reduce unnecessary fungicide use.

Materials and methods

Maturation of pseudothecia was assessed on stubble collected immediately after harvest and incubated in trays outdoors. Periodically, five pseudothecia from each of five stubble sections were excised, placed on slides and examined microscopically. Pressure was applied to the coverslip to squash the pseudothecia and reveal their contents which were graded according to the scale of maturation used by CETIOM; class A = undifferentiated, B = immature asci present but spores are undifferentiated, C = <8 spores per ascus and < 4 cells per spore, D = 8 spores per ascus or > 4 cells per spore.

Furthermore, the numbers and pattern of ascospore discharge, detected by Burkard spore samplers located next to sources of inoculum, were compared with the leaf spot incidence observed over two seasons and two sites.

Results

Maturation of pseudothecia

Maturation of pseudothecia was found to be very variable (Table 1); e.g. at St Pathus, pseudothecia of class D were observed before class A, which means that regular assessments of relatively large numbers of pseudothecia would be required for a reliable forecasting scheme. Additionally, the time period between observation of class D (i.e. mature) pseudothecia and the first measurement of spores from the air differed between sites and seasons. This variability is partly attributed to differences in pseudothecial maturation due to the position of pseudothecia on the stem debris. The first pseudothecia were observed at the sites of stem cankers on the stem base; later infections (upper stem lesions) tended to produce pseudothecia later in the season. Pseudothecia of the B group (Tox^0) on Polish stubble, matured at a similar time to the mainly A group (Tox^+) pseudothecia on UK stubble.

Ascospore discharge

Comparisons of records of airborne ascospores trapped and leaf spot incidence observed over several seasons (e.g. Fig. 1) revealed that leaf spotting was noticed before any major releases of spores were detected. It appears that the first spores released, although relatively few in number, lead to a relatively high incidence of leaf spotting, because they are deposited when the plant is at its most vulnerable stage.

Table 1. Pseudothecial maturation, spore release and disease incidence in France and the UK.

Site	1st A class pseudothecia	1st D class pseudothecia	1st spores trapped	1st leaf spots observed
Nancy, France	7/9/97	18/9/97	9/10/97	10/10/97
Surgères, France	4/9/97	17/9/97	21/10/97	27/10/97
St Pathus, France	12/8/97	5/8/97	25/8/97	17/10/97
St Florent, France	28/8/97	3/9/97	9/9/97	25/10/97
Rothamsted, UK	4/9/97	26/9/97	1/10/97	20/10/97
Rothamsted, UK	5/7/98	23/7/98 *	30/9/98	22/10/98

(* on plants with severe cankers only)

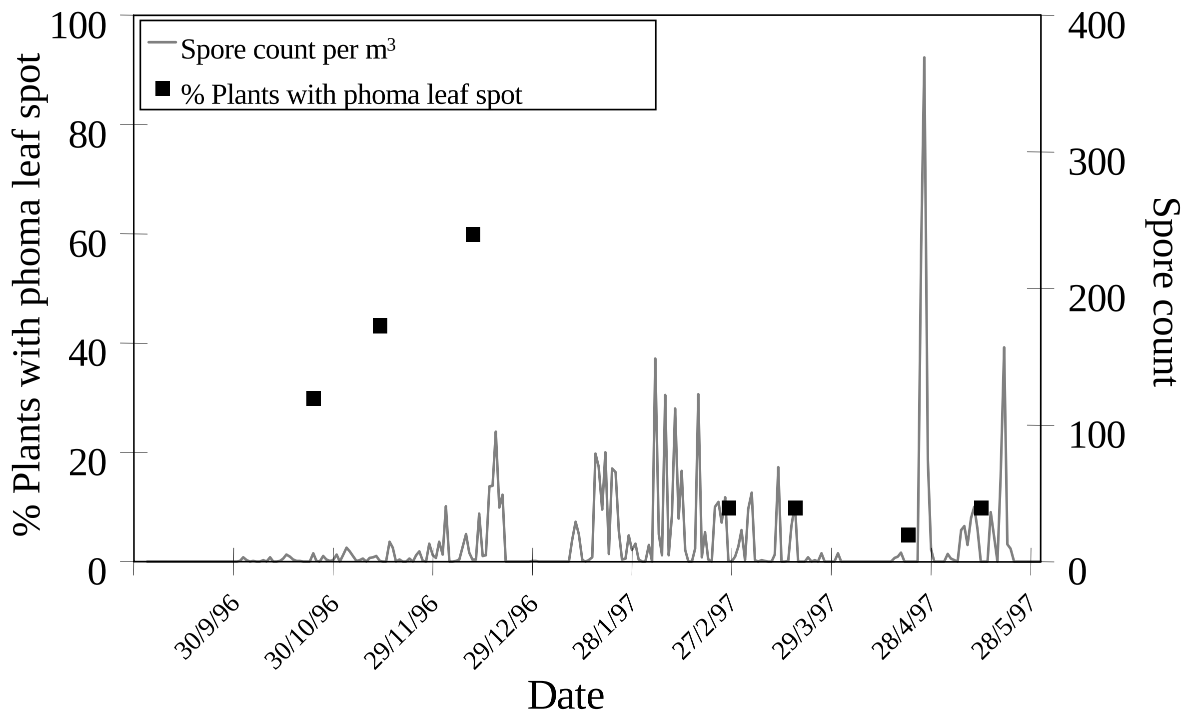

Figure 1. The pattern of ascospore numbers in the air and leaf lesion incidence in a winter oilseed rape crop at Rothamsted, 1996/97

In the example (Fig. 1) leaf spotting was first noticed on 22nd October and was most prevalent that autumn. This led to the appearance of stem cankers from mid-April (data not shown). As in other seasons/sites, a decrease in the incidence of leaf spotting (in untreated plots) was noticed in the winter, despite the presence of spores in the air at this time. Although at low temperatures it takes over a month from infection for leaf lesions to appear, and older (diseased) leaves are shed in response to shading and frost, the reduction in leaf infection in midwinter is also a confirmation that host resistance increases with age. Spores were released for up to a few days after rain or dew, with a general increase in numbers released determined by the maturation of the majority of the pseudothecia present on the debris and by temperature.

Discussion

An early sowing of the crop can be used to evade infection at the most sensitive period for the crop (LePage and Penaud, 1995) but there can be problems of early flowering and the formation of too dense a crop canopy. The crops in this study were sown in late August, as standard practice. Improving host resistance remains an important aim in the control of stem canker. As some leaf lesions are confined while others may lead to stem infections, knowledge of the aggressivity/compatibility of local populations of the pathogen on the predominant cultivar grown in a region would aid spray decisions. Work on this is in progress as part of the IMASCORE project (Balesdent and Rouxel, 1998; Balesdent *et al.*, 1998). Large differences appear to occur in symptom expression between cultivars and between crop growth stages and this may have reduced scientific understanding in the past. Additionally, further work on the importance of secondary infections by conidia should be established.

In addition to climatic factors immediately prior to spore release, it appears that the position and severity of infection in the previous crop influences pseudothecial maturation; the first pseudothecia occurring at the stem base at sites of severe stem cankers, while pseudothecia at sites of upper stem lesions are produced later. There is potential for the population structure of the pathogen to change during the season as pseudothecia from different parts of the inoculum residue mature. This has also been suggested by Thürwächter *et al.* (1999) and Hammond (1985). It appears that spore trapping is a more consistent and convenient measurement than pseudothecial maturation for modelling with climatic conditions in an attempt to predict the first spore releases. Pseudothecial maturation depends upon temperature, wetness and humidity (Poisson, 1997) and the maturation time may also be influenced by host resistance (Pérès and Poisson, 1997) and any applied chemicals. An investigation of pseudothecial maturation (Pérès and Poisson, 1997) indicated that the first emissions of ascospores occurred 16-19 rain-days after harvest and when the average temperature had dropped to 14ºC. A combination of the date of first spore capture and weather conditions was used to produce a preliminary model to advise on spray timing at different sites.

Initial modelling of climatic conditions with spore release data from spore trapping gives a more accurate prediction of leaf infection than the monitoring of pseudothecial maturation. Both monitoring of pseudothecial maturation, and modelling the severity of disease the previous season combined with the weather in late summer/early autumn, are possible methods for predicting the risk of spore release and the first occurrence of leaf spotting. A Burkard spore sampler could augment a forecasting service, giving a warning of spore release in indicator areas.

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Forecasting light leaf spot of winter oilseed rape in the UK

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Abstract: Light leaf spot (*Pyrenopeziza brassicae*) is a serious disease of winter oilseed rape crops in Britain. Wind blown ascospores from crop debris of the previous season are thought to initiate infection in newly sown crops. The pathogen enters a hemibiotrophic phase during which infections are not readily visible. Localised spread in the spring occurs through splash-dispersed conidia. Assessment data from regions of the UK were used to produce a model to predict the risk of a crop in a specific region of the UK developing light leaf spot. The forecast is based on crop and weather factors. At the start of the season, a prediction is made for each region using the average weather conditions expected for that region. This forecast is then updated periodically to take account of deviations in actual weather away from the expected values. Three factors form the basis of the model: amount of pod disease the previous summer, autumn temperatures and the number of winter rain days above the regional average. The model is currently in a second year of evaluation and has been made available to growers over the Internet.

Key words: Forecasting, light leaf spot, *Pyrenopeziza brassicae*, risk prediction, winter oilseed rape

Introduction

Oilseed rape is the most important arable crop in the UK after cereals (>400,000 ha *per annum*). Although losses differ greatly from season to season (Fig. 1), it is estimated that diseases can cause up to £80M of losses per season in winter oilseed rape (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). Light leaf spot is a polycyclic disease, which infects leaves, stems, flowers and pods (McCartney & Lacey, 1990). It is thought that epidemics are initiated by air-borne ascospores of *P. brassicae* which are produced in apothecia on stem and pod debris. Splash-dispersed conidia produced in acervuli on volunteer oilseed rape seedlings and vegetable brassica crops may also be involved in epidemic initiation, although conidia probably play a greater role in localised spread of the disease from initial foci. The disease spreads up plants through secondary spore dispersal and through extension of stems with infected meristematic tissue (Paul & Rawlinson, 1992). Evidence from experiments in which fungicides have been used to manipulate the development of epidemics suggests that light leaf spot generally decreases yields through killing leaves, and sometimes plants, in winter (Rawlinson *et al.*, 1978; Fitt *et al.*, 1998), although occasionally flowers and pods are damaged later. Fungicide timing for the control of light leaf spot has not been optimal and often crops requiring treatment have been left unsprayed at the appropriate time whilst others have been sprayed unnecessarily (Hardwick & Turner, 1994).

For both economic and environmental 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. Retrospective estimates of losses can be used to determine the importance of the disease in a particular season. Forecasting schemes, based on empirical relationships between measured disease incidence and earlier disease incidence or weather factors (e.g. temperature, rainfall) have been developed over a number of years at IACR – Rothamsted. The model is continually being improved by the incorporation of information on the epidemiology of light leaf spot (e.g. infection conditions, ascospore release). This paper describes work on improving the forecast for light leaf spot, and the development of Internet based support systems which allow growers to optimise fungicide use.

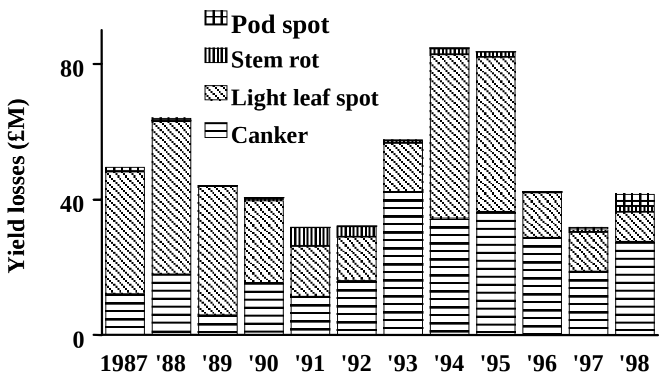

Figure 1. Winter oilseed rape disease losses in England and Wales, estimated from ADAS/CSL survey data for seasons from 1986/87 to 1997/98 and yield loss formulae for each disease.

Model development

An equation for the prediction of yield loss from light leaf spot incidence on leaves at growth stage (GS) 3,3 (flower buds visible; Sylvester-Bradley & Makepeace, 1985) was developed (Su *et al.*, 1998). The relationship was based on results from experiments in Scotland, but also fitted reasonably well to data from experiments in England with the same cultivar. The work has since been extended and tested using data collected in England and Wales and stored on the CSL/ADAS winter oilseed rape disease and pest survey database. The resulting model has been incorporated into a forecasting scheme with different parameter values for different cultivars and regions of the UK (Welham *et al.*, 1998).

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.*, 1996; Welham *et al.*, 1998). Seasonal, regional risk indices, predicting the % crops in a region with light leaf spot in the following March (Fig. 2), have now been issued in October 1996, 1997 and 1998. Spring disease survey data (i.e. March 1997 and 1998) were used to validate predictions made the previous autumn (i.e. October 1996 and 1997). 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 had been sprayed with fungicide.

Development of a WWW-based system

In 1998, web pages were produced and the forecast was issued as a map showing the risks in different regions of the UK (URL http://www.res.bbsrc.ac.uk/molbio/Lls/). The regional forecasts were based on survey data collected in the July before they are issued in October. Regional forecasts can now be updated twice during the autumn/winter (in January and February) by the addition of factors dependent on autumn rainfall and winter temperature (deviations from 30-year mean values). Recently, the addition of active web pages has allowed the development of an interactive component into the model. The pages now contain three information fields; cultivar (chosen from a list of cultivars), sowing date (early/late) and whether or not autumn fungicide has been applied (yes/no) (Fig. 3). Growers input information relative to their situation, press “Submit query” and are presented with a risk prediction for their specific area of the country under the cultural practises used on their farm (Fig. 4).

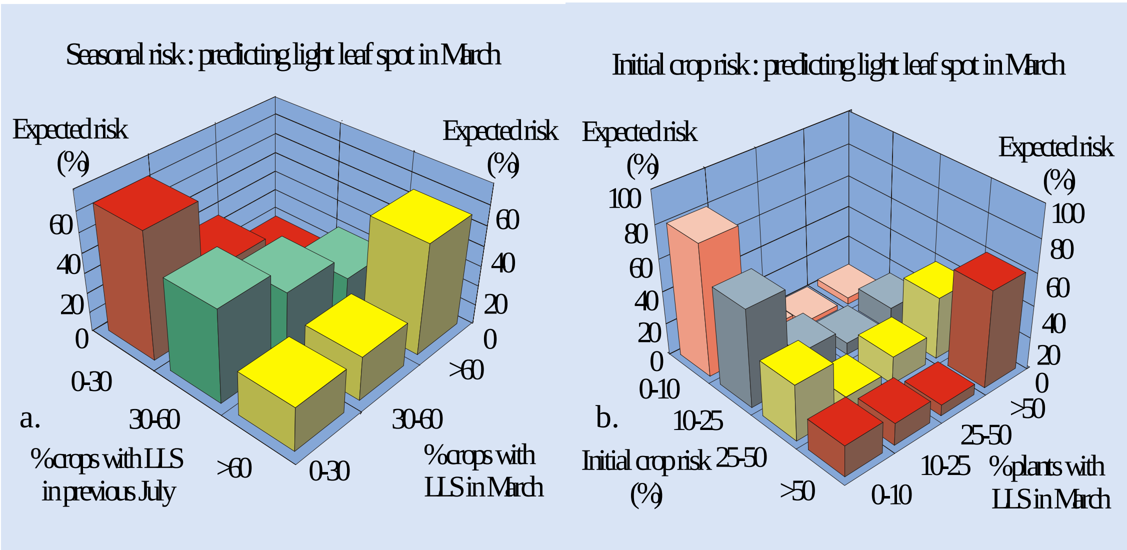

Figure 2. Prediction of light leaf spot risk in October for the following March: a) regional risk, based on survey data from the previous July (% crops in a region with light leaf spot); b) crop risk, based on sowing date, cultivar and ADAS region (Fitt *et al.*, 1996).

Discussion

The aim of crop risk indices is to provide growers with information about the risks of severe light leaf spot epidemics in their own crops. Recent advances in this study have increased the potential of the model through direct interaction with the grower. This allows cultivar resistance ratings, sowing date and early fungicide applications to be taken into account during calculation of the risk indices and further refines the 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.

Use of the interactive model will also provide the grower with a useful tool prior to sowing the crop. Because information about many commonly grown cultivars has been included in the interactive web site, a grower has the opportunity to assess the relative merits of cultivars of different resistance ratings. From this, it is easy to ascertain the potential risk from light leaf spot for a particular cultivar in a given region of the UK in a particular season. More resistant cultivars are likely to require less fungicide treatment; e.g. tebuconazole increased yields of cv. Capitol (rating 8 for resistance to light leaf spot) less than those of cv. Bristol (resistance rating 2) in experiments where severe light leaf spot developed (Sutherland *et al.*, 1998). Nevertheless, the estimates of losses from diseases of winter oilseed rape (Fig. 1), despite expenditure of up to £12M per season on fungicides (Turner *et al.*, 1999), suggest that there is considerable scope to further improve timing of fungicide applications.

Surveys suggest that the incidence of light leaf spot in England and Wales has decreased since 1995 (Fig. 1), as the proportion of crops sprayed in autumn has increased (CSL/ADAS data) (Fig. 5), suggesting that the light leaf spot forecasting scheme has helped to improve farming practice. Ultimately, there is a need to construct a decision support system for integrated management of all major diseases in 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 priorities now must be to obtain accurate biological data about the development of stem canker and other important disease, to construct accurate models to describe these data and then to develop a combined regional risk and crop risk forecast system.

Acknowledgements

This work was funded by the UK Ministry of Agriculture, Fisheries and Food, the Biotechnology and Biological Sciences Research Council and the Home-Grown Cereals Authority. We thank Stewart Souter and Dr John Antoniw for development work of the web pages and CSL/ADAS for providing survey data.

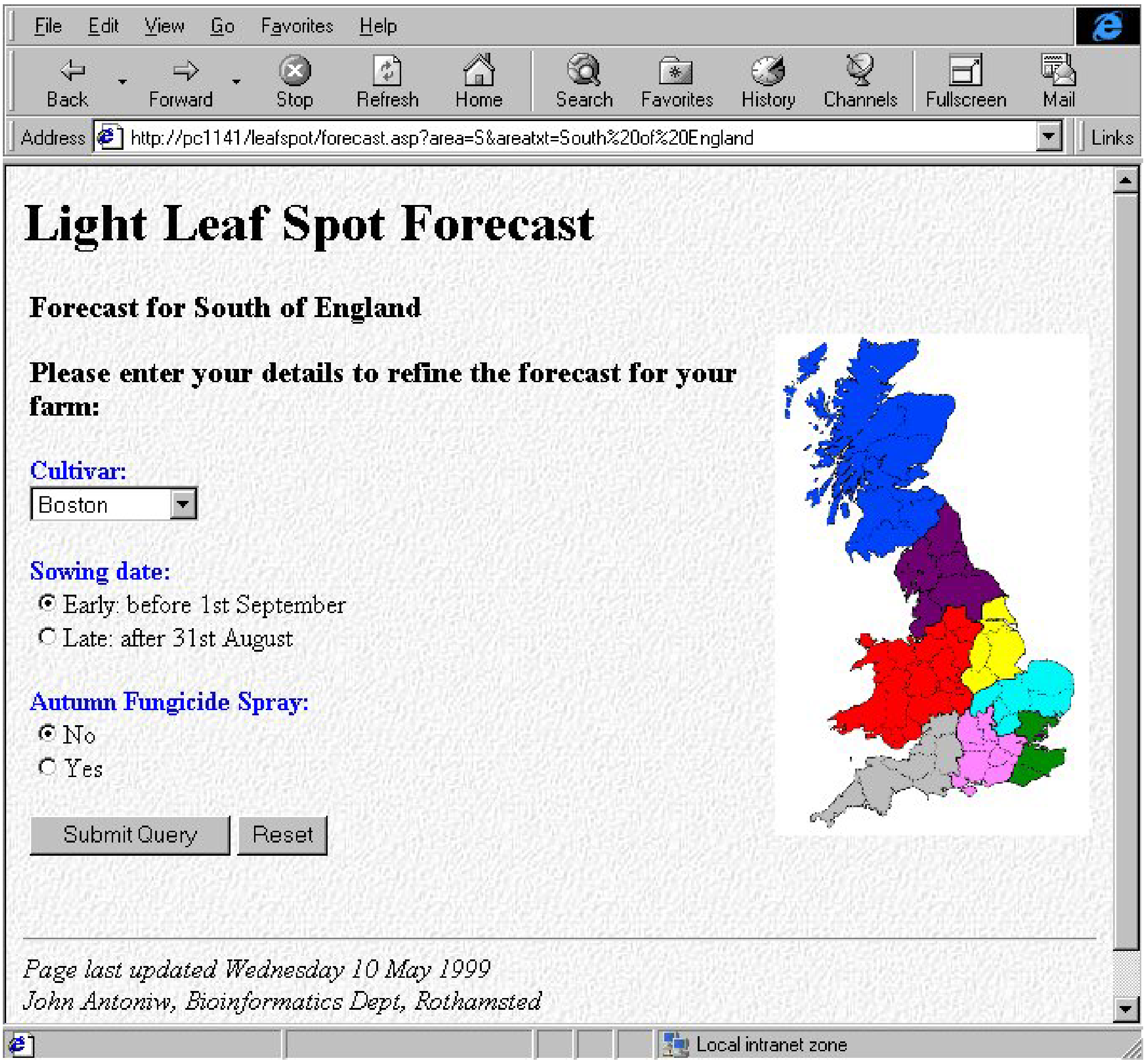

Figure 3. A web-based input page allowing oilseed rape growers to enter cultivar, sowing date and fungicide application information into an interactive model to determine risk assessment of light leaf spot epidemic development.

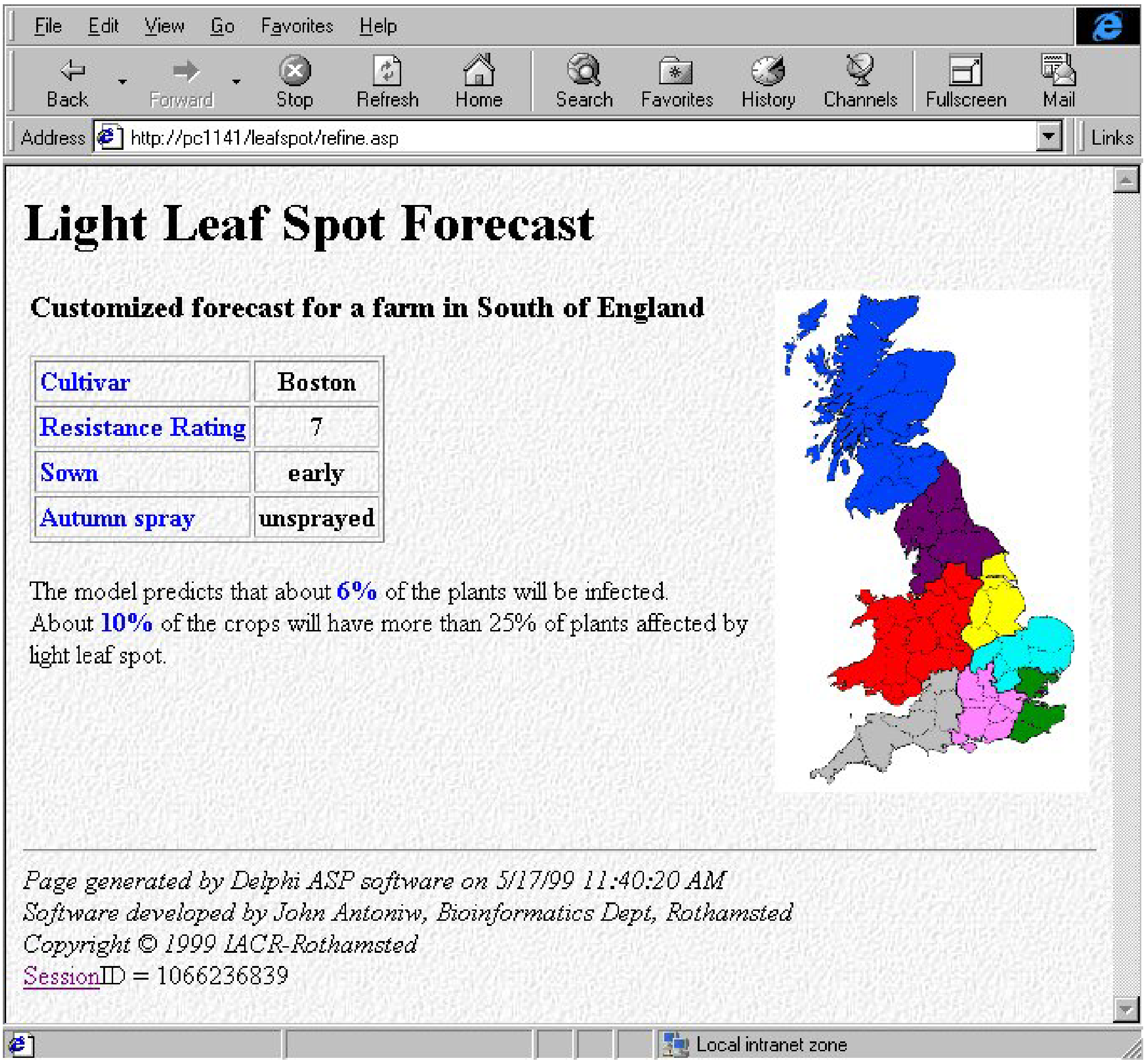

Figure 4. Output page providing an oilseed rape grower with a risk assessment for light leaf spot under the particular parameters on the farm.

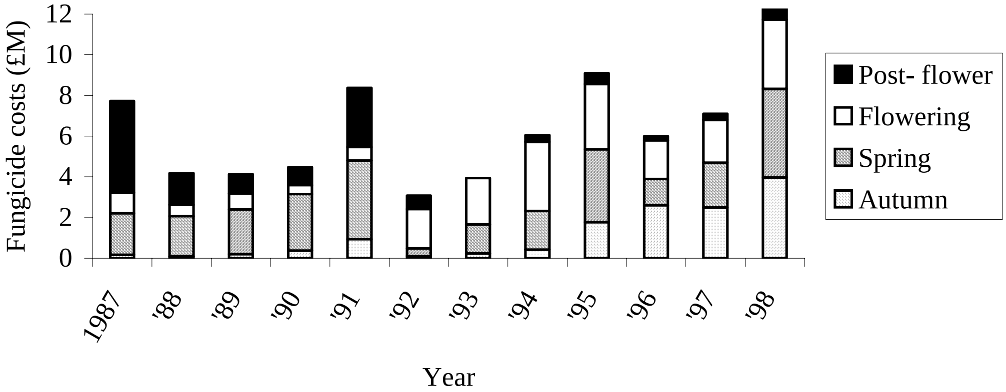

Figure 5. Changes in the pattern of expenditure on fungicides for the control of diseases of winter oilseed rape in the UK (Source CSL/ADAS survey data).

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Monitoring of *Verticillium* in oilseed rape in Germany

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Abstract: Verticillium wilt has been reported to cause disease problems on oilseed rape in Germany since the 1980s. The disease can cause major yield losses with the development of severe epidemics being dependant on crop rotation factors and weather conditions. The identification of *Verticillium longisporum* (syn. *V. dahliae*, var. *longisporum*) is problematic, particularly to the species level and is only possible at the end of the vegetative period. BA-ELISA was used to detect *V. longisporum* in symptomless plant tissue and to quantify the disease attack. In 1996-1998, samples from several locations in Germany were tested for *Verticillium* infections. The occurence of the disease varied between the sites. There was a clear tendency for a high abundance of *Verticillium* in regions with intensive levels of oilseed rape cultivation.

Keywords: oilseed rape, *Verticillium*, microsclerotia, ELISA, monitoring

Introduction

Verticillium longisporum (syn. *V. dahliae*, var. *longisporum*; Karapapa *et al.*, 1997), often connected to *Phoma lingam*, is an important pathogen in oilseed rape causing vascular wilt and "early ripening". Average annual yield losses from the disease are usually in the region of 10 %, but in some cases may be up to 50 %. The most important source of the disease is soil-borne inoculum which is dependant on previous cropping. The first observations of *Verticillium* on oilseed rape in Germany were reported in the 1980s from Schleswig-Holstein and Mecklenburg-Vorpommern (Northern Germany), regions with intensive oilseed rape cultivation (Daebeler *et al.*, 1988, Krüger, 1989).

The current standard techniques used to screen rape plants for *Verticillium* infection are based on symptom development. The disease severity of stems and crop debris is evaluated by assessment of microsclerotia quantity in the plant tissue. These method is open to human error and requires mycological expertise to produce precise results. A new serological test (ELISA) was developed (Cernusko & Wolf, 1997) that can be used for the rapid detection and quantification of *Verticillium* spp. The test has been used for the past three years in Göttingen (1996-1998) to study the occurence of *Verticillium* in oilseed rape plants from different locations of Germany.

Material and methods

Sample preparation

25-30 oilseed rape plants from field trials at growth stages EC 69 and EC 85/87 were collected and air dried. Ground stems (Bottom 20 cm of hypocotyl) were each extracted 1:100 (w/v) overnight with extraction buffer. The resultant supernatant was used in for the ELISA test.

ELISA

The B-A ELISA (Cernusko & Wolf, 1997) was conducted in microtitre plates (Maxisorp U, Nunc) using the following procedure: 100 µl of IgG in coating buffer (Casper & Meyer 1981, Clark & Adams 1977) (1 µg/ml) was incubated overnight. After every step the plates were rinsed 3 times with ½ PBST buffer (PBST = PBS+ 0.05 % Tween 20). Plates were blocked by adding 200 µl coating buffer with 0.2% BSA for 2 hours at room temperature. 100 µl of prepared sample was incubated overnight at 4°C. 100 µl biotinylated IgG fraction (1:1000 dilution) was incubated overnight at 4°C. 100 µl streptavidin-alkaline-phosphatase conjugate (0.01 U, Boehringer Mannheim) was added and incubated for 1 hour at room temperature. 100 µl substrate solution (*p*-nitrophenyl phosphate, 1 mg/ml in substrate buffer) was added. After incubation for 2-4 hours at room temperature, the reaction was quantified by measuring the absorbance of each well at λ=405 nm in a Spectra plate reader. A negative reaction was determinated by $E \leq 0.1$, a positive reaction was determinated by $E \geq 0.2$.

Results and discussion

In the BA-ELISA, the antiserum did not cross-react with mycelial proteins from other fungal pathogens, e.g. *Phoma lingam*, *Sclerotinia sclerotiorum*, *Fusarium* spp., *Alternaria* spp., respectively. Cross reactivity was detected with *Verticillium dahliae*, *V. albo-atrum*, *V. tricorpus*, *V. tenerum*, but not with *Verticillium nigrescens*.

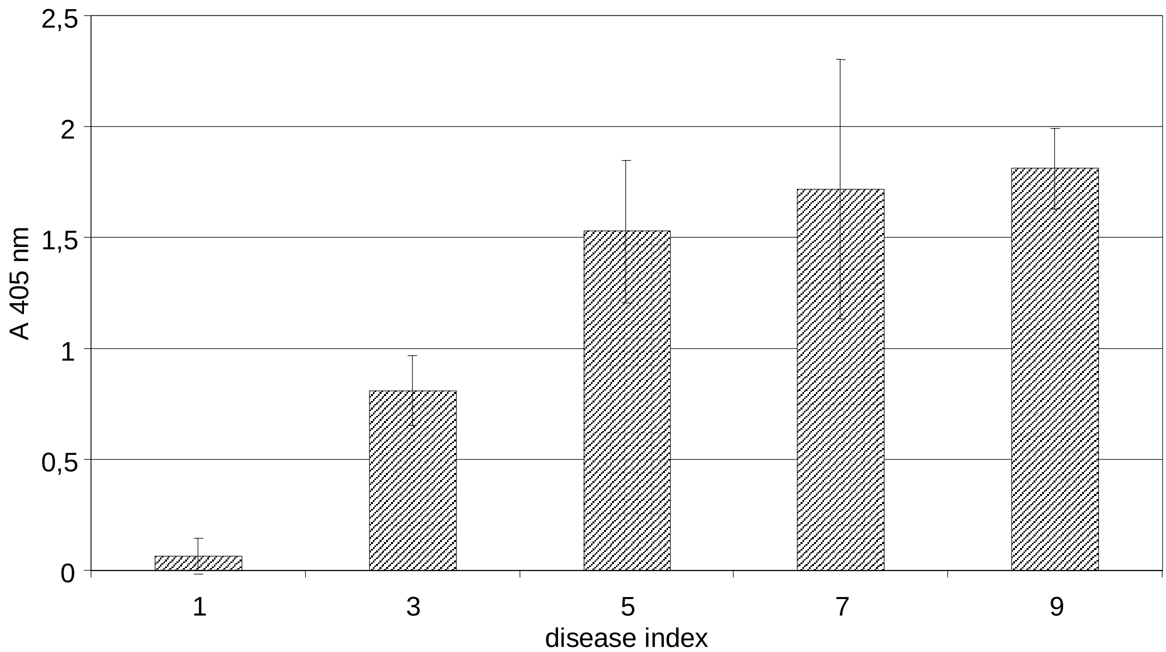

Figure 1. ELISA and optical disease assessment of *Verticillium* in oilseed rape at growth stage EC 69.

The disease severity in plant tissue was estimated with optical disease assessment by microsclerotia quantity in the plant tissue. To evaluate the BA-ELISA the stems were divided into disease index classes from 1 to 9 (1= no microsclerotia) and investigated simultaneously by BA- ELISA (Fig. 1).

Generally, *Verticillium* started a dramatic increase in mycelial growth between oilseed rape development stages EC 69 and EC 85 (Figs.2-4). During this period the amount of fungal mycelium increased rapidly, as can be seen from the ELISA results. There was some variation in *Verticillium* incidence between single plants from the same field (Fig. 2), but the differences between various locations and regions were more significant. The highest level of *Verticillium* infections was observed in Mecklenburg-Vorpommern which represents a typical region within which intensive oilseed rape cultivation has taken place for many years. In addition, the humid climate supports the development of *Verticillium longisporum*. However, *Verticillium* was found in other Federal Lands, especially in Bavaria (Fig. 4), although the infections in these regions have not reached the level of Mecklenburg-Vorpommern.

To prove the influence of soil managment on the disease incidence, three locations in Mecklenburg-Vorpommern were cultivated by plough and cultivator (ripper). Plants from plough treatments showed higher levels of *Verticillium* attack then from cultivator treatments (Fig. 5). This observation contrasts observations on other pathogens such as *Drechslera tritici-repentis* and *Fusarium* in cereals, where ploughing decreases the amount of inoculum.
In the case of *Verticillium* on oilseed rape, the risk of yield losses remains a constant and annual problem, because of the soil-borne inoculum. As the importance of this pathogen depends on crop rotation (cultivation intensity) and climate conditions, a new possible infection parameter is soil managment.

This study was indicates that current problems of *Verticillium* for oilseed rape cultivation in Germany and highlights the need for permanent monitoring of the disease.

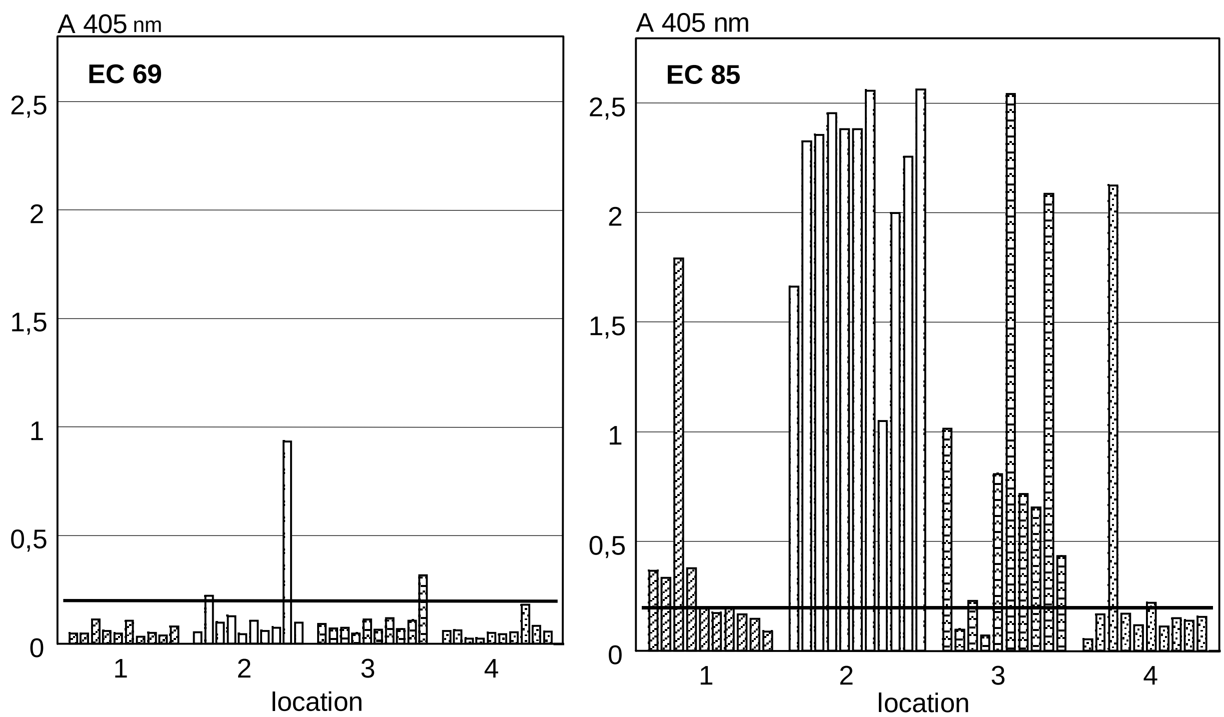

Figure 2. Occurence of *Verticillium* in oilseed rape at growth stages EC 69 and EC 85 in 1996, detected by ELISA (single plants, 4 field trials).

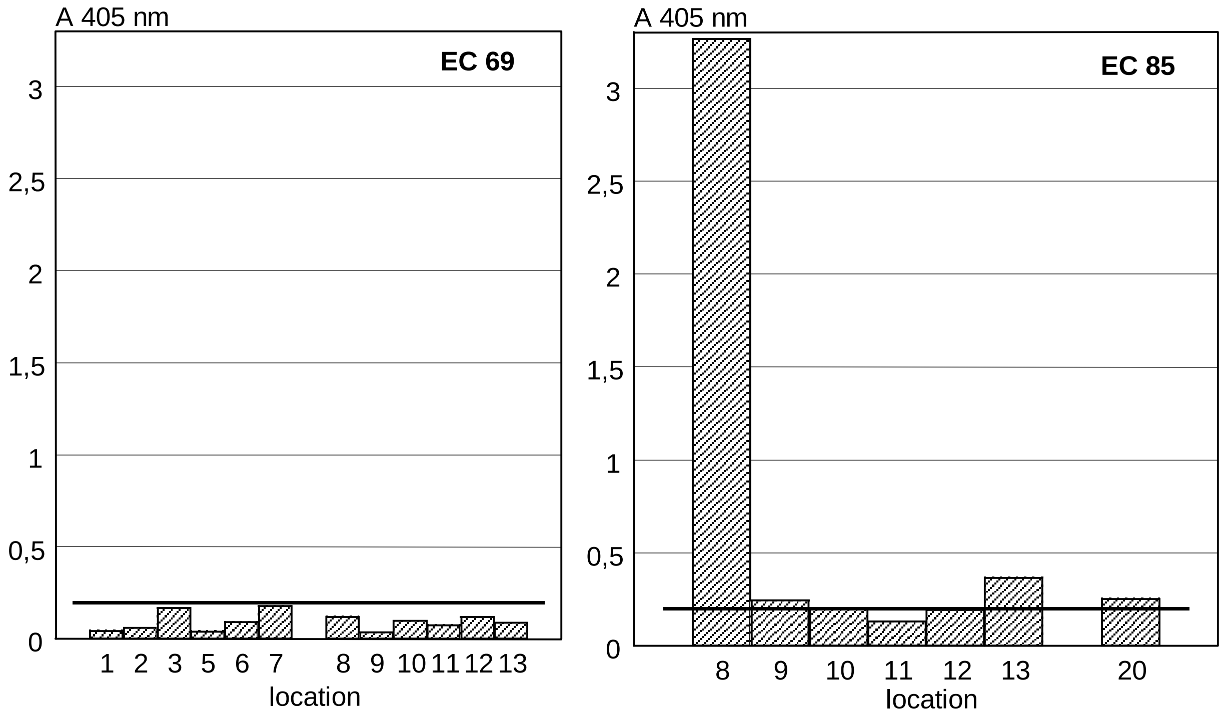

Figure 3. Occurence of Verticillium dahliae in oilseed rape at growth stages EC 69 and EC 85 in 1997, detected by ELISA.

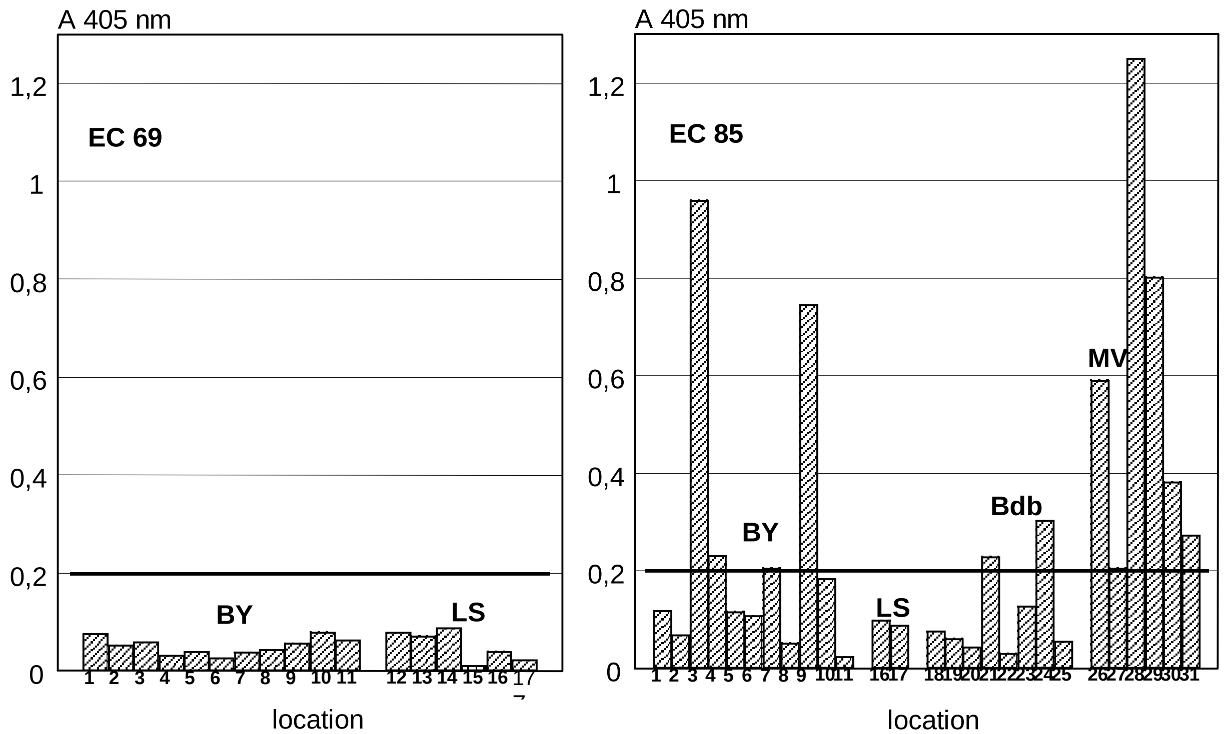

Figure 4. Occurence of *Verticillium* in oilseed rape at growth stages EC 69 and EC 85 in 1998, detected by ELISA (BY= Bavaria, LS= Lower Saxony, Bdb= Brandenburg, MV= Mecklenburg-Vorpommern).

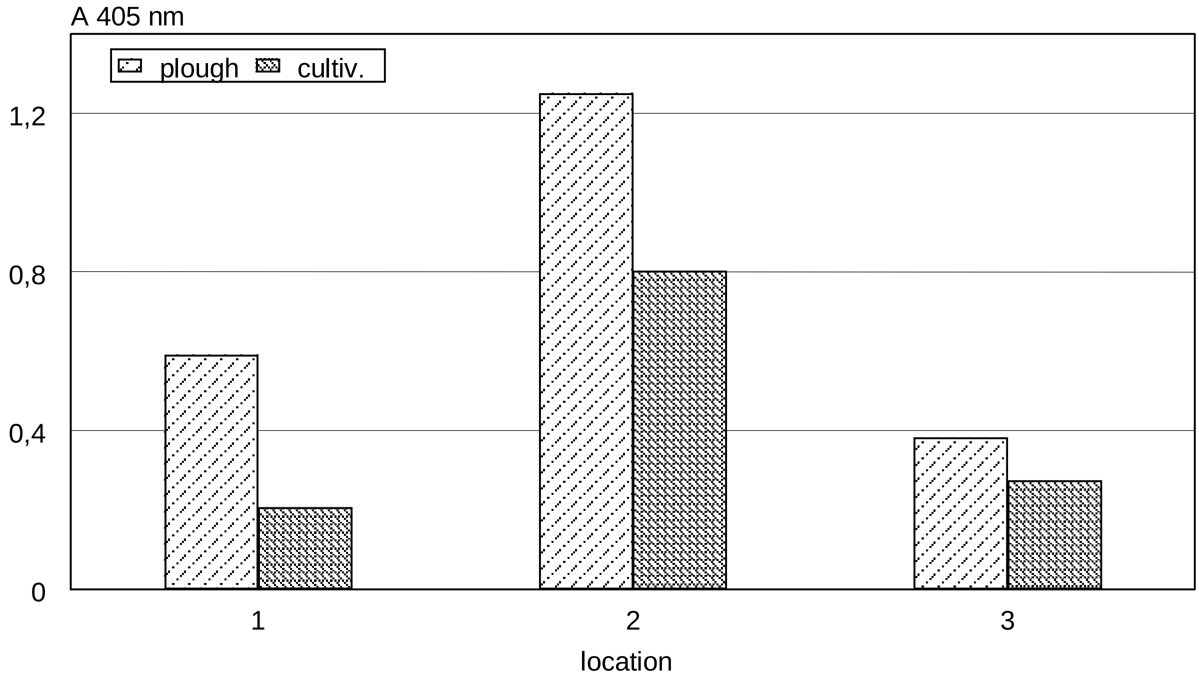

Figure 5. Influence of soil cultivation (plough and cultivator) on *Verticillium* occurence in oilseed rape, growth stage EC 85 (1998, Mecklenburg-Vorpommern).

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Results on the occurrence and seed-borne nature of the new disease bacterial blight (*Pseudomonas syringae* pv. spec) on false flax (*Camelina sativa*)

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Abstract: In the warm summer of 1995, between 30 and 75 % of the false flax grown in different sites of Germany was infected by *Pseudomonas syringae* pv. spec. in the field. In the summers of 1996 and in 1997 between 0 - 68.6 % and 0 - 52 % of the plants were affected, respectively. In 1998 false flax grown on one site in Germany showed an average of 52.2 % affected plants. Seed samples were analysed for infestation with *Pseudomonas syringae* pv. spec. No *Pseudomonas syringae* pv. spec. was found in the seed samples of 1995. From the seed samples of 1996, *Pseudomonas syringae* pv. spec. could only be isolated from 3 seeds of 6000 seeds tested. The infested seeds came from field plots in Merklingsen which was the most severely infested site in 1996.

Key words: False flax (*Camelina sativa*), diseases, Bacterial blight (*Pseudomonas syringae* pv. spec.)

Introduction

Bacterial blight, caused by *Pseudomonas syringae* pv. spec., is a diseasethat was first observed on false flax in Germany in 1995 at two different sites (e.g. Rohrbach [Thüringen] and Merklingsen [Nordrhein-Westfalen]). Bacterial blight can affect leafs, stems and pods of false flax and causes small to elongated black oval lesions. The severity of disease attack differed between seasons and regions and was highest in warm summers.

Since it is known that bacteria can be transmitted by seeds, seed samples of false flax harvested at the different sites in 1995 and 1996 were analysed for infestation by *Pseudomonas syringae* pv. spec in 1997.

Material and methods

Plants

False flax (10 varieties) was grown in ring field trials (Table 1) in the years 1995 to 1998. At the different sites, false flax was assessed for its disease susceptibility at different growth stages. Special attention was paid to a symptom that had not been described in the literature.

Infected plants, which showed small black lesions on leaves and black oval to elongated lesions on stem and pods, were collected from different sites. The isolation of the pathogen responsible for infection was carried out using a number of methods:

1) Infected plant parts were placed in a moist chamber to encourage sporulation.
2) Infected plant parts were surface sterilised (1 min. 70% Isopropanol, 2 min. sodium hypochlorite 3%, 1 min. sterile H_2O) and placed on different fungal nutrient media (with and without antibiotics) and incubated under different environmental conditions.
3) Infected plant parts were examined under a microscope for the detection of conidia or fungal hyphae.

