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Working Group "Integrated Control in Oilseed Crops"

OILB / SROP

Groupe de Travail "Lutte Intégrée en Cultures d'Oléagineux"

Proceedings of the meeting

Comptes-rendus de la réunion

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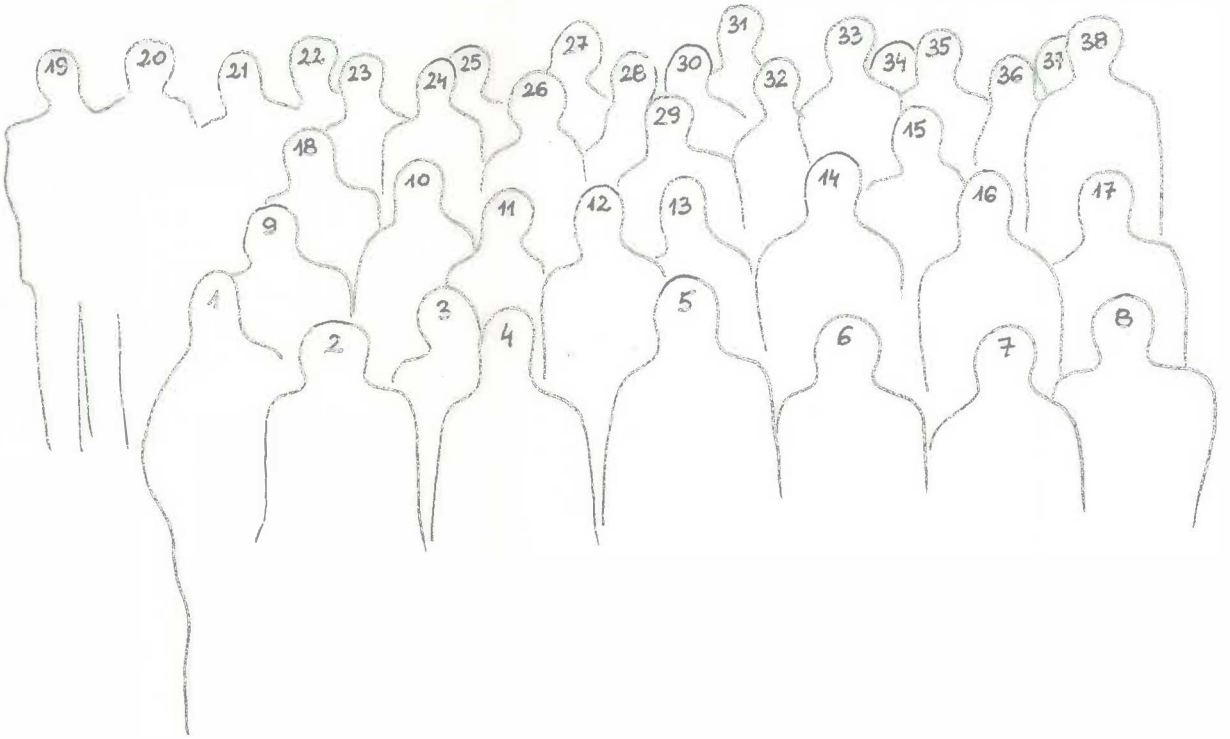
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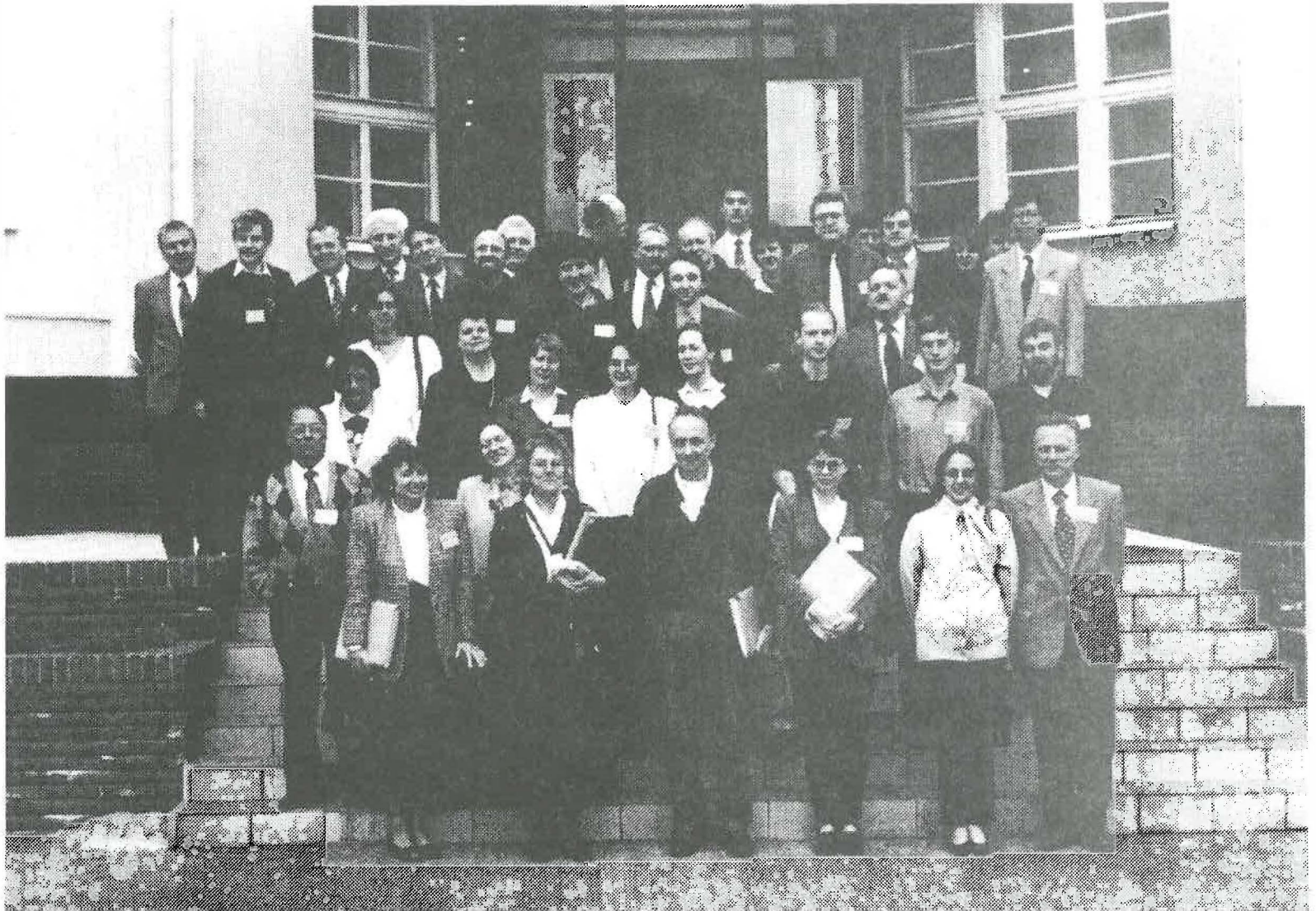