4) Cutting of sterilised stem lesions in a sterile water droplet, squeezing of the tissue and examination under a microscope for bacterial infection.
5) Reinfection with the isolated pathogens to fulfil Koch´s Postulates.

Table 1. Sites and states of the false flax ring field trials from 1995-1998 in Germany.

Site	**State**	**Frequency of bacterial blight in % in:**			
		1995	**1996**	**1997**	**1998**
Merklingsen	Nordrhein-Westfalen	+	+	+	+
Thüle	Nordrhein-Westfalen	+	+	+	-
Kleinmachnow/Dahnsdorf	Brandenburg	+	+	+	-
Rauischholzhausen	Hessen	+	+	+	-
Groß Gerau	Hessen	+	+	+	-
Kritzkow	Mecklenburg-Vorpommern	+	+	-	-
Groß Lüsewitz	Mecklenburg-Vorpommern	+	-	-	-
Rohrbach	Thüringen	+	-	-	-
Thyrow	Mecklenburg-Vorpommern	+	-	-	-
Lübeck	Schleswig-Holstein	-	+	+	-

Seeds
After harvest, seed samples of false flax were obtained from the same sites used for the ring field trials. Seeds were tested for fungal and bacterial infection. One hundred seeds per variety and site were surface sterilised (1 min. 70% Isopropanol, 2 min. sodium hypochlorite 3%, 1 min. sterile H_2O) and placed on King's B Medium. Plates were incubated at room temperature for 4-5 days.

Media

PDA	**SNA**	**YDC**	**KINGS B (KB)**
39 g PDA (Merck)	KH_2PO_4 1 g	Hefeextrakt 5g	33,5 g KB-Agar
1000 ml dist. water.	KNO_3 1 g	Glucose 10g	(Merck)
	$MgSO_4$ x 7 H_2O 0,5 g	$CaCO_3$ 10g	10g Glycerol (Merck)
	KCl 0,5 g	Agar 8g	1000 ml dist. water.
	Glucose 0,2 g	500 ml dist. water	
	Sucrose 0,2 g		
	Agar 15-17 g		

Bacteria

The bacteria isolated were identified following Schaad's (1988) identification chart (Fig. 1) and the LOPAT diagnostic scheme (Lelliott et al., 1966) as shown in Table 2 and Table 3.

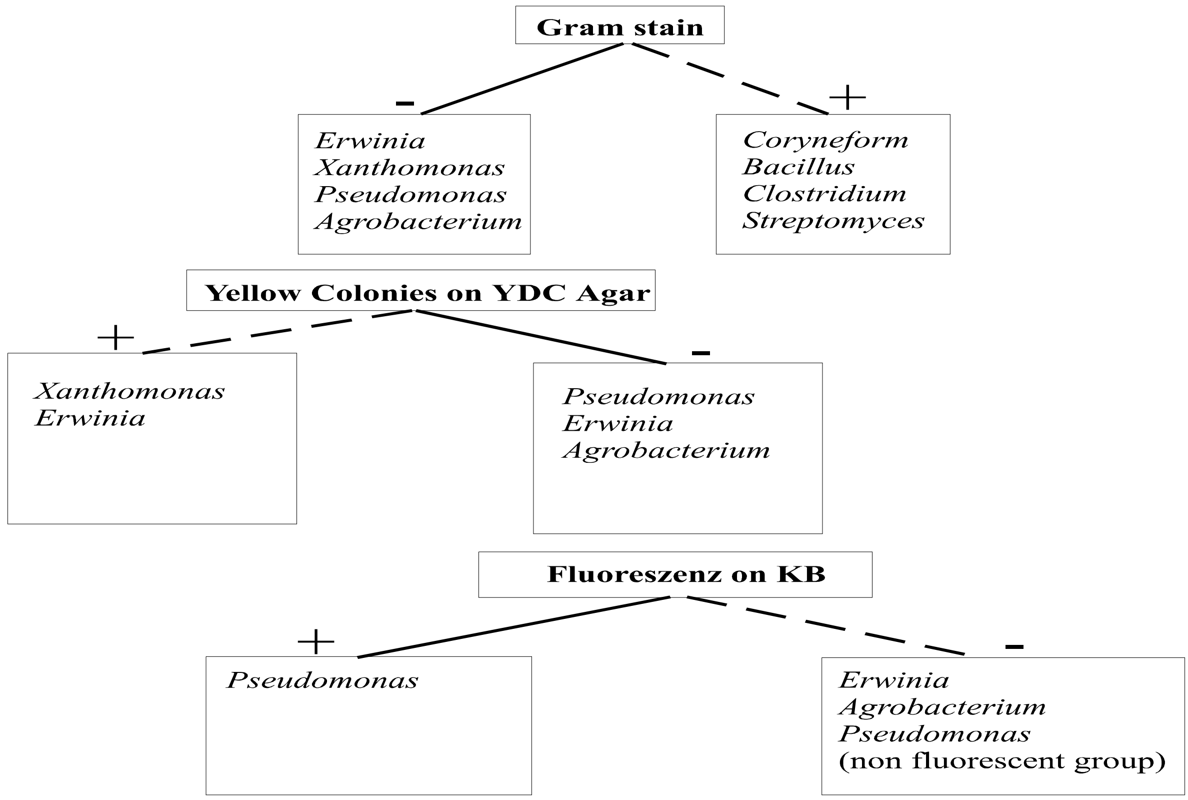

Figure 1. Identification Chart for Bacteria (Schaad, 1988)

Table 2. LOPAT diagnostic scheme for plant pathogenic green fluorescent *Pseudomonas* Species (Lelliott et al., 1966).

Method	Description
L evan	Levan formation is detected on nutrient agar to which 5% sucrose (w/v) has been added. The presence of convex, white mucoid colonies after 3 to 5 days incubation is indicative of levan formation
O xidase	Kovac´s oxidase test is particularly important for differentiation of Gram-negative bacteria and especially useful for Pseudomonas species. *Pseudomonas syringae* pathovars give a negative reaction whereas the saprophytic species are positive
P otato rot	The production of a soft rot in potato slices is used for differentiating soft rot erwinias and pseudomonads from their relatives which lack such capacity like *Pseudomonas syringae*
A rginine dihydrolase	The arginine dihydrolase enzyme system permits certain pseudomonads to grow under anaerobic conditions. The test is mainly of importance for the differentiation of *Pseudomonas* spp.
T obacco hypersensitive reaction (HR)	Determination of a hypersensitive reaction in tobacco leaves is a rapid means of differentiating between the *P. syringae* pathovars, which, except for pv. *tabaci*, give a positive reaction, from the saprophytic which usually give a negative reaction

Table 3. Identification of plant pathogenic green fluorescent *Pseudomonas* spp. (Lelliott, 1966).

Group	**LOPAT characteristics**					**Species**
	Levan	Oxidase	potato rot	Arginine dihydro-lase	HR	
1a	+	-	-	-	+	***P. syringae* pathovars**
1b	-	-	-	-	+	***P. syringae* pv.**[1)]
2	-	-	+	-	+	*P. viridiflava*
3	-	+	-	-	+	*P. cichorii, P. agarici*
4a	+	+	+	+	-	*P. marginalis*
4b	-	+	+	+	-	*P. fluorescens*
5a	-	+	-	+	-	*P. tolaasii* [2)]
5b	+	+	-	+	-	*P. fluorescens* and other saprophytic *Pseudomonas*

[1)] *savastanoi*, *P.delphinii*
[2)] and some other saprophytic *Pseudomonas*
+ = at least 80 % of the strains positive; - = at least 80% min. 80 % of the strains negative

Results

Field trial

A very high infestation with *Pseudomonas syringae* pv. spec. was found in the 1995 field trials. The infestation levels ranged from 30 % of plants infected at Rauischholzhausen to 75 % of plants infected at Rohrbach (Table 4). In 1996, generally infestation level were lower with ≤1 % of plants infected at Kleinmachnow, Rauischholzhausen, Groß Gerau and Lübeck. The highest infestation level observed was found at Merklingsen where 68.6 % of plants were infected. In 1997 the lowest infestation level observed was 2.6 % of plants infected (Thüle and Lübeck) whilst the highest infestation level was 52.0 % of plants infected at Rauischholzhausen. In 1998, false flax was only grown at one site. Here the bacterial blight infestation level was 52.2 % of plants infected (Table 4).

Table 4. Occurrence of bacterial blight (*Pseudomonas syringae* pv. spec.) at different sites in Germany in 1995-1998.

Site	**State**	**Frequency of bacterial blight in % in:**			
		1995	**1996**	**1997**	**1998**
Merklingsen	Nordrhein-Westfalen	59	68.6	14.7	52.2
Thüle	Nordrhein-Westfalen	42	3.4	2.7	-
Kleinmachnow/Dahnsdorf	Brandenburg	65	1	7.3	-
Rauischholzhausen	Hessen	30	1	52.0	-
Groß Gerau	Hessen	37	<1	29.3	-
Kritzkow	Mecklenburg-Vorpommern	44	37	-	-
Groß Lüswitz	Mecklenburg-Vorpommern	45	-	-	-
Rohrbach	Thüringen	75	-	-	-
Thyrow	Mecklenburg-Vorpommern	47	-	-	-
Lübeck	Schleswig-Holstein	-	<1	2.6	-

Bacteria

Using Schaad's (1988) identification chart for bacteria as shown in Figure 1, the bacteria isolated from *C. sativa* was identified as *Pseudomonas* sp., since it was gram negative, had no yellow colonies on YDC Agar and showed fluorescence on KB. Further tests, following the Lopat diagnostic scheme from Lelliott et al. (1966), indicated that the bacteria was Levan positive, Oxidase negative, Potato rot negative, Arginine dihydrolase negative and HR positive (Figure 1, Table 2, and Table 3). These results indicate that the isolated bacteria was therefore *Pseudomonas syringae* pv. spec. The fist taxonomic identification of this P. syringae pv. spec. was carried out by Prof. Rudolph and Dr. Mavrides from Göttingen and the results were later confirmed by Prof. Tewari (Canada) and by the CBS (Netherlands).

Plants and Seeds

From the infected plants and from three seeds out of 14.000 analysed, (6.000 seeds in 1996 and 8.000 seeds in 1995) bacteria could be isolated and these were identified as *Pseudomonas syringae* pv. spec. (s. a.)

The three infected seeds came from the site Merklingsen (1996) which, in 1996, had the highest infection level of all of the field sites (68.6 % of plants infected) (Table 5).

Table 5. Occurrence of pathogens in seed samples of 10 false flax cultivars/breeding lines (*Camelina sativa*) from different sites in 1996 (infestation per 100 seeds/variety).

Site	Groß-Gerau		Rauisch-holzhausen		Merk-lingsen		Klein-machnow		Kritzkow		Lübeck	
Variety	*P.sy.* *	Fungi #	*P.sy.* *	Fungi #	*P.sy.* *	Fungi #	*P.sy.* *	Fungi #	*P.sy.* *	Fungi #	*P.sy.* *	Fungi #
1	0	0	0	0	0	0	0	2	0	0	0	0
2	0	0	0	0	0	0	0	4	0	0	0	0
3	0	0	0	0	0	0	0	1	0	0	0	0
4	0	0	0	0	0	0	0	3	0	0	0	0
5	0	1	0	0	0	0	0	5	0	0	0	1
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	**2**	0	0	1	0	0	0	0
8	0	0	0	2	0	0	0	1	0	0	0	0
9	0	0	0	0	**1**	0	0	1	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
in the field (%)	**<1**		**1**		**68.6**		**1**		**37**		**<1**	

* *P. sy.* = *Pseudomonas syringae* pv. spec.

\# Fungi = different genera and species

Discussion

This paper is the first report of the identification of a new bacteria, isolated from false flax. In addition, Koch's postulates were fulfilled and the seed borne nature of the *Pseudomonas syringae* pv. spec. was also established.

The levels of seed infection were very low in most cases, with seeds from only one of the field sites studied, (Merklingsen 1996) having an infestation level of 0.3 %. In comparison to peas, were an infestation level of 0.1 % can lead to an epidemic (Holloway et al., 1996), the 0.3 % observed for *C. sativa* would appear to be very high. This result is interesting, since the *Pseudomonas syringae* isolates of false flax have a high physiological-biochemical affinity to *Pseudomonas syringae* pv. *pisi* (Mavrides et al. 1998) and this would suggest that an infestation level of 0.3 % infected seeds could lead to the onset of an epidemic in the case of *C. sativa*.

The source of the primary infection of false flax is unknown. An investigation with cross inoculations (Mavrides et al. 1998) with different Brassica species (shepherd's purse [*Capsella bursa-pastoris*]; field pennicress [*Thlaspi arvensis*]; mustard species [*Sinapis* sp.] and oilseed rape [*Brassica napus* ssp. *napus*]) showed that no cross infection took place, whereas cross inoculations with different *Camelina* sp. lead to an infection. Therefore two inoculum sources for primary infections are conceivable, the most probable source being infected seeds, but infection from wild forms of *Camelina* are also a possibility.

Another point of interest is the fact that no *Pseudomonas syringae* could be isolated from seed samples from the 1995 season although a high infestation level was observed in the field. Since all seed samples were analysed in 1997, seed samples from 1995 and 1996 were stored in paper bags at room temperate for the interim period of two years. It could be the case that the bacteria were unable to survive under these storage conditions. Neergard et al. (1977) reported that bacteria located on the seed surface can only survive for a period of 1-2 years and, as such, the results of this study suggest that a closer investigation of the location of *Pseudomonas syringae* on false flax seeds is needed.

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Inhibition of oospore development in *Albugo candida* by *Pseudomonas syringae*

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Abstract: *Albugo candida* causes the white rust and staghead disease in several economically important cruciferous crops, including the oil-yielding crops such as *Brassica rapa* (rapeseed, Polish canola or turnip rape) and *B. juncea* (brown or oriental mustard). The stagheads (malformed inflorescence axes) contain most of the overwintering/survival spores, the oospores, produced by this pathogen. The bacterium, *Pseudomonas syringae*, causes a soft rot disease preferentially of the green developing stagheads, under field conditions in Canada on *B. rapa* and in India on *B. juncea*, leading to abortion of the developing oospores. This natural biological control of *A. candida* may regulate its overwintering population in the field and may have implications towards developing planned biological control strategies for this pathogen.

Key words: *Albugo candida*, *Pseudomonas syringae*, biological control, canola, *Brassica rapa*, mustard, *B. juncea*

Introduction

Substantial yield losses in *Brassica* crops have been reported as a result of *Albugo candida* infection (Saharan & Verma, 1992). The disease is of special concern on *Raphanus sativus*, *Brassica juncea*, and *B. rapa* in seed production. The staghead phase accounts for most of the yield losses. It also accounts for most of the oospore production responsible for perennation of the pathogen. Many studies have reported that *Peronospora parasitica* preferentially colonizes plant tissues infected with *A. candida* (Saharan & Verma, 1992). Also, many necrotrophic secondary-invading fungi are found growing on plant tissues infected by *A. candida* (Petrie & Vanterpool, 1974).

Host resistance, cultural, and chemical control measures are used for reducing the severity of disease caused by *A. candida* (Saharan & Verma, 1992; Kharbanda & Tewari, 1996). In spite of all these efforts, the disease caused by *A. candida* continues to be rampant in many parts of the world. The pathogen is variable and new races infecting previously resistant cultivars, have been reported in the recent past (Petrie, 1994). There is a need to develop further control methods so that an integrated disease control program could be implemented.

A study of microbial interactions under field conditions can provide information on strategies that result in regulation of pathogen populations in Nature. These could then be developed into biological control strategies to supplement other control measures in an integrated disease management program. With this approach in mind, a bacterial disease of the stagheads caused by *A. candida* on *Brassica* spp. was studied and the results are reported here. A preliminary report on this work has been presented (Tewari *et al*., 1998).

Materials and methods

Stagheads caused by *A. candida* on *B. rapa* (rapeseed, Polish canola, or turnip rape) under field conditions were collected and studied in Alberta, Canada starting from the summer of 1974. Stagheads on *B. juncea* (brown or oriental mustard) were collected during January, 1998 from New Delhi, India.

Particular emphasis was placed on collecting stagheads with a bacterial disease. The bacterial exudate was streaked on nutrient agar plates which were incubated at room temperature (about 22° C). Different kinds of bacteria were isolated and grown in pure culture. Young (succulent and green) stagheads without bacterial infection were collected from the field and placed in 250 ml flasks with their stalks dipped in distilled water. Wooden tooth picks were soaked in ethyl alcohol overnight and dried. The bacteria were picked-up from the nutrient agar with tooth picks which were then inserted in stagheads to depths of about 2 mm. Each staghead was inoculated with bacteria at two locations, about 3 cm apart. The flasks were placed in the greenhouse, with the stagheads kept covered with polyethylene bags for 48 h. The inoculated stagheads were observed after 4-5 days. Each kind of bacterium was inoculated on 3 stagheads. Detached green fruits of *B. rapa* (cultivar Torch) and *B. napus* (cultivars Midas and Tower) were also inoculated similarly and placed in petri dishes on moist filter paper. Proper controls were run in all cases. The bacterium causing disease in the stagheads was kindly identified by Mr. Jim Letal of Alberta Agriculture, Food, and Rural Development, Government of Alberta, Edmonton, Alberta, Canada.

Discs cut from younger (succulent and green) and older (hard and light green) stagheads with and without bacterial infection were fixed in form-acetic alcohol. These materials were routinely dehydrated, embedded in paraffin wax, sectioned using a rotary microtome, and stained with safranin and fast green.

Results

Stagheads on *B. rapa* showing a soft rot disease were collected every year from fields in Alberta, Canada since 1974. Similar specmens were collected from New Delhi, India on *B. juncea* during January, 1998.

Symptoms of bacterial infection on younger (green and succulent) stagheads on *B. rapa* included extensive water soaking, soft rotting, and bacterial exudation. Stagheads which were not infected with bacteria remained plump but those infected with bacteria dried-up and became shrunken as they became older. The older (hard and light green) stagheads infected with bacteria showed only limited lesioning and no soft rotting.

Two kinds of bacteria were isolated on nutrient agar plates from the infected stagheads. One had milky white colonies and the other had dull white colonies. Only the former bacterium caused symptoms in stagheads on *B. rapa* in inoculation experiments and was identified as *Pseudomonas syringae*. This bacterium also caused soft rot symptoms on the green fruits of *B. rapa* and *B. napus* upon inoculation. Koch's postulates were satisfied in both sets of experiments.

Sections of younger stagheads on *B. rapa* infected with *P. syringae* showed extensive growth of bacteria and maceration of tissues. Many young oogonia and antheridia were present but they appeared to be degenerating. The bacterium appeared to be affecting only the host cells directly, as the cell walls of fungal structures were apparently intact. The abortion of antheridia and oogonia was, therefore, caused indirectly as the host tissue was degenerating.

Sections of older stagheads on *B. rapa* infected with *P. syringae* revealed much less distortion of the host tissue and had morphologically normal oospores. It seemed that the oospores were already well mature before the bacterial infection took place.

Sections of both the younger and older stagheads not infected with bacteria showed normal antheridia/oogonia and oospores, respectively.

Discussion

The soft rot disease of stagheads on *B. rapa* is widespread and was consistently found in Alberta, Canada every year since 1974. This disease was also found on stagheads on *B. juncea* in New Delhi, India in 1998. The inoculation experiments proved that *P. syringae* was causing this disease of stagheads caused by *A. candida*.

Examination of the sections of younger stagheads infected with bacteria revealed abortion of oogonia and antheridia indicating that *P. syringae* may be causing reduction in numbers of oospores of *A. candida* under field conditions. This is an example of regulation of pathogen population under field conditions through a "natural enemy" (DeBach & Rosen, 1991). There are many examples of direct regulation of plant pathogen populations by activities of other biotic agents through phenomenon such as hypovirulence and hyperparasitism. However, the system reported here is unique in regulation of an obligate pathogen population through the effect of another microbe on their common host.

Pseudomonas syringae is also a pathogen of *B. rapa* and *B. napus* (Henry & Letal, 1977), hence this bacterium cannot be used as a biological control agent for *A. candida*. However, field infections by *P. syringae* are not common in Alberta, Canada and are noticeable only on some older cultivars of *B. rapa* such as R-500 (similar to the var. *yellow sarson* from India) which is a high erucic acid oil cultivar grown only on contract. In view of the field tolerance of the currently-grown *Brassica* cultivars in Canada, field trials should be conducted to ascertain the potential usefulness of *P. syringae* in the biological control of *A. candida*.

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Traditional and innovative methods to detect seed-transmitted pathogens in linseed and rape

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Abstract: The quantities of linseed (*Linum usitatissimum* L.) and rape (*Brassica napus* ssp. *oleifera* D.C.) seeds exchanged between countries has greatly increased and this increases the risks of introducing new pathogens or races to disease-free areas if infected or contaminated seeds are exchanged. The use of healthy seeds, produced under certification programs, is therefore considered a prerequisite for success in efficient crop production. Seed health testing of seed lots, carried out with suitable methods, can play a very important role in Integrated Pest Management. Although some traditional and well developed tests are available to detect viruses, bacteria and fungi in linseed and rape seed lots, the new emerging techniques based on DNA analysis (e.g. polymerase chain reaction) could be developed and used as alternative methods. These techniques have the potential advantages of greater speed and specificity as well as being more amenable to automation. However, before applying them on a large scale, it is necessary to compare the results obtained on the same sub-samples in different laboratories. As the twentieth century draws to a close, it is very important to underline that seed health and quality has become increasingly important with regard to the marketing of seed products throughout the world.

Key words: integrated control, linseed, oilseed rape, seed pathology

Introduction

The increasing importation of seed and other propagative materials heightens the risk of the introduction of new plant pathogens or races to disease-free areas. Seed-transmitted pathogens, which for many diseases often represent the primary source of inoculum, will increase in economic importance during the next century, mainly because markets have become increasingly globalised as trade barriers have been removed. When pathogens are introduced into an area, they can survive in alternative sites, such as soil, weeds and other susceptible hosts. Weeds, in particular, may act as a reservoir of primary inoculum and perpetuate the pathogen through their own seed; they also act as "green bridges" for the infection of industrial crops with important biotrophic pathogens. The diffusion of new pathogens and/or new races can also occur when seed imported for experimental purposes (i.e. breeding programmes, research), are infected/contaminated and are sown in open fields without containment.

The use of pathogen-free seed lots is therefore a fundamental strategy in Integrated Pest Management and the spread of seed-borne pathogens must be reduced or avoided in new areas where susceptible crops are grown (Neergaard, 1979).

Important pathogens are known to be seed-transmitted in different oilseed crops. For oilseed rape (*Brassica napus* ssp. *oleifera* D.C.) and linseed (*Linum usitatissimum* L.) the most frequent and dangerous pathogens are catalogued by Richardson (1990) and summarised in Table 1.

Table 1. Seed transmitted-pathogens of linseed and rape.

Pathogen	Disease
Linseed	
Alternaria linicola Groves & Skolko	Alternaria blight (Malone, 1982a; WS n. 46)
Botrytis cinerea Pers. *ex* Pers.	Grey mould (Anselme and Champion, 1981; WS n.10)
Colletotrichum lini (Westerd.) Tochinai	Anthracnose
Fusarium oxysporum f. sp. *lini* (Bolley) Snyder & Hansen	Fusarium wilt
Guignardia fulvida Sanderson	Stem break
Mycosphaerella linicola Naum.	Pasmo
Phoma exigua var. *linicola* (Naum. & Vass.) Maas	Foot rot (Malone, 1982b; WS n. 47)
Sclerotinia sclerotiorum Lib. De Bary	Sclerotinia disease
Verticillium dahliae Kleb.	Verticillium wilt
Rape	
Alternaria brassicae (Berk.) Sacc.	Grey leaf spot (ISTA, 1966b)
Alternaria brassicicola (Schw.) Wilts.	Black spot (ISTA, 1966a)
Botrytis cinerea Pers. *ex* Pers.	Grey mould
Phoma lingam (Tode *ex* Schw.) Desm.	Blackleg (ISTA, 1993)
Rhizoctonia solani Khün	Damping off
Sclerotinia sclerotiorum (Lib.) De Bary	Watery soft rot
Verticillium dahliae Kleb.	Verticillium wilt
Xanthomonas campestris pv. *campestris* (Pammel) Dowson	Black rot (Schaad, 1982a;WS n. 50)

Traditional methods are available to detect the above mentioned pathogens in seed lots. However, in several public and private laboratories, some innovative seed tests have been developed, often based on biotechnological techniques, which detect traces of inoculum and the location of the inoculum in the seed. To date, protocols for the novel tests have not been described in the literature.

The importance of seed-transmitted pathogens of linseed and oilseed rape were emphasized at the previous Meeting of the Working Group of IOBC/WPRS (International Organisation for Biological Control of Noxious Animals and Plants West Paleartic Regional Section) (Cappelli, 1995; Cappelli *et al.*, 1998; Fitt *et al.*, 1998). For this reason, this paper highlights the advantages and disadvantages of traditional and innovative methods for the detection of pathogens in seed, including those currently used for oilseed rape and linseed and those that could be used in the future.

Traditional methods

Traditional seed health tests, the majority of which are listed in Table 2, have been used for many years to detect viruses, bacteria and fungi in seed lots. Although the tests can be very effective and appropiate, in some contexts they have a number of disadvantages. Incubation tests and assays based on semi-selective agar media which are commonly used for detecting seedborne fungi and bacteria, are time and space consuming and require expert technicians for pathogen identification. In addition, the target pathogen may be obscured by other microorganisms growing on seeds or agar plates. Serological tests commonly used for detecting viruses and bacteria, although often quite useful for rapid testing, can provide false positives and false negatives and do not distinguish vital from non vital inocula. Antisera can be too specific and sometimes do not react with all strains of the pathogen or are not specific enough and cross-react with other organisms.

Traditional tests are also used for detecting seedborne pathogens in linseed and oilseed rape seed lots. For these pathogens, ISTA's Plant Disease Committee (PDC), has standardised specific routine methods, some of which are available as ISTA's Working sheets or have been reported in other ISTA publications (Table 1). For pathogens for which a working sheet has not been established, many methods can still be used as they have been reported in the international literature.

Table 2. List of routine methods used to detect seed-transmitted pathogens

Methods	**Viruses**	**Bacteria**	**Fungi**	**Reference**
1 - Direct inspection	X	X	X	De Tempe and Binnerts, 1979
2 - Washing tests			X	Neergaard, 1979
3 - Incubation methods				
Agar plate			X	Anselme and Champion, 1981
Agar plate selective media		X	X	Schaad, 1982b
Blotter			X	Neergaard, 1979
2,4 D blotter			X	Neergaard, 1979
Freezing blotter			X	Limonard, 1968
Rolled blotter			X	De Tempe and Binnerts, 1979
4 - Growing-on tests	X	X	X	De Tempe and Binnerts, 1979
5 - Histopathological or staining tests		X	X	De Tempe and Binnerts, 1979
6 - Serological methods				
Immunofluorescence		X		Schaad *et al.*, 1990
Immuno Sorbent Electron Microscopy	X			Derrick, 1973
Immunoblot			X	Gwinn *et al.*, 1991
Enzyme Linked Immuno-Sorbent Assay	X	X	X	Clark and Adams, 1977

Within the ISTA-PDC, virology, bacteriology and mycology working groups have been created with the aim of planning and performing comparative tests for a number of host-pathogen combinations, to revise current methods and to prepare new working sheets. These groups are also working on oilseed rape and linseed pathogens, such as *Alternaria* *brassicicola* (Schw.) Wilts., *Colletotrichum lini* (Westerd.) Tochinai, *Phoma lingam* (Tode *ex* Schw.) Desm. and *Xanthomonas campestris pv. campestris* (Pammel) Dowson.

Innovative methods

Over the last decade, DNA-based techniques have increasingly been used in seed health testing. These techniques have the potential advantages of greater speed and specificity as well as being more amenable to automation. Among them, the most powerful is the polymerase chain reaction (PCR), which relies on two specific DNA primers, a thermostable DNA polymerase and temperature cycling to amplify discrete regions of DNA. By amplifying the DNA of a target pathogen several million times, it is possible to detect the DNA from a single pathogen cell. PCR has successfully been used to detect seedborne fungal, bacterial and viral pathogens (see Hutchins and Reeves, 1997). A number of different approaches have been used for designing primers, the key step of PCR. To detect *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young *et al.* in bean seeds, Prosen *et al*. (1993) designed primers which amplified a fragment within the biosynthetic gene cluster of phaseolotoxin, a toxin specifically produced by this bacterium. Amplification of species-specific fungal mitochondrial DNA sequence have been used to detect teliospores of *Tilletia indica* Mitra and *Tilletia barclayana* (Bref.) Sacc and Sydow contaminating wheat and rice seed, respectively (Ferreira *et al*., 1996; Smith *et al*., 1996). Internal transcribed space sequences within the rDNA cluster have been used to design primers for detecting *Xanthomonas oryzae* pv. *oryzae* in rice seed (Alvarez *et al*., 1997). A highly conserved motif in coat protein was used to design specific primers to detect Lettuce Mosaic Virus in lettuce seed (Van der Vlugt *et al.*, 1997).

There are a number of problems with PCR when samples extracted from seed are used. The greatest problem is the presence of PCR inhibitors in plant samples, that can cause false negatives. Another problem is that PCR lacks sensitivity simply due to the use of such a small sample size (10-20 µl) as well as the problem that it is not possible to distinguish vital from non vital inocula. To overcome these problems a highly sensitive PCR technique, named BIO-PCR, was developed for fungal and bacterial pathogens (Schaad *et al.,* 1995). Aliquots of the soaking liquid obtained from seed during the extraction of the target organism are plated on generic agar media suitable for fungi or bacteria. PCR is then carried out using DNA extracted from the microbial growth. As few as 20 cells per ml of original seed washing can be detected. For viral seedborne pathogens, some of the above mentioned problems associated with PCR have been overcome using immunocapture PCR (Nolasco *et al.,* 1993). Briefly, this technique consists in trapping viral particles on the wall of an antiserum-coated tube and plant extracts are removed by washing. PCR is performed after decapsidation of the viral particles and reverse trascription in the case of RNA-viruses.

To the best of our knowledge, innovative procedures such as PCR have never been developed to detect pathogens in linseed and oilseed rape seeds. Among the different strategies, random amplified polymorphic DNAs (RAPDs) could be used to identify DNA fragments specific to the pathogens contaminating or infecting linseed or oilseed rape seed. Subsequently, bands identified in RAPD profiles which are specific for the target organism can be sequenced and primers may be designed to amplify the DNA fragment by PCR. To detect *Xanthomonas campestris* pv. *campestris* in oilseed rape seed, PCR could be used with primers that amplify DNA fragments of the *hrp* gene cluster (Leite *et al*., 1995).

Another objective for the future is the development of the multiplex PCR, that is a test in which more than one pathogen can be detected from one seed extraction sample.

Conclusion

Several plant diseases are caused by seed-transmitted pathogens including a number of those affecting linseed and oilseed rape. For this reason, the use of healthy seed is considered a prerequisite for successful and efficient crop production.

For the efficient production of healthy seed, seed tests must be simple, quick, inexpensive, reproducible within a laboratory and between laboratories, not dangerous to the health of technicians and they also must provide information on plant performance in the field. Although it is important in practice to detect only the amount of vital inoculum present in seed lots, from which an epidemic disease can start, many traditional and innovative methods do not distinguish vital from non-vital inoculum. Another disadvantage of many seed tests is that they do not provide information on inoculum location within the seed, which is very important for the majority of seedborne viral diseases. For these diseases the transmission of the infection from seed to plant is assured only when the virus is located in the embryos.

One of the major problems in the international phytosanitary system is the lack of standardized seed health tests. This has generated serious problems for the seed industry since importing and exporting countries, using different protocols to carry out a test, provide different results. For each host-parasite combination, it is therefore necessary: i) to select a standard seed health method, which must be internationally accepted and approved, ii) to understand the sensitivity and limitations of this test and iii) to determine the minimum inoculum amount in seeds that can generate disease in plants grown from those seeds.

It is to be hoped that more advanced seed health methods, especially those based on DNA techniques, will be developed to directly detect the presence of only vital pathogen cells in seeds or to identify and characterize the pathogens extracted from seeds at the intraspecific level (formae speciales, pathovar, race).

To reduce the risk of using seed lots infected or contaminated at low levels, it is necessary to reproduce seeds under certification programs, which, besides field inspection of seed crops to reveal disease symptoms, could include the detection of pathogens in symptomless plants using sensitive diagnostic tests.

For polycyclic plant diseases, in particular, healthy seed can be conveniently obtained from non-infected seed crops located in areas where climatic conditions are dry during and after flowering, as normally occurs in some areas of South Europe for linseed, barley and other crops (Cappelli, 1992; 1996; Cappelli and Buonaurio, 1995). When the results of the seed tests predict plant behaviour in the field it is possible to decide before sowing whether seed disinfection is necessary or not, or to reject seed in cases of high infection levels.

The final success of points raised in this paper will strongly depend on international cooperation between all the laboratories involved in seed pathology, as an international agreement on innovative methodology and the interpretation of results still remains an unrealized goal. Finally it is essential that sufficient financial support be provided.

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Disease Resistance and Integrated Control of Diseases

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Resistance to *Alternaria brassicae* in Chinese cabbage

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Abstract: There were significant differences in resistance to *Alternaria brassicae* among 18 cultivars, hybrids, and inbred lines of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) from P.R. China. The TLC Cladosporium-bioassay revealed about seven types of anti-fungal spots from the leaves of Chinese cabbage challenged with *A. brassicae*. Resistance in two lines of Chinese cabbage, but not in the third one, was associated with accumulation of relatively large amounts of anti-fungal compounds. One susceptible line also elicited appreciable amounts of anti-fungal compounds. These exceptions need to be studied further with respect to their resistance/susceptibility to *A. brassicae*. Some of these anti-fungal compounds may be phytoalexins. Phytoalexin production has been shown to be associated with host resistance in many crucifers.

Key words: *Alternaria brassicae*, Chinese cabbage, resistance, anti-microbial compounds, phytoalexins

Introduction

The blackspot disease of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) caused by *Alternaria brassicae* is economically important in P.R. China, especially in the northern areas. The disease is also important on oil-yielding *B. rapa* and some other Brassicas in many parts of the world including Canada, P.R. China, India, and certain parts of Europe. Screening for resistance and study of the mechanism of resistance are important in development of the resistant cultivars.

Production of anti-microbial compounds including phytoalexins is one of the important mechanisms of disease resistance in plants. Many such compounds have been described from cruciferous plants (Browne *et al*., 1991; Gross, 1993; Conn *et al*., 1994; Pedras *et al*., 1997; Jimenez *et al*., 1997). The first report on isolation and characterization of phytoalexins in crucifers was from Chinese cabbage. Takasugi *et al*. (1986) described three sulphur-containing indole phytoalexins produced in Chinese cabbage when inoculated with *Pseudomonas cichorii* or *Erwinia carotovora*. This was also the first report on isolation and characterization of unique indole ring- and sulfur-containing phytoalexins.

Eighteen cultivars, hybrids, and inbred lines of Chinese cabbage from P.R. China were screened for resistance to *A. brassicae* in this investigation. The inoculated host material was processed for determining the presence of anti-fungal compounds.

Materials and methods

Plant materials

Eighteen cultivars (Xiaoza-56, 83-24, Xiaoza-60, Yuqing, Taiyuanerqing, Jicai-3, Shandong-4, Fengkang-70, and Qingmaye), hybrids (HN-6x28, HN-10x11, HN-27x29, HN-4x22, and HN-4x24), and inbred lines (HN-20, HN-25, HN-27, HN-29) of Chinese cabbage from P.R. China were grown in a greenhouse at approx. 18/12 C (day/night) temperatures and a light intensity of 400-600 µE/m/s. After 7-10 days, the seedlings were inoculated with the conidial suspension of *A. brassicae* (isolate # AB-6 from P.R. China) for disease resistance evaluation. Detached leaves of some lines kept in a humid chamber were also inoculated with droplets of the spore suspension. For determining the presence of anti-fungal compounds, 30-45 day old plants were inoculated with the mycelial suspension of *A. brassicae*.

Disease resistance evaluation

Alternaria brassicae was grown on V8 juice agar at room temperature in dark. Conidia were washed-off the plates with distilled water, washed twice by low speed centrifugation, and resuspended in distilled water. To evaluate the resistance of Chinese cabbage, drops of conidial suspension were placed on the leaves of 12 seeedlings of each genotype replicated thrice. The 6-category rating scale of Li (1990) was used for evaluation of resistance.

Determination of the presence of anti-fungal compounds

Alternaria brassicae was grown in V8 juice liquid medium at room temperature in dark for 10-14 days, after which the mycelium was harvested and washed with distilled water. The mycelium was then blended in a homogenizer and inoculated on to the leaves of Chinese cabbage. Distilled water was applied on to the control treatment. The inoculated plants were incubated in a humid chamber for 4 days. The anti-fungal compounds were extracted from the leaves in 70 % (v/v) aqueous methanol for 3-5 min using a facilitated diffusion technique (Keen, 1978). The extracts were filtered, evaporated to dryness at 45 C, and redissolved in methanol. These fractions were then spotted on thin-layer chromatography (TLC) plates (K5 silica gel, 250 µm thickness, Whatman) and developed in chloroform/methanol (49:1). To determine the anti-fungal activity of the compounds separated, a bioassay was done on the TLC plates. A thick conidial suspension of a *Cladosporium* sp. in double-strength Czapek-Dox broth (Difco) was sprayed on the plates and the plates were incubated in a humid chamber at room temperature in dark. After 2-3 days, the plates were examined for zones of inhibition indicated by clear areas surrounded by growth of *Cladosporium* sp. To test the antifungal activity against *A. brassicae*, the paper disc method described by Conn *et al.* (1988) was followed and the effect on the growth of *A. brassicae* observed after 2 weeks.

Authentic samples of three phytoalexins (brassinin, methoxybrassinin, and cyclobrassinin), originally reported from Chinese cabbage (Takasugi *et al.*, 1986) and obtained from Dr. M. Takasugi, were used for comparison with the anti-fungal compounds isolated during this investigation.

Results

Disease resistance evaluation

Seedlings of eighteen cultivars, hybrids, and inbred lines of Chinese cabbage were evaluated for their resistance to *A. brassicae*. The disease reactions ranged from highly susceptible to highly resistant (Table 1) and included extensive necrosis and chlorosis to hypersensitive reaction to no symptoms. The four lines, HN-25, HN-27, Qingmaye, and Fengkang-70, were highly resistant while others were moderately resistant to highly susceptible.

Determination of the presence of anti-fungal compounds

Three susceptible (HN-29, HN-20, and HN-6x28) and three resistant (Fengkang-70, HN-27, and HN-25) lines of Chinese cabbage were challenged with *A. brassicae* in order to study the relationships between presence of anti-microbial compounds and resistance/susceptibility.

Various lines showed the presence of upto seven anti-fungal inhibition spots in samples challenged with *A. brassicae* using the TLC Cladosporium-bioassay (Figures 1-3) while no such spots were resolved in the control samples. Each of these inhibition spots presumably contained at least one anti-fungal compound. Two inhibition spots were noticed at Rf values 0.75 and 0.86. These Rf values were similar to those of the phytoalexins, brassinin and cyclobrassinin, respectively (Figure 2). The other compounds are being identified and some of them have proved to be preformed compounds and thus not of phytoalexin nature (Deng, F., Trifonov, L.S., Tewari, J.P., and Ayer, W.A., unpublished data not given in this paper).

Table 1. Reactions of the cultivars, hybrids, and inbred lines of Chinese cabbage to *Alternaria brassicae* (isolate # AB-6 from P.R. China).

Cultivars	Disease index
HN-29	62.1 A
HN-20	61.0 A
HN-6x28	59.6 AB
HN-27x29	53.9 BC
Xiaoza-56	52.9 C
HN-10x11	52.5 C
83-24	47.9 CD
Xiaoza-60	47.9 CD
Yuqing	46.1 DE
HN-4x24	43.1 DE
Taiyuanerqing	42,7 DE
Jicai-3	40.7 EF
Shandong-4	35.3 F
HN-4x22	34.9 F
Fengkang-70	28.2 G
Qingmaye	27.4 G
HN-27	18.2 H
HN-25	11.5 I

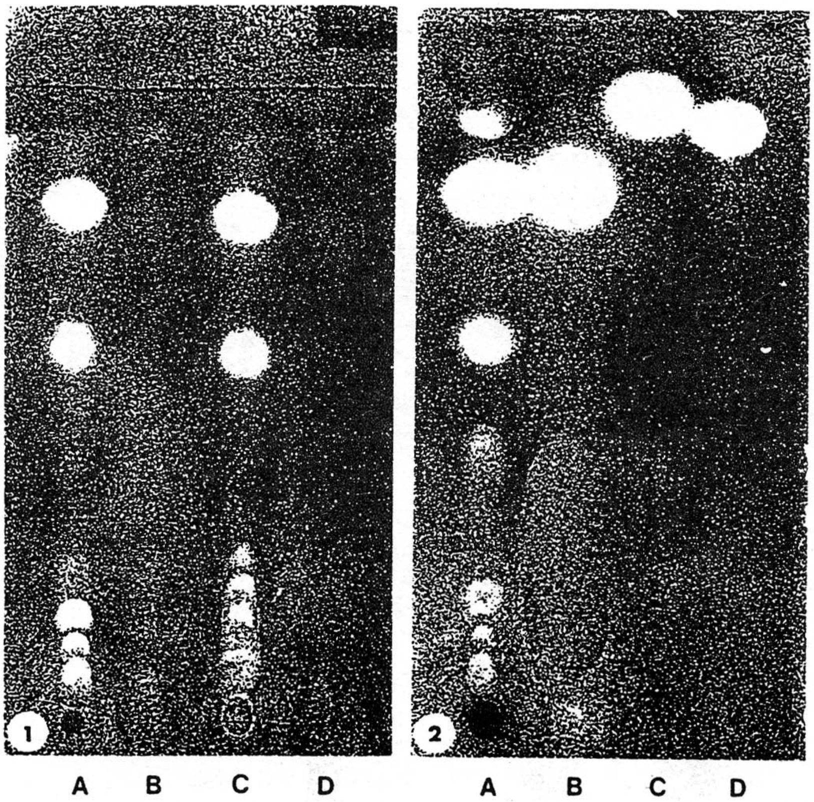
Means with the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test.

Two resistant lines, HN-25 and HN-27, produced greater quantities of anti-fungal compounds than another line, Fengkang-70 (Figure 3). Among the three susceptible lines, HN-20 and HN-29 produced both lesser types and quantities of anti-fungal compounds than the resistant lines HN-25 and HN-27. However, the susceptible line HN-6x28, produced greater quantity of anti-fungal compounds than the resistant line Fengkang-70 (Table 3).

Compounds eluted from three principal anti-fungal spots in Cladosporium-bioassay thin layer chromatograms of resistant Chinese cabbage (challenged with *A. brassicae*) were tested against *A. brassicae* using the paper disc method. *Alternaria brassicae* grew on discs with control fractions but not on those on which the anti-fungal compounds were spotted.

Discussion

There was considerable variation in responses of the cultivars, hybrids, and inbred lines of Chinese cabbage to *A. brassicae*. Depending on the resistance level of lines, the symptoms ranged from no effect to hypersensitive reaction to extensive necrosis and chlorosis. While reactions at the fruiting stage have so far not been studied, leaves of both seedlings and adult plants (30-45 day old) showed similar symptoms. Oil-yielding cultivars of *B. rapa* are highly susceptible to *A. brassicae* (Tewari, 1991) and sources of resistance identified in this study may have use in genetic improvement of this crop. Selection and domestication of leafy Brassicas has been ongoing in P.R. China since ancient times and availability of high degrees of resistance in *B. rapa* ssp. *pekinensis* in that country to *A. brassicae* is a testament to these continuing efforts.



Figures 1 and 2. Cladosporium-bioassay thin layer chromatograms (TLC) of extracts from the resistant inbred line HN-25 of Chinese cabbage. Figure 1. A and C. Extracts from Chinese cabbage challenged with *Alternaria brassicae* (isolate # AB-6 from P.R. China). Note the clear inhibition spots where anti-fungal compounds have separated. B and D. Controls. Figure 2. A. Extract from Chinese cabbage challenged with *A. brassicae* (isolate # AB-6 from P.R. China). B-D. Chromatograms of the phytoalexins brassinin, methoxybrassinin, and cyclobrassinin, respectively. Note that Rf values of two inhibition spots in the lane A are similar to those of brassinin and cyclobrassinin.

Phytoalexins are defense compounds elicited by many plants in response to disease and many other biotic and abiotic stresses. Indole ring- and sulfur-containing phytoalexins were reported for the first time by Takasugi *et al*. (1986) from Chinese cabbage. This first report included the phytoalexins brassinin, methoxybrassinin, and cyclobrassinin. Later, they isolated several other phytoalexins from Chinese cabbage (Takasugi *et al*., 1988; Monde *et al*., 1990). Two TLC Cladosporium-bioassay inhibition spots in extracts of Chinese cabbage challenged with *A. brassicae* corresponded to the Rf values of brassinin and cyclobrassinin. Phytoalexins are "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to micro-organisms" (Paxton, 1981). Compounds present in other inhibition spots are being studied and some of them have proved to be preformed compounds of varied chemistries and thus not of phytoalexin nature (Deng, F., Trifonov, L.S., Tewari, J.P., and Ayer, W.A., unpublished data not given here).

High degrees of resistance to *A. brassicae* in two inbred lines of Chinese cabbage (HN-25 and HN-27) were associated with elicitation of large quantities of anti-fungal compounds, while one resistant cultivar (Fengkang-70) elicited appreciably lesser quantities of these compounds. This indicated dual strategies for regulating resistance to *A. brassicae* in Chinese cabbage. Lesser numbers and quantities of anti-fungal compounds were produced in the susceptible inbred lines HN-20 and HN-29. The suseptible hybrid line HN-6x28 produced greater amounts but lesser numbers of anti-fungal spots in TLC Cladosporium- bioassay plates than the resistant cultivar Fengkang-70. There is a need to study the mechanism of resistance/susceptibility further in Fengkang-70 and HN-6x28.

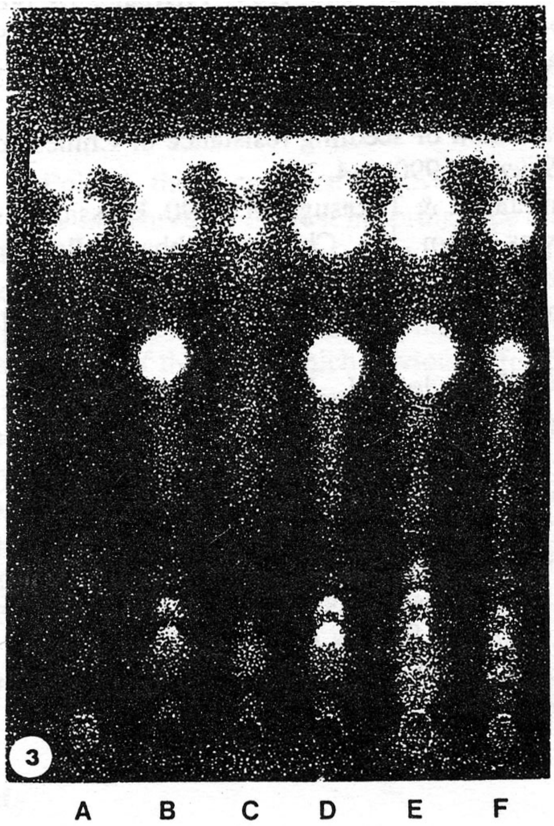


Figure 3. Cladosporium-bioassay thin layer chromatogram (TLC) of extracts from various lines of Chinese cabbage challenged with *Alternaria brassicae* (isolate # AB-6 from P.R. China). A-C. Susceptible lines HN-20, HN-6x28, and HN-29, respectively. D-F. Resistant lines HN-25, HN-27, and Fengkang-70, respectively.

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Effect of sulphur fertilisation on spring rape health status and fungi composition on harvested seeds

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Abstract: A study was carried out on the effect of soil and foliar applied, elementary and ionic sulphur fertilisation treatments, on disease occurrence in spring rape and after harvest, the effect of this on the pathogenic fungal colonisation of seeds was assessed. During the vegetatative growth period, there was a high occurrence of dark leaf and pod spot, and one year powdery mildew was also observed. A slight increase in the occurrence of stem canker, grey mould and stem rot were also noted. Sulphur fertilisation, regardless of the chemical type and method of application, decreased leaf infection by A*lternaria* spp. in comparison to the control. Sulphur fertilisation had no influence on *Erysiphe cruciferarum*. Of the pathogenic fungi, species of the *Alternaria* genus were isolated the most often. No relationship was observed between sulphur fertilisation and the composition of fungi on seeds.

Key words: spring rape, sulphur, fertilisation, diseases, fungi, seeds

Introduction

Over the last few years in Poland, the occurrence of severe winters and subsequent frozen winter oilseed rape crops have increased an interest in spring rape cultivation. Spring rape was often sown on fields after winter rape crops had frozen. As with other Brassicaceae plants, oilseed rape has a high demand for sulphur for proper growth and development. Thus, in order to obtain high yields, it is necessary to fertilise with sulphur. However, too much sulphur can increase the glucosinolate content of the seed, which obviously for double improved varieties, is an undesirable characteristic. The Plant Cultivation Department of our University carries out research on the influence of varied nitrogen and sulphur fertilisation treatments on yield and subsequent rape oil quality.

Different fertilisation regimes can greatly influence plant health. Recently there has been a great interest in the impact of sulphur fertilisation on the occurrence of fungal pathogens, both on plants during vegetative growth and on their harvested seed. Sulphur has been used as a leaf spray to control diseases for many years. However, there are very few reports that highlight the influence of sulphur on improving plant resistance through the stimulation of bio-chemical processes in primary and secondary metabolism (Schnug and Haneklaus, 1994; Booth *et al*., 1995).

Materials and methods

Plot experiment was set up on a suitability complex, good wheat soil at Witosław near the city of Bydgoszcz during 1997 and 1998 seasons. The split-plot design contained four replicate trial plots of 18 m^2. Spring wheat was the previous crop. The content of SO_4 in the soil was about 1.84 mg/100 g of the soil. The sulphur rate was 20 and 60 kg/ha, applied with foliar or soil fertilisation as elementary and ionic sulphur in the form of sodium sulphate**.** In the fertiliser combinations applied to the soil, the total sulphur dose was applied in spring, prior to the sowing of seed. Foliar fertilisation treatments were applied as follows: 20kg/ha combination, sulphur was applied at the rosette stage; 60 kg/ha combinations, applied in three doses of 20 kg each, at the rosette, stem extension and at the onset of flowering. Nitrogen was applied in 60, 120 and 180kg/ha doses. In both years the variety "Star" was sown.

The incidence of disease was assessed during vegetative growth and after harvest, seed was analysed for fungal infection. In both years, analysis was carried out by plating 4 x 100 seeds from each replicate on PDA medium. Before being plated, the seeds were rinsed for 30 min with tap water and then 3 times with sterile water. Additionally in 1998, the filter paper method (Whatman No.1), described by Cappelli *et al*. (1998), was used. In this case, seeds were disinfected with 0.5% NaOCl and those which were not surface disinfected were only rinsed with tap water for 5 min then 3 times with sterile water. In each combination, and for both methods, the occurrence of fungi was being assessed for 1000 seeds.

During vegetative growth, the health of the plants was examined twice; leaves were assessed at the end of flowering, and stems and pods were assessed at ripening. The results reported in the present paper only refer to one dose of nitrogen fertilisation, 120 kg/ha, and sulphur at 60 kg/ha. Dark leaf and pod spot was the main disease observed and infections were evaluated using a 0-4 scale. The first evaluation was carried out on the four lower leaves of 25 plants selected at random from a plot. For the later assessment at the ripening stage, infected stems and pods were assessed. Mean degree of infection and the number of plants analysed were used to calculate the infection index (DI).

In 1998, a high level of powdery mildew was observed and the occurrence of this pathogen was also assessed using a 0-4 scale. Low levels of *Peronospora parasitica* and *Phoma lingam* were evaluated as per cent of the leaves infected, and *Sclerotinia sclerotiorum* was also assessed on the stems. The results obtained were statistically analysed using variance analysis and the Tukey test.

Results and discussion

During vegetative growth, dark leaf and pod spot was the main disease observed on plants (Table 1). Symptoms of the disease occurred both on leaves as well as on stems and pods. In both years the pathogen showed a high intensity of occurrence. The leaf infection index (max. 100), depending on the treatment, ranged from 18.3 to 34.7 in 1997, and from 18.1 to 38.6 in 1998. In both years, leaf infection was significantly higher on plots that had not been fertilised with sulphur. However, sulphur fertilisation did not significantly affect the pathogen occurrence on stems and pods, although in both years, a higher levels of infection was observed on plots that had not been fertilised with sulphur. There was no effect of the form of sulphur being applied (elementary or ionic) or of the application method (foliar or soil).

In 1998, a high occurrence of powdery mildew was noted. The disease was observed on all plants assessed, and the DI ranged from 34.4 to 43.8. Despite high disease occurrence, no relationship between sulphur fertilisation and disease intensity was observed, as differences were not statistically significant.

In both years the occurrence of *Phoma lingam, Peronospora parasitica* and *Sclerotinia sclerotiorum* was observed. However the intensity was not considerable and did not differ between treatments.

There are numerous reports on the influence of fertilisation on oilseed rape health status. However, most reports concern differentiated NPK fertilisation (Sharma and Kolte, 1994; Sadowski *et al*., 1995; Sadowski *et al*., 1998) and only a few deal with sulphur. The impact of sulphur in the environment on rape health status has been reported by Schnug & Ceynowa

(1990) and Schnug *et al.*, (1995). Schnug & Ceynowa (1990) showed that, sulphur deficiency in the environment resulted in a higher intensity in the occurrence of light leaf spot in oilseed rape.

Table 1. Occurrence of spring rapeseed diseaes in response to sulphur fertilisation (DI, max =100).

Treatment with sulphur kg/ha [elementary (e.)] [ionic (i.)]	1997		1998		
	Alternaria spp.		*Alternaria* spp.		Powdery mildew
	Dark leaf	Pod spot	Dark leaf	Pod spot	
S – 0 control	34.7 a*	26.0 a	38.6 a	61.3 a	43.7 a
S – 60 kg soil-e.	24.3 b	22.9 a	20.3 b	53.1 a	41.3 a
S – 60 kg foliar-e.	18.6 b	21.3 a	18.1 b	53.1 a	43.8 a
S – 60 kg soil-i.	25.4 b	22.3 a	20.2 b	43.8 a	40.6 a
S - 60 kg foliar-i.	18.3 b	20.3 a	18.8 b	42.5 a	43.7 a

*/ Values in the same column followed by different letters are significantly different

During the present study, *Alternaria brassicae* was the most prevalent pathogenic fungi and was isolated from all treatments. However, taking into consideration both years, it can be observed that there was no significant influence of sulphur fertilisation on its occurrence. Also *A. alternata*, *A. brassicicola* and, sporadically, *A. consortiale* were isolated (Tables 2 and 3).

Table 2. Occurrence of fungi on seeds of spring rape fertilised with sulphur [%] – PDA medium – 1997.

Fungi	S - O Control	S - 20 kg/ha				S - 60 kg/ha			
		Soil		Foliar		Soil		Foliar	
		Elem.	Ionic	Elem.	Ionic	Elem.	Ionic	Elem.	Ionic
A. brassicae	2.3	1.3	7.5	2.0	3.3	1.3	8.5	11.5	6.3
A. brassicicola	0	0	1.0	0	1.5	0	0	0	1.0
A. alternata	7.8	7.5	0	3.3	16.0	7.5	6.0	2.0	4.5
A. consortiale	0	0	1.0	0	2.3	1.0	0	0	0
Cladosporium herbarum	80.0	4.0	20.3	26.0	46.0	75.0	7.0	25.3	18.0
Penicillium spp.	1.3	89.3	6.0	14.5	34.3	11.8	3.5	14.0	7.5
Others*	12.5	18.8	2.0	8.0	9.0	16.3	4.0	8.3	1.0

*/ elementary

**/- *Arthrinium phaeospermum, Botrytis cinerea, Epicoccum nigrum, Fusarium avenaceum, Fusarium* spp.*, Mucor* spp.*, Rhizopus nigricans*

Table 3. Occurrence of fungi on seeds of spring rape fertilised with sulphur [%] – PDA medium – 1998

Fungi	S - O Control	S - 20 kg/ha				S - 60 kg/ha			
		Soil		Foliar		Soil		Foliar	
		Elem.*	Ionic	Elem.	Ionic	Elem.	Ionic	Elem.	Ionic
Alternaria brassicae	13.3	6.8	3.3	2.5	4.5	0	3.3	0	0
A. brassicicola	8.5	4.0	5.5	0	0	1.0	0	1.3	3.0
A. alternata	2.0	1.5	0	2.0	1.0	0	1.5	0	2.5
Cladosporium herbarum	33.5	61.0	0	2.3	2.3	0	0	0	0
Penicillium spp.	47.0	27.3	98.0	96.5	93.0	100.0	100.0	99.0	97.3
Others*	11.5	4.0	21.0	4.0	1.0	1.5	1.0	5.5	8.0

*/ elementary
**/- *Fusarium avenaceum, Botrytis cinerea, Mucor spinosus, Mucor* spp., *Aspergillus niger, Aspergillus* sp., *Aureobasidium pullulans, Rhizopus nigricans*

Table 4. Occurrence of fungi on seeds of spring rape fertilised with sulphur [%] (filter paper, disinfected with NaOCl – 1999)

Fungi	S - O Control	S - 20 kg/ha				S - 60 kg/ha			
		Soil		Foliar		Soil		Foliar	
		Elem.*	Ionic	Elem.	Ionic	Elem.	Ionic	Elem.	Ionic
Alternaria brassicae	17.0	17.8	14.8	14.2	16.2	19.6	17.4	18.8	15.6
A .brassicicola	1.8	3.0	0.4	2.0	1.4	2.8	1.6	2.4	2.6
A .alternata	2.6	3.8	1.0	2.6	1.4	5.8	6.6	2.4	4.2
Cladosporium herbarum	1.8	0.6	0	1.8	1.4	2.8	0.8	1.0	1.8
Penicillium spp.	58.0	1.6	7.6	40.6	77.4	43.4	53.0	80.2	78.0
Others**	5.4	3.6	7.6	32.2	2.8	29.8	13.6	3.6	10.2

*/ elementary
**/- *Fusarium avenaceum, Fusarium* spp., *Gonatobotrys simplex, Cylindrocarpon* spp*., Aspergillus niger, Aspergillus* spp., *Rhizoctonia solani, Botrytis cinerea, Aureobasidium pullulans, Mucor spinosus,Trichoderma* sp*., Stachybotrys* sp., *Chaetomium* sp.

Alternaria spp fungi isolated on filter paper (Tables 4 and 5) show that the occurrence of fungi of *Alternaria* genus on spring rapeseed was common. Most often *A. brassicae* was noted, whereas *A. alternata* and *A. brassicicola* were less numerous. There was no direct relationship between seed infection caused by these fungi and sulphur fertilisation, either with regard to the sulphur dose, sulphur form or application method. Analysis of the respective combinations of treatments required the use of 20 Petri dishes each with 50 seeds. A comparison of the fungi grown from dishes produced using the two methods indicated that the method described by Cappelli *et al.* (1998), to define seed infection caused by *Alternaria*, was very applicable in this case. This was particularly true in this case as, apart from *A. brassiceae*, seeds also contained fungi of *Penicillium* genus and on PDA medium, these quickly growing saprophytes make it impossible to isolate *A. brassiceae*. Of the seeds tested, such cases were common, and for that reason, the agar test consistently gave the result of a lower percentage of seeds infected with *A. brassiceae* in comparison with the filter paper method. The number of seeds in respective dishes with specific fungal species was similar. The results indicate that this method is useful for defining the occurrence of fungi of *Alternaria* genus, especially as it is cost-effective and is not labour-intensive.

Table 5. Occurrence of fungi on seeds of spring rape fertilised with sulphur [%] (filter paper, non-disinfected – 1999)

Fungi	S - O Control	S - 20 kg/ha				S - 60 kg/ha			
		Soil		Foliar		Soil		Foliar	
		Elem.*	Ionic	Elem.	Ionic	Elem.	Ionic	Elem.	Ionic
Alternaria brassicae	16.6	17.0	17.0	12.0	17.4	23.4	18.2	11.2	14.4
A .brassicicola	2.8	1.2	3.6	2.6	1.8	2.7	2.6	2.8	1.4
A .alternata	9.0	4.2	5.0	4.2	7.2	3.6	3.0	5.4	8.2
Cladosporium herbarum	49.0	73.0	45.2	54.2	21.4	4.6	44.8	34.8	40.0
Penicillium spp.	22.8	14.2	51.4	67.8	88.4	80.2	86.8	90.0	27.8
Others**	16.4	9.4	11.6	11.6	13.4	11.2	7.0	2.4	8.8

*/ elementary
**/- *Fusarium avenaceum, Fusarium* spp., *Gonatobotrys simplex, Cylindrocarpon* spp., *Aspergillus niger, Aspergillus* spp., *Rhizoctonia solani, Botrytis cinerea, Aureobasidium pullulans, Mucor spinosus,Trichoderma* sp., *Stachybotrys* sp., *Chaetomium* sp.

Gugel & Petrie (1992) report that canker (*Phoma lingam*) is common pathogen of spring rapeseed cultivated in Canada. In contrast, our experiments indicate that the main disease was dark leaf and pod spot (*Alternaria* spp.). As many of the seeds tested were infected with fungi of the *Alternaria* genus, the seeds could have been the source of the infection. According to data published in international reviews, the *Alternaria* are one of the major winter rape pathogens (Sadowski & Klepin, 1991; Cappelli et al., 1998; Kennedy & Graham, 1995). During the course of the present study, *Peronospora parasitica*, *Phoma lingam*, *Sclerotinia sclerotiorum* were among other pathogens that were observed at a low intensity of occurrence and these fungi are common in winter rape crops in Poland (Sadowski & Klepin, 1991). However, no effect of sulphur fertilisation on the intensity of these diseases was observed.

There are very few reports to date on spring rape disease status in Poland. An increase of the rape cropping area will probably lead to an increase in the occurrence of many pathogenic fungi. Therefore it is necessary to study their intensity as well as the possible future threat posed by the diseases to the spring crop.

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Accelerated stubble decomposition using *Cyathus olla*. A strategy for control of stubble-borne diseases of canola

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Abstract: The development of innovative and integrated control strategies for plant disease can originate from a thorough study of the microenvironment inhabited by the pathogen. Ecological investigations in these areas may reveal microbial interactions that can be used to develop biological control strategies. In Alberta, Canada, field observations initiated research focused on a white-wood rotting, bird's nest fungus, *Cyathus olla*, and its potential of being developed into a microbial inoculant for stubble-borne disease control. Increased populations of this naturally occurring fungus may accelerate stubble decomposition, thereby eliminating the overwintering site of stubble-borne pathogens. The objectives of this ongoing research are to collect basic biological and biochemical information on *C. olla* in the stubble microenvironment. Scanning electron microscopy, energy dispersive X-ray microanalyses, and biochemical analyses of infested canola stubble and mycelium from culture identified *C. olla* as a calcium oxalate crystal producing fungus, and morphological and molecular characterization studies led to the distinction of a new form of this species. *Cyathus olla* has been successful at degrading a model lignin compound and furthermore, solid state fermentation of canola root material resulted in a significant reduction in lignin content. This proposed control strategy could have a broad application to many stubble-borne diseases in a variety of crops, but accelerated stubble decomposition may be especially beneficial to stubble-borne diseases of canola considering the woody, resistant nature of the basal part of the stem and root.

Key words: white wood-rotting fungus, blackleg, biological control

Introduction

An integrated approach to plant disease control can encompass many different methods including cultural, chemical, and biological strategies. Of these methods, biological control methods are the least developed but are gaining interest due to public concerns over pesticide use, and soil and environmental degradation linked to agricultural practices. These concerns have generated an increased interest in the agroecosystem and the movement toward ecologically based pest management. The development of innovative, biological control strategies requires a knowledge-based system and a comprehensive understanding of the agroecosystem. Building a strong foundation of knowledge in these areas may improve the success of biological control agents.

One unique ecological niche in an agroecosystem is the stubble microenvironment. The stubble or residue that is incorporated into the soil, or left on the field surface following the production of a crop may appear to be waste material, but in actuality, it is a valuable source of plant and microbe nutrients that become available as the stubble is recycled during the process of decomposition. This residue microenvironment is a niche for many diverse organisms, and the interactions that occur among them (Metting, 1993). These organisms vary with respect to saprophytic ability and include microbial antagonists, parasites, hyperparasites, and plant pathogens. Due to these many diverse crop stubble inhabitants, a study of this niche may reveal many naturally occurring interactions that could be used as biological control strategies targeted toward plant diseases. Such is the case with stubble-borne plant pathogens. The purpose of this paper is to discuss an innovative strategy for stubble-borne disease control using a white wood-rotting fungus isolated from canola stubble.

Field observations and development of a control strategy

In western Canada, blackleg and blackspot of canola (*Brassica napus, B. rapa*) incited by the pathogens *Leptosphaeria maculans* and *Alternaria brassicae* respectively, can be a destructive stubble-borne disease. If canola stubble is infested, the pathogens can continue to sporulate as long as the infested stubble remains in the field. For canola stubble, this may be as long as 5 years following production of the crop, since the lower stem and root is very woody and resistant to decomposition (Petrie, 1995). For this reason, a four-year crop rotation is recommended for canola. Cultural and chemical control methods are available, but more recently, research has been initiated to evaluate a white wood-rotting bird's nest fungus, *Cyathus olla* (Nidulariaceae), and its potential of being developed into a microbial inoculant for stubble-borne disease control. The research began following observations of this fungus naturally growing and fruiting on canola stubble in agricultural fields. Colonized stubble had substantial hyphal growth with hyphal cords emanating from it and growing into the soil, and residues were soft and macerated, indicative of enzymatic activity (Tewari & Briggs, 1995). It is anticipated that increased populations of this fungus may accelerate stubble decomposition, resulting in destruction of the nutrient source and overwintering site of the pathogens, and ultimately reduce inocula. With a comprehensive study of the fungus-substrate interaction and subsequent effects on pathogen life cycles, an integrated disease control strategy could be developed.

The bird's nest fungus, *Cyathus olla*

Cyathus spp. are ecologically specialized to degrade plant debris, and are commonly found growing in both tropical and temperate environments (Brodie, 1975). *Cyathus olla* is generally a temperate species, and is commonly found growing in agricultural fields. Isolates have been collected from year to year in the same field, indicating the ability of this fungus to withstand agricultural practices such as tillage and herbicide treatments. Some species are known to exhibit substrate preferences (Abbott & Wicklow, 1984; Wicklow *et al.,* 1984), and in preliminary experiments, *C. olla* colonized wheat, barley, and canola residue, but appeared to have a preference for the woody roots of canola (Tewari & Briggs, 1995). *Cyathus* spp. are biochemically active, and have been shown to degrade lignin (Wicklow *et al.* 1980; Abbott & Wicklow, 1984); and reduce the dry weight and improve digestibility of many different crop residues (Kuhad & Johri, 1991; Akin *et al.,* 1995; Karunanandaa *et al.,* 1992; Agosin *et al.,* 1985, Chen *et al.,* 1995). These attributes, in conjunction with field observations, make *C. olla* a potentially useful candidate for a microbial inoculant intended to accelerate crop stubble decomposition, and ultimately reduce the incidence of stubble-borne diseases of canola.

The objectives of this ongoing research are to ascertain basic biological and biochemical information on *C. olla* in the stubble microenvironment, and determine the usefulness of this fungus for the proposed application. Research is in progress, and some results are presented in the following sections.

Scanning electron microscopy

Scanning electron microscopy (SEM) was a useful tool to provide information on the physical interactions taking place between the colonizing fungus and the canola substrate. *Cyathus olla* field-infested canola stubble was collected and examined using scanning electron microscopy and energy dispersive X-ray microanalysis. These studies revealed heavy crystalline encrustations on the colonizing hyphae, and the substrate had exposed xylem elements indicating structural deterioration. Further FT/ IR and ^{13}C- NMR spectroscopy determined that the crystals were calcium oxalate (Tewari *et al.,* 1997). The presence of these crystals suggest that *C. olla* was involved in the decomposition process. Crystal formation is an indicator of oxalic acid secretion that results in the sequestration of calcium from the cell walls of the host, and the precipitation of calcium oxalate crystals. This structurally compromises the substrate, leaving it susceptible to enzymatic attack (Bateman & Beer, 1965).

Calcium oxalate crystal forming fungi are involved in calcium recycling in the environment, and calcium oxalate can also improve availability of P for root uptake by increasing the solubility of soil iron and aluminum, and chelating these metals (Graustein *et al.,* 1977). Oxalic acid and oxalate may also be important factors involved in the stimulation of the ligninolytic enzyme system present in wood-degrading fungi (Kuan & Tien, 1993; Urzua *et al.,* 1998).

Isolate collection and characterization

Basidiocarps of *C. olla* were collected from northern and central Alberta, Canada. Morphologically these basidiocarps were distinctly different and variable. A taxonomic key of Brodie (1977) was used to identify isolates of *C. olla* f. *olla* and *C. olla* f. *anglicus,* but a third basidiocarp type could not be identified using this key. For these reasons, a detailed morphological and molecular characterization was carried out to assess the variability within the isolate group.

All isolates were characterized according to 13 morphological parameters, as well as polymerase chain reaction based random amplified polymorphic DNA (RAPD) analysis. Morphological and molecular data were analyzed using cluster analyses and used to construct two phenetic trees. Results determined that the unidentified isolates clustered together and were distinctly separate from the identified isolates in both the morphological and molecular cluster analyses. From this study, a new form of *C. olla* was named and described as *C. olla* f. *brodiensis* Shinners et Tewari *f. nov.* (Shinners & Tewari, 1998a).

Identifying any sources of variation within the isolate collection is an important aspect of this project since it is not known if similar variation may also be exhibited with respect to stubble colonization and decomposition capabilities.

Lignin degradation from canola root material

In order to determine if *C. olla* was capable of degrading lignin, a plate-screening assay was conducted on 42 *C. olla* isolates, using a model lignin compound as a substrate. Results indicated that most isolates were able to degrade the model lignin compound, but to different degrees (Shinners & Tewari, 1998b). No correlation was shown between the form of *C. olla* tested and the ability to degrade the model lignin compound. Based on these results, 15 isolates were selected for solid state fermentation on canola root material. Following incubation for 45 days, the canola substrate was harvested and the fiber content (hemicellulose, cellulose, and lignin) was determined using the Goering-Van Soest method (1970).

Preliminary results have shown that *C. olla* is capable of significantly reducing the lignin content of canola root material. All canola material incubated with a *C. olla* isolate had a significantly lower percentage of lignin (10.30-13.71%) when compared to the non-inoculated control (15.50% lignin). There were significant differences observed among the isolates, indicating that there is variability with respect to stubble decomposing capabilities. Research in this area is ongoing, and will eventually lead to the selection of the most effective isolates for stubble decomposition.

Conclusions

The biological control strategy using accelerated stubble decomposition described in this paper represents an innovative approach to stubble-borne disease control. To date, the results obtained are encouraging, but much more research is needed before a microbial inoculant could be developed. This proposed strategy is not disease or crop specific, but may be especially beneficial to stubble-borne diseases of canola considering the woody, resistant nature of the basal stem and root of this plant. In addition to the obvious benefits for disease control, *C. olla* may also be beneficial to plant nutrition and soil fertility through release of nutrients from the stubble during the decomposition process. These attributes would make this strategy an excellent component to an integrated crop management system.

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First results on the effect of different fungicidal oilseed-rape dressings against the emergence diseases Phoma damping off and downy mildew with special regard to two sowing dates in Fall 1998

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Abstract: In a joint field trial of the University of Paderborn (Location: Soester Börde, Haus Düsse Chamber of Agriculture, Dr. Block) with the Federal Biological Research Centre for Agriculture and Forestry (Dr. V. Garbe) 7 different fungicidal seed-coatings and leaf treatments (1 = Control; 2 = SZX 0722 + Thiram; 3 = Thiram + 0.75l/ha Folicur [Tebuconazol]; 4 = SZX 0722 + Thiram [TMTD]+ 0,75l/ha Folicur; 5 = Dimetthomorph [DMM]; 6 = Caramba [Metconazol]; 7 = Folicur) sown at a "normal" (sown 01.09.98) and at a "late" sowing date (sown 08.09.98) are compared for their effect on emergence diseases, with special regard to downy mildew (*Peronospora parasitica*) and the Phoma-damping off/Root- and stem canker (*Phoma lingam*, Teleomorph: *Leptosphaeria maculans*).

During the first assessment, downy mildew was the most relevant disease and differences between the different seed dressings and the different sowing dates were found. At the second assessment, stem canker had a higher occurrence, but no differences between the different seed dressings were found.

Introduction

In oilseed rape cultivation, diseases can appear as early as emergence, can cause considerable damage and can lead to a build up of infection the following spring (Paul et al., 1998). Within the group fungi that cause damping off are *Phytium* spp., *Fusarium* spp. and *Rhizoctonia solani*, but also other species such as downy mildew (*Peronospora parasitica*), and Phoma (*Phoma lingam,* Teleomorph: *Leptosphaeria maculans*) also cause damping off (Paul, 1992; Klodt-Bußmann, 1995). In the field trial results presented here, new fungicidal seed-coatings are assessed for their effect on different fungal oilseed rape diseases, especially *Phoma*-damping off and downy mildew. A special point of interest is the joint effect of sowing date and seed coating, and the influence of these factors on disease frequency. On account of the high precipitation in the fall of ´98 the "normal" sowing date (01.09.98) was one week later than usual in the Soest Area.

Material and methods

In a field trial, six different fungicidal seed-coatings were compared with an untreated control at a "normal" and at a "late" sowing date. Treatments 3 and 4 were additionally treated with the foliar fungicide Folicur (0.75l/ha) at the 6-8 leaf stage (Table 1). The field trial was set up using a plot size of 30 m^2 with four replicate plots per treatment in the Soester Börde at the Chamber of Agriculture, Westfalia (Haus Düsse), and assessed for disease occurrence and plant development at different growth stages. The sowing, treatment and assessment dates are shown in Table 2. The assessment after emergence in the "late" sowing date was accomplished on the 15th October 1998. The assessment was carried out later than planned since it had rained excessively after sowing (9.4 mm precipitation one day and 79.6 mm precipitation within the first week after sowing), and very few plants had emerged. For this reason no Folicur treatment and no assessment at tenth true leaf stage took place.

Table 1. Seed coating treatments used in the field trial.

Treatment label	Treatment
1	Control
2	SZX 0722 + Thiram (TMTD)
3	Thiram **+ 0.75l/ha Folicur**
4	SZX 0722 + Thiram **+ 0.75l/ha Folicur**
5	Dimethomorph (DMM)
6	Metconazol (Caramba)
7	Tebuconazol (Folicur)

Table 2. Sowing-, treatment- and assessment dates in 1998.

	"normal" sowing date	**"late" sowing date**
Sowing date:	01.09.98	08.09.98
Assessment at Emergence:	20.09.98	15.10.98
Assessed for:	Diseases and number of plants per metre res. m^2	
Assessment at 10th true leaf stage:	06.10.98	-
Assessed for:	Number of plants per metre resp. m^2 and plant development	
Folicur treatment:	15.10.98	-
Assessment pre winter:	25.01.99*	25.01.99
Assessed for:	Plant development and diseases	

* this late date was chosen on account of the mild weather

Results

"Normal" sowing date, plant development

At the first assessment (emergence stage), the number of plants per metre and the diseases that were present (downy mildew [*Peronospora parasitica*] and stem canker [*Phoma lingam*; Teleomorph*: Leptosphaeria maculans*]) were determined (Table 3). The number of plants per metre ranged from 8 plants for treatment 7 and 10.8 for treatment 3. The control treatment had 9.1 plants. This corresponds with a plant density of 50 plant/m^2 for treatment 8 and 75 plants/m^2 for treatment 3. At the tenth true leaf stage, the number of plants per metre and the plant development was assessed. A reduction in the number of plants was found between the first and second assessment. For treatments 2 and 3, the number of plants decreased by an average of 2.5 plants (from 9.5 to 7.0 plants/metre in treatment 2 and from 10.8 to 8.1 plants/metre in treatment 3). The control treatment (from 9.1 to 8.4 plants/m) and treatment 4 (from 9.0 to 8.6 plants/m) showed the least change with an average reduction of 0.5 plants. In general, plants were well developed by this stage. Treatment 5 had the worst assessment rate (a.r.) with an a.r. average of 3.3 (a.r. from 1-9; 1 = very well developed) (Table 3). On account of the high levels of precipitation, plant development was poorer at the second assessment date (pre winter). The average assessment rating was 4 (a.r. from 1-9; 1 = very well developed) and the plant population was irregular. On average, plant density only reached 65 % of the expected number of 65 plants/m^2 before winter. By the assessment date following winter, the a.r. for plant development ranged from 2.3 (treatments 1, 2 and 4) to 2.8 (treatments 3, 5 and 7) and by the assessment at the end of flowering it ranged from 1.5 (treatment 2) and 2.0 (all other treatments) (Table 4).

"Normal" sowing date, diseases

The infestation level of downy mildew in the control treatment was 46.3 %, with an a.r. of 4.8 (a.r. from 1-9; 1 = no attack). Compared with the control, treatments 2, 4 and 5 showed very good control of downy mildew. The frequency of infection was reduced to 10 % with an a.r. of 3.5 for treatment 2, to 6.7 % with an a.r. of 3.5 for treatment 4 and to 10.3 % with an a.r. of 2.0 for treatment 5. Treatment 6 also had an effect on downy mildew, but not to such a marked degree (17.5 %). Phoma leaf spot (*Phoma lingam*, Teleomorph *Leptosphaeria maculans*) was observed, but at a very low level. The infection frequency for this pathogen was observed to be about 1 % with an average a.r. around 1.5 (Table 3). By the second assessment (before winter), downy mildew infection was reduced in comparison to levels observed at the first assessment, while levels of stem canker (Phoma leaf spot) had increased. The highest frequency of downy mildew was observed in both the control and treatment 3 with 5.0 % and an a.r. of 1.8 and 1.5 respectivly. Downy mildew was not observed in treatment 5. These results reflect those of the first assessment. By the third assessment following winter, only stem canker (Phoma leaf spot) was found. The disease incidence ranged from 14.4 (Control) to 18.8 (treatment 6) (Table 4)). By the assessment carried out at the end of flowering, downy mildew (*Peronospora parasitica*), stem canker (*Phoma lingam*), dark leaf and pod spot (*Alternaria brassicae*), grey mold (*Botrytis cinerea*) and light leaf spot (*Cylindrosporium concentricum*) were observed. The results are shown in Table 4.

"Late" sowing date, plant development

At the first assessment date (emergence), the assessment of the number of plants/metre was changed to plants per m^2, since very few plants had emerged at the "late" sowing date (Table 5). The number of plants ranged from 16 to 22 plants/m^2. In a good population the number of plants should be 65 plants/m^2 before winter. The development of the plants was much poorer than at the "normal" sowing date. Treatment 4 was observed to have the best assessment rating with an a.r. of 3.5 (assessment rate 1-9; 1 = plants very well developed). Treatment 5 showed the lowest a.r., with an a.r. of 5.0. In the "normal" sowing date, treatment 5 had the poorest development in comparison to these results, with an a.r. of 3.3 (Table 3 and Table 5). By the assessment date following winter, the a.r. for plant development ranged from 4 (treatment 2) to 4.8 (treatments 5 and 6). Plants were better developed by the assessment at the end of flowering, with assessment ratings ranging from 1.5 (treatment 2) to 2 (all other treatments) (Table 5).

"Late" sowing date, diseases

At the first assessment of the "late" sowing date treatments, downy mildew was the most prevalent disease. The lowest infestation levels were observed on treatment 3, with 18.8 % of plants infected (a.r. = 2.8) and also in the control, with 21.3 % of plants infected (a.r. = 4.3). By the second assessment date, the infestation level had been reduced to 0 to 1.3 % of plants infected (Table 5). The frequency of stem canker at the first assessment date ranged from 0 % of plants attacked (treatment 3) to 3.8 % of plants attacked (untreated control and treatment 2). By the second assessment date, levels had increased to 58.5 % of plants attacked (treatment 3) and 67.5 % (treatment 2)(Table 5). By the assessment following winter, as was

Table 3. Results of the effect of different fungicidal oilseed-rape seed-coatings on fungal diseases and plant development; Location: Soester Börde, Haus Düsse Chamber of Agriculture 1998/99 (“normal“ sowing date).

Seed-coating treatments	Emergence					10th leaf stage		pre winter							
		Diseases								Diseases					
	Pl. per	P. p.		Ph. l.		Pl. per	Pl.	Pl.	Den.	P. p.		Ph. l.		A. b.	
	met	%	R.	%	R.	metre	dev.*	dev.*	rel.	%	R.	%	R.	%	R.
1 = Control	9.1	46.3	4.8	0.8	1	8.4	2.5	4.5	78.8	5.0	1.5	86.3	1.6	5.0	1.0
2 = SZX 0722* + Thiram	9.5	10	3.5	1.3	2	7.0	2.8	4.3	75.0	1.3	0.5	81.3	1.6	7.5	1.5
3 = Thiram + 0.75l/ha Folicur	10.8	45	5.3	0.5	1	8.1	2.8	3.8	75.0	5.0	1.0	63.8	1.0	7.5	1.5
4 = SZX 0722* + Thiram + 0,75l/ha Folicur 4	9.0	6.75	3.5	1.3	1.5	8.6	3.0	3.5	75.0	1.3	0.5	86.3	1.5	3.8	1.0
5 = Dimethomorph (DMM)	9.8	10.3	2.0	0.8	1	7.3	3.3	4.0	76.3	0.0	0.0	92.5	1.7	3.8	1.0
6 = Metconazol (Caramba)	8.9	17.5	3.0	0.5	1	7.6	2.3	4.0	72.5	2.5	0.5	93.8	2.1	3.8	1.0
7 = Tebuconazol (Folicur)	8.0	26.3	4.8	0.0	0	6.9	2.5	4.8	73.8	3.8	1.5	86.3	1.3	5.0	1,0

P.p. = Downy mildew (*Peronospora parasitica*)
Ph. l. = Stem Canker (*Phoma lingam*)
A. b. = Dark Leaf and Pot Spot (*Alternaria brassicae*)
R.: Assessment Rate (a.r.) from 1 = no attack to 9 = 100 % attack
Pl. dev. (Plant development) = Assessment from: 1 = plants very well developed to 9 = plants very poorly developed; Den. = Density
* experimental product from the Bayer AG

Table 4. Occurence of different oilseed rape diseases and plant development at the assessments after winter and end of flowering ("normal" sowing date).

	after winter			end of flowering											
		Diseases			Diseases										
Treatments	Pl. Dev.	Ph.l.	Rate	Pl. Dev.	P.p.	Rate	Ph.l.	Rate	A.b.	Rate	B.c.	Rate	C.c.	Rate
1 = Control	2.3	14.4	2.0	2.0	97.5	2.5	6.3	1.5	7.5	1.4	2.5	1.3	0.0	1.0
2 = SZX 0722 + Thiram (TMTD)	2.3	15.6	2.0	1.5	97.5	2.8	6.3	1.4	0.0	1.0	12.5	1.5	0.0	1.0
3 = Thiram + 0.75l/ha Folicur	2.8	15.6	2.1	2.0	95.0	2.3	2.5	1.3	6.3	1.4	7.5	1.5	0.0	1.0
4 = SZX 0722 + Thiram + 0,75l/ha Folicur	2.3	16.3	2.4	2.0	96.3	2.5	2.5	1.3	1.3	1.1	8.8	1.4	0.3	1.1
5 = DMM (Dimethomorph)	2.8	18.1	2.3	2.0	92.5	2.5	5.0	1.4	3.8	1.4	5.0	1.3	0.0	1.1
6 =Caramba (Metconazol)	2.5	18.8	2.0	2.0	93.8	2.3	5.0	1.4	1.3	1.1	11.3	1.5	0.0	1.0
7 = Folicur (Tebuconazol)	2.8	13.8	2.1	2.0	96.3	2.9	2.5	1.3	1.3	1.1	16.3	1.8	0.0	1.0

P.p. = Downy Mildew (*Peronospora parasitica*)
Ph. l. = Stem Canker (*Phoma lingam*)
A. b. = Dark Leaf and Pod Spot (*Alternaria brassicae*)
B.c. = Grey Mold (*Botrytis cinerea*)
C.c. = Light Leaf Spot (*Cylindrosporium concentricum*)
Rate: Assessment rate from: 1 = no attack to 9 = 100 % attack
Pl. Dev. = Plant Development; Rate from: 1 = Plants very well to 9 = Plants very poorly developed

observed for "normal" sowing date treatments, stem canker was the only disease found. Disease incidence ranged from 6.9 % of plants infected (treatment 5) to 13.1 % (treatments 3 and 4). By the end of flowering, downy mildew, stem canker, dark leaf and pod spot and grey mold were all observed. The data for the different disease are shown in Table 6.

"Late" sowing date, diseases
At the first assessment of the "late" sowing date treatments, downy mildew was the most prevalent disease. Lowest infestation levels were observed on treatment 3 with 18.8 % of plants infected (a.r. = 2.8) and on the control treatments, with 21.3 % of plants infected (a.r. = 4.3).

By the second assessment date, infestation levels had reduced and ranged from 0 to 1.3 % of plants infected (Table 5). At the first assessment date, the frequency of stem canker infection ranged from 0 % of plants attacked (treatment 3) to 3.8 % (untreated control and treatment 2). By the second assessment date, level were observed to increase to 58.5 % of plants attacked (treatment 3) and 67.5 % (treatment 2)(Table 5). By the third assessment date following winter, as observed for the "normal" sowing date treatments, only stem canker was found. The disease incidence ranged from 6.9 % of plants infected (treatment 5) to 13.1 % (treatments 3 and 4).

By the end of flowering, downy mildew, stem canker, dark leaf and pod spot and grey mold were all observed. The data for these diseases are shown in Table 6.

Table 5. Results on the effect of different fungicidal oilseed-rape seed-coatings on fungal diseases and plant development; Location: Soester Börde, Haus Düsse Chamber of Agriculture 1998/99 (“late“ sowing date).

Seed-coating treatments	**Emergence**					**pre Winter**							
		Diseases						**Diseases**					
	Pl.	P. p.		Ph. l.		Pl.	Den.	P. p.		Ph. l.		A. b.	
	m^2	%	Rate	%	Rate	dev.	rel.	%	Rate	%	Rate	%	Rate
1 = Control	22.8	21.25	4.3	3.8	1,0	4.25	17.5	0	0	63.75	2.12	3.75	1.5
2 = SZX 0722* + Thiram	17.8	65	6.5	3.8	1.5	3.75	16.25	1.25	0.5	67.5	2.12	6.25	1
3 = Thiram	25.3	18.75	2.8	0.0	0	4.5	13.75	0	0	58.5	2.12	5	1
4 = SZX 0722* + Thiram	24.5	57.5	5.0	1.3	0.5	3.5	15	0	0	63.5	2.12	5	1
5 = Dimethomorph (DMM)	16.0	58.75	5.0	1.3	0.5	5	8.75	1.25	0.5	61.25	2.12	7.5	1.5
6 = Metconazol	16.8	27.5	5.5	1.3	0.5	4.25	8.75	0	0	63.75	2.25	1.25	0.5
7 = Tebuconazol	18.5	27.5	5.3	1.3	0.5	4.25	11.25	1.25	0.5	61.25	2.25	1.25	0.5

P.p. = Downy Mildew (*Peronospora parasitica*) – Ph. l. = Stem Canker (*Phoma lingam*) – A. b. = Dark Leaf and Pod Spot (*Alternaria brassicae*)

Rate: Assessment Rate (a.r.) from 1 = no attack to 9 = 100 % attack

Pl. dev. (Plant development) = Assessment from: 1 = plants very well developed to 9 = plants very poorly developed; Den. = Density

*experimental product from the Bayer AG

Table 6. Occurence of different oilseed rape diseases and plant development at the assessments after winter and end of flowering (“late” sowing date).

	after winter			end of flowering								
		Diseases			Diseases							
Treatments	Pl. Dev.	Ph.l.	Rate	Pl. Dev.	P.p.	Rate	Ph.l.	Rate	A.b.	Rate	B.c.	Rate
1 = Control	4.5	7.5	1.8	2.0	5.0	1.4	23.8	2.0	1.3	1.1	1.3	1.1
2 = SZX 0722 + Thiram (TMTD)	4.0	12.5	1.9	1.5	6.3	1.5	22.5	1.9	0.0	1.0	2.5	1.1
3 = Thiram + 0.75l/ha Folicur	4.5	13.1	1.8	2.0	10.0	1.9	15.0	1.9	0.0	1.0	0.0	1.0
4 = SZX 0722 + Thiram + 0,75l/ha Folicur	4.5	13.1	1.9	2.0	10.0	1.6	20.0	1.9	0.0	1.0	1.3	1.1
5 = DMM (Dimethomorph)	4.8	6.9	1.6	2.0	15.0	1.8	18.8	1.9	0.0	1.0	0.0	1.0
6 =Caramba (Metconazol)	4.8	8.1	2.0	2.0	11.3	1.8	20.0	2.0	0.0	1.0	2.5	1.1
7 = Folicur (Tebuconazol)	4.3	11.3	1.9	2.0	5.0	1.4	22.5	1.9	0.0	1.0	0.0	1.0

P.p. = Downy Mildew (*Peronospora parasitica*)
Ph. l. = Stem Canker (*Phoma lingam*)
A. b. = Dark Leaf and Pod Spot (*Alternaria brassicae*)
B.c. = Grey Mold (*Botrytis cinerea*)
C.c. = Light Leaf Spot (*Cylindrosporium concentricum*)
Rate: Assessment rate from: 1 = no attack to 9 = 100 % attack
Pl. Dev. = Plant Development; Rate from: 1 = Plants very well to 9 = Plants very poorly developed

Comparison of the two sowing dates

The “normal” and “late” sowing date treatments produced opposite effects with regard to the levels of downey mildew control produced by the different seed treatments. Whereas, the level of infection was lowest for treatments 2, 4 and 5 at the normal sowing date, these treatments had the highest incidence at the “late” sowing date (Fig. 1).

The lowest levels of stem canker infection were observed at the first assessment date for both “normal” and “late” sowing dates,. For the “normal” sowing date, the highest infection was observed for treatments 2 and 4, with 1.3 % of the plants showing symptoms. For the “late” sowing date, the highest levels of infection were observed for treatment 2 and the control, with 3.8 % of plants attacked. Both assessments are compared in the Figure 2. At the second assessment, a higher incidence of infection was present. In the “normal” sowing date, the highest infection incidence was 93 % of plants infected (treatment 6), whilst for the “late” sowing date, 67.5 % of plants were infected (treatment 2). It is interesting to note the levels of control achieved by treatments 3 and 4 which both received an additional foliar treatment of Folicur. In treatment 3, an effect on the stem canker infection level was observed. For the “normal” sowing date, the infection level was reduced from 86 % for the untreated control to 63 % and for the “late” sowing date from the reduction was from 63 to 58 %. Conversely, no effect was observed for treatment 4, where the incidence of infection was the same as the untreated control for both sowing dates.

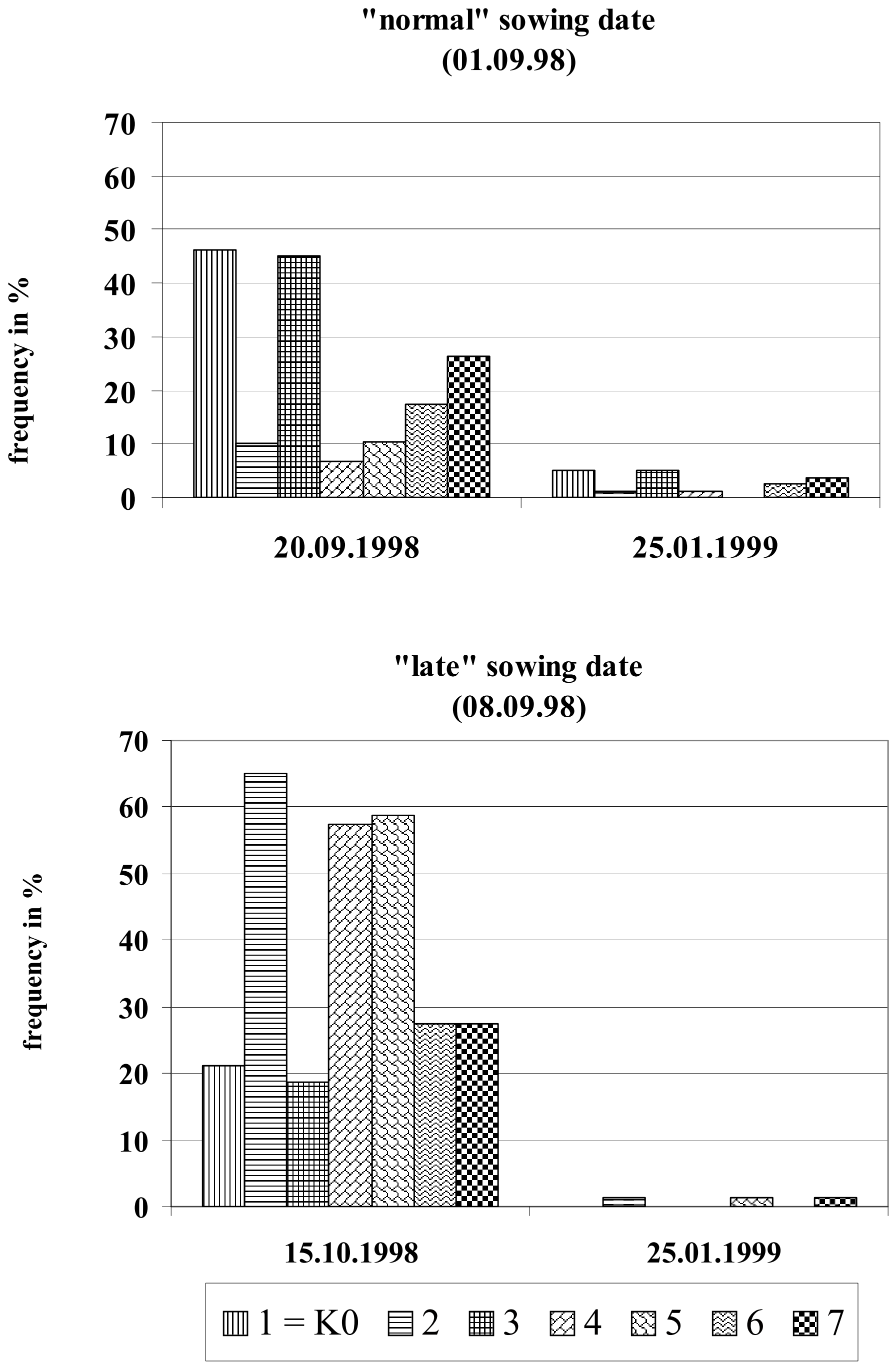

Figure 1. Effects of different fungicidal seed treatments on downy mildew (*Peronospora parasitica*) on winter oilseed rape at different sowing dates.

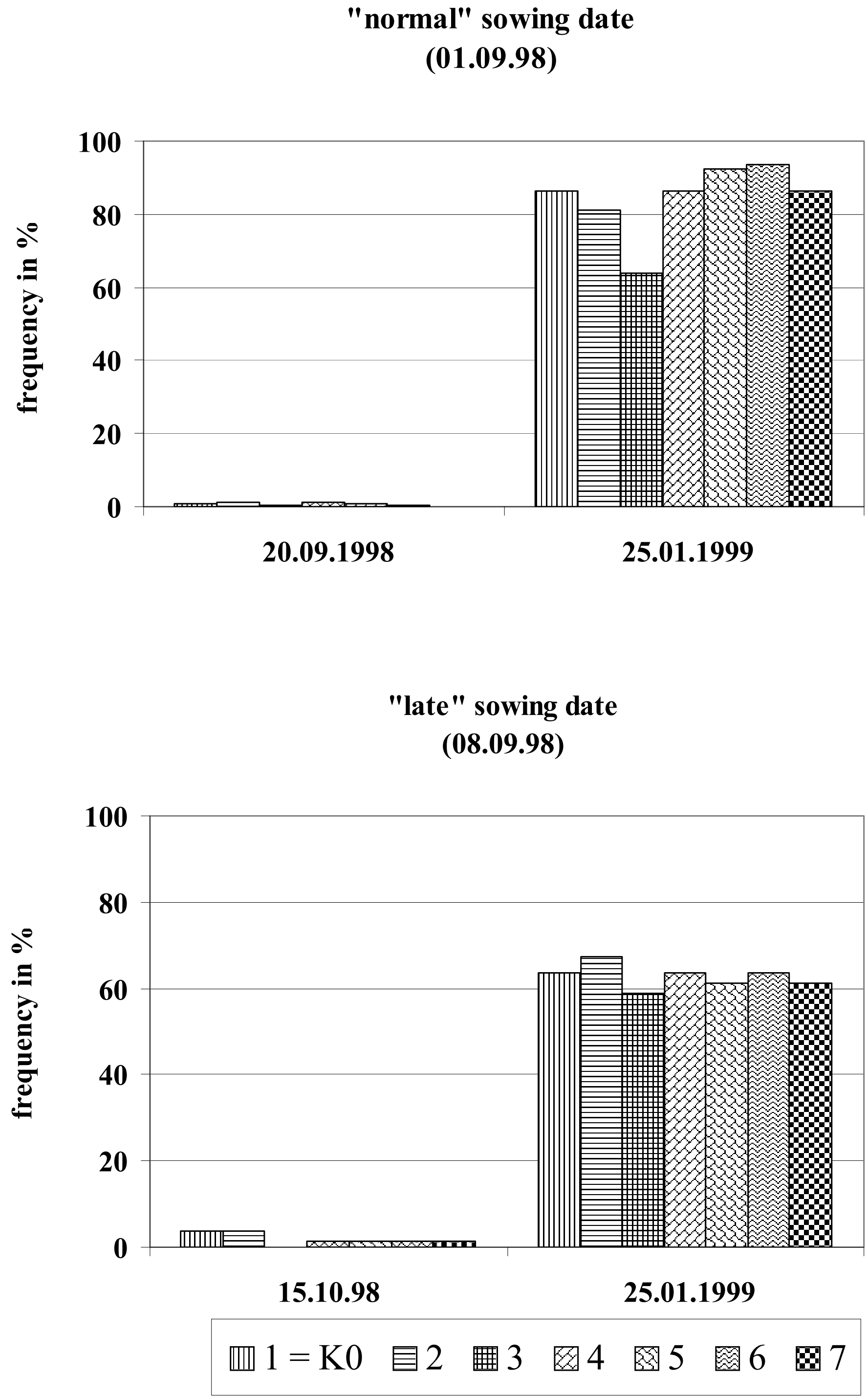

Figure 2. Effects of different fungicidal seed treatments on stem canker (*Phoma lingam*) on winter oilseed rape at different sowing dates.

Discussion

Both sowing dates showed a high infection level of downy mildew. For the “normal” sowing date, the control treatment and treatment 3 (standard seed treatment: Thiram) were severely infected (46.3 % and 45 % of plants infected, respectively) while the opposite occurred for the “late” sowing date (21.2% and 18.7 % of plants infected, respectively). Treatments 2 (SZX 0722 + Thiram), 4 (SZX 0722 + Thiram) and 5 (Dimethomorph = DMM) also showed a similar result. These treatments gave better control of downy mildew for the “normal” sowing date and less control for the “late” sowing date (Table 3 and Table 5 and Fig. 1). This result cannot be explained to date as the DMM seed treatment was developed for the control of downy mildew during the emergent and juvenile stages of oilseed rape growth. It may be possible that the plants were weakened due to the unfavourable weather conditions encountered during the fall of 1998, or possibly even by the trial product (SZX 0722) and the DMM treatment, such that infection conditions conducive to the downy mildew fungus were produced. Conversely, the seed treatment Thiram is known to be well tolerated by plants (Heitefuß, 1987) and even though it has no effect on downy mildew, the chemical does not weaken the plants which are therefor more resistant to downy mildew.

As foliar fungicides, treatments 6 (Metconazol) and 7 (Tebuconazol) do have a good effect on stem canker and stem rot. However, due to the low stem canker infection level (0.8 % of plants infected in the untreated control) for the “normal” sowing date, no effects of seed treatment against stem canker could be observed in the field trial. For the “late” sowing date treatments, where the infection level was slightly higher (3.8 % of plants infected in the untreated control), a small reduction in infection was recorded for treatments 6 and 7 (reduction of 2.5 %).

For the “normal” sowing date treatments, plant density before winter reached between 73-92 % of the desired density of 65 Pl./m^2 , with 48 Pl./m^2 for treatment 7 and 60 Pl./m^2 for treatment 4. For the “late” sowing date treatments, plant density was very low at the assessment prior to winter (assessment on 25th January) due to low emergence through the heavy, wet soil surface was only 8-17 % of the expected normal population. Nevertheless, the emergent plants had a medium development rate, due to the otherwise mild weather conditions (Table 4). The assessment of plant development indicated assessment rates ranging between 3.5 for treatment 4 and 5.0 for treatment 5.

Summary

Taking into account the very moist weather in the fall 1998, “normal” sowing date crop emergence was relatively good, with 50-75 Plants/m^2. The “late” sowing date crop emerged very poorly, with 17-25 Plants/m^2.

For the “normal” sowing date treatments, 46.3 % of the plants in the control plots were infected by downy mildew at the two-leaf stage with an average assessment rate of 4.8. Compared to the control, treatments 2, 4 and 5 showed very good control of downy mildew. The frequency of infection was reduced to 10 % with an a.r. of 3.5 for treatment 2, to 6.75 % with an a. r. of 3.5 for treatment 4 and to 10.3 %, with an a.r. of 2 in treatment 5. Treatment 6 also controlled downy mildew to a degree, but not as well as treatments 2, 4 and 5.

For the “late” sowing date, the results outlined above were completely reversed. The control treatment had a lower frequency of downy mildew (21.25 % with an a.r. of 4.3) than treatment 2 (65 % with an a.r. of 6.5), treatment 4 (57.5 %with an a.r. of 5.0), or 5 (58.7 % with an a.r. of 5.0). Similar results, with lower disease occurrence, were obtained at the assessment date before winter.

The Phoma-damping off did not occur, but a low frequency of stem canker (as Phoma leaf spot) was assessed in both sowing dates in the fall at the two leaf stage.

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First results on the effects of the new triazole fungicide Caramba® against oilseed rape diseases and plant growth in regard to application dates in 1998/99

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Abstract: In Germany, the triazole fungicide Folicur, which contains the active ingredient *tebuconazole*, has been used for about 5 years to control pathogens of oilseed rape. In 1999, another triazole fungicide with the active ingredient *metconazole* (Caramba) has become available for use on oilseed rape Table 10. This paper reports the results of an investigation of the effects of the two triazole fungicides against oilseed rape diseases using *in vitro* tests and growth chamber and field plot experiments. Furthermore, the effects of Caramba on plant growth of winter oilseed rape were investigated.

In laboratory tests, three concentrations of both fungicides were used in the culture medium. The mycelium growth rates of different fungal pathogens of oilseed rape were measured. Except for stem rot (*Sclerotinia sclerotiorum),* the pathogens showed no difference in reaction to the two fungicides tested. In a more detailed study, different reactions to the two fungicides were observed in 6 different stem rot isolates.

In growth chamber experiments, winter oilseed rape plants were protectively treated with different concentrations of the fungicides and inoculated with three different isolates of stem rot. The results indicated that there were differences in the control of stem rot with regard to the different concentrations of fungicide used and the different stem rot isolates with which plants had been inoculated.

In field trials, the effects of fungicide, fungicide concentration and application date were evaluated. The evaluation of the fungicides showed a correlation between plant parameters and the frequency of disease attack with stem canker (*Phoma lingam*) with regard to fungicide concentration and application date.

Key words: Oilseed rape, fungal diseases, plant growth, triazole, Caramba (metconazole), Folicur (tebuconazole)

Introduction

The cultivation of oilseed rape in Germany has been continuously increasing over the last twenty years. Furthermore, the cultivation of this plant has since extended to other parts of the world. Today, oilseed rape cultivation is an established practise in Europe, USA, Asia, and Eastern European countries. Depending on the growth region, the exact growing conditions and the occurrence of diseases differ. Therefore, the cultivation methodology is specific for the location of the crop. The cultivation of oilseed rape is very intensive and to be economically viable, requires crop treatment with growth regulators, fungicides and insecticides. A treatment with growth regulator is necessary to achieve a stem shortening effect on winter oilseed rape as this improves the winterhardness and steadfastness of the crop. Furthermore, many growth regulators have fungicidal effects. In Germany, the triazol

Folicur (containing tebuconazole) has been available for a number of years and has been used as a growth regulator and fungicide. A new compound, the triazol Caramba (containing metconazole), has recently become available in Germany and this product also has growth regulation and fungicidal properties. In the following experiment, the two triazole compounds are compared with regard to their effects on plant growth and their antifungal effects in a series of field experiments and growth chamber and *in vitro* tests.

Materials and methods

Screening of oilseed rape pathogens in vitro

To get a first impression of the effects of the two fungicides against oilseed rape pathogens we carried out a screening experiment with 7 different oilseed rape pathogens: *Alternaria brassicae, Botrytis cinerea, Cylindrosporium concentricum, Phoma lingam, Rhizoctonia solani, Sclerotinia sclerotiorum and Verticillium dahliae*. For this purpose petri dishes with PDA containing concentrations of 10 and 100 ppm of each product (according to C.C. Reilly) were used. We used 5 replications of each concentration, pathogen and fungicide. To start the experiment pieces of a defined size were cut from well growing PDA-culture and transferred to new petri dishes (diameter of 5 cm) with PDA-medium supplemented with different concentrations of the fungicides. Growth rates of the fungal mycelium growth were measured in centimeter [cm].

Screening of Sclerotinia sclerotiorum isolates in vitro

The same method as described above was used. Six *S. sclerotiorum* isolates from different locations in Germany were used for this experiment.

Growth chamber test

For this test, oilseed rape were grown to the cotyledon stage before use. The method of the test is shown inTable 1 and 2. Here, 3 isolates (DK 97, Rhh 95, GG 95) were taken from the isolates of the *in vitro* screening with *S. sclerotiorum*.

Table 1. Inoculation of *Brassica napus* (cotyledon stage) with *Sclerotinia sclerotiorum* in growth chamber test (according to Schmidt).

Plant material	Winter oilseed rape at the cotyledon stage, fungicide application 3 days before inoculation
Material for inoculation	6 different stem rot (*S. sclerotiorum*) isolates
Inoculation method	1. *S. sclerotiorum* was grown on PDA medium 2-3 days, then a piece of defined size was cut out
	2. The pieces were put on the surface of the cotyledons (with mycelium on the leaf surface)
Incubation	14/10 h day/night with a temperature of 18/10° C and 6000 Lux
Assessment	Infested leaf surface in %, 4 days after inoculation according to the scheme shown in Table 2**.**

Table 2. Assessment scheme for infestation with stem rot.

Rate	% infested leaf surface
1	no infestation
2	1-12
3	13-25
4	26-37
5	38-50
6	51-63
7	64-76
8	77-88
9	89-100

Field experiment

The field trial was carried out in a block construction with 5 replicates, including one replication allowing sampling of plants in the field (plot size = 30 m^2). The 14 treatments are shown in Table 3. Fungicides were applied at four different growth stages, two in autumn at the 3-4 leaf stage and the 6-8 leaf stage and two the following spring at stem extension and flowering. Assessments were carried out as shown in Table 3. Two assessments were used to determine the effects of the fungicides. Twenty plants per plot were visually assessed in the field, while twenty plants were taken from the field for closer assessment in the laboratory.

Table 3. Application rates and application dates of the field trial at the experimental station of UGH Paderborn in Merklingsen, in 1998/99

Treatment	**Product**	**Concentration [l/ha]**	**Application date**
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/spring2
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 = 3-4 leaf stage; autumn 2 = 6-8 leaf stage; spring 1 = stem extension; spring 2 = flowering, plot size: 30 m^2, n=4

Table 4. Assessment dates and the assessments methods carried out at a field trial on the experimental station of UGH Paderborn in Merklingsen, in 1998/99.

Assessment date	Growth stages/fungicide application	Assessments
in autumn		
7.10.98	5-6 true leaf stage field assessment fungicide application: autumn 1.	• infestation with pathogens; stem canker and downy mildew on leaf were found • general plant condition[1]* • effects of product[2]* • length of plant
2.12.98	About tenth true leaf (before winter) field assessment	• leaf infestation with stem canker and downy mildew • general plant condition[1]* • effects of product[2]* • length of plants
17.12.98	laboratory assessment fungicide application: autumn 2.	• stem infestation with stem canker • plant morphological parameters
in spring		
12.3.99	about fifteenth true leaf (after winter) field assessment	• leaf infestation with stem canker and downy mildew • general plant condition[1]* • effects of product[2]* • length of plants
6.4.99	stem extension laboratory assessment	• stem infestation with stem canker • plant morphological parameters
22.4.99	beginning flowering field assessment fungicide application: spring 1	• leaf infestation with stem canker and downy mildew • general plant condition[3]* • lodging

[1]*Covering of ground, positions of leaves, colour of plant, number of wilted leaves per plant
[2]*Shorter petiole, position of leaves near to ground,
[3]*Intensively green coloured, tendency of lodging, condition of the buds, flowers and pods

Results

Laboratory investigations
Screening with oilseed rape Pathogens

Figure 1 shows the results 12 days after inoculation. Some of the pathogens show inhibition of mycelium growth rate on PDA dishes with the two fungicides compared to the control. The growth rate of *R. solani* showed no reaction to either product. The mycelial growth rates of *B. cinerea, C. concentricum* and *P. lingam* were completely inhibited by the concentrations of 10 and 100 ppm of the two products. In the case of *V. dahliae* the higher concentration of both products caused complete inhibition of mycelium growth, whereas the lower product concentration of both products produced a growth rate of approximately 1 cm. The growth rate of the *V. dahliae* was 5 cm and the growth of *A. brassicae* and *S. sclerotinia* was inhibited by the two products at all concentrations. In general, the higher concentration had the greatest inhibitory effect, allowing lower mycelial growth.

With the exception of *S. sclerotiorum,* the pathogens show differences with regard to the two triazole products. The inhibitory effect of Folicur (*tebuconazole*) on mycelium growth was higher than that of Caramba (*metconazole*). Apart from this result, the higher concentration caused a lower mycelium growth and vice versa. To verify the results with *S. sclerotiorum*, a seperate screening experiment, using the same test procedures but with 6 different isolates of *S. sclerotiorum*, was carried out.

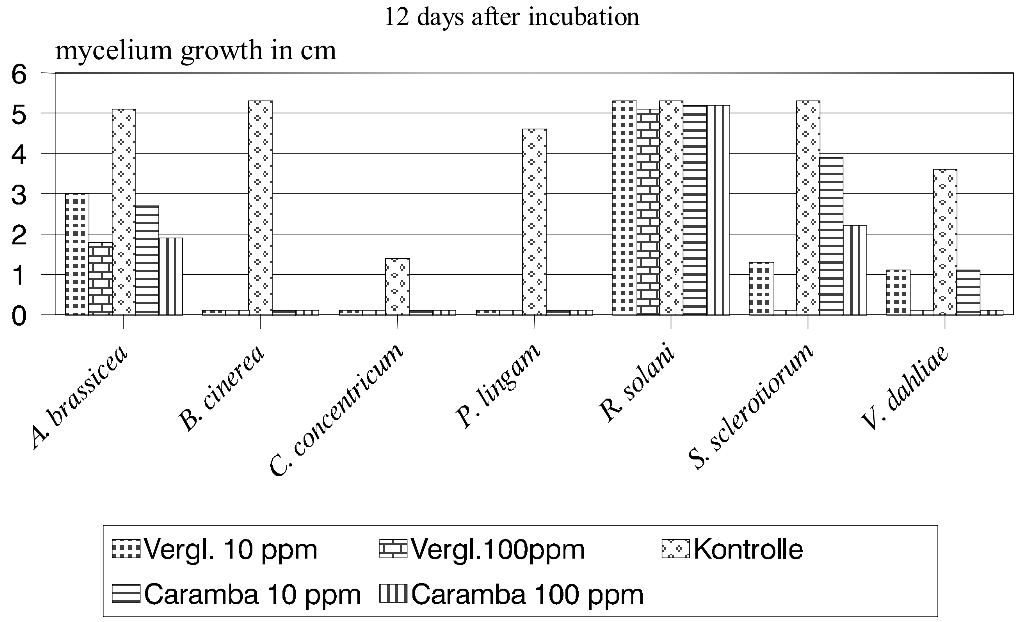

Figure 1. Effect of metaconazole on the growth rate of fungal oilseed rape pathogens *in vitro.* Mycelium growth rates of some oilseed rape pathogens on PDA with different concentrations of tebuconazole and metconazole respectively.

Screening with S. sclerotiorum isolates

Figure 2 shows the mycelial growth rates of six *S. sclerotiorum* isolates from 2 to 28 days after incubation on PDA supplemented with two fungicides. With regard to the concentration of 10 ppm, the isolates can be subdivided in three groups concerning the inhibition of mycelium growth: low inhibition in the case of isolate GG95, medium inhibition in the cases of isolates L 97, RHH 95, DK 97 and high inhibition in the case of isolates Me 97 and Pol 97. Furthermore, there are some differences concerning the effect of the products on growth rate. However, 28 days after the experiment had begun, no more differences were noted. In the case of the 100 ppm treatment, two of the six isolates were totally inhibited by Caramba, whereas the growth of four of the six isolates was totally inhibited on Folicur treated plates.

Growth chamber test

The results of the growth chamber tests are shown in Figure 3. Three different concentrations of each product were used in close correlation to common field applications. Water was used as the control. Both concentrations of the two products caused an inhibition of mycelial

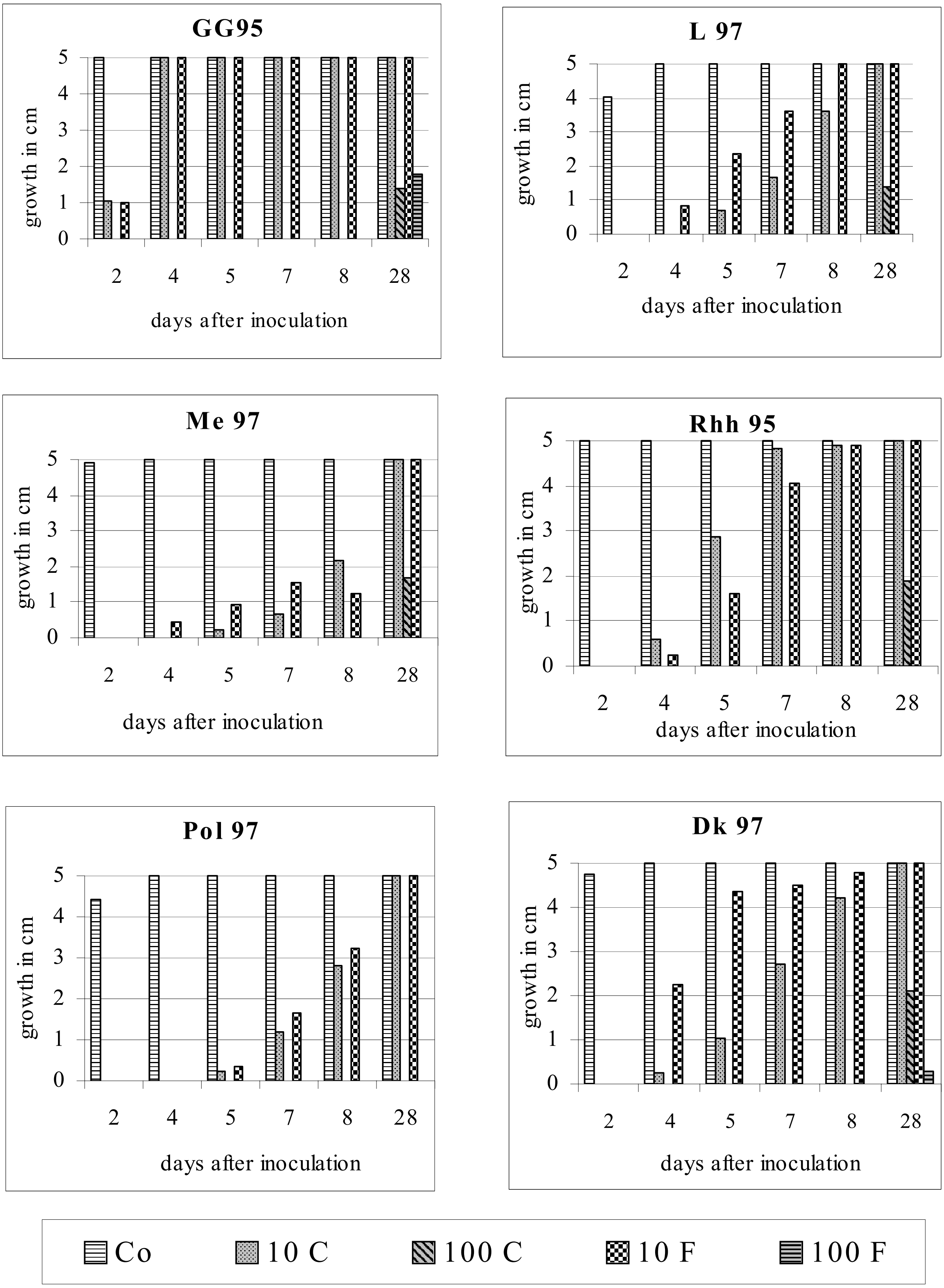

Figure 2. Results of a screening experiment with *Sclerotinia sclerotiorum* tested *in vitro* on PDA containing Caramba=C (metconazole) and Folicur=F (tebuconazole).

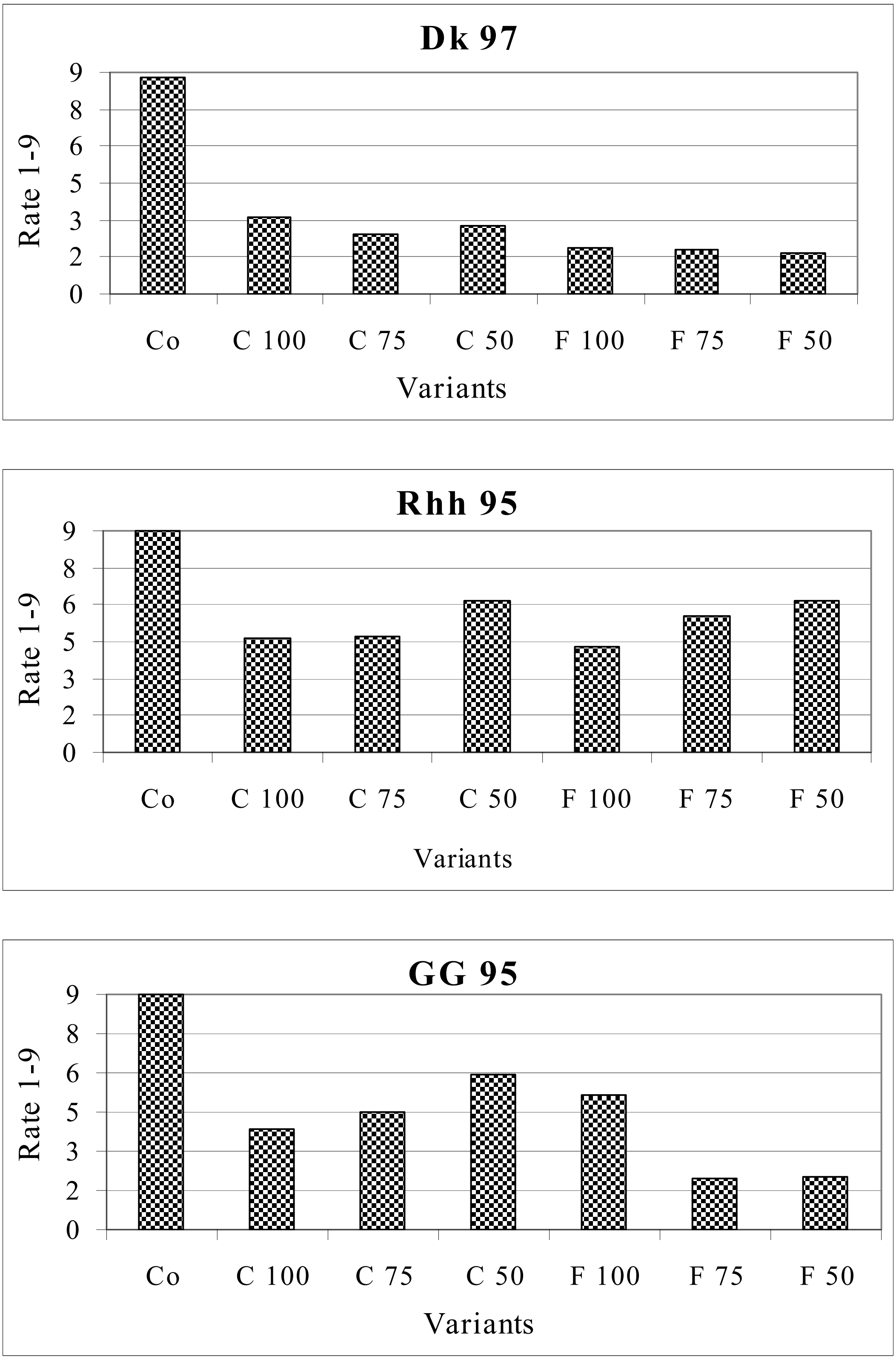

Figure 3. Results of the growth chamber experiment with three different isolates and different concentration of Caramba=C (metconazole) and Folicur=F (tebuconazole).

Table 5. Results of autumn assessments of the oilseed rape field trial at the experimental station UGH Paderborn, in Merklingsen, in 1998/99.

	Diseases				Plant development				
Treat-ment	*P. lingam*, (leaf) infestation in %		*P parasitica*,(leaf) infestation in %		Plant height [cm]	Effect of product[1]* 1-9		General plant condition [2]*1-9	
	07.10.	02.12.	07.10.	02.12.	07.10.	7.10.	12.12.	7.10.	12.12.
1	5	7	7	7	10	2	3	8	8
2	5	7	7	12	14	3	3	6	8
3	7	12	0	12	16	5	4	8	8
4	5	12	0	12	20	8	6	7	6
5	5	12	0	12	20	8	6	7	7
6	5	12	0	20	19	8	6	8	8
7	5	12	0	12	20	8	5	7	7
8	5	20	7	20	19	8	5	8	8
9	5	20	0	20	19	8	6	6	8
10	5	7	7	7	12	2	3	8	7
11	5	12	0	12	18	8	6	8	6
12	5	12	0	20	19	8	7	7	7
13	5	12	0	20	20	8	7	8	8
14	5	20	0	20	18	8	7	8	8

Plant height from the hypocotyl [cm], effect of product (assessment scale from 1-9, 1 =compact/shortening); general plant condition. (assessment rate 1-9, 1=poor), average of 20 plants / plot, n=4;
[1]*Shorter petiole, position of leaves near to ground, intensive green colour
[2]*Covering of ground, sites of leaves, colour of plant, number of wilted leaves per plant
LSD $_{\text{effect product}}$ 1,56.

growth of all the isolates in comparison to the control. There was also some differences between the three *S. sclerotiorum* isolates in response to the different concentrations of the fungicide products. With regard to the isolate GG 95, there was a high inhibition of mycelium growth caused by the lower concentration of the product Caramba.

The results of the growth chamber tests and the *in vitro* tests show no similar reaction of the isolates.

Field experiment
In the autumn, both products have a shortening effect on the height of the plants, length of the petiole, leaf position and the intensity of green colour of the leaves (Table 5). These effects were significantly more distinct with the earlier application date and high concentration and the main root shows a tendency to extend (LSD $_{\text{effect product}}$ 1,56. Table 6) However, the diameter of the hypocotyl shows no differences compared with the control. The fresh and dry mass of roots and plants tend to increase with product application, although significant differences between the application dates and rates were not found. The control showed the lowest masses. Autumn applications reduced the infestation caused by *P. lingam* on leaves

and stems and *Peronospora parasitica* on leaves Table 5 LSD $_{\text{effect product}}$ 1,56 Table 6).

In spring, no differences were found between the parameters assessed with the exception of the height of the plants (Table 7 and Table 8) Here, the height of the plants were reduced with regard to the application dates (Table 7). Snow fell during the night of 15 April 1999 depositing an accumulation of 5cm and contributing to the high lodging score recorded for the control (Table 9). Lodging was reduced by fungicide application.

Table 6. Autumn assessment of Stem canker (*Phoma lingam*) infestation on hypocotyl and of plant morphological parameters (n=20 plants per variant).

	Disease	**Plant morphological parameters**					
Treat-ment	*P. lingam*, hypocotyl 1-9	Length of main root [cm]	Diameter of hypocotyl [cm]	FM of roots [g]	DM of roots [g]	FM of plants [g]	DM of plants [g]
	17.12.98	17.12.98	17.12.98	17.12.98	17.12.98	17.12.98	17.12.98
1	1.2	12.5	1.3	116.4	30.6	508.8	82.4
2	1.6	12.5	1.4	124.5	34.8	509.0	89.4
3	1.6	13.5	1.4	127.7	35.3	479.8	94.0
4	1.5	12.5	1.4	147.2	39.3	696.8	88.2
5	1.5	14.5	1.4	174.9	40.8	545.5	98.4
6	1.6	13.0	1.6	131.1	35.6	717.3	118.4
7	1.7	14.0	1.2	124.6	33.7	580.1	91.9
8	2.0	13.0	1.3	131.7	34.7	581.2	93.7
9	1.9	13.0	1.2	113.0	30.4	550.1	86.0
10	1.2	12.5	1.4	123.5	32.4	576.5	92.4
11	1.1	12.0	1.3	136.4	35.2	560.9	90.0
12	1.3	13.0	1.2	103.3	28.5	423.6	70.4
13	2.2	12.0	1.5	118.3	35.0	465.1	88.4
14	2.4	11.5	1.4	99.7	27.5	446.5	76.4

Assessment of stem canker (*Phoma lingam*): 1=no infestation, 9=67-100% affect; according to Krüger (1991), FM=Fresh mass, DM=Dry mass

Table 7. Results of spring assessment (about fifteen leaf stage) of the oilseed rape field trial at the experimental station UGH Paderborn, in Merklingsen, in 1998/99.

	Diseases		**Plant development**		
Treat-ment	*P. lingam*, (leaf) infestation in %	*P parasitica*,(leaf) infestation in %	Plant length [cm]	Effect of product[1]* 1-9	General plant condition [2]*1-9
	12.03.99	12.03.99	12.03.99	12.03.99	12.03.99
1	1.75	1	11.8	6.8	8.0
2	2	1	12	6.3	8.0
3	2	1	11.5	6.8	8.0
4	1.75	1	13.3	6.5	6.0
5	2	1	14.3	6.8	7.0
6	2	1	13.3	6.3	8.0
7	2	1	13.8	6.8	7.0
8	2	1	14	7.3	8.0
9	2	1	14	7.3	8.0
10	1.75	1	11.8	6.5	7.0
11	2	1	12.3	6.3	6.0
12	2	1	13	7.0	7.0
13	2	1	14.5	6.5	8.0
14	2	1	15.8	6	8.0

Plant lengh from the hypocotyl [cm], effect of product (assessment scale from 1-9, 1 =compact/shortening); general plant condition. (assessment rate 1-9, 1=poor), average of 20 plants / plot, n=4.

[1]*Shorter petiole, position of leaves near to ground, intensive green colour

[2]*Covering of ground, sites of leaves, colour of plant, number of wilted leaves per plant

Table 8. Spring assessment of Stem canker (*Phoma lingam*) infestation on hypocotyl and of plant morphological parameters, (n=20 plants per variant).

	Disease	**Plant morphological parameters**					
Treat-ment	*P. lingam*, hypocotyl 1-9	Length of main root [cm]	Diameter of hypocotyl [cm]	FM of roots [g]	DM of roots [g]	FM of plants [g]	DM of plants [g]
	06.04.99	06.04.99	06.04.99	06.04.99	06.04.99	06.04.99	06.04.99
1	2.0	13.5	1.4	103.1	31.6	772	125.0
2	1.7	15.3	1.6	198.7	47.2	1168	156.5
3	2.3	15.2	1.6	197.9	46.4	1292	169.0
4	2.1	14.8	1.7	216.0	47.9	1214	163.6
5	2.1	14.8	1.7	203.5	46.8	1412	177.2
6	2.0	15.6	1.7	184.3	54.8	1331	178.5
7	1.9	14.7	1.6	179.7	42.1	1240	155.9
8	2.4	17.6	1.7	288.0	61.7	1303	152.7
9	2.2	15.4	1.6	213.9	56.8	987	170.0
10	2.0	16.6	1.6	224.0	60.6	1059	173.2
11	2.1	14.4	1.6	187.1	44.7	1029	158.2
12	1.9	15.8	1.6	213.8	48.6	1260	171.0
13	1.9	15.9	1.6	248.4	50.9	1374	167.3
14	2.2	14.3	1.5	208.5	45.7	1333	175.5

Assessment of stem canker (*Phoma lingam*): 1=no infestation, 9=67-100% affect; according to Krüger (1991), FM=Fresh mass, DM=Dry mass

Table 9. Results of the assessments at flowering stage of the oilseed rape field trial at the experimental station in Merklingsen UGH Paderborn in 1998/99.

	Disease				**Plant development**	
	P. lingam,(leaf)		*P. parasitica,* (leaf)		lodging 2*	General plant condition 1*
Variant	infestation in %	assessment rate 1-9	infestation in %	assessment rate 1-9	assessment rate 1-9	assessment rate 1-9
	21.4.99	21.4.99	21.4.99	21.4.99	21.4.99	21.4.99
1	9.3	2.0	2.3	2.0	3.0	9
2	10.8	2.0	1.8	2.0	3.3	9
3	12.3	2.0	3.3	2.0	3.8	9
4	8.5	2.0	2.5	2.0	4.0	9
5	9.0	2.0	3.5	2.0	5.0	9
6	9.5	2.0	2.5	2.0	5.5	9
7	8.8	2.0	2.0	2.0	6.0	9
8	10.8	2.3	2.3	2.3	6.0	9
9	10.5	2.0	3.0	2.0	6.5	9
10	11.0	2.0	1.5	2.0	3.8	9
11	10.0	2.0	2.8	2.0	4.3	9
12	10.5	2.3	2.5	2.0	5.0	9
13	10.3	2.3	2.5	2.0	6.3	9
14	12.0	2.0	3.0	2.0	6.8	9

Assessment rate lodging and diseases 1-9, 1= low; n=4, 20 plants per plot
1*Colour of plants, number of wilted leaves per plant, conditions of flower buds/flowers/pods, general impression of the plots
2*In the night from 15 to 16.4.1999 there was a snowfall with up to 5 cm fresh snow.
$LSD_{lodging}$ 1,6

Table 10. Concentration of the active substance used in the in vitro tests.

Product	Folicur		Caramba	
Active substance	Tebuconazole		Metconazole	
Concentration of active substance	250 g/l		60 g/l	
Concentration of product in the in vitro test	10 ppm	100ppm	10 ppm	100 ppm
Concentration of active substance in the in vitro test	2.5 ppm	25 ppm	0.6 ppm	6 ppm

Discussion

In vitro test and growth chamber test with oilseed rape pathogens
The *in vitro* test with oilseed rape pathogens was carried out to get a first impression of the effect of the products on oilseed rape pathogens. Both products showed a high inhibition effect on the pathogens *B. cinerea*, *C. concentricum* and *P. lingam*. The fungal pathogens *A. brassicae*, *S. sclerotiorum* and *V. dahliae* showed moderate inhibition whilst *R. solani* showed low inhibition to the triazoles (see Figure 1). The concentrations of the products used during *in vitro* tests and growth chamber experiments were calculated based on the product concentration used in the field. This was done to get a better comparability of the results from the *in vitro* tests with the results of growth chamber tests and those of the field trials.

The concentrations of active substances are shown in Table 10. With the exception of *S. sclerotiorum,* similar inhibition of mycelium growth was obtained with both Caramba and Folicur, particularly in the case of the former of these two products as a quarter of the concentration of the active substance showed similar inhibition to the full concentration. Folicur caused a high inhibition of the different oilseed rape pathogens during the *in vitro* test with the exception of the *S. sclerotiorum* isolate (Table 1). However, this was a specific reaction to the isolate used and the results of the *in vitro* test with several different *S. sclerotiorum* isolates indicates isolate dependant differences (Figure 2). Isolates could be subdivided into high, medium and low inhibition. In the case of the 10 ppm treatment (0.6 ppm a.i. metconazole and 2.5 ppm a.i. tebuconazole), the inhibition effect of the growth rate of the products of each isolate was different. At the 100 ppm treatment, the inhibition effect was higher on average. Overall, the results indicate that a quarter of the amount of metconazole is required to give the same level of inhibition in comparison to tebuconazole. The results of the cotyledon test of *Brassica napus* confirm this (Figure 3). The inhibition effect on *S. sclerotiorum* growth after inoculation correlates to the application rate of the products in %, but the amount of active substance in the case of Caramba is about one quarter that of the active substance of Folicur. Comparing the inhibition effects of the *in vitro* test and of the growth chamber tests with stem rot, the reactions of the isolates do not correlate. This can be explained by the differences in the availability of the active substances in the agar medium and in the plants. The different inhibition rates of the isolates caused by the products (Figure 2 and 3) appear to indicate that some resistance against the compared products may develop. At this stage, it is difficult to predict to what extent these results are transferable to the field situation and indeed, no stem rot infestation has been found in the field on treated plants to date.

Based on the laboratory and growth chamber results outlined in this paper, of the investigation with regard to stem rot, it is very likely that the inhibitiory effects caused by both products can be observed in the field trial in cases where a high infestation level of stem rot occurs. We plan to carry out more screenings in the laboratory and growth chamber with different isolates of fungal oilseed rape pathogens in a same manner as was done in more detail for stem rot.

Field trials
From the field trial data, plant growth effects were observed in autumn, as well as a reduction of stem canker and downy mildew (Table 5 and LSD effect product 1,56 Table 6) In the autumn, only stem canker and downy mildew were observed. Both products regulated the growth of winter oilseed rape and reduced infestation caused by the pathogens. Only stem canker control was observed with both products during the autumn. Infestation caused by

stem canker on leaves and stems was reduced by the products depending on to application rate and application date. The earlier application dates and the higher rates controlled stem canker better than later and lower concentrations by which time, no differences were observed between the products. With regard to the plant development and morphological parameters assessed, a similar situation was found in the autumn. By spring, both products produced similar effects and no differences were observed between the products. In general the concentration of active substance in the case of Caramba (metconazole) was only a quarter of the amount of active substance of Folicur (tebuconazole) and this produced the same effect. At this stage of investigation no further results have been obtained. Some assessments are planed for the summer of 1999 during which, levels of stem rot and stem canker infestation will be assessed and pod and yield assessments are also planned. Identical field trials are planned for the next two seasons in order to confirm the preliminary results outlined in this paper.

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Monitoring Pests –
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Within-field distributions of the seed weevil, *Ceutorhynchus assimilis* (Paykull) and its parasitoid, *Trichomalus perfectus* (Walker), on winter oilseed rape

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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.

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 ($I_a > 1$). 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.

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Weather as a factor determining damage caused by oilseed rape pests

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Abstract The studies were carried out in 1980-1987 and 1991-1993 and were concentrated on three insect species of the greatest economical importance. The cabbage aphid, *Brevicoryne brassicae* (L.), was most numerous when the rainfall in March and April was much below average and the mean temperature in May was higher than 15°C. The increased rainfall in early spring caused a high reduction in the aphid population which wintered in oilseed rape. The highest damage by the cabbage aphid was found during the years with lowest rainfall in May or July. In the case of damage by larvae of the stem weevil *Ceutorhynchus pallidactylus* Marsh., a full compensation in seed yield was found when the rainfall in May and June was higher than the average and exceeded 260 mm during March-July. The negative effect of the feeding by the pollen beetle *Meligethes spp*. on the yield occurred only when the rainfall in July was reduced to 1/3 of the average. The compensation in number of pods was the most distinct when the rainfall in June was above the average. During the periods of drought in July the compensation processes were inhibited and the loss in seed yield occurred. The greatest over-compensation was observed at a yield over 30 dt/ha.

Key words: oilseed rape, pests, damage , compensation , weather conditions

Introduction

The damage caused by phytophagous insects to oilseed rape has always been subject to controversy. In different European countries, the economic threshold of the pollen beetle *Meligethes aeneus* F., which is the most common species, varies from 1 to 20 beetles/plant. Golinowska (1991) studied the damage caused by insect pests to oilseed rape and the effectiveness of control treatments during 10 years. She found that the yield of oilseed rape and its compensation abilities were always negatively or positively correlated with climatic conditions. Lerin (1995) pointed to the necessity of correlating the economic thresholds of a given insect pest with meteorological conditions at the time of its occurrence in the field. We have analysed pest incidence and damage to winter rape in relation to weather conditions over ten years.

Material and methods

The material for study was collected from the Agricultural University experimental field of winter oilseed rape in Pawłowice, near Wrocław, Poland. During the years 1980-1982 the variety Górczański was grown; in 1983-1987-the variety Quinta, and during 1991-1993- the variety Ceres.

Brevicoryne brassicae L.

The studies on *B. brassicae* were carried out during two periods: 1980-1984, and 1991-1993, i. e., for 8 years collectively. The aphid infestation was assessed weekly on 50 randomly chosen plants within 0.5 ha (1980-1984) or 0.3 ha (1991-1993) of an unprotected part of an oilseed rape crop. In early July every year, 60 plants free of aphids and 60 aphid infested plants were selected and labelled. After harvest the yield of seeds was determined for each plant separately and the percentage loss due to infestation was evaluated.

Meligethes spp.
The studies on pollen beetle were carried out during two periods: 1982-1987 and 1991-1992, i.e., for 8 years collectively The 1ha experimental field was divided into two parts: unprotected and protected with insecticide Cymbusz (a.i. cypermethrin). The protected part was sprayed every 5-7 days during the occurrence of *Meligethes* spp. On both parts, 30 plants were labelled along a transect. Before harvest the podless stalks and pods on each plant were counted. The yield of seeds was evaluated and the percentage loss due to infestation determined.

***Ceutorhynchus pallidactylus* Marsh.**
In 1985-1987 and 1991-1993, samples of 60 plants were taken after harvest from the part of field that was protected in early spring against stem weevils with insecticide Cymbusz and the unprotected part. Parameters of plant growth, yield and injury of stem was assessed.

Meteorological data were obtained from the Meteorological Station, in Pawłowice, 300 m from the experimental field.

Table 1. Weather conditions in experimental periods.

Year	Average temperature (°C)						Rainfall (mm)					
	March	April	May	June	July	Total	March	April	May	June	July	Total
1980	2.1	6.2	10.5	15.4	16.7	50.9	14.3	57.2	19.4	51.1	204	346
1981	6.6	7.1	14.8	17.6	17.6	63.7	61.8	39.5	17.9	60.9	135	315
1982	4.8	6.2	13.8	16.9	19.3	61	19.3	15.0	62.6	58.5	45.4	200
1983	4.9	9.9	14.0	17.0	20.4	66.2	23.3	39.7	97.9	106	35.1	302
1984	2.1	7.7	12.9	15.2	15.8	53.7	16.4	27.8	104	65.1	59.4	272
1985	3.6	8.8	15.2	14.6	18.6	60.8	33.0	42.7	53.8	92.8	31.7	254
1986	3.2	8.8	15.4	16.2	17.4	61	35.1	41.1	60.5	61.0	94.3	291
1987	0.8	8.3	11.4	15.8	18.0	52.7	16.5	54.7	70.4	54.0	64.7	260
1991	6.1	8.5	10.3	15.1	19.9	59.9	14.5	28.7	41.6	68.8	37.4	191
1992	4.4	7.0	13.7	19.4	20.0	64.5	89.8	20.3	19.5	50.6	57.6	237
1993	0.7	8.8	17.0	14.9	16.0	57.4	45.2	9.3	49.8	94.0	62.6	260
Average	3.3	8.1	13.4	16.3	18.2	59.2	34.8	41.0	60.5	61	94.0	250

Results

Brevicoryne brassicae
The lowest numbers of *B. brassicae* occurred in 1980 when the total rainfall of the whole spring vegetation period was the greatest (346 mm). Most rain occurred in April, June and July. At the same time, the year 1980 was the coldest one during the whole period of study. Extremely low mean temperatures occurred in April and May (6.2 and 10.5 °C, respectively).

In 1992, the number of *B. brassicae* was also very low. The total rainfall of that year was relatively low, but during March the rainfall was three times greater than the average for this month. At that time, the aphid population, which overwintered in oilseed rape, was reduced by a very high percent (exceeding 95%). The aphids were the most abundant in 1993 when there was almost no rainfall in April (9 mm) and the mean temperature in May was the highest. The aphids were also very numerous in 1982, 1984, and 1991. During all these years, the rainfall in March was below 20 mm (the average being 34.8). The rainfall in April of these years was also below the average (Table 1 & 2).

The damage cause by aphids was the greatest during the years of the lowest rainfall during May (1981) or July (1983). The year 1992 was exceptional. The spring drought lasted for over two months, and before that, the water deficit had occurred during previous fall. Due to such weather the yield was very low. At the same time the aphid numbers were quite low (see earlier), therefore, no difference between the yield of infested and uninfested plants could be found. In 1993, although the weather was very suitable for yielding of oilseed rape, the yield was reduced by 75%. During that year, the aphid colonies were very abundant. The aphid numbers were high also in 1984, but the reduction of yield was negligible. In 1984 an extremely high rainfall occurred in May.

Table 2. Occurence and damage caused by *B. brassicae* in years 1980-84 and 1991-93.

Year	Average no. in colony	Yield losses	Minimum temperature (°C)		Minimum rainfall (mm)		Maximum temperature (°C)		Maximum rainfall (mm)	
		%	Month	Level	Month	Level	Month	Level	Month	Level
1980	39	64	Acc.	50.9	March	14.3	-	-	Acc.	346
1981	122	85	-	-	May	17.9	July	17.6	July	135
1982	161	80	-	-	March April	19.3 15.0	July	19.3	-	-
1983	102	84	-	-	July	35.1	Acc.	66	June May	106 97
1984	159	35	July	15.8	March	16.4	-	-	May	104
1991	147	36	May	10.3	March Acc.	14.9 190	July	19.9	-	-
1992	77	+9	-	-	April May	20.3 19.5	June July	19.4 20.0	March	89.8
1993	307	75	-	-	April	9.5	May	17.0	-	-
Average	139	58								
Total March- July			59.2 (°C)		250 (mm)					

Acc.- accumulated.

Meligethes spp.

The greatest yield occurred in 1991, and the lowest – in 1982, and 1992. The low yield occurred when the spring drought lasted for two months (March-April 1982, and April-May 1992), and the rainfall was less that 50 % of the average.

The compensation of pod number was marked especially in 1983 and 1991, when the June rainfall was above the average. The compensation processes were restrained when July rainfall was below the average, which resulted in the decline in seed yield per plant. The negative effect of pollen beetle on oilseed rape yield occurred in 1983 and 1985, and during these years the July rainfall was only 1/3 of the average. In 1983, in spite of the over-compensation in pod number, the seed yield loss was 18%. The greatest overcompensation (28.2 % in pod number, 9.2 in seed weight) occurred when the yield was the highest (31 dt/ha). Such high yield was probably achieved by agrotechnic management, especially due to higher doses of nitrogen fertilisers (Table 3).

Table 3. Injury and damage caused by *Meligethes spp.* to protected (P) and unprotected (UP) winter rape.

Year	Podless stalks		Pods		Compensation in no. of pods. %	Overcompensation in no.of pods. %	Seed yield loss %	Commercial yield dt/ha
	UP-P no.	Difference %	P-UP no.	Difference %				
1982	59	44.7	2	2.4	94.7	0	5.7	12.8
1983	95	46.2	-6	-10.3	100	10.3	18.0	18.0
1984	103	55.4	16	8.8	84.2	0	7.9	19.1
1985	47	87.1	8	6.4	86.4	0	11.3	26.3
1986	36	81.8	-8	-7.6	100	7.6	3.0	27.2
1987	26	74.3	-8	-7.4	100	7.4	3.3	21.4
1991	27	37	-13	-28.2	100	28.2	+ 9.2	31.0
1992	44	32	-6	-18.5	100	8.5	+ 1.3	15.7

Table 4. Injury and damage caused by *Ceutorhynchus pallidactylus* Marsh. to protected (P) and unprotected (UN) winter rape.

Year	Stem larval tunnelling (cm)			Yield g/plant		Damage %
	P	UP	Difference	P	UP	
1985	27	70	43	19.5	17.2	11.8
1986	19	56	37	15.7	15.7	0
1987	23	56	33	11.9	12.3	+3.3
1991	36	70	34	9.9	7.7	22.3
1992	23	41	18	2.3	2.9	+26.1
1993	12	34	22	12.1	11.9	1.7

***Ceutorhynchus pallidactylus* Marsh.**
The greatest injury to plants occurred in 1985 and 1991. In these years, the mean length of larval tunnel was ca. 70 cm. The damage caused by stem weevil varied during the years of study. In 1991, the yield loss was 32 % and in 1985 – 12 %. In 1991, the total rainfall during the whole vegetation period of oilseed rape was the lowest, and in 1985, the June rainfall was very high. The complete compensation of yield in injured plants occurred in 1986, 1987, and 1993. The total rainfall during these years was above 260 mm, and above the average in May-June (Table 4).

Discussion

The statistical analysis in our previous study (Kelm et al. 1996) revealed that rainfall exceeding 20 mm a week might cause reduction in *B. brassicae* population in oilseed rape. The present data confirm that finding, especially for the early spring period (March) and the final period of oilseed rape development (July). The limiting effect of rainfall did not occur in May, as observed in 1984. At that time, the rainfall was 44 mm above the average and during the remaining months the rainfall was typical. Such effect might be explained by a different structure of oilseed rape plants during these months. In March, oilseed rape is in a rosette stage and the cabbage aphid eggs are most often laid on the under side of leaves. The fundatrices that emerge in March are then very close to the ground, so there is a clear danger for larvae of dropping onto the soil. In summer, the aphids live on smooth surface of pods and stems, from where they might easily be washed off by rain, especially during storms. Additionally, the aphids might be weakened by summer heat. In May, on the contrary, very high temperatures do not accompany the rain, which helps aphids to keep on their plants. Moreover, the petals of flowers might form a kind of an umbrella and therefore be a good protection against the rain. The leaves still exist on plants, which also helps aphids remain on the plant. Similar observation of a reduction of aphid numbers by heavy rains in June was done on cereal aphids by Jones (1979), and on pea aphid by Dunn and Wright (1955). Our results confirm also the opinion by Barlow and Dixon (1980) that weather might be a decisive factor for development of an aphid population. Moreover, our data suggest that in the case of the rainfall effect, also the position of aphid colony on a plant and the morphological characters of a plant are probably also important.

Weather has not previously been analysed in publications dealing with the compensation ability following insect injury. Lerin (1987) suggested that the full compensation might occur in spite of a long-term drought preceding flowering. He reported that in that case the rainfall in April was only 6.2 mm and water deficit occurred during the whole period of development. Muśnicki et al. (1994) found that the difference in yield between protected and unprotected plants was 28% when weather was favourable and oilseed rape was under intensive cultivation. While under the conditions of spring drought the difference reached 60%. According to Ballanger (1987) in southern France where low rainfall is typical, the stem weevil *C. napi* might cause substantial losses. In south-western and central France where rainfall is sufficient, the losses might not occur at the similar plant injury level. In our earlier work we found a significant correlation between the length of larval tunnels of *C. pallidactylus* in stems and the number of podless stalks, this feature indicating the level of plant condition, only in years of water deficit (Kelm, Walczak 1998). It may be presumed that also in the case of stem weevils' injury the plant compensation abilities are determined by weather.

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The effect of stem weevil (*Ceutorhynchus pallidactylus* Marsh.) infestation on oilseed rape yield

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Abstract: Damage due to stem weevil *Ceutorhynchus pallidactylus* infestation is rather underestimated in agricultural practice, which results probably from the lack of visible external injury to oilseed rape plants. The studies carried out in 1985-87 and 1991-93 have shown a significant negative correlation between the length of the feeding tunnels in the stem and the seed yield. The first symptom indicating the presence of the stem weevil larvae in stems was the inhibition of plant growth. The infested plants had reduced leaf area, the leaves were lost sooner and the flowering of plants was delayed. The stems had worse mechanical characteristic, which promoted the lodging of plants. The studies on physiology of yielding show that such plant reactions to larval feeding may cause a considerable loss in seed yield. A compensation of the injury has also been observed during the years with sufficient rainfall (especially when the rainfall exceeded the average in May-June). The injured plants produced more of petiole-less leaves at that time. Such leaves significantly increase the photosynthesis of oilseed rape plants during the pod development.

Key words: oilseed rape, yielding physiology, pests, harmfulness

Introduction

A significant negative correlation between the length of the feeding tunnels of weevils in the stem and the quantity and quality of seed yield occurred in five out of six years of study on harmfulness of oilseed rape phytophages carried out in 1985-87 and 1991-93 in Poland (Kelm, 1999). Dmoch (1959) suggested that this species might cause a significant yield loss. However, the later works did not support this idea, and reported on the lack of negative effect of the stem weevil on the yield of plants that are well-grown (Rudny 1974, Pałosz 1978). Golinowska (1991) analysed the economic relationship between the abundance of the stem weevil and the seed yield during 1979-1986. She found that *C. pallidactylus* caused yield loss in Poland in 1979-1981 and 1984. Walczak et al. (1997) found 37% yield loss due to stem weevil injury. Similar results were obtained by Muśnicki et al. (1994, 1995) and Toboła et al. (1996). Therefore, it might be inferred that *C. pallidactylus* has a more significant economic importance than it has been believed hitherto. The underestimation of the stem weevil harmfulness in the agricultural practice results probably from the lack of visible external injury of plants. Lerin (1995) suggested, that the negative effect of plant injury in early stage, might last until the harvest, which increase the probability of *C. pallidactylus* harmfulness.

The aim of the study in 1993 was to analyse the reaction of oilseed rape plants to internal injury of stem. In the present work, the results were related to literature data on physiology of yielding of oilseed rape. This might reveal the direct cause of loss in seed yield.

Methods

The studies were carried out in *Brassica napus* var. *oleifera* cv. Bolko field on the farm in Świniary, 15 km north of Wrocław in 1993. The field was divided into two parts: protected (P) – 7 ha, where plants were sprayed against stem weevils with Fastac 10EC on 15 April, and unprotected (UP) – 0.5 ha, where no insect control treatments were applied. From each part of the field, 15 randomly chosen plants were collected once a week for measurement of stem weevil tunnels, and for analysis of plant growth parameters and number of flowers and pods. Additionally, at three dates: 9 May (beginning of flowering), 13 June (end of flowering), and 20 July (before harvest), 10 plants were collected from each field for measurements of leaf area, and 50 plants for analysis of physical properties of stems. The leaf area was measured using the computer program Biograf (by Witra Company). The comparative analysis of physical properties of plants from protected and unprotected field was done using Instron (by British manufacturer) and Dynstat (by German manufacturer) at the Institute of Agrophysics of PAS in Lublin. Each plant was divided into three equal sections. In the middle of each section the following parameters were analysed: energy of cutting, maximum force of inflection and stem inflexibility. Injury to the stem was also recorded.

Table 1. Comparison of growth and development parameters between protected (P) and unprotected (UP) plants.

Date	Plant height (cm)		Stem diameter (mm)		Racemes 1^0 (no.)		Flowers (no.)		Pods (no.)	
	P	UP	P	UP	P	UP	P	UP	P	UP
21.04	6.3	8.8	0.9	0.9						
28.04	44.6	38.5	1.5	1.3						
04.05	94.0	76.8	1.4	1.4	7.7	8.1	26.5	0.6	2.4	
12.05	108.2	83.9	1.4	1.2	7.9	7.8	22.4	19.7	37.8	23.8
18.05	108.9	80.0	1.4	1.0	6.8	5.8	38.6	59.2	111.8	31.6
25.05	113.3	80.6	1.1	1.0	6.2	7.0	21.6	19.4	168. 3	14.6
01.06	112.7	79.4	1.3	0.9	6.8	5.6	0.4	0	138.8	72.9
09.06	113.6	87.7	1.0	1.0	6.4	6.6			100.9	132.5
15.06	107.4	81.9	1.2	0.9	6.9	6.6			120.2	109.4
23.06	107.2	88.7	1.1	0.9	6.4	6.0			106.3	90.7
30.06	118.4	86.0	1.3	1.0	6.6	6.4			103.9	64.8
06.07	115.7	83.0	1.1	1.0	5.9	7.2			73.8	59.1
14.07	113.9	87.2	1.3	1.3	6.5	7.2			81.6	86.4

Results and discussion

Protected and unprotected plants differed significantly in the level of injury. The length of the feeding tunnel in control plants exceeded 20 cm in May. In protected plants, the tunnels were less than 10 cm long at that time. In mid June, the tunnel in control plants reached more than 30 cm, and in the control plants, the tunnel length did not change. The differences between protected and control plants manifested before flowering. The reduced growth and reduced leaf area were the first effects caused by stem injury. The maximum growth of rape stems in the field P occurred during the period between 21 April and 4 May, and was 88 cm, while in

Table 2. Number and leaf area on plants uninfested and infested by *C. pallidactylus*.

Plant stage	Plant	No. of leaves per plant	Leaves area (cm^2)
Before flowering 9.05.	Uninfested	6.2	629.1
	Infested	8.6**	618.5
The end of flowering 13.06.	Uninfested	19.9	672.0
	Infested	24.5	382.1**
Maturity of pods 20.07.	Uninfested	13.7	187.8
	Infested	34.4**	396.1**

** – significant difference

Table 3. Stem parameters of plants infested by *C. pallidactylus* in comparison to uninfested ones.

Parameters	Decrease of parameters (%)		
	I date	II date	III date
Plant height (cm)	9.1	19.7	11.2
Height to the first raceme (cm)	0	13.8	7.9
Root neck diameter (mm)	0	6.7	16.0
Cutting energy:			
A	19.9	26.3	2.0
B	29.9	32.4	3.2
C	20.0	34.4	19.9
Maximum inflection energy:			
A	1.1	18.8	38.7
B	3.5	26.9	12.1
C	27.7	34.1	13.5
Stem inflexibility:			
A	44.4	42.3	37.6
B	24.1	33.4	12.7
C	63.9	35.3	14.1
Stem larval tunnelling (cm)	39.0	49.6	51.6

A – Ist section; B – IInd section C – IIIrd section

the field UP – 68 cm. This difference remained until the harvest (Table 1). Such inhibition of growth in injured plants was also observed by Pałosz (1978). According to Woyke (1993 a, b), the growth rate and height of plants are the characters most constantly positively correlated with seed yield. The injured plants, in the period before flowering, had a significantly higher number of leaves, but their total area was significantly lower as compared to the uninjured plants . Also, at the end of flowering, the healthy plants had a leaf area almost twice that of injured ones, which was due to the longer period of their attachment to the main stem (Table 2 & Figure 1). Dmoch (1959) noted the earlier drop of leaves as a cause of deficiency of assimilates in the plant. Leaf drop is a critical point in yield formation because the limitation of photosynthetic area might induce the increased natural reduction of generative organs (Evans 1984, Mendham and Scott 1975, Labana et al. 1987). Flowering started on 4 May. The protected plants had 26.5 flowers, and the unprotected ones – only 0.6 flowers. At the time of full bloom, the protected plants had only 38 flowers, and the unprotected ones – 59. The end of flowering occurred at the same time for protected and unprotected plants (Table 1 & Figure 1). The plants with uninjured stems had more flowers during the first half of the flowering period. According to Tayo and Morgan (1975), the more abundant flowering at the beginning gives a chance of formation of more pods, and the pods, which are formed earlier in the flowering period have more seeds (Williams and Free 1979).

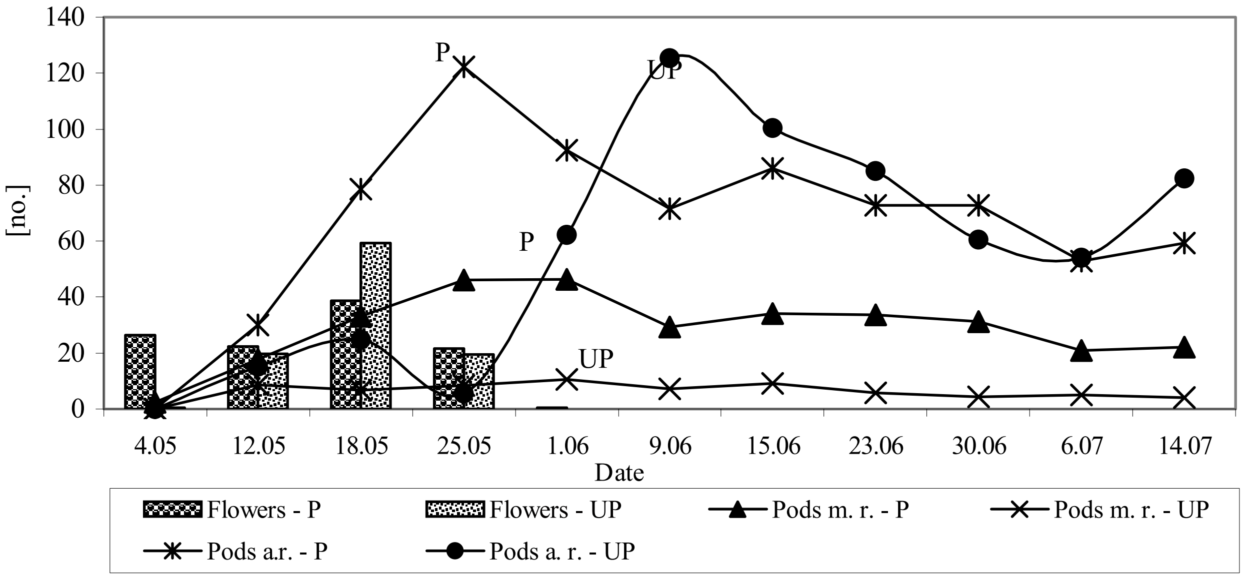

Figure 1. Comparison of flowering and pot setting process for protected (P) and unprotected (UP) plants 1993.

The results on parameters of mechanical properties of stems of oilseed rape show, that the internal injury of stem caused deterioration of physical properties of tissues (Table 3). These parameters were stronger in uninjured plants. The energy of cutting was higher in uninjured plants at each phenophase. The most noticeable differences occurred at the end of flowering. The differences in the force of inflection were observed at the second and the third sampling date. The stems of uninjured plants were always more rigid than those of the injured ones, but this difference lessened with plant age, probably because of stem lignification. It must be stressed that the energy of cutting and the force of inflection decreased towards plant apex. The most prominent decrease in stem inflexibility, occurred in the early plant stage in the lower parts and later in upper parts. Such decrease in rigidity, promotes lodging and may affect yielding. In lodged plants, the assimilating area is reduced almost by a half, which might cause seed abortion (Norton et al. 1991). In consequence, empty pods are formed, and the number of long podless stalks is increased at harvest time. In the year of study the compensation in seed yield occurred. Probably it was due to higher number and area of petiole-less leaves during time of pod formation (Loboda et al.).

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Bibliography of parasitoid species and levels of parasitism of rape stem weevil *Ceutorhynchus napi* Gyll. and cabbage stem weevil *Ceutorhynchus pallidactylus* (Mrsh.)

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Abstract: A survey of the literature showed that *Ceutorhynchus napi* is attacked by two larval parasitoid species, whereas three larval parasitoids and one adult parasitoid were recorded for *Ceutorhynchus pallidactylus* (syn. *C. quadridens*). The Tersilochinae species *Tersilochus fulvipes* (Gravenhorst) and *Tersilochus obscurator* Aubert (Hymenoptera: Ichneumonidae) were identified as predominant parasitoids of *C. napi* and *C. pallidactylus*, respectively, in the Czech Republic, France and Germany. High levels of parasitism have been reported from central European countries. The life histories of the predominant Tersilochinae species are similar.

Key words: bibliography, *Ceutorhynchus napi, Ceutorhynchus pallidactylus*, parasitoids, *Tersilochus spp*.

Parasitism of *C. napi*

In the literature, reference is made to only two species of the sub-family Tersilochinae (Hymenoptera: Ichneumonidae) parasitising the larvae of the rape stem weevil: *Tersilochus fulvipes* (Gravenhorst) and *Tersilochus moderatus* L. (Table 1). In revisions of the taxonomy of the Tersilochinae, Aubert & Jourdheuil (1958) and Horstmann (1971) determined *P. fulvipes*, *T. fulvipes* ssp. *gallicator* and *T. gibbus* to be synonyms of *T. fulvipes*.

The levels of parasitism of *C. napi* generally showed large variations between different years and different locations. In France, Jourdheuil (1960) found 25-95% of *C. napi* parasitised by *T. fulvipes* between 1952 and 1956. Sedivy (1983) studied the level of parasitism of *C. napi* from 1951 to 1982 in the Czech Republic: 79-98% of the larvae were parasitised by *T. fulvipes*. From 1977 onwards, increasing levels of parasitism were accompanied by decreasing levels of infestation of oilseed rape by *C. napi*, which led to the conclusion that this parasitoid species has a significant effect on the population dynamics of the pest. In Switzerland in 1945, 30-40% of the prepupae of *C. napi* in the ground were parasitised by *T. gibbus* (= *T. fulvipes*) (Günthardt 1949). However, at the same site in Switzerland in 1989 and 1990 Büchi & Roos-Humbel (1991) could not find any parasitism of rape stem weevil larvae. In the region of Göttingen in Germany, no parasitism of *C. napi* was detected in 1990 to 1994 as well, in spite of regularly high levels of infestation by *C. napi* and extensive sampling programs. However, in 1995, 1996 and 1997 the levels of parasitism of larvae by *T. fulvipes* increased to 1%, 21% and 6%, respectively (Klingenberg & Ulber 1994, Nitzsche 1998, Nitzsche & Ulber 1999).

Table 1. Larval endoparasitoids (Hymenoptera: Ichneumonidae) of *Ceutorhynchus napi* Gyll.

Species	Country	Reference
Tersilochus fulvipes (Gravenhorst)	France	Jourdheuil (1960)
	Germany	Lehmann (1965), Nitzsche (1998)
	Czech Republic	Sedivy (1983)
syn. =		
Porizon fulvipes Gravenhorst		Horstmann (1971)
Thersilochus fulvipes (Gravenhorst)		
ssp. *gallicator* Aubert	France	Aubert & Jourdheuil (1958)
Tersilochus gibbus Holmgren	Switzerland	Günthardt (1949)
	Germany	Buhl (1952)
	Czech Republic	Kazda (1956)
Tersilochus moderatus L.	Germany	Dosse (1951)

Parasitism of *C. pallidactylus*

Three parasitoid species were found attacking the larvae of cabbage stem weevil. All of them belong to the sub-family Tersilochinae as well: *Tersilochus obscurator*, *Tersilochus tripartitus* and *Tersilochus exilis*. The other species included in Table 2 have proved to be synonyms of these (Horstmann 1971, 1981).

As with *C. napi*, few investigations on the levels of parasitism of *C. pallidactylus* in central Europe have been made in the past. In France, 35% and 54% of cabbage stem weevil larvae were parasitised by *T. tripartitus* spp. *obscurator* in 1953 and 1956, respectively (Jourdheuil 1960). In the Czech Republic, the level of parasitisation of larvae by *T. obscurator* ranged between 6% and 69% during the years 1951 to 1982 (Sedivy 1983). Günthardt (1949) found in Switzerland that 35 - 75% of the prepupae of *C. pallidactylus* in the ground were parasitised by *T. melanogaster*. However, when Büchi (1991) and Büchi & Roos-Humbel (1991) studied the level of parasitism of cabbage stem weevil at the same site in 1989 and 1990 only 6 and 11%, respectively, of the larvae were parasitised by *T. obscurator*. In rearings of cabbage stem weevil larvae sampled from spring rape at three different locations in 1951 in Sweden, Herrström (1964) found 30%, 4% and 0% of parasitism by *T. exilis*.

The adults of cabbage stem weevil have been attacked by the braconid *Microctonus melanopus*. This parasitoid species has been recorded to attack adults of *Ceutorhynchus assimilis* and *Psylliodes chrysocephala* (Jourdheuil 1960).

Life histories of *T. fulvipes* and *T. obscurator*

The most extensive information on the biologies of *Tersilochus* spp. is given by Jourdheuil (1960). The life cycles of these univoltine, solitary endoparasitoids are similar. It is characterised by a koinobiontic development.

The adults emerge from the soil of last year's rape fields and migrate to new oilseed rape crops in April/May. The females of *T. fulvipes* and *T. obscurator* forage on the rape plant and oviposit through the plant tissue into the host larvae while these are mining within the stem or petioles. After hatching from the egg, the parasitoid larva remains in its first instar within the host. The development of the host larva apparently is not affected. However, when the mature host larva leaves the rape plant to pupate in soil, the parasitoid larva develops rapidly and kills the host prepupae. After preparing a silken coocon it pupates within the hollow produced by the host larva in the ground. The adult parasitoid hatches in late summer and overwinters in this coocon.

There is no information so far on alternative host species of *T. fulvipes* and *T. obscurator* besides *C. napi* and *C. pallidactylus*, respectively.

Table 2. Larval endoparasitoids (Hymenoptera: Ichneumonidae) of *C. pallidactylus*.

Species	Country	Reference
Tersilochus obscurator Aubert	Czech Republic	Sedivy (1983)
	Germany	Lehmann (1965)
		Klingenberg & Ulber (1994)
		Nitzsche (1998)
syn. =		
Thersilochus tripartitus Brischke	France	Aubert & Jourdheuil (1958)
ssp. *obscurator* Aubert		
Tersilochus tripartitus Brischke	France	Jourdheuil (1960)
syn. =		
Tersilochus melanogaster Thomson	Switzerland	Günthardt (1949)
		Horstmann (1981)
Tersilochus exilis Holmgren	Sweden	Herrström (1964)
syn. =		
Probles exilis (Holmgren)		Horstmann (1971)

Table 3. Adult endoparasitoids (Hymenoptera: Braconidae) of *C. pallidactylus.*

Species	Country	Reference
Microctonus melanopus Ruthe	France	Jourdheuil (1960)
syn. =		
Perilitus melanopus Ruthe	Germany	Speyer (1925)
	Switzerland	Günthardt (1949)

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Stenomalina gracilis (Walker), a new parasitoid reared from *Ceutorhynchus napi* Gyll. in Poland

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Abstract: The parasitoids of stem weevils are not well studied. Only two species are reported relatively frequently in the literature:
- *Tersilochus fulvipes* (Gravenhorst) from *Ceutorhynchus napi* Gyll., and
- *Tersilochus obscurator* Aubert (= *tripartitus* Brischke spp.) from *Ceutorhynchus pallidactylus* (Marsh.).

For the present study 12 samples each of 50 winter oilseed rape plants, were collected from 22 April until 25 June 1998. The eggs and larvae of stem weevils found in the collected plants were kept for rearing the parasitoids. A total of 371 larvae of *C. napi* and 166 larvae of *C. pallidactylus* were reared. Eighteen individuals of parasitoids emerged, and among them 3 ex. *Stenomalina gracilis* (Walker) (Pteromalidae). Until now, *S. gracilis* has not been known from other weevils on oilseed rape, except *C. assimilis*. Parasitoids of eggs were not found.

Key words: oilseed rape, stem weevil, parasitoid, *Stenomalina gracilis* (Walk.), Pteromalid

Introduction

The parasitoids of the stem weevils *Ceutorhynchus pallidactylus* (Marsh.) and *Ceutorhynchus napi* Gyll. have not been studied in detail hitherto. The scarce literature reports a wide percentage range of host parasitization, usually rather low (Büchi, 1991). The following species have been reported to parasitize *C. napi* larvae (Klingenberg & Ulber, 1994):

- *Tersilochus fulvipes* (Gravenhorst), formerly reported as *Porizon fulvipes* Gravenhorst or *Tersilochus fulvipes* (Grav.) *gallicator* Aubert, or sometimes erroneously as *Tersilochus gibbus* Holmgren. The holotype of this species established by Townes now has the name *Phradis gibbus* (Holmgren) (Günthart, 1949). *Tersilochus fulvipes* (Gravenhorst) has been reported from Bohemia, Moravia, Hungary, Poland, Germany, France, Spain, and Switzerland (Aubert & Jourdheuil, 1958; Lehmann, 1965; Horstmann, 1971).
- *Tersilochus moderatus* L. cited only once by Dosse (1951). Although, it is difficult to be certain which species the author had in mind, it was probably *Porizon moderator* (F.) (=*Ichneumon moderator* L.).

The other three species of the subfamily *Tersilochinae* are endoparasitoids of *C. pallidactylus* larvae:

- *Tersilochus obscurator* Aubert (= *Tersilochus tripartitus* Brischke spp. *obscurator* Aubert), reported mainly from Germany, France, and UK (Jourdheuil, 1960; Lehmann,1965)
- *Tersilochus tripartitus* Brischke (= *Tersilochus melanogaster* Thomson), reported from France and Switzerland
- *Tersilochus exilis* (= *Probles exilis* (Holmgren)) reported only from Sweden (Herrström 1964).

No data have been published on the parasitization of stem weevil larvae by species of the family Pteromalidae.

Methods

The study was carried out in the crop of winter oilseed rape from 22 April until 25 June 1998. Twelve samples, each of 50 plants collected once a week. The stems and the leaf petioles were dissected and the stem weevil eggs and larvae found were reared at 22-25°C and 90% relative humidity (or 90-95% for the eggs). The larvae were transferred to fresh pieces of stem (ca. 4 cm long) twice a week. They were determined to species from head chaetotaxy. From 17 March, samples of adults were also collected from yellow water traps placed in the same crop.

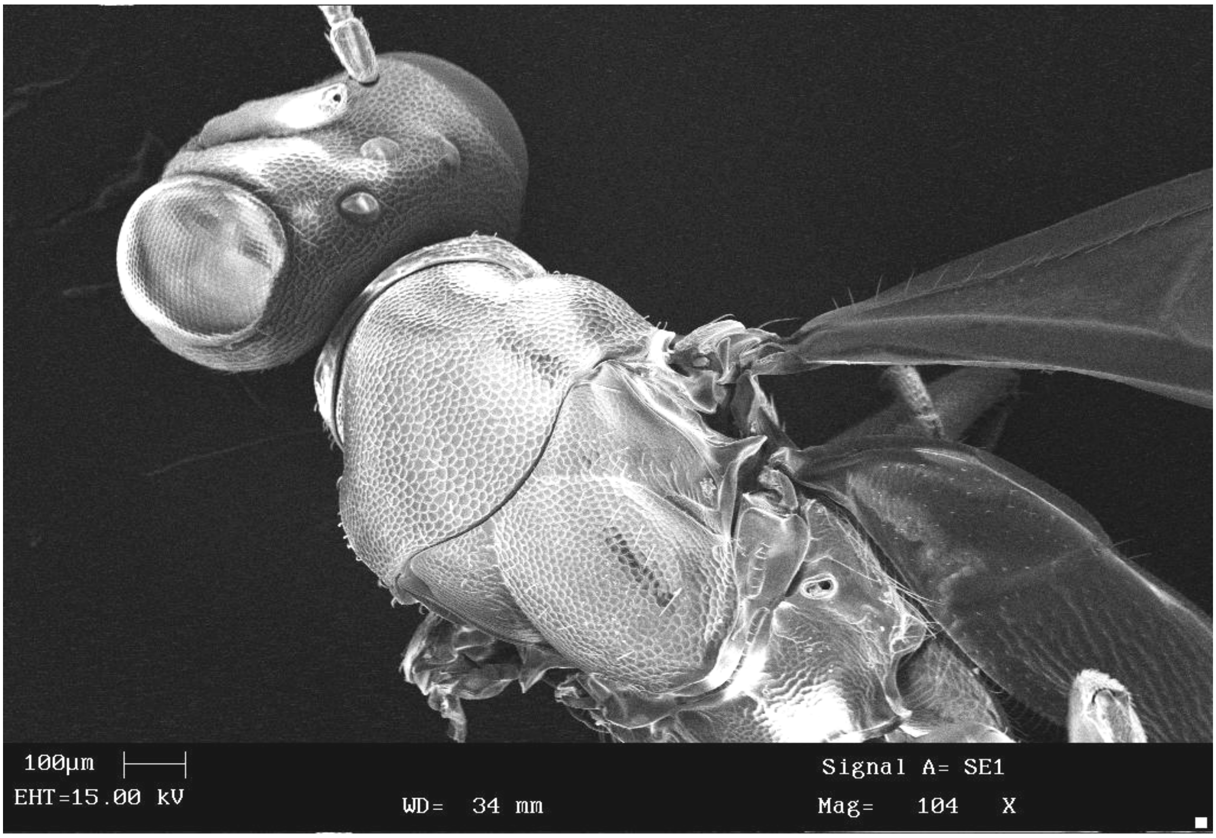

Figure. 1. *Stenomalina gracilis* (Walk.) reared from *Ceutorhynchus napi* Gyll. General view.

Results and discussion

In total, 600 plants were dissected and 371 larvae of *C. napi,* 166 larvae of *C. pallidactylus*, and 178 eggs of both species were collected. From the eggs, 58 L_1 larvae of *C. napi* and 40 larvae of *C. pallidactylus* (i.e., 55%) were obtained. No parasitoids of weevils' eggs emerged. The proportion of unfertilised eggs is estimated at 10-20%.

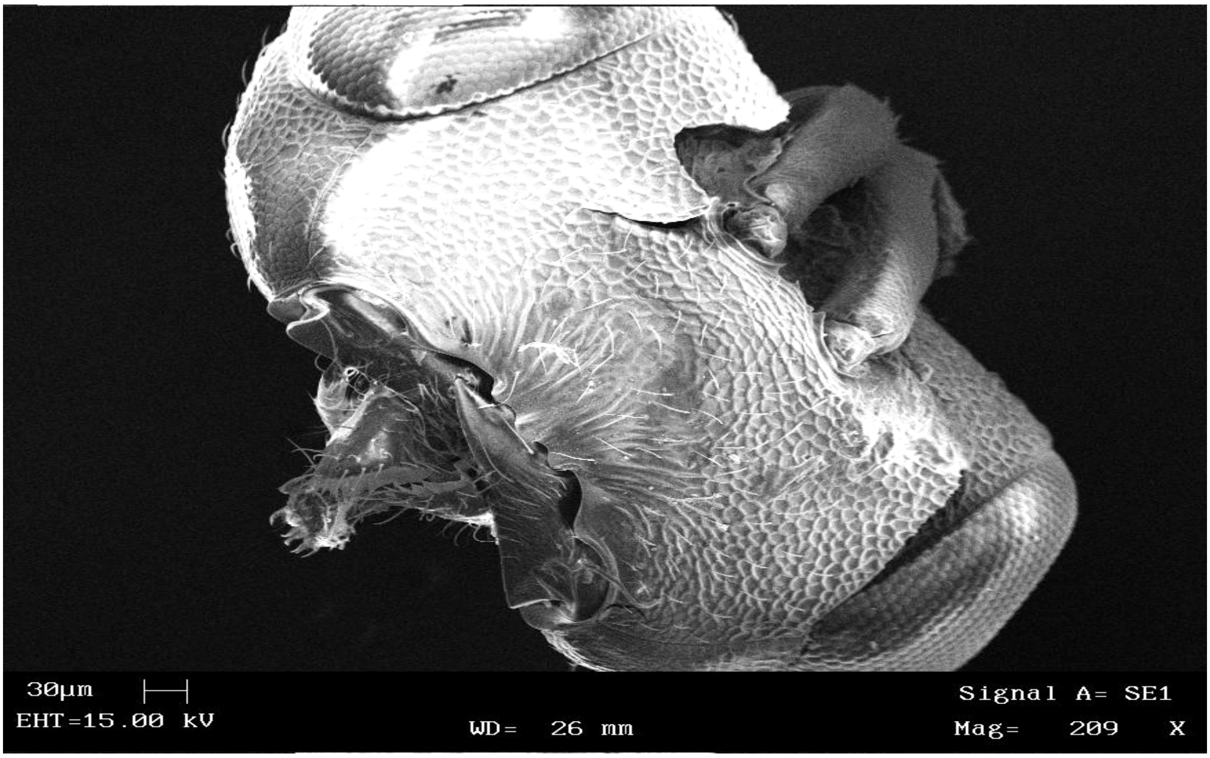

Figure 2. *Stenomalina gracilis* (Walk.) reared from *Ceutorhynchus napi* Gyll. Clypeus.

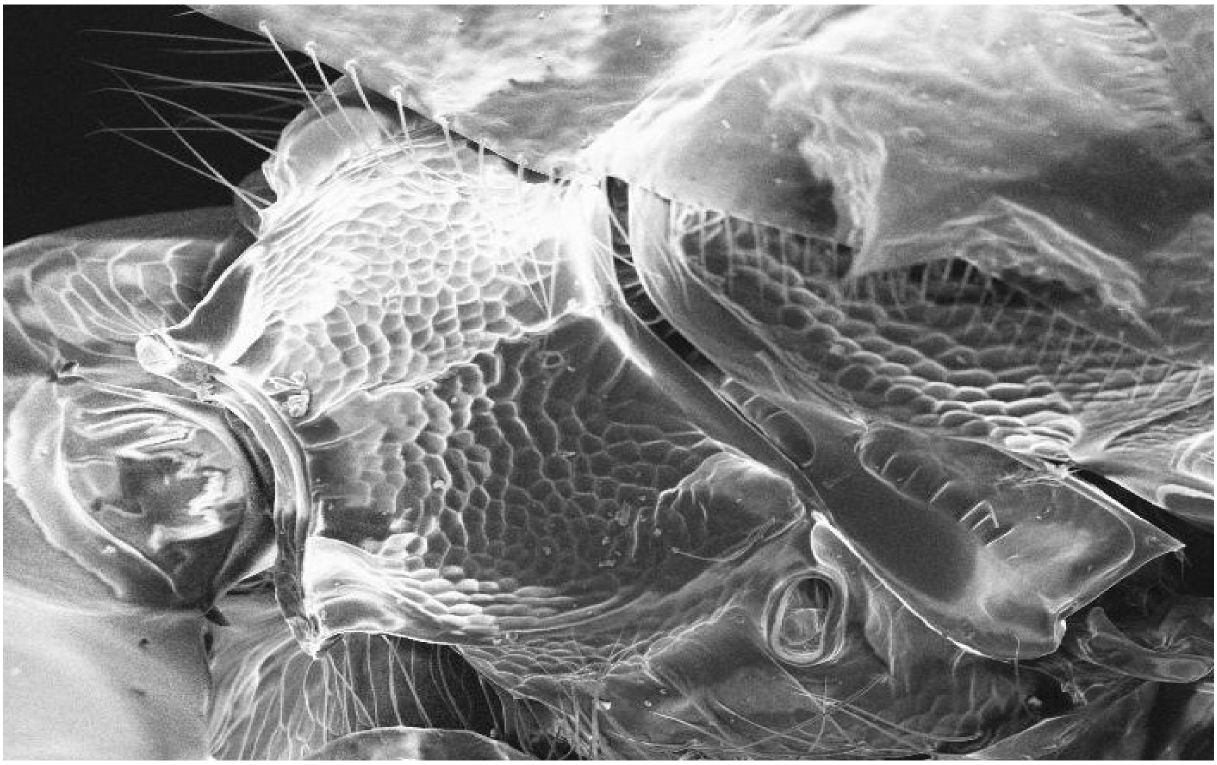

Figure 3. *Stenomalina gracilis* (Walk.) reared from *Ceutorhynchus napi* Gyll. Propodeum.

From the dead weevil larvae of both species, 18 adult parasitoids emerged. Fifteen individuals belonged to *Phoridae* and *Tachinidae* and their species affiliation is to be determined. The remaining three individuals belonged to *Stenomalina gracilis* (Walker) (Pteromalidae) (on *C.napi* parsitoids only).

Two individuals of *S. gracilis* were reared from larvae of different stages collected on 16 June. The parasitoids emerged on 26 June and 3 August, respectively (Fig. 1). The third individual emerged on 4 August, from material collected on 25 June. They pupated next to the body of the host, in the feeding tunnel in the stem. Therefore, it is possible to detect the exarate pupa even before the emergence of the adult parasitoid. The first individuals of *Stenomalina gracilis* were found in yellow traps on 5 April, and appeared in the traps regularly during April and May.

Stenomalina gracilis has previously been was reported only as the parasitoid of *C. assimilis*. As shown here, this parasitoid also develops in *C. napi*, and may explain its early presence in crops of oilseed rape. Further studies on the bionomy of this species are needed.

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Biological and Integrated Control of Insect Pests

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A European Database on Biological Control of Oilseed Rape Pests

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Abstract: In recent years studies of various aspects of biological control of oilseed rape pests have been undertaken in several European countries, with detailed reference to parasitoids, predators and pathogens. These studies have yielded data which may be applied in many parts of Europe and an EC Concerted Action on the Biological Control of Oilseed Rape Insect Pests (BORIS) was convened to provide a mechanism for exchange of information between specialists in various sectors of the agrochemical industry. To ensure efficient collation of detailed information and to safeguard data integrity, a database (BORISBASE) has been developed. The database stores relevant information in a readily accessible format, and will make it widely available via a page on the World Wide Web. This paper describes the structure of the database and the data-types stored, gives details of the information/data sources it contains, provides an example of a typical interrogation of the database to illustrate the user-orientated interface that has been designed, and gives details of the website on which it will be made available for use. The use of similar techniques for collation and dissemination of comparable diverse and disparate data sets in the future is discussed.

Key words: database, natural enemies, biological control, integrated control, oilseed rape, pests

Introduction

Work conducted in several European countries during the last few years has resulted in significant developments in natural, non-chemical methods of controlling oilseed rape pests, and thereby the potential for reducing pesticide use. Even small reductions in pesticide usage on such a major cropped area will result in significant environmental and economic benefits to farmers. Although it is not possible to quantify such benefits, reducing reliance on spraying would reduce the risks to natural enemies (Alford, Walters, Williams & Murchie, 1996), pollinating insects (Corbet, Williams & Osborne, 1991) and other non-target organisms, and so encourage bio-diversity.

To facilitate the efficient transfer of the recent developments in biocontrol of oilseed rape pests to farmers, it is important that the disparate data sets that have been collected in several parts of Europe are collated and disseminated to extension services. To support the effective collation of the results, an EC Concerted Action was formed and first met in 1997 with the objectives of establishing a network amongst European scientists currently working in this field and providing a forum for information exchange between participants. In addition, the group will devise strategies for farmers to exploit natural enemies and thereby minimize and better rationalize pesticide use. To encourage uptake within the EU of the strategies advocated, direct contact with extension services is maintained, principally though extension scientists that are members of the group. The results of the Concerted Action will also enable more reliable prioritization of research funding and better-targeted collaborative research programmes.

The volume and variety of detailed information and data which had to be made available initially to group members and later to users in the agrochemical industry, and the need to enable the efficient selection of the specific elements required for individual applications, presented data handling problems which had to be addressed if the Concerted Action was to succeed. Previous studies have encountered similar data collation problems which have been solved by the development of a computerised database (Theiling & Croft, 1988), and amongst other approaches the group has developed a similar database (BORISBASE) on the natural enemies of oilseed rape pests. The database forms a depository of current information and will be made generally available on the World Wide Web.

This paper describes the structure of BORISBASE and the data-types stored, gives details of the information/data sources it contains, and provides an example of a typical interrogation of the database to illustrate the user-orientated interface that has been designed.

Materials and methods

Information sources

Data were collated from the individual countries represented in the Concerted Action by the consortium members. Data types were agreed and proformas for collecting the information were developed. The database was designed using precise formats for entry of information, to accommodate these proformas.

Database structure

Each data type is represented in a specific storage area within the database system. The database was constructed on an Online INFORMIX database system and was designed as a hierarchical structure with links between tables storing related pieces of information (Figure 1).

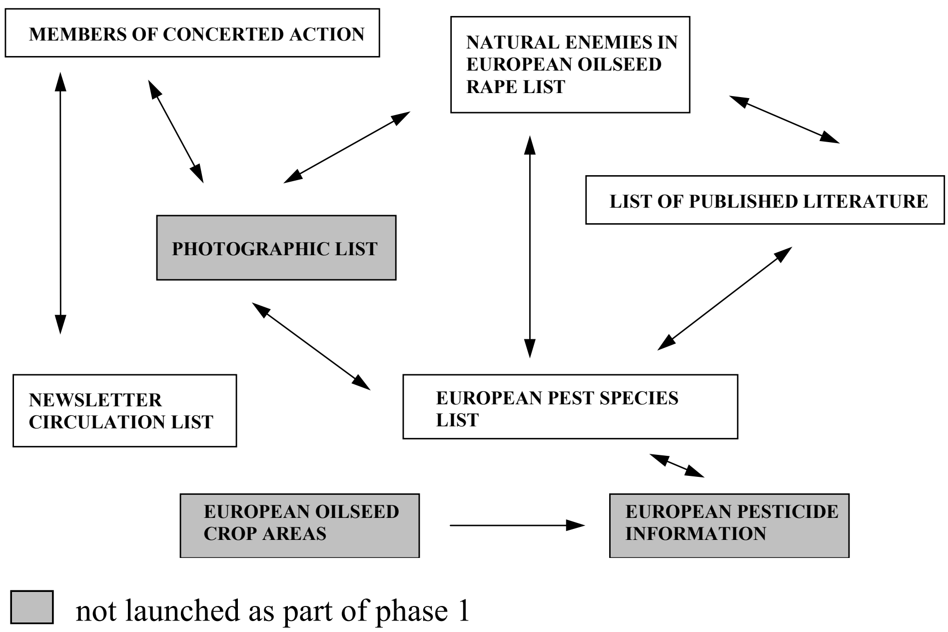


Figure 1. The relational diagram for BORISBASE. Arrows represent the direction of joins.

The use of joins avoided duplication of the data being stored and database indexes were created to maintain data integrity and to aid retrieval of information. To ensure that relevant information was stored in a structure that was amenable to efficient interrogation for the most commonly required output types, the database structure and content were modified through group consultation and feedback. To aid input verification and ensure data integrity, the database will be launched on the World Wide Web in two phases.

Data types

The data types selected for inclusion on the database can be divided into eight main groupings. Amongst those scheduled for launch in phase 1 (Figure 1), two relate mainly to the effective administration on the EC concerted action group which developed the database, including a list of members and details of the recipients of the Concerted Actions' regular newsletters. Within the membership list area of the database, the name and full contact details for each individual is stored, together with information of the members specialist expertise and their role within the Concerted Action. The information on the newsletter circulation list is constrained to that which is needed to automate the regular posting of the document to recipients and access to this area will not be given to general users when the database goes live on the World Wide Web.

The remaining six groupings relate to scientific information which has been collated and stored on the database. A European pest species list provides a comprehensive list of all the currently recognised insect pests of oilseed rape from the countries covered by the database. Within this area details of the current name of each pest and other taxonomic details, synonyms, stage(s) of the life cycle which damage the crop and the geographical range within Europe of the species are recorded. In addition, the names of endoparasites or ectoparasites, or other natural enemies, which attack each life stage of the pest species are recorded and links established via this primary key to another area of the database, the natural enemy list.

The natural enemy list stores detailed data on a wide range of natural enemies that attack pests of oilseed rape crops. This area of the database collates a range of information concerning each natural enemy species, including its current name, details of the taxonomy of the species (including family and order), synonyms, the life stage(s) of the natural enemy which attack each pest, details of key published references, and the name and address of a recognised expert on each species. In addition, the current names and synonyms of all other pests of oilseed rape that are known to be susceptible to the natural enemy, and the life stage of each pest that is attacked, are recorded, together with the host or prey ranges within Europe. To avoid data duplication automatic links to other areas of the database are used to access some of this information, for example geographical ranges of host and prey species are obtained from the European pest species list area.

An important component of the information available on the database is the list of published literature. The database is not intended to provide a comprehensive reference list for each pest or natural enemy species, as this was thought to duplicate a range of other databases that are available commercially. Instead, it highlights key references on each species that have been identified by the development group. This area of the database includes a full reference to each publication, details of the names of the pests and natural enemies addressed by each paper, and key words indicating the content of the paper (e.g. biology or control). A single reference list is held in the database and this is linked to all other relevant database areas, thus avoiding data duplication.

The remaining three datatypes will be launched on the World Wide Web in phase 2. High quality photographs of many of the pests and natural enemies can be difficult to obtain, and a photographic list has been developed to store details of available material. Within this area of the database details of each photograph are made available including the current name of the pest or natural enemy, the life stages represented in the picture, and the name and address of the copyright holder and other copyright details. In addition, a unique reference number is allotted to each photograph to enable its unambiguous identification.

Two areas of the database are designed to contain data on usage and other relevant information on pesticides, and details of the extent of oilseed rape cropping in different parts of Europe. Specific data stored in these areas include the products available, active ingredients, chemical groups, target pests for each active ingredient, crop growth stages at which applications are permitted, average dates of application, and minimum, maximum and mean application rates. Cropping data include areas of the different crop types grown in the European countries represented by members of the development group, and more general pesticide usage data. Differences in the format and extent of data available from different countries necessitated a flexible approach to the development of database structures to house it and this limited the degree of integration of the data that could be achieved

Data entry screens

Information collected and supplied by the BORISBASE development group was added to the database using a number of data entry screens designed for effective input (Figure 2). Each entry field was constrained to allow only a specified type or format of information to be added, providing a degree of automated data verification at the entry stage.

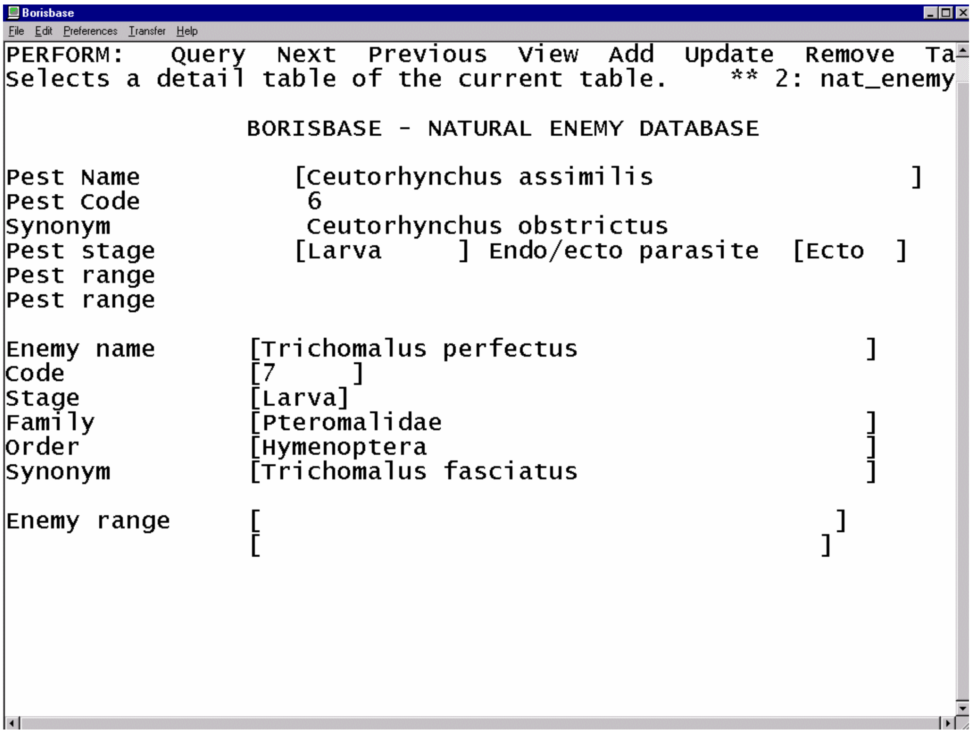


Figure 2. Data entry screen for natural enemy information.

Development of the WWW front-end

Following development of the INFORMIX database for data entry and storage, a front-end was designed to display the information on the Internet. The user-orientated interface for the database was designed as a series of Web pages. The basic Web pages were developed in HTML (Hypertext mark-up language) using Microsoft Front Page, with CFML (ColdFusion mark-up language) added to the pages to make them interact with the INFORMIX database. Pages containing CFML are intercepted by the ColdFusion Web application server and translated into a format understandable by an ODBC (Open Database Connectivity) driver. ColdFusion connects via the ODBC driver to the INFORMIX database where the CFML database queries are carried out. The resulting output is converted by ColdFusion back into HTML and can be viewed through the Web browser. All connections to the database are completely transparent to the Web browser.

The information is accessed through a series of screens via hyperlinked text or drop-down menus.

Results

Data concerning the biological control of oilseed rape pests were received from BORIS members in eight different European countries and entered onto the INFORMIX database. Data were checked by returning outputs from the database to members for verification and update.

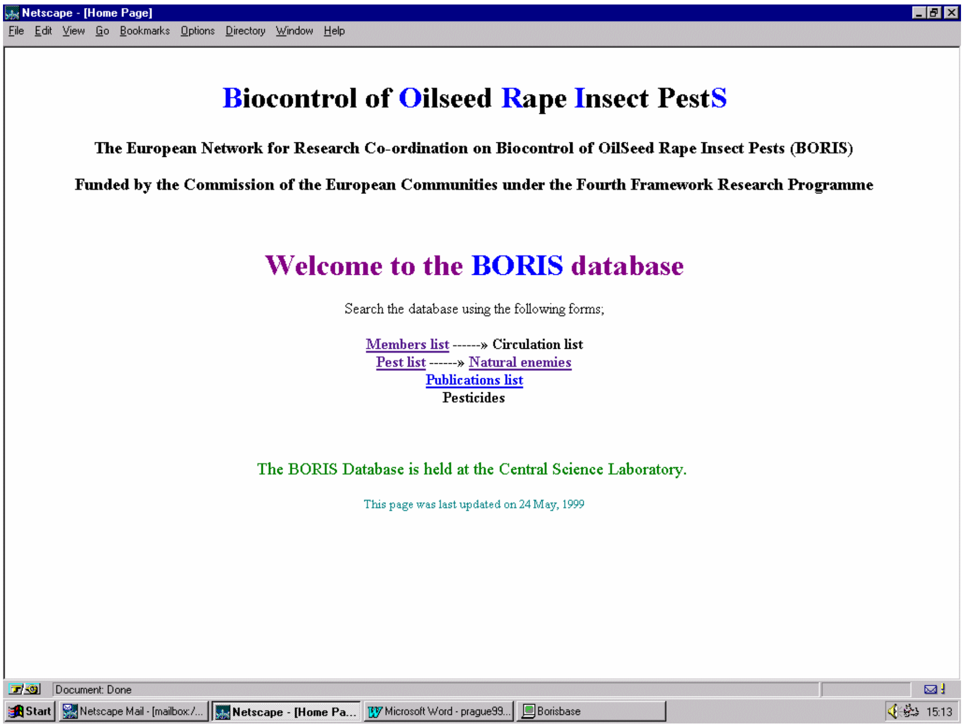
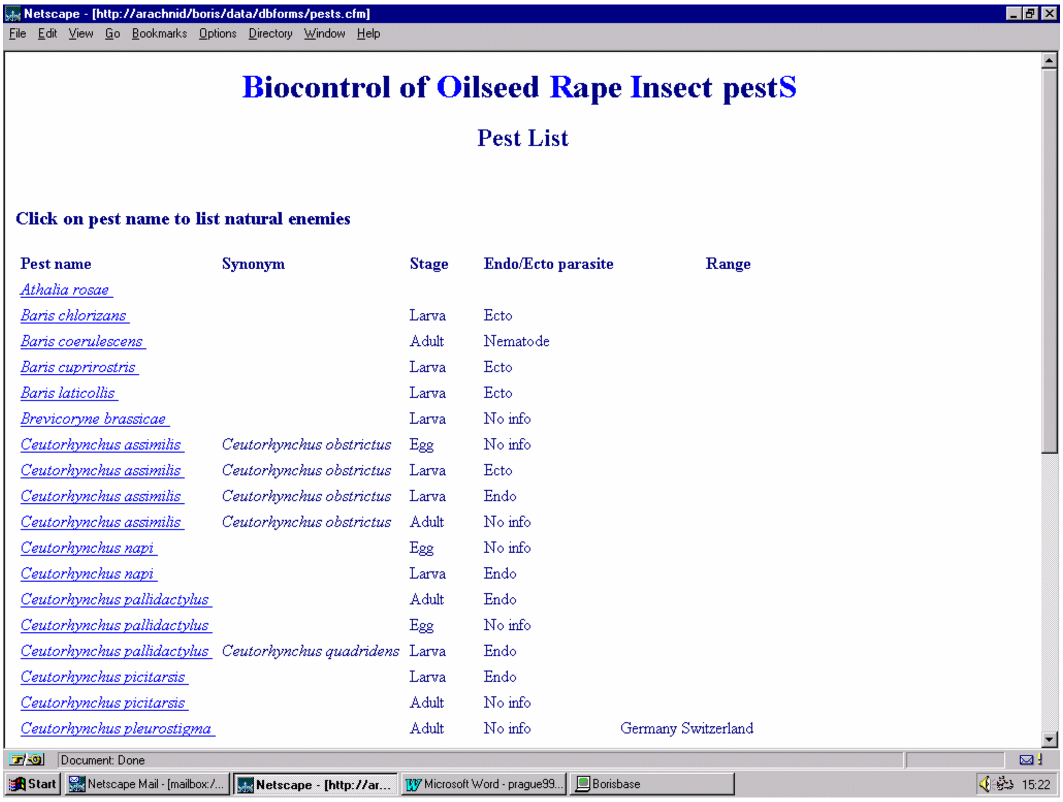


Figure 3. BORISBASE welcome screen on the Internet.

The Web-based front-end was constructed following agreement on preferred formats for data retrieval. The initial screen of the database Web-site is shown in Figure 3. The database can be searched by selecting the appropriate highlighted hypertext.

The database can be interrogated by a user-targeted tiered questioning approach. For example, to retrieve specific information on pests, natural enemies and key publications, the first stage is addressed by selecting the hypertext Pest List on the initial web-site screen. This results in a display of a full current list of pests of the crop in Europe (Figure 4), from which selections of individual pests can be made to retrieve specific information on natural enemies attacking the selected species. These natural enemies are cited as endoparasites, ectoparasites, predators or nematodes (other pathogens will be added) and categorised by the pest stage attacked.



Pest name	Synonym	Stage	Endo/Ecto parasite	Range
Athalia rosae				
Baris chlorizans		Larva	Ecto	
Baris coerulescens		Adult	Nematode	
Baris cuprirostris		Larva	Ecto	
Baris laticollis		Larva	Ecto	
Brevicoryne brassicae		Larva	No info	
Ceutorhynchus assimilis	*Ceutorhynchus obstrictus*	Egg	No info	
Ceutorhynchus assimilis	*Ceutorhynchus obstrictus*	Larva	Ecto	
Ceutorhynchus assimilis	*Ceutorhynchus obstrictus*	Larva	Endo	
Ceutorhynchus assimilis	*Ceutorhynchus obstrictus*	Adult	No info	
Ceutorhynchus napi		Egg	No info	
Ceutorhynchus napi		Larva	Endo	
Ceutorhynchus pallidactylus		Adult	Endo	
Ceutorhynchus pallidactylus		Egg	No info	
Ceutorhynchus pallidactylus	*Ceutorhynchus quadridens*	Larva	Endo	
Ceutorhynchus picitarsis		Larva	Endo	
Ceutorhynchus picitarsis		Adult	No info	
Ceutorhynchus pleurostigma		Adult	No info	Germany Switzerland

Figure 4. Display screen of list of pests stored.

Once a particular hypertext selection has been made, for example ectoparasites of the larval stage of *Ceutorhynchus assimilis*, a further screen of information is displayed (Figure 5) showing all natural enemies on the database which fit the selection criteria. Specific information on a natural enemy can be retrieved by selection of further info. For example, if the natural enemy *Trichomalus perfectus* is chosen then a further screen displays information on the taxonomy of the species and details of a recognised expert on the species (Figure 6). Further links on phase 2 of BORISBASE will allow selection of further information on the natural enemy and give access to the photographic list.

To provide further information sources for detailed investigation of the pest or natural enemy species chosen, the database can also be searched for key references which are retrieved by using specific keywords or by naming individual pest or natural enemy species.

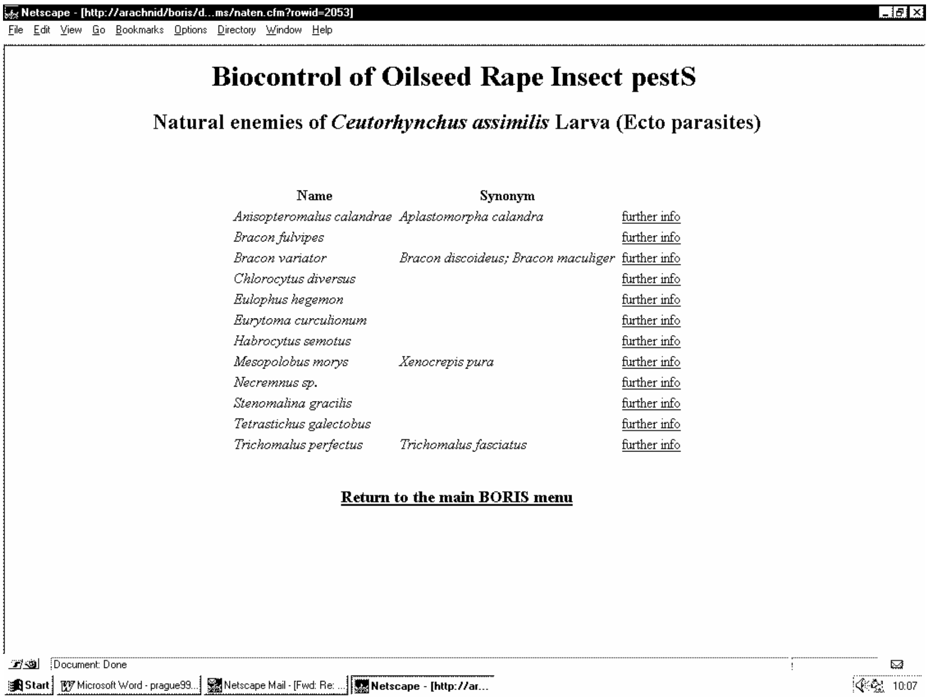


Figure 5. Display screen of natural enemies (ectoparasites) of *Ceutorhynchus assimilis* larvae.

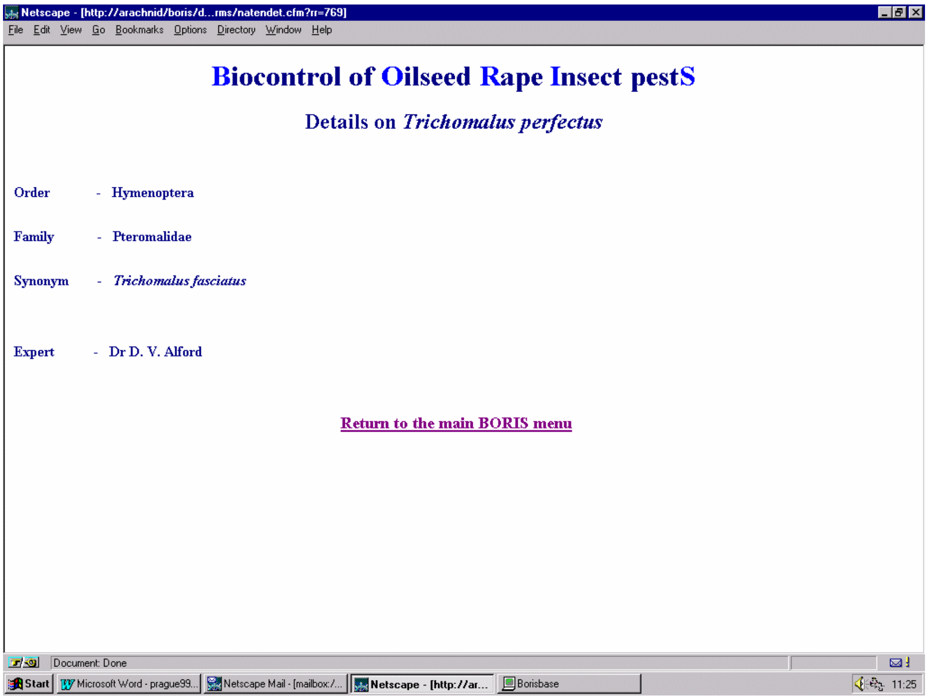


Figure 6. Display screen of further details of a specified natural enemy.

The Publications List option on the initial screen is selected to display the screen shown in Figure 7.

This screen displays the menu system for retrieval of key references according to pest species and in the example a selection for references to *Ceutorhynchus assimilis* is highlighted that retrieves details of twenty-one publications currently held on BORISBASE on this pest (Figure 8).

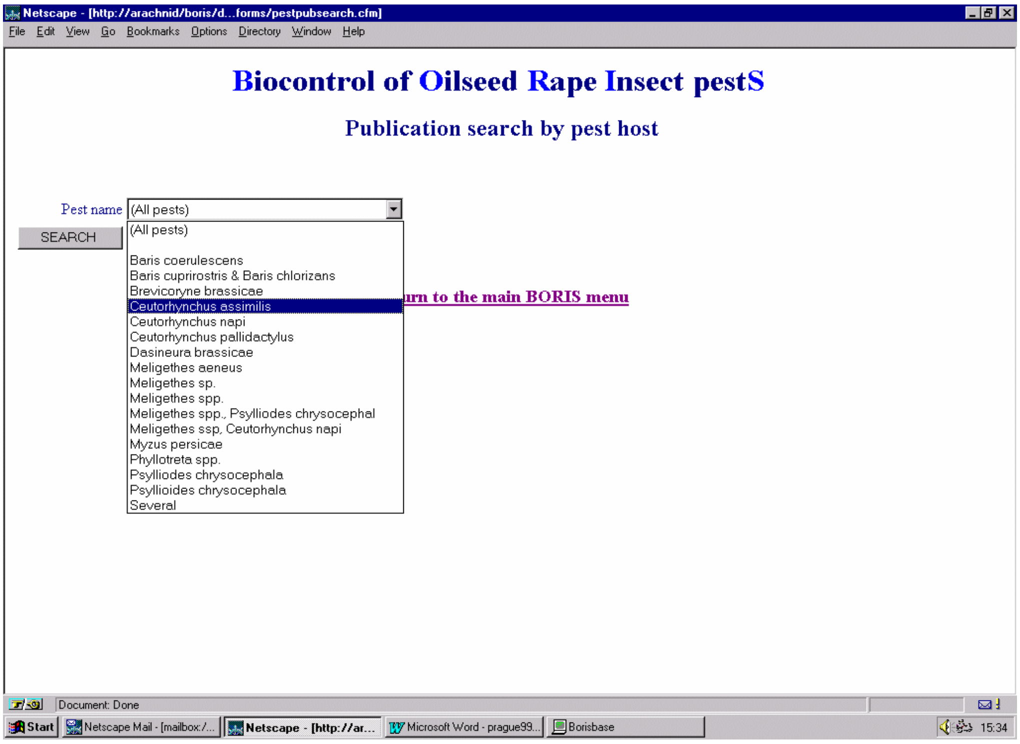


Figure 7. Display screen for selection of key references by pest host.

Figure 8. Display screen of key references for a selected pest species.

Discussion

The design and implementation of the BORISBASE database is at an advanced stage, with an initial launch of the system on the World Wide Web scheduled for mid-1999 and release of the larger database soon thereafter. Access via a web site was found to be the most cost-effective method of enabling a wide range of potential users to benefit from the results of this study, and facilitates maintenance of a single central database. This approach allows immediate updating and release of new information as it is received. To ensure ease of use, help screens and email contacts will be made available.

Quality control of data entered onto the system is essential if the integrity of the system is to be protected after it is launched. To ensure that entry of inappropriate information is avoided, remote data entry via the web site will not be allowed. Instead, new data will be added by the database manager, enabling consultation with members of the development group and validation of input prior to general release. Rapid transfer of information and updates will be facilitated by email connections.

Development of the database system was the result of a close interaction between a group of specialists in biological control of insect pests of oilseed rape. Convening the development group was an essential component of the project, enabling the experience and knowledge of a wide range of individuals to be utilised and ensuring that the database contained information that was applicable across Europe rather than being specific to a small group of countries. Additionally, the need for the experts to meet regularly to agree data types to be included in the system and identify the most common interrogation routes required by end users, thus defining the optimal structure of the database was a central requirement for completion of this work. Any attempts to expand the database by adding new data, or to build analogous systems, would benefit from adopting a similar approach of consultation and liaison with local experts at all stages of development.

Some difficulties were encountered in the format in which analogous data are collected in different countries, and this prevented incorporation of some datasets. For example, the widely differing methodologies employed to collect and collate information on pesticide usage resulted in a series of incompatible datasets. Wherever possible, the structure of the database was developed to accommodate the different data formats, but flexibility was constrained by the need to build standard interrogation approaches which yielded comparable datasets from all areas of Europe. Similar problems will be encountered when building analogous systems in the future, which in some cases will prevent complete datasets being collated. However, in this study it was found that by identifying the principal information types required to achieve the objectives that the system was designed to address, problems arising from data compatibility issues were reduced.

Acknowledgements

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necessarily reflect the Commission's views and in no way anticipates the Commission's future policy in this area.

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First steps to assess the importance of epigaeic active polyphagous predators on oilseed rape insect pests with soil pupating larvae

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Abstract: Larvae of oilseed rape pest insects such as *Meligethes aeneus*, *Ceutorhynchus assimilis* and *Dasineura brassicae* which infest buds, flowers or pods drop to the ground to pupate in the soil. During this period (and also as pupae in the upper soil layers) they are easily available to epigaeic active predators. Here we considered the importance of ground beetles (Coleoptera: Carabidae) as beneficials. In order to quantify their predatory capacity as natural enemies of insect pests in winter oilseed rape three treatments with different abundances of carabid beetles were investigated: 1. Total exclusion; 2. Reduced exclusion; 3. Increased predator abundance. Hatching success of the pest insects was generally reduced accordingly to the densities of the predators and reached values up to 51% for *Ceutorhynchus assimilis*, 56% for *Meligethes* spp., 58% for *Dasineura brassicae* and 82% for *Ceutorhynchus pallidactylus.* Both, reduced and increased abundances of epigaeic active polyphagous predators caused a significant increase of the total mortality of the pollen beetle (*Meligethes* spp.) up to 4%, of the brassica pod midge (*Dasineura brassicae*) up to 10% and of the cabbage seed weevil (*Ceutorhynchus assimilis*) up to 52%.

Key words: predators, Carabidae, Araneae, Staphylinidae, *Meligethes* spp., *Dasineura brassicae*, *Ceutorhynchus assimilis*, *Ceutorhynchus pallidactylus*, winter oilseed rape, prey consumption, biocontrol, enclosures

Introduction

The aim of the experiments presented here was to get information of the importance of epigaeic active polyphagous predators as natural enemies of insect pests of winter oilseed rape (OSR) which infest flowers and pods (*Meligethes* spp., *Ceutorhynchus assimilis*, *Dasineura brassicae*) or stems (*Ceutorhynchus pallidactylus*). The larvae of the mentioned pest species drop to the ground to pupate in the soil.

In several field experiments in oil seed rape crops (e.g. Büchs et al. 1997, Stippich & Krooß 1997, Goltermann 1994) ground beetles, spiders and rove beetles could be identified as the most abundant taxa of epigaeic active polyphagous predators. Whereas some publications give evidence of the carabids as effective antagonists of insect pests in cereals (Ekbom et al. 1992, Chiverton 1988, Poehling et al. 1985, Scheller 1984, Chambers et al. 1983, Griffith 1982, Sunderland & Vickerman 1980, Basedow 1973) there are only a few and mostly old publications of predator-prey relationships in oilseed rape,

- These do not correlate with the current agricultural practice of OSR-growing (e.g. cultivars, tillage, plant protection),
- are based on different methodological approaches, and
- are to some extent contradictionary.

According to Scherney (1959) the carabid beetle *Clivina fossor* caused larval mortality of the Pollen Beetle of 60% to 65%; similar results are stated by Bonnemaison (1957) for the cabbage seed weevil. Basedow (1973) showed that predators might increase the larval mortality of the pollen beetle and the brassica pod midge up to 39% or 65% respectively. Finally Goltermann (1994) explained 25% of the total mortality of the pollen beetle as effect of epigaeic active predators by using only the reduced exclosure-method and pitfall traps. Additionally it has to be considered that the cultivation technique in these experiments was still slightly influenced by the current practice of the former German Democratic Republic. So Nilsson & Andreasson (1987) came to contradictionary results (no effects of epigaeic active polyphagous predators on pollen beetle) in Sweden.

In the field experiments presented here the potential effect of epigaeic active predators on the hatching rate or mortality of the new generation of pest insects was investigated by using different kinds of enclosures. The aim was to design the experiment in a way, that the evaluation of the predatory capacity of certain epigaeic taxa was conducted under field conditions which on the one hand correspond to the current practice of OSR-production, but which on the other hand enable to create different densities of epigaeic predators in order to assess the degree of predatory capacity. As precondition the field experiment should be conducted using natural occurring predator populations and not (as it was done in other experiments) by an artificial releasing of selected (and may be reared) predator species in different densities/numbers into the enclosures.

Materials and methods

The field experiment was conducted between August 1997 and August 1998 on an experimental site of 0.7 ha within a 10 ha OSR-field at Wendhausen (83 m above see level) about 12 km northeast of Braunschweig. No insecticides where applied to the experimental area. It had three treatments:

1. ***”Enhancement”.*** The density of epigaeic active predators was artificially enhanced by using 1m²-metal frames with outer ramps which allowed the predators to enter the enclosed area but not to leave it (especially species which are unable to or do not fly).
2. ***”Reduced” exclusion.*** Barrier systems (1m² metal frames of 36 cm height, dug into soil to a depth of ca. 20 cm) enclosed a defined area (1 m²) of ”natural” field-soil. This treatment dealt with those predators which hatched from the soil within the enclosure naturally. It allowed access by predators and parasitoids which are able to fly or to climb over the barriers.

The treatments ”Enhancement” and ”Reduced exclosure” had two parallel variants:

A) with 5 pitfall traps within each enclosure, controlled weekly

B) without pitfall traps

This experimental design was chosen for the following reasons: to assess the effect of the natural emergence/immigration of predators in a defined area on the hatching success they should have the opportunity to act as predators over the whole period of the experiment. So it seemed not to be appropriate to remove them by pitfall traps. On the other hand it is essential to get informations about the dimension of the natural occurring emergence/immigration of the predators. This is only possible if pitfall traps were installed within the enclosures which catch the hatching/immigrating predators. It is assumed that it is possible to a certain extent to transfer the number of predators which were observed in the treatment A (with pitfall traps) to the area of the parallel treatment B (without pitfall traps).

3. "Total exclusion". Before sowing the OSR, selected areas of 1m² were dug out to a depth of 25 cm. The soil was steamed over night at 110°C to free the soil from remaining predator larvae. The soil was then carried back to the field and replaced into the same areas where it was taken from. The areas were enclosed by metal frames as in the other treatments. The OSR was sown by hand in rows (120 seeds in 6 rows per m²). In each enclosure, pitfall traps were installed to control the occurrence of eventually penetrating predators and remove them from the system as soon as possible. Finally the metal frames were covered by gauze cages. For fertilizer or herbicide applications the frames were opened for a short time so that they were treated as the surrounding crop. After the first peak of flight activity of *Meligethes* spp. (recorded with yellow water traps) the gauze cage was removed to allow infestation of the buds. At that stage the problem arose to let the plant grow as naturally as possible and to make an infestation by the relevant pest insects possible, but to prevent at the same time the uncontrolled immigration of predators into these enclosures. So a horizontal gauze was installed between the plant rows. This allowed infestation and nearly naturally growing of the plants. In order to enable the pest larvae to reach the ground when dropping in order to pupate in the soil, the horizontal gauze strip had to be removed at BBCH-stage 68, when the dropping of petals (including the *Meligethes*-larvae) started in higher abundance.

Figure 1a. Metalframes with outer ramps in order to enhance the density of epigaeic predators within the enclosure.

Figure 1b. Metal frame based enclosure type of the treatment „Reduced exclusion“

Figure 1c. Treatment „Total exclusion“ with horizontal gauze between plant rows during the period of OSR-infestation (buds, flowers, pods) to prevent penetration of epigaeic active predators

Figure 1d. Emergence traps replaced the metal frame based enclosures in all treatments during the hatching of the new generation.

After the infestation of pods by all relevant pests was finished and the beginning of the hatching period of the new generation of pest insects was expected (calculation based on existing knowledge), in all replicates of all treatments the metal frames were removed and replaced by emergence traps which cover the whole area (1 m²) of each enclosure to measure the hatching of the new generation of pest insects. The emergence traps remained until end of August (about four weeks after harvesting).

The number of pest insect larvae dropping from the OSR-flower stands to the ground were recorded by a set of 12 funnel traps (diameter 21 cm) which were distributed groupwise over the experimental field.

The evaluation of the total mortality was based on the comparison of the abundance of dropping larvae, recorded with funnel-traps, and the treatment-related hatching rate of the new generation, recorded with emergence traps.

Results and conclusions

Abundance, dominance structure and species numbers

Table 1 shows for the dominant and subdominant species of carabid beetles, the total number of specimens as well as the frequency and species numbers in each treatment. These values are also given for other epigaeic predators. The ranking of dominance is mainly determined by the relations in the treatment where the carabid were artifically enhanced.

Table 1. Structure of dominance (%) including the most frequent species of ground beetles (Coleoptera: Carabidae) of the treatments „Total exclusion“, Reduced exclusion“, „Enhancement“ (D = dominance; n = no. of specimens; further explanations see text).

Species	**D** total [%]	**n** total	**Total exclusion**	**Reduced exclusion**	**Enhancement**
dominant					
Amara similata	27,7	797	10,7%	15,8%	31,7%
Pterostichus melanarius	13,7	382	37,1%	27,3%	7,8%
Poecilus cupreus	11,7	337	16,6%	10,3%	11,2%
subdominant					
Trechus quadristriatus	9	260	9,5%	22,0%	7,1%
Pseudophonus rufipes	6	173	14,1%	10,6%	4,2%
Loricera pilicornis	5,4	156	1,5%	0,6%	6,7%
Harpalus affinis	5,2	149	2,8%	2,8%	5,9%
Amara aenea	3,2	93	0,3%	0,9%	4,0%
Number of specimens		**2880**	**108,7***	**75,5***	**556,8***
Number of species		**58**	**15**	**26**	**56**
Staphylinidae °		609	62*	68*	479*
Araneae °		843	135*	59*	649*

°: Sampling period 24 March 98 - 2 June 98

*: individuals/m²

In the treatments „Total exclusion“ and „Reduced exclusion“ ground beetles, spiders and rove beetles were recorded in similar numbers. This demonstrates that certain species as for example *Pterostichus melanarius* (well digging) or *Poecilus cupreus* (able to fly) and *Pseudophonus rufipes* can not be avoided. However, it can be assumed that the time period of occurrence of predators in the treatment „Total exclusion“ is clearly limited, because the predators are continously removed by the pitfall traps installed within these enclosures.
In contrast, in the plots without pitfall traps of the treatment „Reduced exclosure“ - which were the basis to record the hatching of the new generation of the pest insects – the carabid beetles were not removed but could be active as predators during the whole period of the experiment.

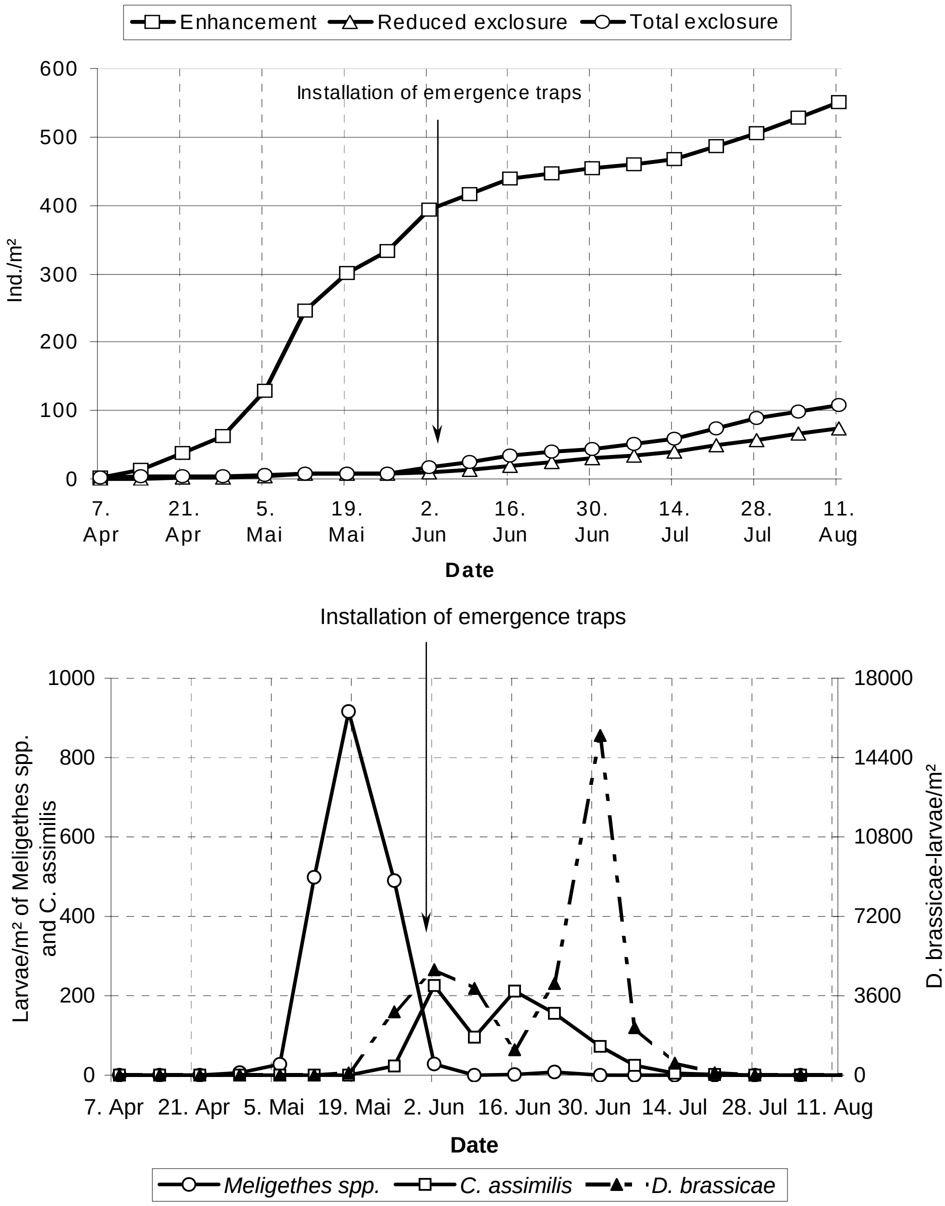

Figure 2 a, b. Phenology of occurrence/immigration of carabid beetles (fig. 2a on the top: cumulative graph) and dropping of insect pest larvae from OSR flower stands onto the soil (fig. 2b at the bottom).

So, although the numbers of individuals recorded in both treatments are fairly similar the predatory effects are assumed to be quite different.

Coincidence of the occurrence of ground beetles and the dropping of pest larvae

Figure 2a,b compare the coincidence in time between the dropping of the larvae to the ground and the occurrence of the carabid beetles within the enclosures. Figure 2a shows the immigration/occurrence of carabid beetles in the metal frames as accumulation over the experimental period, Figure 2b shows the actual number of dropping larvae.

The results demonstrate, that

- in the treatment „Enhancement“ a high accumulated immigration rate (of about 300 beetles/m², starting from 7 of April) was recorded at the time of the maximum dropping of *Meligethes*-larvae at the 19 of May.
- in the treatments „Reduced exclusion“ (and also „Total exclusion“) during the whole period when the *Meligethes*-larvae dropped to the ground, nearly no ground beetles were present up to the time when the metalframes were replaced by emergence traps. So regarding the treatment „Reduced exclosure“ it can be assumed that the abundance of *Meligethes*-larvae was not significantly reduced by predation of carabid beetles (see fig. 4).
- during the main period of larvae dropping of *C. assimilis* and *D. brassicae* however also considerable ground beetle densities were recorded in the treatment „Reduced exclusion“.

Phenological coincidence of selected carabid species and the dropping of pest larvae

To identify potential key species of carabid beetles as natural enemies of the pest insects in Figure 3a-c the phenology of the three most abundant ground beetle species (immigration rates in treatment enhancement) have been compared to the phenology of larvae dropping of *Meligethes* spp., *Ceutorhynchus assimilis* and *Dasineura brassicae*.

Figure 3a demonstrates, that in the time period, when the larvae of *Meligethes* spp. drop to the ground (mostly within the petals of the flowers), especially carabid beetles of the species *Amara similata* and *Poecilus cupreus* are active in the field with maximum densities of ca. 50 or 20 Ind./m² (in the peak time of the dropping of *Meligethes*-larvae). The new generation of *Amara similata*, hatching in early summer, shows coincidence with the period of dropping of larvae of the brassica pod midge (fig. 3b; second generation) and the cabbage seed weevil (Fig. 3c).

Therefore, in comparison to *Poecilus cupreus* and *Pterostichus melanarius*, the species *Amara similata* in general showed the best phenological coincidence with the dropping of OSR insect pest larvae from flowers/pods to the ground. These results could indicate a key role of this ground beetle species as natural enemy of the above mentioned pest insect species. However, generally *Amara*-species are said to be phytophagous. Luka et al. (1998) observed *Amara ovata* and *Amara similata* feeding on OSR-seeds, -pods and -flowers. But it is also known, that *Amara*-species optionally can be also carnivorus, for example, feeding on Diptera-eggs, larvae and pupae (Lindroth 1945, Dobson 1961). However, this question can be answered only by a detailed analysis of their gut contents.

As figures 3a-c demonstrate the activity period of *Poecilus cupreus*-imagines is restricted to that time period, when the *Meligethes*-larvae drop from the flowers to the ground. So this carabid species will have an influence only on the mortality of *Meligethes*-larvae.

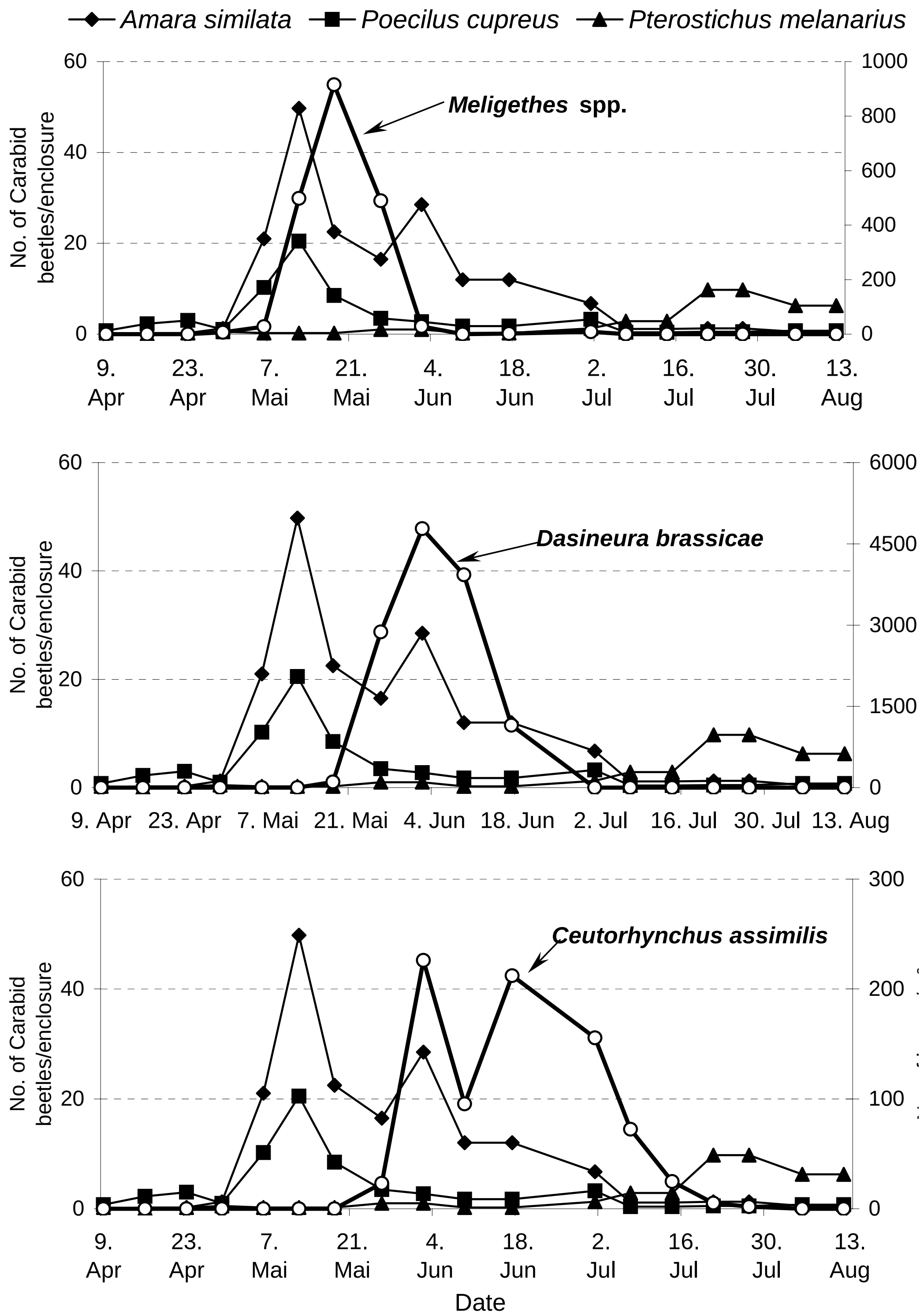

Figure 3a, b, c. Phenological coincidence of the three most abundant carabid species (see tab. 1, treatment „Enhancement“) and the dropping of larvae of *Meligethes* spp., *D. brassicae* and *C. assimilis* from oilseed rape flower stands to the ground (note: larval drop of third generation of *D. brassicae* is not considered in fig. 3b for the reason that no infestation of OSR-plant by the midges of the second generation of *D.brassicae* could occur, because all enclosures where covered by emergence traps since 3 June; further explanations see text).

For *Pterostichus melanarius*-imagines considerable emergence rates were recorded only at the end of July. At that time, dropping of insect pest larvae was already finished and also the hatching of the imagines of the new generation of pest insects was in progress to a high extent. So it can be assumed that this ground beetle species is of lower importance as biocontrol agent of the mentioned pest insect larvae. For larvae of carabid beetles considerable epigaeic or near soil surface activity was recorded only during the dropping of larvae of *D. brassicae* and *C. assimilis*. However, the data of ground beetle larvae presented here rely only on pitfall trap catches, for more exact statements on the activity density of the mostly endogaeic active predator larvae a special trapping equipment has to be used.

Impact of epigaeic active, polyphagous predators on the hatching of the new generation of insect pests

Figure 4 shows the reduction of the hatching success of the new generation of the four relevant OSR insect pests according to the different treatments. The percentage of reduction of the hatching of pest insects (new generation) was always related to the hatching success in the treatment „Total exclusion“, which was set to „zero“.

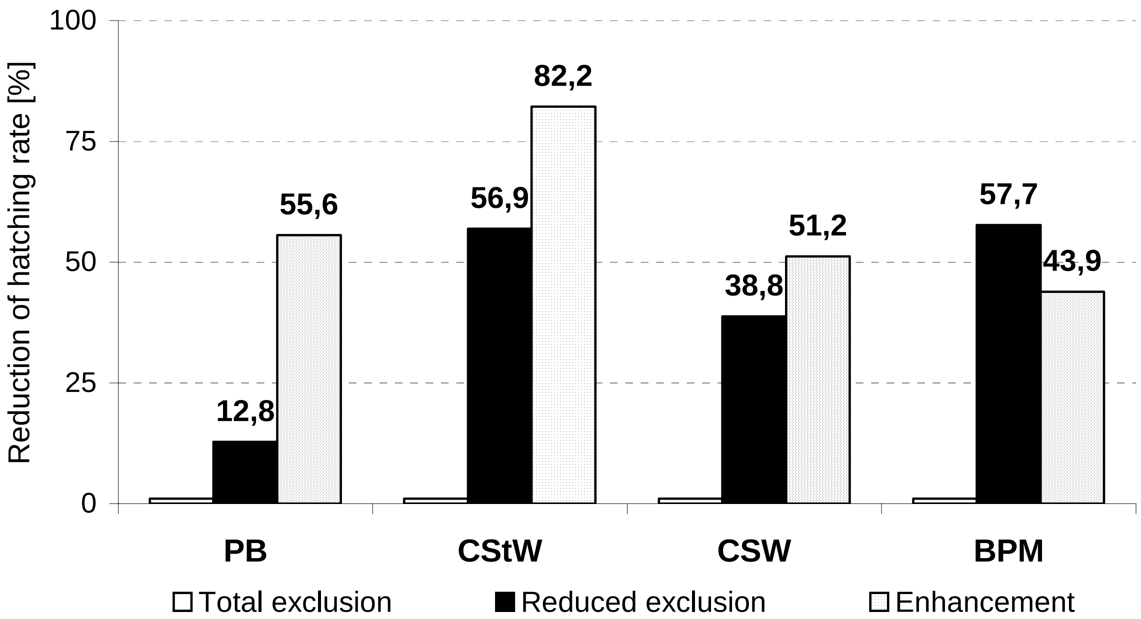

Figure 4. Reduction of hatching rate of the Pollen Beetle (PB), the Cabbage Stem Weevil (CStW), the Cabbage Seed Weevil (CSW) and the Brassica Pod Midge (BPM) according to different densities of epigaeic active predators (further explanations see text).

In comparison to the treatment „Total exclusion“ as reference value for all pests a clear and mostly significant reduction of the hatching rates was recorded if epigaeic predators were present. This reduction increased significantly by increasing densities of predators as it was actually recorded in the treatment „enhancement“. As figure 4 shows the hatching rates are reduced up to 45% and 55% for *D. brassicae, C. assimilis* and *M. aeneus* and even up to app. 80% for *C. pallidactylus*.

Quite striking is the low reduction of 12.8% regarding the hatching rate of the new generation of the Pollen Beetle (*Meligethes* spp.) in the treatment „Reduced exclosure“ in comparison to the treatment „Enhancement“. If this low reduction rate is related to the cumulative occurrence of carabid beetles (see figure 2a), one will be aware to the fact, that in the time period when *Meligethes*-larvae drop to the ground the activity of carabid beetles in the field (which is detected by treatment „Enhancement“) is quite high, the natural emergence of ground beetles however (which determines the predator densities in the treatment „Reduced exclusion“) is nearly not perceptible at that time. From this result (absence of carabids in one and their presence in the other case and accordingly low or high reduction of the pest hatching rate) can be generally derived, that predators have a clear (and in most cases significant) effect on the hatching rate of the new generation of the pest insect species.

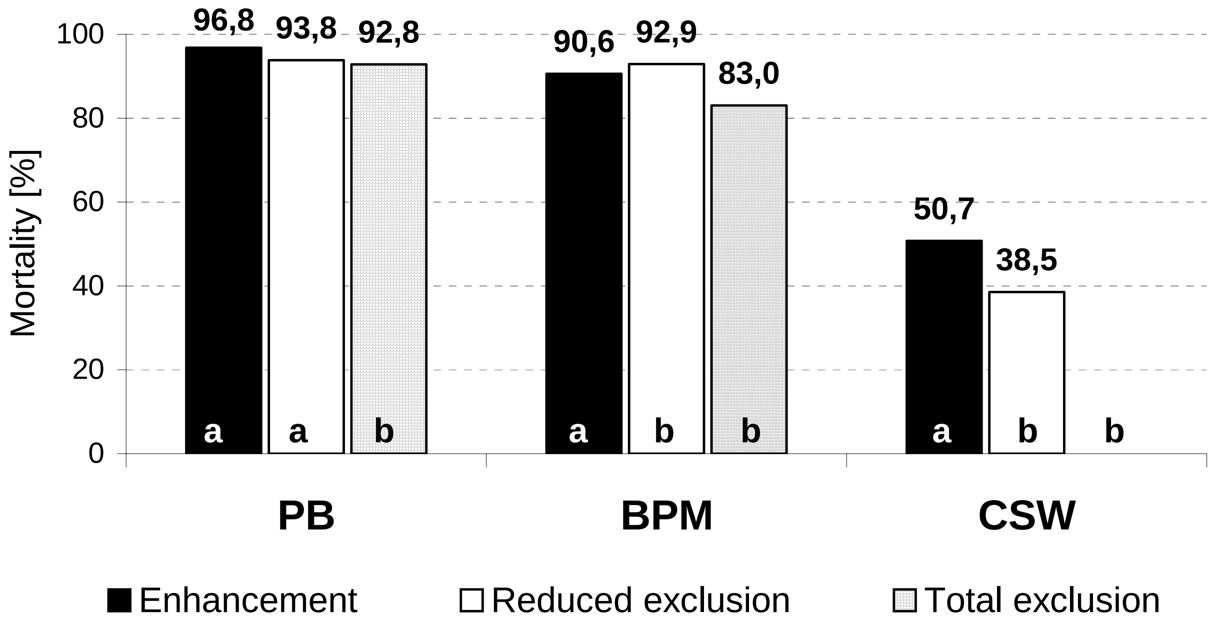

Figure 5. Mortality of larvae and pupae of the pollen beetle (PB), the brassica pod midge (BPM) and cabbage seed weevil (CSW) according to different densities of epigaeic active predators (different letters indicate significant differences; t-test; p = 0,05).

The evaluation of the total mortality in Figure 5 is based on the comparison of the larvae-abundance (recorded by funnel-traps) with the treatment-related hatching-rates of the new generation of pest insects (recorded by emergence traps).

Only regarding *C. assimilis*-larvae, for that nearly no natural mortality was recorded in the soil because attacks usually happen before the larvae drop to the soil surface (e.g. parasitism/predation occurs mostly in the pods, the larvae decay and do not reach the stage that drops to the ground). For the cabbage seed weevil it can be assumed that especially the epigaeic active predators caused a mortality of ca. 40% („Reduced Exclusion“) and 50% („Enhancement“), that was even significant in relation to the control treatment („Total exclusion“).

For all pest insect species already a high „basic“ mortality was recorded, that can not be related to the density of the epigaeic active predators, but refers to other factors, for example soil moisture, temperature, soil chemistry, attacks of larvae/pupae by fungi, bacteria or parasitoids etc.[1]). For example the „basic“ mortality in the treatment „Total exclosure“ reached already a level of about 93% for the Pollen Beetle (PB) and 83,0% for the Brassica Pod Midge (BPM). Therefore the predator-related increase of mortality is only up to 4% for the Pollen Beetle (*Meligethes* spp.) and 7% to10% for *Dasineura brassicae* (in comparison to the treatment „Total exclosure“). Nevertheless these slight differences are statistically significant.

However these and other biotic and abiotic factors which are responsible for the level of „basic“ mortality were equal in all treatments while the density of epigaeic active predators differed. So it can be assumed that the significant differences regarding the hatching reduction or the mortality of *Meligethes* spp., *C. pallidactylus*, *C. assimilis* and *D. brassicae* can be related to the diffferent densities of epigaeic active predators. But nevertheless, those principal methodological difficulties regarding an exact measurement of predatory effects in the field is obviously one of the main reasons for the huge lack of knowledge of the nutrition ecology of otherwise well known beneficials.

If the total mortality is considered, in a first view the importance especially of the epigaeic active predators seems to be fairly low. Apparently this demonstrates that one should not overestimate the regulatory effect of a single beneficial taxon, but among several natural enemies each beneficial taxon contributes to a certain extent to the biocontrol of a pest organism. However, even the observed obviously small increase of mortality of a pest insect should not be neglected, because at the moment one is not able to assess the absolute effect of those minor mortality increases, that means that it is not known which percentage in the increase of mortality is the decisive threshold for an effective biocontrol of the pests; these interrelations might be elucidated by two aphoristic examples as there is the drop that is the trigger for an overflow of water in a vessel, or an only 3-4% increasing mortgage interest which is able to crash a whole finance scheme. So, back to the interrelations of predators and pest insects, in our present knowledge it can not be excluded that even a low increase of mortality of a pest insect leads to a depression of the population development which is relevant for a lower attack level in the next season.

Acknowledgements

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[1] The assessment of the parasitation rate of Pollen Beetle-larvae showed that on average 62% of the second instar-larvae were parasitized by *Tersilochus heterocerus* and *Phradis interstitialis*. For random samples of dropped *Dasineura brassicae*- and *Ceutorhynchus assimilis*-larvae no parasitation could be recorded. The drying of soil because of higher temperatures and a lack of rainfall underneath the emergence traps can also cause higher basic mortalities by making it more difficult that larvae penetrate into the soil or by hindering their hatching. For example Basedow (1973) observed that in a wheat field larvae of the gall midge *Sitodiplosis mosellana* needed only 10 minutes for penetration into a wet soil of sandy loam (after thundery rain), but nearly three hours when the soil was more or less dry (1 mm rainfall). According to Leuchs (1956) heavy soil increases mortality of Pollen Beetle-larvae. There is also a hint on fungal infections of *Meligethes*-larvae and -pupae by Nilsson (1988).

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Integrated Control in Oilseed Crops
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Control of oilseed rape pests with combined application of insecticides and foliar fertilisers – ecological and economical aspects

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Abstract: In 1993–1998, at the Sośnicowice Branch of the Institute of Plant Protection, Poznań, investigations were made of tank-mix application of various agrochemicals in modern production technologies of rape. This paper reports results from synchronous application of insecticides to control pollen beetle (*Meligethes aeneus* Fab.) and cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) with foliar fertilizers, applied at green bud. Tank-mix application of insecticides and foliar fertilizers were found to be more economical than single applications of these agrochemicals and the biological effectiveness of particular agrochemicals, when jointly applied, did not differ from conventional application.

Key words: oilseed rape, cabbage stem weevil, pollen beetle, control, insecticides, foliar application of fertilisers

Introduction

An important task in oilseed rape crop protection is the control of pests that attack this crop at all growth stages. In Poland, crop losses due to pest feeding can amount to 15-50%, and in extreme cases may be as high as 80% (Mrówczyński at al. 1993; Muśnicki at al. 1994).

In Southern Poland, where the investigations were conducted, cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) and pollen beetle (*Meligethes aeneus* Fab.) are the most numerous and damaging pests of rape. However, opinions differ as to the economic significance of the damage caused by cabbage stem weevil. Some authors report that they cause loss of seed yield (Dmoch 1959: Witkowski 1977) while others report that they cause little injury provided that rape cultivation is correctly managed (Pałosz 1978).

Pollen beetle adults appear at nearly the same time as the cabbage stem weevil. Again, opinions differ as to the injury caused by pollen beetles. Some studies have demonstrated the considerable ability of oilseed rape to compensate for damage caused by this pest (Szulc 1959; Winfield 1962; Starzyński at al. 1989), while others demonstrate yield loss when just 2-5 beetles per plant are present (Sylven et al. 1976), with damage increasing if, due to low air temperature, the start of flowering is delayed prolonging feeding time on the buds.

Rape is a plant with large nutritional requirements. It gives a substantial increase in seed crop not only to artificial fertilisers applied to the soil, but also to additional foliar feeding with major and microelements. Foliar emergency feeding aims to supply nutrients at the time of maximum need, i.e. during the vegetation period of bud formation. At that time, deficiency of major and micronutrients in rape can occur even on rich soils, particularly during adverse weather conditions.

The steadily increasing number of plant pesticides permitted for use, along with an extended plant protection programme, more frequent use of liquid fertilisers for foliar emergency feeding, and the search for less energy-consuming and more economical cultivation, have aroused interest in the possible combined application of various agrochemicals.

Combined application is possible whenever the recommended application times are synchronised.

Studies of tank-mix application of agrochemicals in oilseed rape were initiated in 1987-1988 (Pałosz 1987; Sikora et al. 1988). These investigated combined applications of insecticides to control pollen beetle and cabbage stem weevil with foliar fertilisers. Tank-mix application of agrochemicals is frequently more effective both in respect of crop yields obtained and their profitability compared with a separate application (Grala at al. 1991; Mrówczyński at al. 1993; Seta and Sikora 1993).

Here we report further investigations conducted at the Sośnicowice Branch of the Institute of Plant Protection, Pozna of mixtures of new insecticides recommended to control cabbage stem weevil and pollen beetle along with new fertilisers for rape on-leaf emergency feeding.

Materials and methods

The investigations were conducted on winter rape (pollen beetle and cabbage stem weevil) and spring rape (pollen beetle) in 1993-1998. Experimental plots were sprayed at greed bud, i.e. crop growth stage 51-57 according to Schütte (Muśnicki Cz. 1995). Insecticides were used individually or mixed with fertiliser. Table 1, lists the insecticides and fertilisers tested and their active ingredients. Table 2, lists the mixtures of insecticides and fertilisers tested.

Table 1. The types of insecticides and foliar fertilisers, tested on oilseed rape in 1993–1998, and their active ingredients.

Insecticides	**active ingredients**	**Foliar fertilisers**	
Bancol 50 WP	bensultap	Basfoliar 12-4-6	microelements
Bulldock 025 EC	betacyfluthrin	Basfoliar 36 Extra	N+Mg+microelements
Cyperkill 25 EC	cypermethrin	Basfoliar 34	N+Mg+microelements
Danacap 450 CS	methyl parathion	Boraks	B
Enduro	betacyfluthrin	Florogama R	microelements
	d'oxydemeton-methyl	Insol 5	Mg + microelements
Fastac 350 WG	alfacypermethrinl	Mocznik	N
Fastac 10 EC	alfamethrin	Solubor DF	B
Fury 100 EC	zetacypermethrin	Wuxal TOP N	N-P-K + microelements
Karate 025 EC	lambdacyhalothrin		
Karate 10 CS	lambdacyhalothrin		
Regent 200 SC	fipronil		
Sumi Alpha	esfenvalerate		
Trebon 10 EW	ethophenprox		

Pollen beetles were counted on 25 rape plants along the plot diagonal, before the application and 1, 3, 7 and 14 days afterwards. At the same time, plants were examined for possible phytotoxic effects of mixtures. At the seed formation and plant ripening growth stages, i.e. 69–92 according to Schütte, 25 plants were sampled from each plot and their stems examined for tunnels produced by cabbage stem weevil larvae. On those plants the pods were counted and their lengths were measured.

Table 2. The insecticides and their mixtures with foliar fertilisers used in tank-mix applications to oilseed rape in 1993–1998.

Insecticides	**Foliar fertilisers**
	Insol 5; mocznik; mocznik + Florogama R; mocznik + Insol 5; Solubor DF; Wuxal TOP N; Basfoliar 36 Extra; Basfoliar 34; Basfoliar 12-4-6
Bulldock 025 E +	Insol 5; mocznik + Insol 5; mocznik + Florogama R; mocznik; Wuxal TOP N; mocznik + Wuxal Combi; Florogama R; Boraks; Solubor DF; Basfoliar 36 Extra; Basfoliar 34; Basfoliar 12-4-6
Cyperkill 25 EC +	Basfoliar 36 Extra; Basfoliar 34; Basfoliar 12-4-6; Solubor DF
Danacap 450 CS +	Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34; Solubor DF
Enduro 258 EC +	Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34; Insol 5; Solubor DF
Fastac 350 WG +	Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34; Insol 5; Solubor DF
Fastac 10 EC +	Mocznik; mocznik + Insol 5; mocznik + Florogama R; Florogama R; mocznik + Wuxal Combi; Insol 5; Boraks; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34
Fury 100 EC +	Wuxal Combi; Insol 5; Boraks; Solubor DF; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34
Karate 025 EC +	Mocznik; mocznik + Insol 5; mocznik + Florogama R; Insol 5; Wuxal Combi; mocznik + Wuxal Combi; Solubor DF; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34
Karate 10 CS +	Solubor DF; Wuxal Combi; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34
Regent 200 S.C. +	Solubor DF; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34;
Sumi Alpha 050 EC +	Insol 5; Solubor DF; Basfoliar 36 Extra; Basfoliar 12-4-6; Basfoliar 34

The seed yields (t/hectare) from respective experimental plots and the profitability of the tank-mix application of insecticides and foliar fertilisers were computed, taking the profit from the increased yield and the market price of applied agrochemicals and labour costs.

Results and discussion

Table 3, presents data from 1998 trials showing the effect of mixed application of insecticide and foliar fertiliser in pest incidence, seed yield and profitability.

In none of the trials were any phytotoxic effects seen. Any observed effects on plants were of short duration and had no effect on the plant growth and yield. Moreover, all combinations were subjected to physical and chemical tests and the following parameters: stability of water suspension, water emulsion, pH of water suspension, foam stability, surface tension and leaf wettability were evaluated. The effects gained as a result of combined application were manifold.

Extremely important, as seen from Table 3, is the increased yield from rape crops resulting from combinations of insecticides and fertilisers, due to synergetic effect of mixtures on the effectiveness of plant pesticides in use. Further, fertilisers applied in this way are applied more uniformly and accurately thus excluding the entry of major- and microelements into water reservoirs and resultant environmental degradation.

Table 3. The effect of combined application of insecticide and foliar fertilisers on pest incidence, seed yield and crop profitability in 1998.

Treatments	Dose kg/ha	Density of *M. aen.* 1 day after spraying	Damage caused by *C. pallidact.* tunnel, cm	Yield dt/ha	Increased Yield dt/ha	Net Profit $/ha
CONTROL		0,28	12,05	3,23 a	-------	-------
Trebon 10 EW + Basfoliar 36 Extra	0,50 + 10,0	0,09 abc	2,90 abcd	4,34 cde	1,11	261
Bancol 50 WP + Basfoliar 36 Extra	0,08 + 10,0	0,08 abc	2,98 abcd	3,54 abc	0,31	61
Trebon 10 EW + Wuxal TOP N	0,50 + 5,0	0,04 abc	6,00 bcdef	4,26 bcde	1,03	240
Bulldock 025 EC + Basfoliar 12-4-6	0,25 + 10,0	0,04 abc	2,48 ab	4,06 abcde	0,83	194
Trebon 10 EW + Solubor DF	0,50 + 1,0	0,02 ab	6,45 cdef	4,16 bcde	0,93	215
Karate 10 CS + Solubor DF	0,08 + 1,0	0,02 ab	2,05 a	4,07 abcde	0,84	197
Bulldock 025 EC + Wuxal TOP N	0,25 + 5,0	0,01 a	3,48 abcde	4,03 abcde	0,80	187
Bulldock 035 EC + Basfoliar 36 Extra	0,25 + 10,0	0,01 a	3,35 abcde	4,26 bcde	1,03	245
Trebon 10 EW + Basfoliar 34	0,50 + 10,0	0	3,30 abcde	3,78 abcde	0,55	120
Karate 10 CS + Wuxal TOP N	0,08 + 5,0	0	0,78 a	4,45 de	1,22	293
Karate 10 CS + Basfoliar 34	0,08 + 10,0	0	1,55 a	4,04 abcde	0,81	190
Karate 10 CS + Basfoliar 12-4-6	0,08 + 10,0	0	1,60 a	4,33 cde	1,10	263
Karate 10 CS + Basfoliar 36 Extra	0,08 + 10,0	0	2,48 ab	4,14 bcde	0,91	215
Bulldock 025 EC + Solubor DF	0,25 + 1,0	0	2,58 ab	4,38 cde	1,15	275
NIR 0,05		0,10	3,86	0,86		

Fuel and labour costs were reduced by about 40%. Other benefits included the possibility of using equipment for other work, a reduction in soil compaction and mechanical damage to plants and improved labour organisation. Savings resulting from reducing the number of operations should be included among the measurable economical advantages. So, for example, in 1998 the cost of technology (machinery + labour costs) of using plant pesticides or spreading fertilisers amounted in Poland, at the average, from 9 to 13 dollars/hectare. If no additional operation was performed, that particular sum has been saved.

Conclusions

1. Tank-mix application of insecticides and foliar fertilisers during green bud growth stage of oil seed rape increases of effectiveness of control of cabbage stem weevil and pollen beetle.
2. Application of different agrochemicals in one spraying operation permits the production costs of spring and winter rape to be appreciably reduced.

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Table 3. The effect of combined application of insecticide and foliar fertilisers on pest incidence, seed yield and crop profitability in 1998.

Treatments	Dose kg/ha	Density of *M. aen.* 1 day after spraying	Damage caused by *C. pallidact.* tunnel, cm	Yield dt/ha	Increased Yield dt/ha	Net Profit $/ha
CONTROL		0,28	12,05	3,23 a	-------	-------
Trebon 10 EW + Basfoliar 36 Extra	0,50 + 10,0	0,09 abc	2,90 abcd	4,34 cde	1,11	261
Bancol 50 WP + Basfoliar 36 Extra	0,08 + 10,0	0,08 abc	2,98 abcd	3,54 abc	0,31	61
Trebon 10 EW + Wuxal TOP N	0,50 + 5,0	0,04 abc	6,00 bcdef	4,26 bcde	1,03	240
Bulldock 025 EC + Basfoliar 12-4-6	0,25 + 10,0	0,04 abc	2,48 ab	4,06 abcde	0,83	194
Trebon 10 EW + Solubor DF	0,50 + 1,0	0,02 ab	6,45 cdef	4,16 bcde	0,93	215
Karate 10 CS + Solubor DF	0,08 + 1,0	0,02 ab	2,05 a	4,07 abcde	0,84	197
Bulldock 025 EC + Wuxal TOP N	0,25 + 5,0	0,01 a	3,48 abcde	4,03 abcde	0,80	187
Bulldock 035 EC + Basfoliar 36 Extra	0,25 + 10,0	0,01 a	3,35 abcde	4,26 bcde	1,03	245
Trebon 10 EW + Basfoliar 34	0,50 + 10,0	0	3,30 abcde	3,78 abcde	0,55	120
Karate 10 CS + Wuxal TOP N	0,08 + 5,0	0	0,78 a	4,45 de	1,22	293
Karate 10 CS + Basfoliar 34	0,08 + 10,0	0	1,55 a	4,04 abcde	0,81	190
Karate 10 CS + Basfoliar 12-4-6	0,08 + 10,0	0	1,60 a	4,33 cde	1,10	263
Karate 10 CS + Basfoliar 36 Extra	0,08 + 10,0	0	2,48 ab	4,14 bcde	0,91	215
Bulldock 025 EC + Solubor DF	0,25 + 1,0	0	2,58 ab	4,38 cde	1,15	275
NIR 0,05		0,10	3,86	0,86		

Integrated Crop Protection

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Report of the concerted Action "Research for the adaption of oilseed crops to the new requirements of the common agricultural policy: crop competetivity, seed quality, environment"

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Abstract: An EU financed concerted action between 1995 to 1998 investigated different aspects of integrated crop protection (ICP) for the main oilseed crops grown in Europe: oilseed rape, sunflower and linseed. Overall, it was concluded that ICP had to be improved and had to be introduced into common agricultural practice, that survey information also had to be improved and that new technologies (GPS, GIS, GMO's) should be introduced as quickly as possible into ICP. Regulation of plant protection (for example, law and the registration of pesticides and varieties) differed significantly between the participant countries. This in itself can have a major influence on ICP and the overall environmental effect of plant protection practices. ICP has to be supported by these legislative tools. Most of the information obtained during the concerted action relates to oilseed rape data, with less data available on sunflower and linseed. The following summary is therefore restricted to oilseed rape.

Key words: Oilseed rape, diseases, importance and distribution of diseases and pests, threshold, forecasting, control, integrated crop protection

Introduction

During an EU financed concerted action between 1995 to 1998, a working group of integrated crop protection (ICP) investigated different aspects of ICP on the main oilseed crops grown in Europe, oilseed rape, sunflower and linseed. The main topics, with regard to environmental aspects were, the importance of pests, diseases and weeds in oilseed crops in Europe, the current knowledge of ICP, available control measures, the status of ICP (thresholds, forecasting systems & decision support systems), the acceptance of ICP, regulations influencing ICP, possibilities of improving ICP, gaps in ICP, research requirements and further research priorities.

Results

The importance and control of oilseed rape pests and diseases in the EU was reviewed using quantitative survey information (where available) and general experience in research and advisory work.

Table 1. Importance of diseases of winter oilseed rape in Europe.

Diseases	**UK**	**D**	**S**	**DK**	**F**	**B**
Phoma leaf spot, canker - *(Leptosphaeria maculans,* anam.: *Phoma lingam)*	+	+	+	+	+	+
stem rot - (*Sclerotinia sclerotiorum)*	+	+	+	+	+	+
light leaf spot - *(Pyrenopeziza brassicae,* anam.: *Cylindrosporium concentricum)*	+	(+)	(+)	(+)	(+)	(+)
verticilium wilt *(Verticillium dahliae)*	0	(+)	+	(+)	(+)	n.i.
dark leaf spot, alternaria *(Alternaria brassicae)*	(+)	(+)	(–)	+	(+)	(+)
downy mildew *(Peronospora parasitica, P. brassicae)*	(+)	(+)	–	–	(+)	(+)
grey mould (*Botrytis cinerea)*	(+)	(+)	–	+	–	–
clubroot *(Plasmodiophora brassicae)*	(+)	(+)	(+)	(+)	–	–
damping off (*Pythium spp.)*	–	–	–	–	–	–
rhizoctonia root rot (*Rhizoctonia solani)*	–	–	–	–	–	–
powdery mildew grey *(Erysiphe cruciferarum)*	–	–	–	–	(+)	–
ring spot (*Mycosphaerella brassicicola)*	–	–	–	–	–	–
white leaf spot (*Mycosphaerella capsellae, Pseudocercosporella capsellae)*	–	–	–	–	(+)	–
beet western yellow virus	(+)	–	–	–	(+)	
cauliflauer mosaic virus	–	–	–	–	(+)	–
turnip mosaic virus	–	n.i.	n.i.	n.i.	(+)	–

+ Often damaging
(+) Occasionally or locally damaging
– Disease is present but of no importance
0 Not present
n.i. No information

Importance of diseases (Tab. 1)

Canker (L*eptosphaeria maculans*) was often the cause of yield loss in all the main areas of production and was considered the most important disease. Attacks of stem rot (*Selerotinia sclerotiorum*) occurred in all countries but severe epidemics occurred perhaps 1 or 2 years in every 10. Light leaf spot (*Pyrenopeziza brassicae*) was reported from all production areas but serious attacks only occurred regularly in northern England, Scotland and maritime regions of France. Verticillium, which is now recognised in the UK as being caused by the pathogen *Verticillium longisporum,* has long been a problem in Sweden and was found to be a developing problem in France and Germany. Dark leaf and pod spot (*Alternaria brassicae)* had potential to cause serious yield losses, but recently, severe attacks have only been of local importance in the EU. Downy mildew *(Peronospora parasitica*) was found to be ubiquitous on seedlings and during stem extension, but only occasionally caused economic damage. Grey mould (*Botrytis cinerea)* causes leaf, stem and pod rotting and was notably important in the more northerly parts of the UK and Scandinavia. Patches of clubroot (*Plasmodiophora brassicae*) could be serious in Germany, Scandinavia and occasionally elsewhere, and the persistent soil-borne nature of the disease represents a long term threat to the crop. Powdery mildew (*Erysiphe cruciferarum)* has be found in early sown crops in the autumn and near to harvest in some years. Occasionally powdery mildew did reduce yield but this effect was only observed in France. A range of damping off and foot rot problems affected crops at the seedling stage but damage was generally slight. Early dying syndrome may be due to soil-borne pathogens but the disease had not been fully investigated. Foliar diseases such as ring spot (Myco*sphaerella brassicicola)* and white leaf spot (*Mycosphaerella capsellae)* have occurred but only the latter merits control. Virus diseases have been important during some years, particularly the aphid-borne beet western yellows virus which has been a problem in France and the UK and localised outbreaks of cauliflower mosaic virus and turnip mosaic virus have also occurred.

Table 2. Thresholds and forecasting systems or prediction available for diseases in oilseed rape in Europe.

Diseases	**UK**	**D**	**S**	**DK**	**F**	**B**
Phoma leaf spot, canker - *(Leptosphaeria maculans* anam.: *Phoma lingam)*	(+)	0	0	0	(+)	(+)
stem rot - *Sclerotinia sclerotiorum*	(+)	(+)/–	(+)	(+)	(+)	(+)
light leaf spot - *Pyrenopeziza brassicae* anam.: *Cylindrosporium concentricum)*	(+)	–	0	–	(+)	(+)
verticilium wilt *(Verticillium dahliae)*	0	(+)	0	0	0	0
dark leaf spot, alternaria *(Alternaria brassicae)*	(+)	–	0	–	(+)	(+)
downy mildew *(Peronospora parasitica, P. brassicae)*	–	0	0	0	0	0
grey mould (*Botrytis cinerea)*	–	0	0	0	0	0
clubroot *(Plasmodiophora brassicae)*	0	0	0	0	0	0
damping off (*Pythium spp.)*	0	0	0	0	0	0
rhizoctonia root rot (*Rhizoctonia solani)*	0	0	0	0	0	0
powdery mildew grey *(Erysiphe cruciferarum)*	0	0	0	0	(+)	(+)
ring spot (*Mycosphaerella brassicicola)*	0	0	0	0	n.i.	n.i.
white leaf spot (*Mycosphaerella capsellae)*	–	0	0	0	+	n.i.
beet western yellows virus	–	0	0	0	–	–
cauliflauer mosaic virus	–	0	0	0	–	–
turnip mosaic virus	0	0	0	0	–	–

+	available based on experimental datas	0	none available
(+)	available based on insecure datas	n.i.	no information
–	some information for orientation available		

Forecasting and thresholds for diseases

The development of forecasting systems and thresholds for chemical control of diseases is a developing area of research as new fungicides have become available. The use of forecasts for diseases is much more rudimentary than schemes that are currently available for pests of oilseed rape (Tab. 2)

Preliminary schemes to forecast canker have been developed for eastern England and France. Thresholds for treatment are poorly defined as chemical control measures have generally shown limited success. Risk assessment methodology and use of "depots" of sclerotina play a part in decision making for stem rot. Petal testing techniques are showing promise in the UK and these could be developed for wider use in the EU. An agrometeoroligcal method is currently being tested throughout Germany. A regional forecast for light leaf spot risk is being developed in the UK and is combined with a crop risk index. Elsewhere guidelines are available for fungicide use. Dark leaf and pod spot, Verticillium wilt, powdery mildew and white leaf spot have received some attention, but robust thresholds or forecasts are not yet available. There is limited information available for virus disease control.

Table 3. Importance of pests in oilseed rape in Europe.

Stem Borers	**UK**	**D**	**S**	**DK**	**F**	**B**
cabbage stem flea beetle - *Psylliodes chrysocephala*	+	+	+	+	+	+
cabbage stem weevil - *Ceutorhynchus quadridens*	(+)	+	–	–	–	–
oilseed rape stem weevil - *Ceutorhynchus napi*	0	+	0	–	+	(+)
rape winter stem weevil - *Ceutorhynchus picitarsis*	(+)	(+)	0	–	+	–
Inflorescence/Bud Pests						
pollen beetle – *Meligethes aeneus*	(+)	+	+	+	+	+
cabbage seed weevil - *Ceutorhynchus assimilis*	+	+	(+)	+	(+)	+
brassica pod midge - *Dasineura brassicae*	(+)	+	(+)	+	(+)	(+)
Other Pests						
Slugs - eg *Derocerus reticulatum, D. agreste, Arion hortensis*	+	+	(+)	(+)	+	+
aphid virus vectors - eg *Myzus persicae*	(+)	(+)	–	–	(+)	–
cabbage aphid - *Brevicoryne brassicae*	(+)	(+)	–	–	(+)	–
Field thrips - *Thrips angusticeps*	–	–	–	–	–	–
Flee beetles - *Phyllotreta spp.*	–	–	–	–	–	–
Cabbage root fly - *Delia radicum*	–	(+)	–	–	(+)	–
mice	–	(+)	–	–	–	–

+ often damaging   0 not present
(+) occasionally or locally damaging   n.i. no information
– of little or no importance , but pest is present

Importance of pests

Tab. 3 shows the importance of pests in Europe. Cabbage stem flea beetle (*Psylliodes chrysocephala)* was observed to be widely distributed throughout northern Europe and as such, was regarded to be the most important of the stem boring pest of oilseed rape Cabbage stem weevil (*Ceutorhynchus quadridens)* was present in most countries, with the exception of Germany, but was of little or no importance. Oilseed rape stem weevil (*Ceutorhynchus napi)* was a significant pest in Germany and France. Rape winter stem weevil (*Ceutorhynchus* *picitarsis)*, a potentially damaging pest of winter rape was economically important only in France, although local damage was also observed in UK and Germany.

Pollen beetle (*Meligethes aeneus)* was the most abundant pest of oilseed rape throughout the EU. The pest was recognised as being very damaging to spring oilseed rape but it's relevance to winter rape varied between countries. Cabbage seed weevil (*Ceutorhynchus assimilis)* and the associated brassica pod midge (*Dasineura brassicae)* were also common in most countries but again, economic significance varies between countries.

Slugs (e.g. *Deroceras reticulatum)* are regarded as important pests of oilseed rape in most countries and the importance of this pest was acknowledge to be increasing.

Aphids (*Myzus persicae* and *Brevicoryne brassicae)* as vectors of virus diseases are important in some years in UK, Germany and France. Damage caused by aphid feeding was usually only important in spring rape.

Table 4. Thresholds of pests of oilseed rape in Europe.

Stem Borers	**UK**	**D**	**S**	**DK**	**F**	**B**
cabbage stem flea beetle - *Psylliodes chrysocephala*	+	+/(+)	+	+	+	+
cabbage stem weevil - *Ceutorhynchus quadridens*	–	(+)	0	0	–	–
oilseed rape stem weevil - *Ceutorhynchus napi*	–	(+)	0	0	+	+
rape winter stem weevil - *Ceutorhynchus picitarsis*	–	–	0	0	(+)	(+)
Inflorescence/Bud Pests						
pollen beetle - eg *Meligethes aeneus*	(+)/+	+	+	+	(+)	(+)
cabbage seed weevil - *Ceutorhynchus assimilis*	+	+	0	+	–	–
brassica pod midge - *Dasineura brassicae*	(+)	–	0	0	–	–
Complex pod midge/seed weevil	+	(+)	+	+	(+)/–	(+)/–
Other Pests						
Slugs - eg *Derocerus reticulatum, D. agreste, Arion hortensis*	–	0	0	0	–	–
aphid virus vectors - eg *Myzus persicae*	–	0	0	0	(+)/–	–
cabbage aphid - *Brevicoryne brassicae*	–	(+)	0	0	(+)/–	–
Field thrips - *Thrips angusticeps*	–	0	0	0	0	0
Flee beetles - *Phyllotreta* spp.	–	0	0	0	0	0
Cabbage root fly - *Delia radicum*	–	0	0	0	0	0
Mice	0	–	0	0	0	0

+ available based on experimental datas (economic threshold with references)
(+) available based on insecure datas
– some information for orientation available (guideline)
0 none available
n.i. no information

Thesholds and forecasting of pests (Tab. 4)

Control strategies for the cabbage stem flea beetle are generally based on water trapping for adult flea beetles and/or later larval infestations within plants. There are some similarities in treatment thresholds currently used for the control of larvae. Treatment thresholds for cabbage stem weevil and oilseed rape stem weevil have been established in Germany and France and sampling methods exist.

Pollen beetle treatment thresholds are available for most countries of the EU and are well established. Nevertheless, monitoring methods and treatment thresholds differ significantly between countries. Seed weevil treatment thresholds also exist for most EU countries. However, information on thresholds for the pod midge are lacking.

Crop damage caused by slugs is unpredictable. At present, there are no reliable damage forecasting methods available and treatment thresholds have not been established. Nevertheless, there is an urgent need for the development of thresholds and forecasting systems for slug control.

Aphid infestations and subsequent virus infection are difficult to forecast and aphid treatment thresholds developed to date are unreliable.

Discussion

The results indicate the importance of diseases and pests of oilseed rape in the EU through the analysis of quantitative survey information and the interpretation of general experience in research and advisory work. The results show that, in most EU countries, there was, and remains, a lack of survey data, particularly with respect to continuous sampling on a long-term basis. For many pests there are thresholds or forecasting methods available, but these differ between EU countries and between sampling methods. Comparing and optimising these could be advantageous. Threshold and forecasting models are not available for most diseases. There was a strong need to develop such tools for the further improvement of ICP.

Apart from the essential advances outlined above, there were further priorities areas of research which were highlighted by the members of the working group. Priorities For Future Research focussed on the development of pest control strategies for new oilseed rape genotypes and the development and use of new technologies, e.g. GIS, GPS for yield mapping and differential pesticide application. Also, it was recognised that there was a need to aid decision making for pest control in oilseed rape as this would result in more effective targeting of pesticide inputs. Extensive research in this area was required at both the regional and farm/crop level on within area/field distribution of pest infestations.

A further priority for research was the development or improvement of an expert system for oilseed rape pests and diseases. In all EU countries, further evaluation of the systems was needed.. In order to co-ordinate activities that aim to improve decision making for pest control in oilseed rape between member countries, further evaluation of existing systems was required using common key pests e.g. cabbage stem flea beetle, pollen beetle, cabbage seed weevil/brassica pod midge. The development of expert systems for diseases was also targeted as a major priority.

Forecast and threshold models are being developed within individual regions or countries with little exchange of information or standardisation of epidemiological records between developing groups. Disease development varies considerably within Europe, particularly where there are contrasts in temperatures and rainfall. Collaborative studies would provide more diverse information on the effect of variance in weather factors on disease epidemics than could be obtained within a single country and would thus enhance the development of forecasting systems. Contrasting results from chemical control studies would be understood more readily if a standardised protocol were to be used to record disease progress. A further point for discussion was the transfer of ICP tools at the farm level. This was recognised by the members of the working group as an area of ICP that required improvement.

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Integrated weed control in winter oilseed rape by the use of economic thresholds

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Abstract: An economic threshold model for weed control in winter oilseed rape has been developed and evaluated at the University of Göttingen. Losses due to weeds can be predicted by taking weed densities and weed cover into account. The threshold model was tested in state-wide trials of the German Advisory Service for plant protection, the "Task-group Oilseed Rape" of the German Phytomedical Society, the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig and the University of Göttingen. The primary objective of the trials was the examination of the state-wide applicability of the model.

The reduction in herbicide treatments, as dictated by the model, in comparison to "normal" treatment, did not produce problems at harvest or reduce quality or yield of oilseed rape. The model accounted for necessary treatments at sites. Predicted losses and relative weed coverage are the two most important criteria of the model on which decisions are made. Therefore, there is a need to define individual thresholds which are adapted to specific situations. The probability of losses caused by weeds can be reduced by different measures of crop farming.

Key words: Weed control, winter oilseed rape, economic threshold, model, prediction of loss, relative weed coverage

Introduction

In various regions of Europe, excessive and unspecific use of herbicides has severely impacted agro-ecosystems and the environment. However, the farmer is under considerable pressure to critically evaluate the yield-increasing or -protective measures necessary for economic crop production. As part of the crop production process, this also applies to chemical weed control, which has to be reduced to the absolute minimum in accordance with the principles of Integrated Plant Protection (Heitefuss *et al*. 1993). An important approach to the realisation of the reduction in usage is the utilisation of weed economic thresholds.

Winter oilseed rape is a crop with considerable variation in vigour. The variation depends on a variety of factors, such as the type and quality of tillage (Lutman, 1989), the sowing date and the ground conditions at sowing (Jenkins & Leitch, 1986; Bowerman, 1989) and climatic conditions and the timing of weed germination in comparison to the crop (Küst 1989). For these reasons, it has not been possible to develop thresholds that depend on linear weed density / yield loss - relationships (Walker *et al*. 1990, Wahmhoff 1990, Lutman & Dixon 1991) such as those that have been developed for cereals.

Some predictive success for weed control in oilseed rape has been achieved in Germany. Küst (1989) and Munzel (1992) developed a complete threshold model that included different information about the weeds and also about the crop in terms of loss prediction. Werner (1996) simplified this model and demonstrated that it works under field conditions in the south of Lower Saxony.

The state-wide applicability of the threshold model has been tested in co-operative trials of the German Advisory Service for plant protection, the “Task-group Oilseed Rape“ of the working group “Integrated Plant Protection“ of the German Phytomedical Society, the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig and the University of Göttingen.

Material and methods

Description of the economic threshold model

Küst (1989), Klostermyer (1989) and Munzel (1992) carried out the fundamental research on the development of the model. The model (Figure 1) consists of three stages. In the early season from the germination of weed species until the rape has reached the 4 - 6 true leaf stage, weed density and the condition of the crop are recorded in the field. Cleavers and chickweed are counted separately whilst the other weeds are integrated into three groups: other dicotyledons, grass weeds and volunteer cereals. Monetary losses per ha are calculated by multiplying the weed densities by the competitive index, the factor for the condition of the crop, the expected yield loss and the price of rape. If the sum of predicted losses exceeds the threshold, which depends on the costs for herbicide application, weed control is necessary.

From oilseed rape growth stage BBCH 16 to late autumn, the ground cover by weeds (WCO) and by crop (CCO) are estimated to calculate the relative weed coverage (rWCO). Under the current economic conditions in Germany the threshold for weed control is 20 % relative weed coverage.

The third part of the model refers to *Galium aparine*, which is a particularly damaging weed in oilseed rape. The ability of cleavers to grow up, through the canopy of the crop leads to combine harvester problems at harvest. Additionally, cleavers influences seed contamination and can increase the moisture content of the rape grain. For this reason, special thresholds have been created for cleavers and these depend on the condition of the crop.

Field trials

The economic threshold model has been tested over a four year period (1993/94 - 1996/97). A series of 89 trials have been carried out in different regions of Germany usually on a randomised block design with 3 or 4 replicates. Some of these trials have been combined with other field trials of the different participant research groups.

Densities and coverage of crop and weeds have been counted at a number of dates in autumn and spring (Table 1). The first four dates had relevance for decisions about specific weed control. Agronomic factors, such as sowing date or tillage method, have also been studied. It has to be noted, that the number of sites (n) changed between the different analyses because, for different reasons the participants scientific groups were unable to record all of the information requested. Rape crops were harvested at maturity and yield, grain moisture and weed seed contamination was recorded.

The economic threshold model for weed control in winter oilseed rape

From germination stage of weeds to BBCH 16 of rape:
The densities of different weeds/m² are counted and the losses due to weeds has to be predicted. The threshold for weed control is 80 DM/ha predicted losses at the germination stage of weeds and 120,- DM/ha between BBCH 14 and 16 of rape.

Weed species	Density (plants/m²)	x Competitive index	x Factor A	B	C	Predicted losses (DM/ha)
Galium aparine		60				
Stellaria media		0,12				
Other dicotyledons		0,04				
Grass weeds		0,08				
Volunteer cereals		0,15				
			Sum of predicted losses (DM/ha)			

Factor A: Condition of crop

very good 0,5
good 1,0
middle 1,5
bad 2,0

Factor B:

Expected yield

dt/ha:100 0 ,

Factor C:

Price of rape

DM/dt

From BBCH 16 of rape to late autumn:
Weed coverage and crop coverage are estimated, the relative weed coverage has to be calculated.The threshold for weed control is 20 % relative weed coverage.

$$\text{Relative weed coverage} = \frac{\text{Weed coverage}}{\text{Weed coverage} + \text{Crop coverage}} \times 100$$

Galium aparine
The density of *Galium aparine* has to be counted from germination stage of the weeds to the start of vegetation in spring.
The threshold for plants/m² of *Galium aparine* is depending on the condition of crop.

Condition of crop	very good	good	middle	bad
Galium aparine (plants/m²)	2	1	0,7	0,5

Figure 1: The economic threshold model for weed control in winter oilseed rape

Table 1. Dates of assessment.

Date	Description	BBCH-code-rape
G*	Germination stage of weeds	10-12
A1*	4.-6. leaf of rape	14-16
A2*	End of vegetation - autumn	19-31
S1*	Start of vegetation - spring	19-31
S2	Rape with first buds	51-53
S3	Maturity	85-87

* : relevant dates for weed control

Results

Table 2 shows the initial weed densities in weed density classes and the total weed density on the sites. Differences between sites and years were marked. Reasons are for example different tillage or different ground conditions on the sites and changing climatic influences in the different years. The total weed density as average of all sites was high with 229.5 plants/m². Only 28.6 % of the sites had a weed density below 100 plants/m².

Table 2. Frequency (%) of weed density classes and total weed density (plants/m²) at sites (n).

		Weed density classes (plants/m²)					
Year	n	0-50	50 - 100	100 - 150	150 - 200	>200	Weed density total
93/94	32	15.6	9.4	6.3	15.6	53.1	257.1
94/95	21	19.0	23.8	14.3	9.5	33.3	152.9
95/96	14	0.0	21.4	14.3	14.3	50.0	216.9
96/97	17	23.5	17.6	17.6	11.8	29.4	282.6
Total	84	15.5	16.7	11.9	13.1	42.9	229.5

The different initial weed densities, in combination with other criteria included in the model, gave the following decisions in accordance with the threshold model: 7 of the 89 sites (7.8 % of the sites), did not require a herbicide treatment. A further 5 sites (5.6 %) only required herbicide application for the control of monocotyledons.

Table 3 indicates weed and crop development in the untreated plots of the sites depending on the herbicide application decision produced by the model. At sites where the decision was not to spray, strong rape plants developed and weeds were not competitive. On the untreated plots of these sites the ground cover of the crop at the end of the vegetative period was more than 77 % in the autumn and thus rape covered most of the weeds at this time. At sites where the decision from the threshold model was that a herbicide treatment was necessary, non treatment led to the development of a highly competitive weed population and by autumn, ground cover by weeds reached 25 % on the untreated plots. A strong influence of the weeds on yield of rape could be expected on these sites.

Table 3. Weed and crop development on the untreated plots of the sites (n) depending on the decision in accordance with the model.

	Decision in accordance with the model	
	Untreated	Treated
Weed density (plants/m²)	62.6 (7)	244.7 (77)
G. aparine (plants/m²)	0.2 (7)	4.3 (77)
Weed coverage - A2 (%)	5.7 (5)	25.0 (68)
Crop coverage - A2 (%)	77.2 (5)	50.7 (68)
Relative weed coverage - A2 (%)	6.8 (5)	34.3 (68)

Different parameters of harvest and yield have been recorded in the field trials and many of these are of high economic importance for the farmer. Lodging and the growth of weeds up through the rape canopy can cause problems at harvest. Increases in grain moisture and / or weed seed contamination are reflected in higher grain drying and cleaning costs. The influences of different control decisions on these parameters have been analysed.

Figure 2 shows data from the treated and untreated plots of sites that had not been treated in accordance with the output from the model. For these sites, there were no differences between the treated and untreated plots with respect to the remaining weed cover on the ground or growing through the crop, lodging, grain moisture, weed seed contamination or yield. On the other hand, at sites where the decision of the model was that herbicide treatment was necessary (Figure 3), the different treatments had substantial effects on the parameters measured. Of these, only lodging did not show significant differences between treated and untreated sites. Where a spray was applied, as suggested by the model, the average yield increase was 9.8 dt/ha. Thus, it can be concluded that on the one hand, the decisions not to spray saved herbicides and costs and conversely, the decision to spray prevented subsequent yield loss and harvest problems caused by the weeds.

If herbicides that can be sprayed later than at weed germination become available, the relative weed coverage would be an important tool of integrated weed control. Ground cover of weeds and crop can be recorded and analysed, and relative weed coverage analysis can lead to more reliable control decisions. The relative weed coverage and the differences in yield between the treated and untreated plots has been compared using linear regression (Figure 4). At all dates tested, relative weed coverage and the differences in yield were significantly correlated. It can be shown that, at the early date when rape has 4-6 leaves, relative weed coverage can be used as a criterion for control decision. In the spring, the use of relative weed coverage has to be used with caution. If the rape was frozen over the winter, ground cover of the crop could be underestimated and thus the relative weed cover would be overestimated.

In conclusion, the results of the co-operative trials throughout the whole of Germany indicate that the economic threshold model for weed control in winter oilseed rape operates successfully under field conditions. The omission of herbicide treatments in accordance with the threshold criteria did not lead to combining problems at harvest or a loss of quality or yield of rape. Furthermore, the model determined the necessity of treatment at the sites.

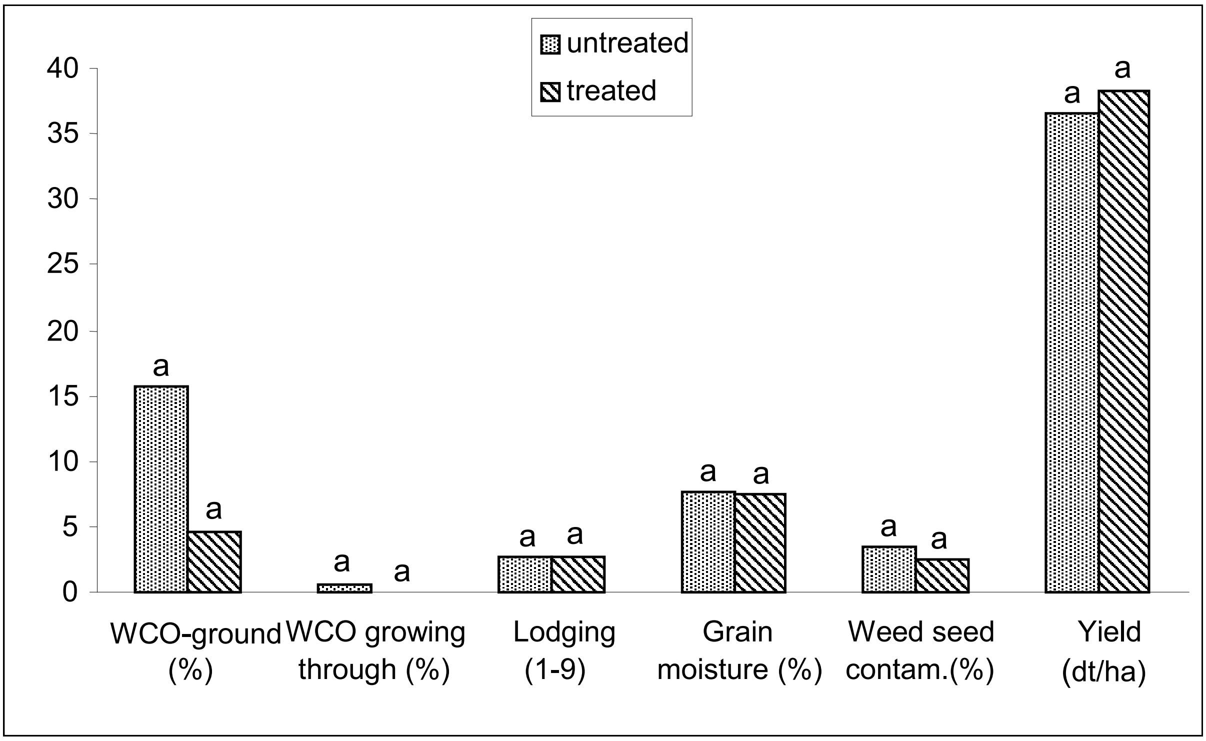

Figure 2. Influence of herbicide treatments on parameters of harvest and yield on the untreated sites depending on the decision in accordance with the model (Tukey, $p < 0.05$).

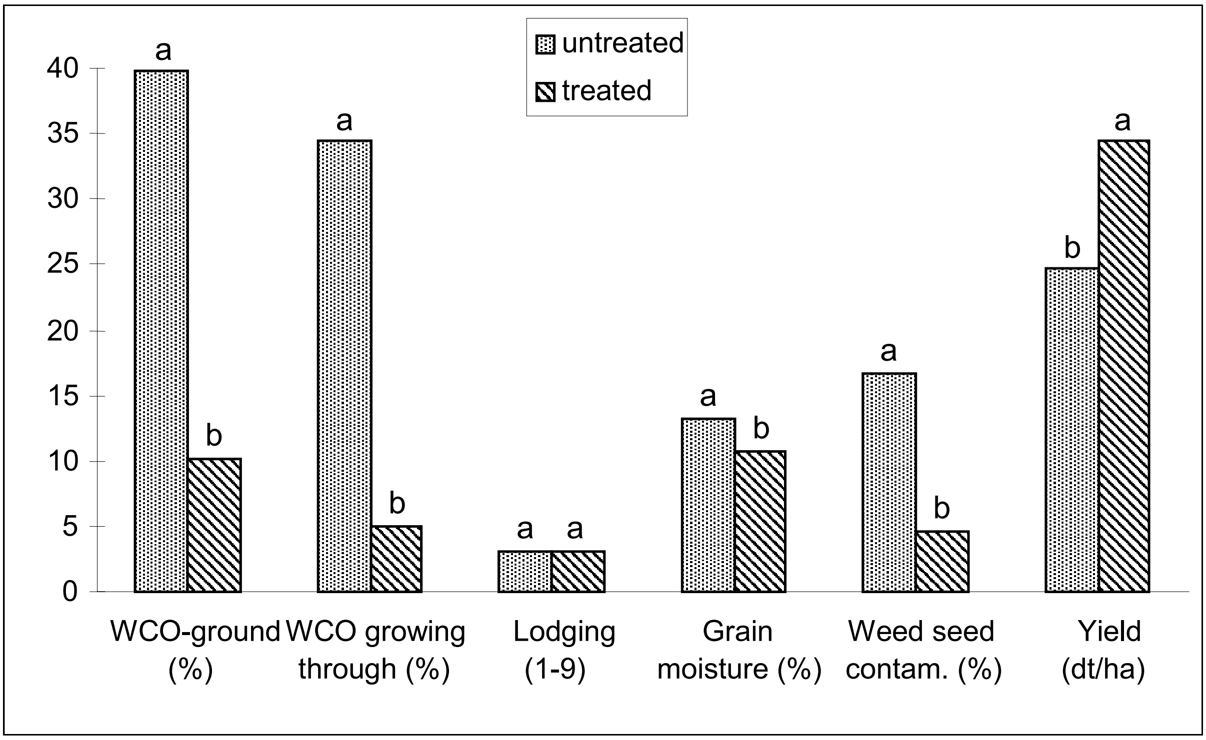

Figure 3. Influence of herbicide treatments on parameters of harvest and yield on the treated sites depending on the decision in accordance with the model (Tukey, $p < 0.05$).

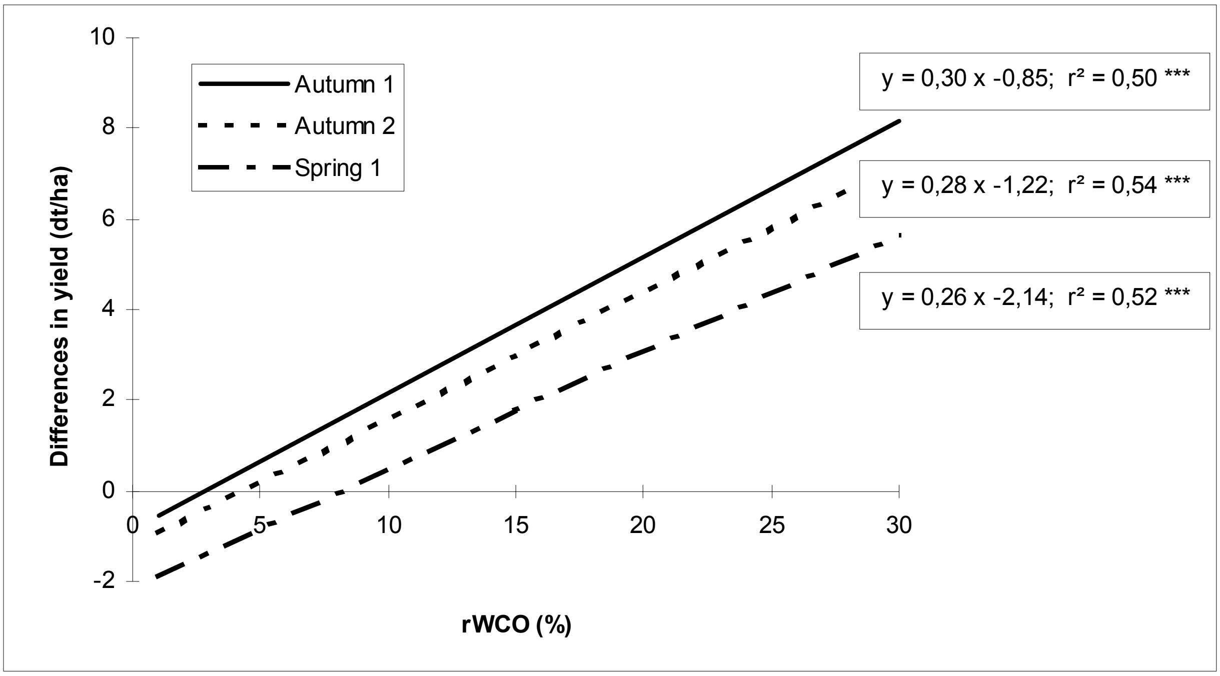

Figure 4. Influence of the relative weed coverage (rWCO) at different dates on differences in yield (dt/ha) between treated and untreated plots by means of linear regressions ($p < 0.001$).

Discussion

Under the premise of reducing herbicide inputs in oilseed rape, an economic threshold model developed in Göttingen was tested under different regional conditions throughout the whole of Germany. The results from the use of the model indicated that loss predictions based on weed densities or relative weed coverage can lead to correct spray application decisions for weed control in winter oilseed rape. However, of the total number of sites where the model was used, the number of sites where it was decided herbicide treatment was not necessary (7.8 %) and the number of sites where only a monocotyledon control application (5.6 %) was necessary were not as high as suspected. In different studies it has been pointed out that the amount of uneconomic herbicide treatment in winter oilseed rape is obviously higher. For example, Wahmhoff (1990) evaluated 249 herbicide trials to determine the efficacy and cost effectiveness of various herbicide treatments in winter oilseed rape. Of these, 23.5 % of all treatments caused yield reductions and a total of 44.1 % of the treatments were not economic. Some of the present trials have been combined with other herbicide trials of the different participant scientific groups. Many of these trials have therefore been carried out on fields with higher weed densities than normal and this could be one reason for the lower amount of untreated sites in the common trials. For example, in other field trials in Lower Saxony (Werner and Heitefuss, 1996), the utilisation of the threshold model led to the decision to leave 20.8 % of the fields without herbicide treatment.

The model has been tested in a multitude of field trials (Kees & Lutz, 1992; Broschewitz & Goltermann, 1994; Gehring, 1997) and it can be recapitulated that, with high precision, the model permits decisions for or against chemical weed control applications in oilseed rape. Thresholds for predicted losses and relative weed coverage have to be adapted to specific situations like, for example, changing economic conditions. To reduce herbicide treatments considerably, the economic threshold model has to be included in an integrated system of weed control in winter oilseed rape. This would involve a long-term strategy of weed management that also considers agronomic measures such as appropriate soil tillage, optimal seedbed preparation and sowing date, mechanical weed control and weed patch management.

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Impact of soil cultivation on weeds, diseases and slugs in winter oilseed rape

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Abstract: The occurrence of diseases, weeds and slugs was investigated in field trials with different tillage systems in a crop rotation consisiting of winter wheat, winter barley and winter oilseed rape. Non-inversive and zero tillage were compared with conventional ploughing. In direct-drilled oilseed rape, damage caused by slugs increased, especially if the ground was wet at the time around the sowing date. Experiments with different strategies of slug control in oilseed rape under conservation tillage indicated that tine cultivation followed by seedbed preparation using a rotating harrow can reduce slug activity and plant losses in contrast with zero tillage. The application of slug pellets directly after sowing decreased slug damage considerably in both tillage systems. The growth of oilseed rape in direct-drilled plots was retarded through a combination of low temperature and wetness in the autumn. The weed suppression ability of the oilseed rape in these plots was diminished due to the retarded growth and plant losses. Because of this, weed control by herbicides was not so effective as in plots with competitive plants. No differences in plant growth were found between tine cultivation and ploughing. Over the two year investigation period, there was no effect of soil tillage on *Sclerotinia sclerotiorum*. However, in some cases, direct-drilling reduced canker (*Phoma lingam*) at both the root collar and on the stem.

Key words: non-inversive tillage, zero tillage, conventional tillage, slug control, weed control, *Sclerotinia sclerotiorum*, canker, *Leptosphaeria maculans* anamorph: *Phoma lingam*

Introduction

A growing interest in non-inversive tillage and zero tillage can be observed in Germany as in other countries, especially in regions with a high risk of soil erosion. Under such conditions, ploughless tillage can help stabilise the physical structure and fertility of soils and can help reducing fertiliser and pesticide runoff. On heavy soils the increasing demand to reduce costs by saving energy and time is also an important factor. However, in many cases the use of non-inversive or zero tillage led to plant protection problems and this is the main reason why many growers still hesitate to use ploughless tillage. It is well known that ploughless tillage can lead to an increase in several monocotyledonous weeds, for example *Alopecurus myosuroides*, *Agropyron repens* and *Bromus species.* (Knab 1988, Balgheim & Kirchner 1998, Arnold-Reimer 1994, Bräutigam 1994). Also, the common occurrence of slugs on oilseed rape prompts growers to intensify chemical control through the use of molluscicides (Garbe 1994). The effect of soil tillage on diseases in oilseed rape has not been thoroughly investigated. Generally the degree of impact of tillage depends on the biology of the pathogen and its mechanism for dispersal and survival. Minimum or zero tillage tend to delay the decay of crop residue on the soil surface and thus may extend the time pathogens can survive on infested host material (Kharbanda & Tewari 1994).

Economically important diseases of oilseed rape that occur in the field study area are canker (*Leptosphaeria maculans*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*). Ascospores of *Leptosphaeria maculans* produced on infested stubble are responsible for the primary infestation of oilseed rape with canker. Thus, it was expected that during the course of the field trials, an increase in canker would be observed following the long term establishment of non-inversive tillage or zero tillage, in contrast to conventional ploughing. Similar to canker, a higher risk of sclerotinia was also expected due to an accumulation of sclerotia at or near the soil surface in tillage systems without ploughing.

A large research project was begun in 1996 in the region near Braunschweig in eastern Lower Saxony, Germany. The study monitored weed, disease and slugs in a crop rotation of oilseed rape followed by winter wheat and winter barley under different soil tillage and different levels of plant protection intensity. The purpose of this article is to give a short overview of the preliminary oilseed rape results.

Materials and methods

Site characteristics, experimental set-up and management

The experiments were located on soils ranging between sandy loams and loamy clays (< 100 m elevation). The area had an 8-year-average rainfall of 622 mm and a mean temperature of 9,4 °C. The central trial was established in a crop rotation of winter oilseed rape-winter wheat-winter barley in the autumn of 1995 with three different soil tillage systems. Conventional tillage (25-30 cm deep ploughing) was compared to non-inversive tillage (15-20 cm deep tine cultivation) and zero tillage using a Amazone Primera direct-drill. Under conventional ploughing and non-inversive tillage, seedbed preparation and sowing was carried out by a rotating harrow-drill-combination. Direct-drilled plots were prepared by applying 2 l/ha Roundup Ultra® (glyphosate 360 g/l) before sowing. All crops of the crop rotation were present during each season and under all the three tillage systems with two replicates. Trial plots measured 120 m long and 18 m wide. Since 1996, three levels of insecticide and fungicide treatment with four replicate per treatment were tested on oilseed rape under all tillage systems. To achieve this, main plots were split up into subplots 20 m x 18 m in size. The levels of insecticide and fungicide treatment were (1) untreated (2) application of insecticides and (3) application of insecticides and fungicides. In each season 0.15 kg/ha KARATE WG® (lambda-cyhalothrin 50 g/kg) was sprayed once in spring. The fungicide treatment was also carried out in spring (BBCH 39-55) with 0.8 l/ha FOLICUR® (tebuconazol 251.2 g/l). For oilseed rape plots, all tillage systems received a herbicide application between 10 - 14 days following sowing date with 1.75 l/ha BUTISAN TOP® (metazachlor 375g/l; quinmerac 125g/l). In order to control barley, especially under reduced or zero tillage, 0.8 l/ha FUSILADE ME® (Fluazifop-P 107 g/l) was applied during the autumn as a separate control measure. Under conventional tillage this treatment was not necessary or application rates could be reduced. All plots received an application of nitrogen in the autumn (40 kg N/ha). Sowing rate for zero tillage treatments was increased generally by 25 %. Applications of molluscicides with metaldehyde-based and carbamate-based molluscicides were carried out uniformly across all plots.

Severe slug damage in direct-drilled oilseed rape either with or without molluscicide treatment provided the incentive to carry out a extra experiment in the autumn of 1998. Under non-inversive tillage and zero tillage, different treatments were implemented with slug pellets to show the effect of molluscicides and stubble cultivation on slug activity and plant density of oilseed rape. The seed rate was the same under each tillage system. Each plot measured

30m long and 24 m wide and was divided into two subplots where slug activity and plant density were monitored. Plots where slug pellets were applied directly after sowing of oilseed rape were compared to untreated plots and plots with a treatment three days before harvest of the pioneer crop (winter barley). A combination of treatment before harvest of barley and after the sowing of oilseed rape was also tested. Each application was carried out with a mixture from metaldehyde-based (4%) and methiocarb-based (4%) slug pellets. The application rate was 2.0 kg/ha for the metaldehyde-based and 1.5 kg/ha for the methiocarb-based molluscicide.

Data collection

The total number of weeds was determined before herbicide application in the autumn. The efficiency of the herbicide treatments was investigated the following spring by estimating the weed coverage on the soil surface prior to the onset of flowering of the oilseed rape.

Roots, the root collar, the stem and leaves of oilseed rape were sampled for the assessment of *Phoma lingam.* Before harvest (BBCH 81), the scheme of Krüger (1983) was used to assessment canker symptoms at the root collar. The scheme uses a scoring system from 1 to 9 (1 – healthy plant, 9 – plant is dead). In order to assess sclerotinia stem rot, the frequency of damaged plants was determined at BBCH 79. Slug activity was monitored by using slug mats (Bayer Schneckentest), which consisted of an insulating fabric enclosed by silver foil at the top and a black perforated layer at the bottom. The mats were 50 cm x 50 cm in size. To improve the attraction of the mats, the soil surface below was moistened. Furthermore, slug pellets were spread under the mats. The mats were placed in the centre of each plot and were moved to a new position weekly. Distances of at least 8 m were kept from plot margins. In autumn 1998 sampling began 5 weeks before sowing and ended 5 weeks after sowing of oilseed rape.

Results and discussion

Crop establishment and plant growth

In the autumn of 1996 and 1998, the growth of winter oilseed rape in plots with zero tillage was considerably decreased through a combination of low temperatures and wet conditions at the sowing date and during the following weeks. In both years, slug damage was exceptionally high under zero tillage and led to lower plant densities and crop coverages (Table 1) in comparison to ploughed plots or plots under non-inversive tillage. Only in the autumn of 1997 were conditions favourable for oilseed rape to be direct-drilled. During this year, a long growing season in the autumn and a temperate winter favoured the lush growth of vegetation in all crops and under all tillage systems. However, under non-inversive tillage, the emergence of oilseed rape was impeded at first due to reduced moisture availability in the seed-zone. In this case the crop coverage assessed at the end of October was lower than under ploughing tillage or zero tillage. In all years plant density in plots with non-inversive tillage was similar to ploughed plots and both treatments had only minor slug damage.

Slug control

The observed increase in slug damage on winter oilseed rape under zero tillage was confirmed by the side experiment initiated during the autumn of 1998 on a clay soil near Braunschweig. The results indicated that stubble incorporation by 15 cm deep tine cultivation over two passes and seedbed preparation using a rotating harrow both contributed to reduce slug activity in comparison to zero tillage (Table 2). This agrees with the observations of other groups who suggest that thorough stubble cultivation can help prevent the accumulation of mulch layers on the soil surface and high weed coverage results in the destruction of refuge habitats and food for slugs. Also, slugs and slug eggs might be damaged directly through the mechanical action of the cultivator and rotating harrow.

Table 1. Effect of soil tillage on plant density and crop coverage of winter oilseed rape before winter.

		Plant density (plants/m^2)	Crop coverage (%)
1996/97	Ploughing	41 A	66 A
	Non-inversive	38 A	62 A
	Zero tillage	21 B	29 B
1997/98	Ploughing	56 A	91 A
	Non-inversive	61 A	58 B
	Zero tillage	57 A	77 C
1998/99	Ploughing	85 A	71 A
	Non-inversive	81 A	79 A
	Zero tillage	38 B	38 B

In each year, different letters indicate significant differences based on Kruskal-Wallis-test ($p \leq 0.05$).

In each tillage system, slug activity was reduced significantly through the application of slug pellets directly after sowing of oilseed rape (Table 2). The results confirmed that the main damage to oilseed rape occurs during the germination and seedling stage and thus, slug pellet treatment should be applied as soon as possible after sowing. Assessment of plant density 60 days after sowing indicated that an additional application of slug pellets before the harvest of the pioneer crop did not increase the level of slug control (Table 3). Differences between an application just after sowing and the combined treatment, observed 20 days after sowing, were not constant.

In plots with more than 90 plants/m^2 a strong reduction in the number of plants occurred between the first assessment date and the second as a result of high competition between plants. In plots where slug pellets were applied just before the harvest of the barley, slug activity and plant losses were similar to untreated plots.

The main reason for low control effects from slug pellet applications in barley in this case could be the long period between the pellet application and the sowing date of the oilseed rape. Voss *et al* (1998) could only detected a control effect from a metaledyde-based molluscicide for a period of 2 to 3 weeks following application. In autumn 1998, there was an interval of 5 weeks between the barley harvest and the oilseed rape sowing date. However, an advantage of treatment a short time before pioneer crop harvest could be the placement of slug pellets underneath the crop residue which , at harvest, is chopped and spread on the soil surface by the combine harvester. In this case, slugs resting under the straw during dry periods may be better controlled by the molluscicide and this would certainly be expected under zero tillage. It was also expected that intake of slug pellets would be higher during periods of slug food deficit. More experiments are necessary to verify these issues.

Table 2. Effect of soil tillage and molluscicide treatment on the average number of slugs (*Arion sp*, *Deroceras s*p.) per mat over a period of 5 weeks after ploughless sowing of oilseed rape.

Slug pellet application	Non-inversive	Zero tillage
	Individuals/slug mat*week	
1 Untreated	10.6 A, W	19.0 B, W
2 Application before harvest of barley	12.6 A, W	20.0 A, W
3 Application directly after sowing of oilseed rape	2.4 A, X	1.3 A, X
4 Combination of 2 and 3	0.7 A, X	3.1 A, X

Different letters indicate significant differences between soil tillage (A-B) and slug pellet application (W-Z) based on Kruskal-Wallis-test ($p \leq 0.05$).

Table 3. Effect of soil tillage and molluscicide treatment on plant density of oilseed rape, recorded 20 days and 60 days after sowing.

Slug pellet application	Non-inversive	Zero tillage	Non-inversive	Zero tillage
	Plants/m^2			
	20 days after sowing		60 days after sowing	
1 Untreated	50 A, W	3 B, W	25 A, W	1 B, W
2 Application before harvest of barley	89 A, X	8 B, W	37 A, W	4 B, W
3 Application directly after sowing of oilseed rape	102 A, X	63 B, X	78 A, X	77 A, X
4 Combination of 2 and 3	95 A, X	112 A, Y	81 A, X	76 A, X

At each assessment point, different letters indicate significant differences based on Kruskal-Wallis-test ($p \leq 0.05$); A-B for differences between soil tillage and W-Z for differences between slug pellet application.

Weed control

The assessment of weed coverage prior to oilseed rape flowering (Table 4) indicates that the efficiency of the herbicide treatment decreased considerably under zero tillage treatment, where crop coverage was reduced due to low plant density and impeded growth. In these cases, oilseed rape did not have the ability to supress the growth of weed species. Monocotyledon species increased in particular and were able to go to seed which lead to a higher density of weeds in the following crops, for example, *Alopecurus myosuroides* became more prevalent under zero tillage at our sites. This confirms the results of Amann *et al* (1992) who suggested that the effective control of *A. myosuroides* by herbicides is a necessary prerequisite on land where zero tillage or non-inversive tillage is practiced constantly.

Table 4. Effect of soil tillage on crop coverage and weed coverage in herbicide treated winter oilseed rape, recorded in spring (BBCH 55).

		Crop coverage	Weed coverage (all species)
		%	
1996/97	Ploughing	68.9 A	5.0 A
	Non-inversive	68.8 A	2.1 A
	Zero tillage	38.7 B	53.8 B
1997/98	Ploughing	100.0 A	0 A
	Non-inversive	90.5 B	8.3 B
	Zero tillage	89.0 B	8.5 B
1998/99	Ploughing	98.2 A	2.0 A
	Non-inversive	99.0 A	2.9 A
	Zero tillage	68.3 B	23.5 B

In each year, different letters indicate significant differences between soil tillage based on Kruskal-Wallis-test ($p \leq 0.05$).

Diseases

The incidence of sclerotinia stem rot in our field trials was low during the first cropping season and differences between tillage systems were not significant (Table 5). Under non-inversive tillage and zero tillage, an increase of sclerotinia stem rot had been expected due to the accumulation of sclerotia in the upper layers of the soil. Knowledge of the biology of *S. sclerotiorum* suggests that the opposite effect may occur. Investigations by Abawi and Grogan (1979) on the epidemiology of diseases caused by Sclerotinia species indicated that sclerotia of *S. sclerotiorum* are functional only in the top 2-3 cm of soil as apothecia with stipes longer than 3 cm are rarely produced under field conditions. This study suggests that the risk of Sclerotinia in oilseed rape may decrease if non-host crops follow oilseed rape and shallow cutivation or zero tillage is carried out. Depending on the crop rotation, this procedure may contribute to considerably lower the number of functional sclerotia near soil surface during seasons when host crops are absent.

Important factors affecting disease are soil moisture and microbial activity. In irrigation experiments, Teo *et al* (1989) have shown that the degradation of sclerotia was accelerated in soils with high moisture availability. Thus, the increased soil moisture and microbial activity that would be expected to develop in zero-tilled fields may reduce the risk of Sclerotinia (Bailey 1996).

Table 5. Effect of soil tillage on incidence of sclerotinia stem rot (%) in plots without fungicide treatment.

	Ploughing	Non-inversive	Zero tillage
		% infested plants	
1996/97	5.7 A	4.8 A	1.8 A
1997/98	3.5 A	3.1 A	4.3 A

In each year, different letters indicate significant differences based on Tukey-test ($p \leq 0.05$).

Canker root collar symptoms (*Phoma lingam*) were not significantly influenced by soil tillage (Table 6). Canker infestation on the stem was considerably lower in direct-drilled plots in 1996/97, and this was significantly different to levels found in non-inversive tillage and conventional ploughing plots (Table7). Many plants assessed from zero-tilled plots were characterised by large diameter stems and root collars and in these cases canker symptoms were limited to the root collar and stem surface. Significant interactions between soil tillage and plant protection intensity were not detected. Insecticide treatment had a low effect on canker disease index and in a few cases infestation decreased. Effects on the stem were higher than at the root collar. Additional fungicide application generally led to lower infestation in all tillage systems but effects were not significant. The highest reduction of infestation on the stem was observed for fungicide treatment under zero tillage cultivation.

Two years results have shown that canker has not increased in our field trials after the establishment of non-inversive tillage or zero tillage. Early infestation of leaves in the autumn, caused by ascospores of *Leptosphaeria maculans* produced on infected stubble, was low in the past two years. In the case of *L. maculans*, many authors recommend that infested residue should be buried by ploughing or deep cultivation and that shallow cultivation or direct-drilling should be practised in crops following oilseed rape to avoid re-exposing the buried residue (Gugel and Petrie 1992). To answer the question on impact of soil tillage on diseases in oilseed rape, long-term experiments are necessary. The effects caused by growing system and cultivation practices on the epidemiology of certain diseases can be masqued by site characteristics especially climate conditions.

Table 6. Effect of soil tillage and plant protection on root collar symptoms caused by canker at BBCH 81 (scoring system from1-9).

	Plant protection	Ploughing	Non-inversive	Zero tillage
1996/97	Untreated	6.1 A, X	5.6 A, X	5.1 A, X
	Insecticide	5.9 A, X	5.4 A, X	5.3 A, X
	Insecticide and fungicide	5.3 A, X	5.0 A, X	4.1 A, X
1997/98	Untreated	6.1 A, X	6.5 A, X	5.8 A, X
	Insecticide	5.8 A, X	6.1 A, X	6.2 A, X
	Insecticide and fungicide	5.4 A, X	5.5 A, X	5.4 A, X

In each year, different letters indicate significant differences based on Tukey-test ($p \leq 0.05$); A-C for differences between soil tillage and X-Z for differences between plant protection intensity.

Yield response

Insufficient winter oilseed rape crop establishment after zero tillage led to significant yield losses in comparison to other tillage systems in 1996/97 (Table8). No differences were found between non-inversive tillage and conventional ploughing. In the following year, the yield achieved under zero tillage was comparable to the other systems. Response to an insecticide treatment increased yield by 0.9-2.7 dt/ha. In plots with additional fungicide treatments, yield increased by 3.6-11.5 dt/ha in contrast to untreated plots. Compared with untreated plots, the effects of fungicide treatment, in addition to the insecticide applications, was especially high in 1997/98 under conventional ploughing (+11.5 dt/ha) and in 1996/97 under zero tillage (+6.1 dt/ha). The application of triazole-based fungicide produces advantageous yield effects as diseases are reduced diseases and the chemicals have growth regulatory activity. In ploughed plots, which were characterized by lush vegtation, lodging was reduced considerably. In zero-tilled plots where plants were initially under-developed, we suppose that plant growth was stimulated through an increase in stress tolerance (Siefert and Grossmann 1996). The interaction between plant protection intensity and soil tillage were not significant.

Table 7. Effect of soil tillage and plant protection on canker stem symptoms at BBCH 81 (scoring system from 1-9).

	Plant protection	Ploughing	Non-inversive	Zero tillage
1996/97	Untreated	5.3 A, X	5.9 A, X	4.4 B, X
	Insecticide	5.6 A, X	5.1 A, X	4.3 B, X
	Insecticide and fungicide	5.4 A, X	5.4 A, X	3.1 B, X
1997/98	Untreated	5.2 A, X	4.9 A, X	4.3 A, X
	Insecticide	5.0 A, X	4.4 A, X	3.8 A, X
	Insecticide and fungicide	4.4 A, X	4.3 A, X	3.3 A, X

In each year, different letters indicate significant differences based on Tukey-test (p≤0.05); A-C for differences between soil tillage and X-Z for differences between plant protection intensity.

Table 8. Effect of soil tillage and plant protection treatments on yield response.

	Plant protection	Ploughing	Non-inversive	Zero tillage
1996/97	Untreated	37.3 A, X	38.7 A, X	23.1 B, X
	Insecticide	40.2 A, XY	39.6 A, X	24.9 B, XY
	Insecticide and fungicide	42.3 A, Y	42.3 A, X	29.2 B, Y
1997/98	Untreated	37.4 A, X	41.4 A, X	41.3 A, X
	Insecticide	39.4 A, XY	44.1 A, X	42.3 A, X
	Insecticide and fungicide	48.9 A, Y	45.4 A, X	45.4 A, X

In each year different letters indicate significant differences based on TUKEY-test (p≤0.05); A-C for differences between soil tillage and X-Z for differences between plant protection intensity.

Conclusions

Initial results of our field trials indicate that crop establishment under zero tillage was more difficult than in other growing systems with a higher intensity of soil tillage. Zero tillage lead to increased layers of crop residue on the soil surface. The consequence of this was an increase of slug activity. Recently, field mouse damage has also become important. Growers practising extreme reductions in soil tillage intensity should monitor slug and field mouse activity continuously. Research of more effective controls is necessary. At our site the main cause of weed problems was the lowered competitive ability of the oilseed rape crop. Also, the efficiency of herbicide treatments was reduced because of low plant density and poor crop coverage. Effects of cultivation treatments on diseases of oilseed rape have been low so far. Non-inversive tillage before oilseed rape by tine cultivation achieved yields similar to conventional ploughing. Results of other groups have shown that a higher diversity of crops in the crop rotation can reduce plant protection problems in systems were soil tillage intensity is reduced.

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Investigations on fungicide application in oilseed rape in systems of integrated pest management

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Abstract: The control of diseases of oilseed rape with fungicides in Germany has increased during recent years in a situation of high prices for oilseed rape. The most important diseases which have needed control are *Sclerotinia sclerotiorum*, *Leptosphaeria maculans,* (asex.: *Phoma lingam*), *Pyrenopeziza brassicae* (*asex.: Cylindrosporium concentricum*), *Alternaria brassicae* and *Peronospora parasitica*. Field trials in Germany in the region of Braunschweig were carried out with different susceptible varieties, different sowing dates and different fungicide treatments. For the control of canker it was important to grow a variety with low susceptibility. Applications with azoles (e. g. tebuconazole) were effective in situations with high infestation. The economical gain from fungicide use was more beneficial for susceptible varieties than in less susceptible varieties. The growth regulator effect of tebuconazole from a spring application gave an economic advantage, although an autumn application led to better control of canker. Seed treatment, for the control of downy mildew, highlighted the effectiveness of dimethomorph against this pathogen, particularly in the situation of highly infected seed and a late sowing date. The results indicate that there is a strong need for forecasting systems or thresholds models for the support of integrated pest management with regard to the control of diseases in oilseed rape.

Key words: Canker, *Phoma lingam, Leptosphaeria maculans*, Downy mildew, *Peronospora parasitica,* variety, susceptibility, fungicide, integrated pest management, threshold, forecasting system

Introduction

Control of diseases on oilseed rape in intensive production regions of Germany has been a normal practice during the last few years as the high oilseed rape prices has made it profitable for growers to do so. Fungicides were used principally for the control of stem rot (*Sclerotinia sclerotiorum*), canker (*Leptosphaeria maculans,* asex.: *Phoma lingam*), light leaf spot (*Pyrenopeziza brassicae asex.: Cylindrosporium concentricum*), dark leaf and pod spot (*Alternaria brassicae*) and in some situations downy mildew (*Peronospora parasitica*). For most diseases in Germany, there is much scope for improvement with respect to targeting application timing.

For stem rot, rainfall, temperature, soil moisture, humidity, plant development and the infestation of the soil with sclerotia of *S. sclerotiorum* are all factors of importance with regard to infection by the fungus. These factors form the basis for a agrometeorological model, which was developed by Friesland (1998). The model has been tested for it's suitability as a forecasting system.

Factors which favour the infection and disease development of canker, light leaf spot, dark leaf and pod spot and downy mildew are commonly understood. However, in Germany thresholds or forecasting systems are not yet available for these pathogens. Therefore, in most cases, the application of fungicides is aimed at different factors e.g. previous experience at a particular field site, to include other cropping systems, the susceptibility of the variety and the sowing date. The experiments described in this paper were carried out over several years in order to show the inportance of the each of the factors described.

Materials and methods

Field trials of winter oilseed rape (four replicates) were carried out in Germany in the region of Braunschweig between 1993 and 1998. Different susceptible varieties were grown, different sowing dates were used and different fungicide treatments were applied, with 1.2 l/ha FOLICUR® from 1993 to 1995, 1.0 l/ha and 1.2 l/ha FOLICUR® from 1996 to 1998 (tebuconazol 250 g/l, autumn and spring applications) and with 1.5 l/ha RONILAN FL® (vinclozolin 500 g/l, application at flowering). Experiments for the control of downy mildew using seed treatments were carried out with a number of fungicides and these are described below. The plot size was 25 m². Disease assessment was carried out several times during each season, depending on the appearance of disease. Scores of disease incidence were recorded in addition to disease severity. For canker root neck disease, the assessment scheme of Krüger (1983) was used, with a scoring system from 1 - 9 (1 - healthy plant, 5 - root neck shows a damage of 50%, 9 - plant is dead). As well as disease assessment, the development of the plants, plant density and plant height were also scored and the yield was determined at harvest.

Results

The investigations show no or only a small effect of sowing date on canker. The effect of the fungicide application is indicated in figure 1. With increasing fungicide concentration the degree of canker was reduced. The strongest effect on canker could be gained by an autumn application of FOLICUR. Over all varieties this effect was similar. The degree of canker infestation was different between the varieties, with SAMOURAI being the most susceptible. The effect of reduction of canker infestation by applying a fungicide increased with the degree of variety susceptibility.

The effect of fungicide treatments on yield is shown in figure 2. Yield response increased with the canker susceptibility of the variety. The strongest yield response was achieved with fungicide applications in the autumn, spring and at flowering (AUT, SPR & FLW). The spring application produced the greatest economical effect. This could be explained because of the low infestation level of canker during the years of the study, the low level or absence of other diseases and the strong side effects of tebuconazole on lodging, overwintering and root growth of the plants.

Economic calculations indicated that the application of fungicides for the control of canker under the low to middle canker infection rates that occurred in Braunschweig was only economically viable on the susceptible variety SAMOURAI. There was an economic benefit from the spring application in all varieties and both described years. Because stem rot was not present in the field trials, the application of Vinclozolin at flowering was not economical. The economic effect of the autumn application varied strongly depending on the year. Whether the autumn application was economical or not depended on the calculated oilseed price.

Figure 3 shows the effect of a seed treatment for the control of downy mildew in Braunschweig. Besides the control, the oilseed rape was treated with thiram (THR), a new fungicide for the control of downy mildew (TEST), dimethomorph (DMM), metconazol

(MET) and tebuconazol (TEB). The infestation with downy mildew was strongly dependent on the sowing date. The late sowing date favoured disease development but the results clearly show that the disease could be controlled by the application of fungicides. The best control was achieved by the application of DMM, with slightly less control by the TEST compound. MET and TEB had little or no effect on controlling downy mildew.

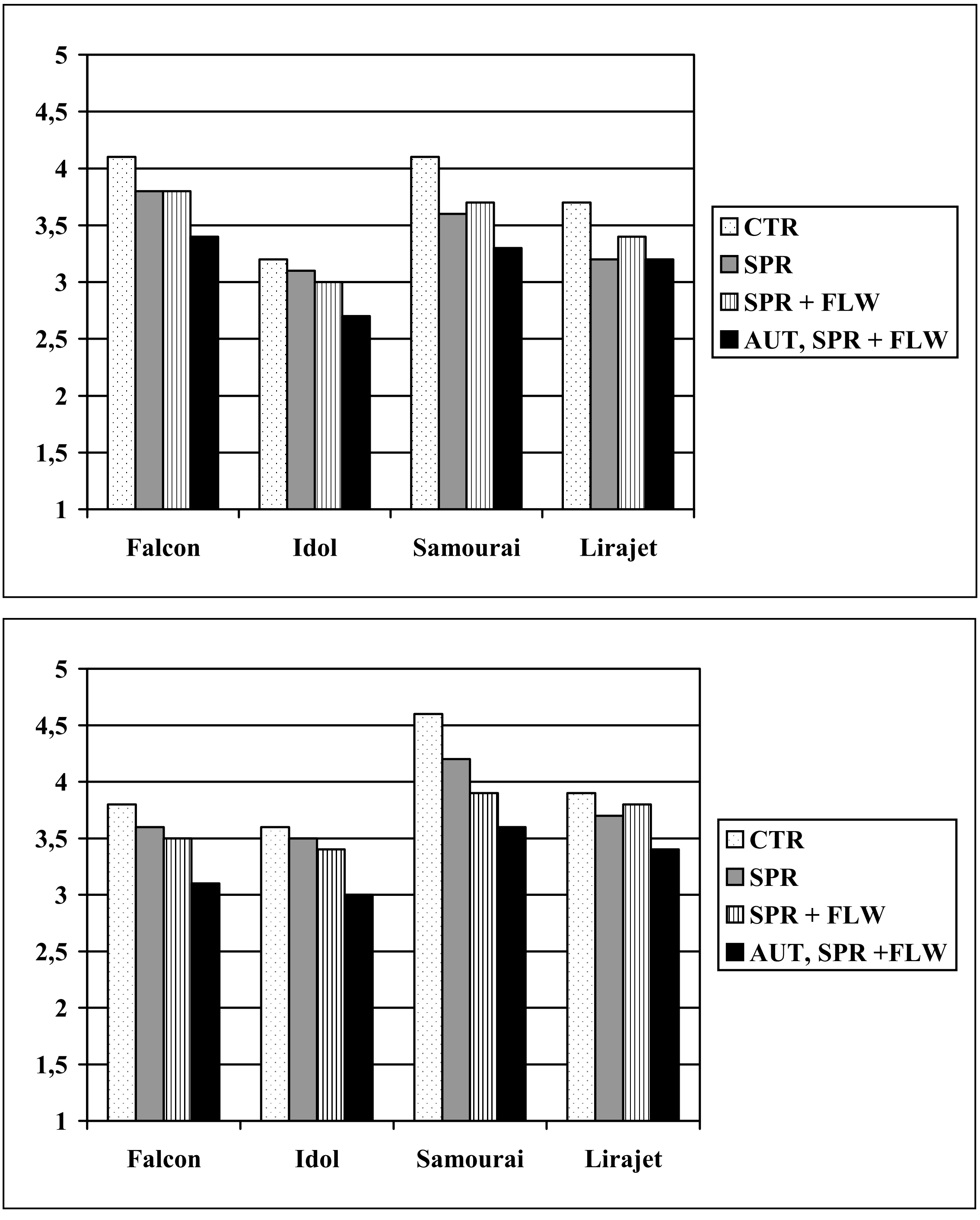

Figure 1. Effect of different fungicide treatments on canker (scoring 1-9) in 1994 and 1995.

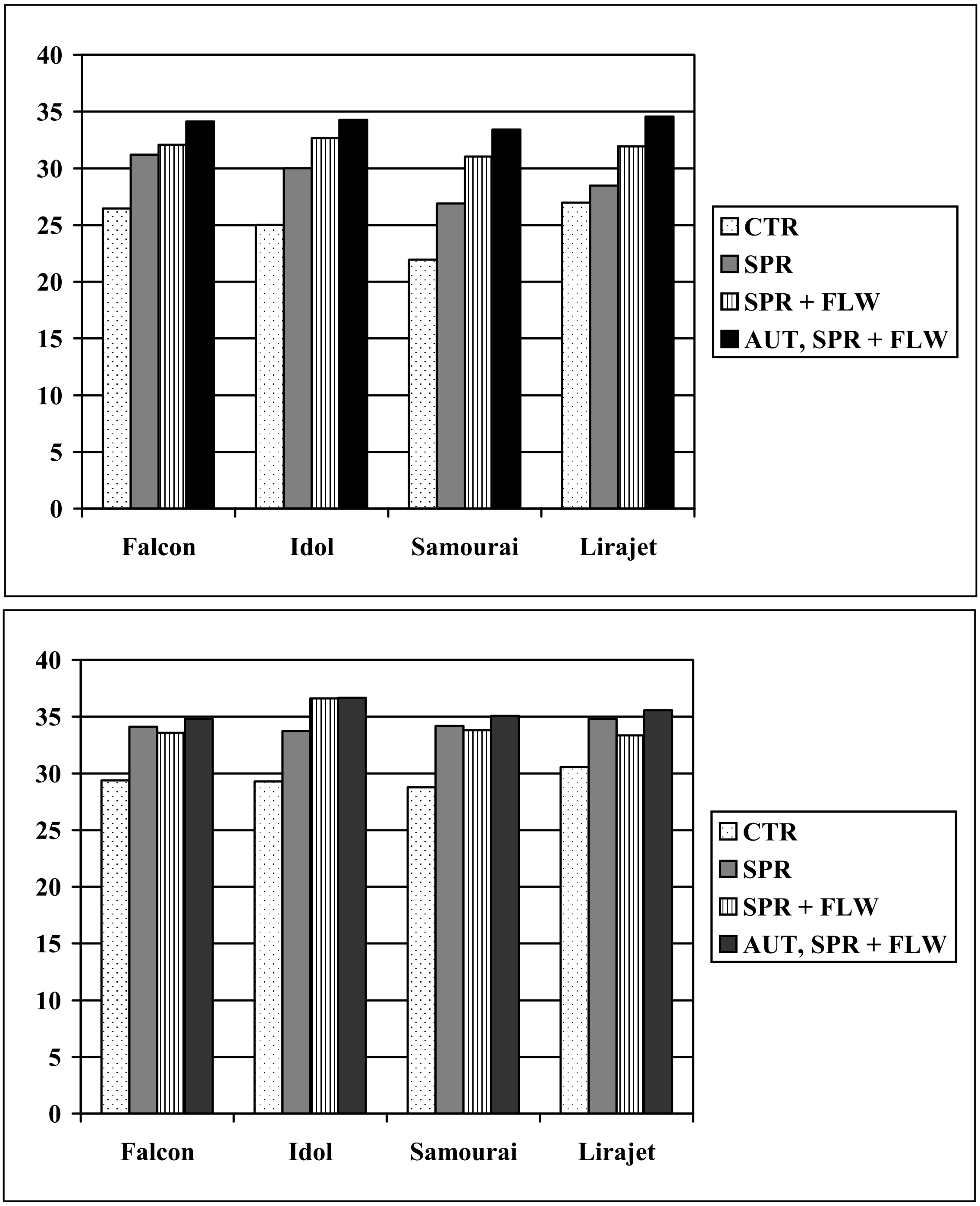

Figure 2. Effect of different fungicide treatments on the yield of oilseed rape in 1994 and 1995.

The results shown in figure 4 indicate that an application of DMM to oilseed rape can be useful in late sowing date situations. An application of DMM increased the fresh weight of the leaves and the roots by over 40%. A similar but smaller effect was achieved by an application of TEST. Applications of THR, MET and TEB had no significant effect on fresh weight. The results indicate that in addition to the fungicide effect of a DMM seed treatment, there seems to be an positive physiological effect on the plant following application of this compound.

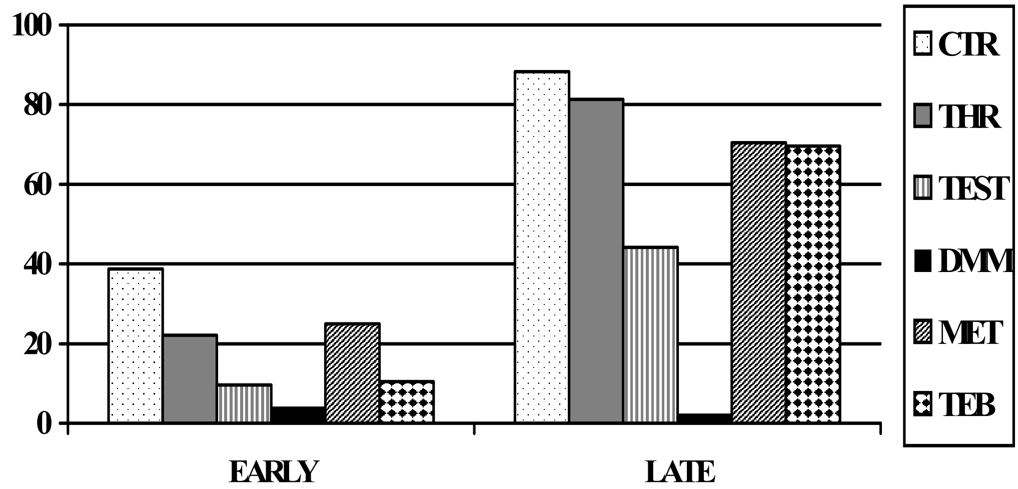

Figure 3. The effect of seed treatment and sowing date on the incidence of downy mildew [%] in the autumn.

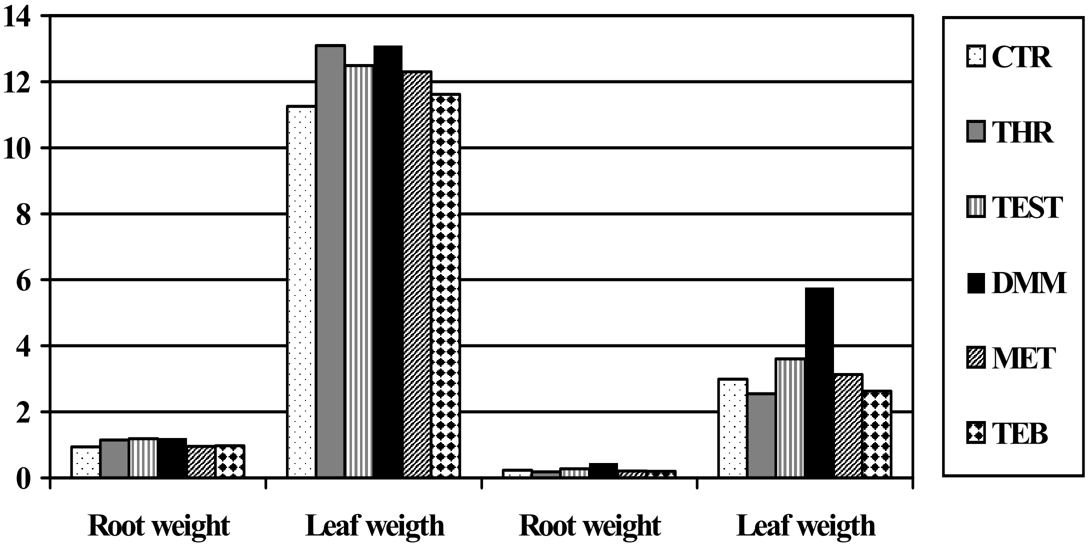

Figure 4. Effect of seed treatment for the control of downy mildew on the fresh weight [g] of oilseed rape at an early sowing date (two column blocks on the left side) and late sowing date (two column blocks on the right side).

Discussion

For the control of diseases of oilseed rape, there is a strong need for the development of economic thresholds or forecasting systems. Preliminary results with the agrometeorlogical stem rot model of Friesland (1998) appear to be quite promising. However, for the other diseases that occur in Germany, fungicide application for the control of canker, light leaf spot and dark leaf and pod spot are based on cropping systems and low susceptible varieties (Garbe 1994, 1995, 1996). The results presented in this paper show that for canker control, there are possibilities of reducing fungicide application. Garbe (1998) showed a correlation between the incidence of symptoms of canker on the leaves in December and subsequent yield losses could only be used to target spring applications of fungicides. However, for the control of canker, an autumn application appears to be more effective and therefore, these results are of limited use. For the control of light leaf spot, the results of Fitt et al. (1998)

indicate that under the climatic conditions that occur in the UK possibilities exist for targeting applications against this disease. However, the transferability of these results and practicability of use have to be examined under different climatic conditions.

The results and experiences of the use of fungicides for the control of diseases in oilseed rape in Germany during the last few years indicate that there is a strong dependency on oilseed rape prices. For example, prices have shown strong fluctuations during recent years and this must be taken into serious consideration in forecasting systems or variable thresholds for canker.

Seed treatment applications for the control of downy mildew seem to be highly effective. Initial results show that the need for treatment is limited to sites with high infection pressure and/or a late sowing date. For the purpose of Integrated Pest Management, further research should be focussed on the possibilities of limiting the need for treatment.

Summary

Field trials were carried out in Germany in the region of Braunschweig between 1993 and 1998. Different susceptible varieties were grown, different sowing dates were used and different fungicide treatments were applied. For the control of canker, the importance of growing a low susceptibility variety was highlighted. Fungicide applications with tebuconazole were effective and in situations with high infestation, these applications were found to be economically viable, more so when susceptible varieties were grown. The growth regulatory effect of tebuconazole led to a further economic advantage from the spring application, although autumn application led to better control of canker. A seed treatment test for the control of downy mildew indicated that dimethomorph was highly effective, particularly in situations with high infection and a late sowing date. There is a strong need for the development of forecasting systems or threshold models for the control of diseases in oilseed rape, particularly as part of an integrated pest management strategy.

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Joint field experiment on cultivation, yield, and diseases of false flax (*Camelina sativa*) in Germany and Canada

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Abstract: Ten cultivars and lines of summer false flax (*Camelina sativa*) were grown at the experimental farm of the Universität - GH-Paderborn in Merklingsen/Nordrhein-Westfalia, Germany in 1995 and 1996 gave seed yields of 7-25 dt/ha. The yield was 11-20 dt/ha at the Edmonton Research Station, University of Alberta, Edmonton, Alberta, Canada in 1996. In Edmonton, in addition to these, 8 other lines from the Regional Plant Introduction Station, Iowa State University, Ames, Iowa, U.S.A. were also grown. In Edmonton, in 1996 the yields of check cultivars of *Brassica napus* (Westar and Quantum) were between 21 and 23 dt/ha. In Germany, the diseases/pathogens found in 1995 and 1996 were a bacterial disease (*Pseudomonas* sp.) stem rot (*Sclerotinia sclerotiorum*), powdery mildew (*Erysiphe* sp.), and white rust (*Albugo candida*). In addition, in 1996 sore shin damping-off (*Rhizoctonia solani*) and downy mildew (*Peronospora parasitica*), were also found in Germany. In Canada, in 1996 a bacterial disease, stem rot, powdery mildew, white rust and staghead, and a phytoplasma (MLO) disease were present. It appears that both the bacterial and phytoplasma diseases have so far not been reported on *C. sativa*.

Key words: *Camelina sativa*, false flax, diseases

Introduction

Camelina sativa (L.) Crtz. (false flax, gold-of-pleasure) is an ancient crop that is staging a come-back (Schuster & Friedt, 1995). This plant is resistant to *Alternaria brassicae* (Berk.) Sacc., *Leptosphaeria maculans* (Desmaz.) Ces. & De Not., and *Rhizoctonia solani* Kühn (Salisbury 1987; Conn *et al.*, 1988; Browne *et al.*, 1991; Jejelowo *et al.*, 1991; Conn *et al.*, 1994) and could be a good gene-source for *Brassica* crop improvement. Hybridization attempts between cultivated Brassicas and *C. sativa* have, however, failed due to reproductive barriers (Narasimhulu *et al.*, 1994). A few workers have attempted somatic hybridization between crop Brassicas and *C. sativa* (Narasimhulu *et al.*, 1994; Hansen, 1997; Sigareva & Earle, 1997).

This paper reports observations on the diseases and crop yields of *C. sativa* gathered during a joint field experiment in Germany and Canada.

Materials and methods

Plots of 10 cultivars and lines of *C. sativa* were grown at the experimental farm of the Universität-GH-Paderborn in Merklingsen/Nordrhein-Westfalia, Germany in 1995 and 1996 and at the Edmonton Research Station, Edmonton, Alberta, Canada in 1996. In Edmonton 8 additional lines from the Regional Plant Introduction Station, Iowa State University, Ames, Iowa, U.S.A. and two cultivars, Westar and Quantum of *B. napus* as check, were also grown. Seed yields in 10 lines and cultivars from Germany were monitored at both locations.

Diseases appearing at both locatons were monitored through direct observations or culturing techniques. Some materials were fixed in 3% glutaraldehyde and 2% osmium tetroxide, both in 0.1 phosphate buffer (pH 7.0), dehydrated in ethanol-water series, and embedded in araldite. The ultrathin sections were stained with uranyl acetate and lead citrate before observation in a transmission electron microscope.

Results and discussion

Yields of 10 cultivars and lines of *C. sativa* from Germany during 1996 grown at Merklingsen and Edmonton are shown in Table 1. They ranged from 7-12 and 11-20 dt/ha from the two locations, respectively. In Edmonton, the two check cultivars, Westar and Quantum of *B. napus*, had yields of 21 and 23 dt/ha, respectively. The yields of *C. sativa* in Germany during 1995 calculated from a plot size of 12.5 m^2 were considerably higher and ranged from 15-25 dt/ha (data not shown here).

Table 1. Seed yields in 1996 (dt/ha).

Locations	*Camelina sativa* cultivars/lines										*Brassica napus*	
	1	2	3	4	5	6	7	8	9	10	Westar	Quantum
Merklingsen (n=6)	8.6	11.6	11.4	9.9	9.6	7.1	9.0	7.3	8.5	7.3	–	–
Edmonton (n=1)	15.1	15.6	19.8	11.3	12.9	14.2	13.6	15.2	16.2	13.0	20.6	23.0

Several diseases were observed at both locations. These included a bacterial disease caused by *Pseudomonas* sp., stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, powdery mildew caused by *Erysiphe* sp., and white rust and stagehead disease caused by *Albugo candida* (Pers.) Kuntze. At Merklingsen, in addition during 1996 sore shin damping-off caused by *R. solani* and downy mildew caused by *Peronospora parasitica* (Pers.:Fr.) Fr. were also observed. At Edmonton, in 1996 a phytoplasma disease was also present.

Finding sore shin damping-off caused by *R. solani* in Merklingsen may be consistent with only partial resistance of *C. sativa* to this pathogen (Conn *et al*., 1994). This is also consistent with detoxification of the phytoalexin, camalexin, produced by *C. sativa* upon challenge by *R. solani* into less toxic compounds (Conn *et al*., 1994; Pedras & Khan, 1997). Isolates of *R. solani* from *C. sativa* should be studied for their anastomosis group so that proper cultures become available for disease screening.

This investigation revealed two new diseases of *C. sativa*. The first new disease was caused by a bacterium and was in the form of small necrotic somewhat sunken spots on the stem and fruits. These spots were dark brown to almost black and without any obvious bacterial exudate. Isolates from these spots have been tentatively identified as a new pathovar of *P. syringae* van Hall (Professor Klaus Rudolph, personal communication).

The second new disease was caused by a phytoplasma and showed typical proliferative and bronzing symptoms. Ultrathin sections of phloem elements from diseased plants revealed characteristics phytoplasma cells in the sieve tubes upon examination by transmission electron microscopy.

Camelina sativa continues to be a source of high degrees of resistance to *A. brassicae*. Infection with *L. maculans* was not observed in our fields. These attributes make *C. sativa* as an attractive alternative crop in areas where these pathogens are important.

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Satisfying Koch's postulates and histopathological studies on the pollen necrosis disease of *Brassica* leaves

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Abstract: Earlier we reported localized necrosis under deposits of pollen grains on the leaves of *Brassica rapa*, *B. napus*, and *B. juncea*. Koch's postulates have now been satisfied for this pollen necrosis disease using a rapid-cycling *B. napus* (Acaacc, Rcb), catalog # 5-1 from the Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin-Madison, Madison, Wisconsin, 53706, USA. High humidty was required for the causation of this disease and that inoculations with autoclaved pollen grains and talcum powder did not cause this disease. Several microbes were isolated from lesions caused by the pollen necrosis disease on greenhouse-grown plants. None of these microbes caused lesions on leaves upon inoculation. The results indicated that the pollen necrosis is caused by a thermolabile component of pollen grains. The lesions were studied by light, scanning electron, and transmission electron microscopy. The pollen grains did not germinate on the leaf surface. Ultrathin sections revealed necrosis of epidermal and superficial mesophyll cells close to the deposit of pollen grains.

Introduction

Leaves of *Brassica rapa* L., *B. napus* L., and *B. juncea* (L.) Czern. et Coss. undergo localized necrosis at places where the pollen grains are shed (Tewari *et al*., 1994, 1995). This was called as the pollen necrosis disease. This unique reaction of *Brassica* leaves to pollen grains was widespread geographically and was observed both in the field- and greenhouse- collected materials of various species in Canada, Germany, and India (Tewari *et al*., 1995). It was stipulated that the leaves perceive some-component(s) of the pollen grains as an alien substance and react to it hypersensitively. The necrotic leaf areas thus produced, may predispose the plant to infection by other pathogens.

The relationship of pollen grains with the pollen necrosis disease had so far been reported only through association. This paper reports on proving Koch's postulates for this disease. Also, effects of some factors on the pollen necrosis disease were studied. Isolations of microbes resident on the leaf surface were made and their interactions, if any, with this disease studied. The diseased material was also examined by light, scanning electron, and transmission electron microscopy.

Materials and methods

Rapid cycling *B. napus* plants (Acaacc, Rcb), catalog # 5-1 from the Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin-Madison, Madison, Wisconsin, 53706, U.S.A. were grown in 2" pots in the greenhouse. Fresh or autoclaved pollen grains from mature anthers were applied on the leaves by brushing. Other leaves were dusted with talcum powder or kept without any application. The plants were covered with unperforated or perforated polyethylene bags or left uncovered. They provided treatments with different relative humidity values. Observations were recorded 4 days after inoculation with pollen grains. Three plants (and 2-4 leaves in each plant) were used for each treatment. The experiments were repeated twice.

Pieces of leaves with or without pollen necrosis were surface sterilized with 1% bleach solution for 4 min, washed twice with sterile distilled water, and plated on nutrient agar (4.0 g Difco nutrient broth, 7.5 g Difco agar, 500 ml distilled water) or potato-dextrose agar (12.0 g Difco potato-dextrose broth, 7.5 g Difco agar, 500 ml distilled water). Fungi and bacteria growing on these plates were isolated, grown in pure culture and inoculated on the leaves. Each leaf was gently scratched at 3 points and a 10 µl suspension of 10^5-10^7/ml fungal spores or bacterial cells in sterile distilled water placed on them. Four such leaves were used for each microbe. Controls were run with application of sterile distilled water.

The diseased material was examined by light microscopy. Pieces of leaves with or without pollen necrosis lesions were processed for scanning electron microscopy (SEM) using the CO_2 critical point-drying technique and coated with gold before examining in the SEM. The material for transmission electron microscopy (TEM) was routinely fixed in 3% glutaraldehyde and 2% osmium tetroxide, both in 0.1 phosphate buffer (pH 7.0), dehydrated in ethanol-water series, and embedded in araldite. The ultrathin sections were stained with uranyl acetate and lead citrate before examination in the TEM.

Results and discussion

Consistent results were gathered in the pollen grain inoculation experiment and this data are summerized in Table 1. Necrosis of the leaves was consistently observed when the fresh pollen grains were inoculated and the plants covered with unperforated polyethylene bags. Results were negative in all other treatments.

Table 1. Results of pollen grain inoculation experiments.

Application	Plants covered with polyethylene bag		Uncovered plants
	Unperforated	Perforated	
Fresh pollen grain	+	–	–
Autoclaved pollen grain	–	*	–
Talcum powder	–	*	–
No application	–	–	–

+Lesions present.
-Lesions not present.
*No data.

The results clearly proved that pollen grains were the cause of the pollen necrosis disease and that high humidy was required for development of necrosis. High humidity and perhaps the associated condensation of water droplets may be crucial for an intimate contact between the pollen grains and the leaf, leading to recognition of the active causal component of pollen grains by the leaf cells. The active component is also thermolabile as the autoclaved pollen grains did not cause the disease (Table 1). Also, the disease was not caused by the physical presence or light shadding by the pollen grains as the talcum powder did not cause this disease (Table 1). None of the microbes isolated from healthy and necrotic lesions of leaves caused necrosis in the leaves ruling out the possibilty of microbial origin of this disease.

Scanning electron microscopy revealed groups of ungerminated pollen grains immediately surrounded by collapsed epidermal cells corresponding to the superficial necrotic cells. Transmission electron microscopy of ultrathin sections showed necrotic epidermal, and palisade and spongy mesophyll cells with electron-dense contents in the region where the pollen grains were placed. The surrounding cells had normal ultrastructure and appeared to be healthy.

Future research will focus on identifying the active component(s) of pollen grains that causes pollen necrosis and studying the interactions between pollen necrosis and other diseases of Brassicas, such as the blackspot disease caused by *Alternaria brassicae* (Berk.) Sacc.

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References

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