Preface

For the first time since the existence of the working group on Integrated Control in Oilseed Crops the biannual workshop meeting took place in Central Europe. We met in the new Conference Centre of the Institute of Plant Protection in Poznan, Poland, from April 10 to 12, 1997.

On this 7th workshop 38 scientists from 8 countries attended. Pleasing was the numerous participation of 17 east European colleagues and from young scientists.

On behalf of the working group I would like to thank Mrs. M. Jedryczka, Mr. P. Kachlicki and Mr. Cz. Sadowski for the excellent local organisation that was a much appreciated contribution to the successful progress of the conference.

It is a very satisfying fact that our mutual efforts for financial support by the European Union finally seems to bear fruits. This can be seen in the fact that members of our working group are involved in three EU research projects. Those are

⇒ Biocontrol of Oilseed Rape Insect Pests (BORIS)
⇒ Integrated Strategies for the Management of Stem Canker of Oilseed Rape in Europe (IMASCORE)
⇒ Alternative Oil-seed Crop *Camelina sativa*

A very promising success that should encourage future mutual efforts to apply for further financial support by the EU.

Volker H. Paul

Convenor

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Monitoring Diseases – Biology of Pathogens

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Detection of seed-transmitted pathogens of rape (*Brassica napus* ssp. *oleifera* D.C.)

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To prevent yield losses, growers must always battle against plant diseases. Different control strategies are available to them: crop rotation, chemical treatments of soil sterilization, seed treatments (physical or chemical), protective or curative sprays during crop growth, use of resistant cultivars and healthy seeds produced under certification programs. All these strategies are each useful to reduce the incidence of disease and improve the quality of the products when used in different combinations under integrated pest management.

Interest about diseases caused by seed-transmitted pathogens has increased during the last 30 years. Today seed pathology is an important part of epidemiology; often the amount of the seed inoculum is sufficient to start an epidemic. Seed-borne pathogens may be introduced in new areas of cultivation by seed and can remain viable in the soil, weeds, other hosts etc. Often experimental fields where seeds of imported cultivars untreated with chemicals have been sown are Trojan horses for the introduction of new pathogens and/or new races.

Actually for each crop we know at least one destructive seed-transmitted pathogen. Viruses, bacteria and fungi were initially detected in seeds with traditional methods normally applied to the other plant parts (leaves, stems and roots). However, new methods have been developed to detect the pathogens present only in traces or located in particular sites of seeds.

In the case of diseases caused by seed-transmitted pathogens, especially for polycyclic diseases, prevention by using pathogen-free seeds is the best solution and treatment is not necessary. Healthy seeds can be conveniently obtained from non-infected seed crops located in areas where climatic conditions are dry during and after flowering. Unfortunately in different countries of central and northern Europe where the weather is quite humid diseases develop and sometimes infected seeds can be produced by symptomless infected plants. In these situations it is very important to avoid or reduce the spread of the pathogens by improving the health of the seeds and germplasm exchanged between seed companies and scientists. To achieve this goal before sowing seeds in the field, it is important to analyze seed samples using specific and simple laboratory methods.

When the results of the seed tests predict the behaviour of the plants in the field it is possible to make a decision before seeding, in particular if seed disinfection is necessary or not, or rejection of seeds in the case of high levels of infection.

Rape crops may be affected by some destructive diseases shows in Table 1.

Table 1. Fungal diseases of oilseed rape (*Brassica napus* var. *oleifera*)

Disease	Causal agent	Seed transmission
Clubroot	*Plasmodiophora brassicae*	N
Downy mildew	*Peronospora parasitica*	P
Powdery mildew	*Erysiphe cruciferarum*	N
Stem canker	*Phoma lingam*	Y
Stem rot	*Sclerotinia sclerotiorum*	Y
Dark leaf and pod spot	*Alternaria brassicae*	Y
	Alternaria brassicicola	Y
Grey mould	*Botrytis cinerea*	Y
Typhula rot	*Typhula gyrans*	N
Verticillium wilt	*Verticillium dahliae*	Y
Light leaf spot	*Cylindrosporium concentricum*	P
White leaf spot	*Pseudocercosporella capsellae*	N
Ramularia leaf spot	*Ramularia armoraciae*	N
White rust	*Albugo candida*	N
Ring spot	*Mycosphaerella brassicicola*	N
Sore shim and damping off	*Rhizoctonia solani*	Y

N= no; Y= yes; P= probably, it must be demonstrated

It has been demonstrated that important pathogens such as fungi, bacteria and viruses are seed-transmitted (14, 20). According to the data published in international reviews *Phoma lingam* and some species of *Alternaria* are the main pathogens of this important crop. Some methods to detect pathogens in the seeds have already been published by the ISTA (International Seed Testing Association). The "CMI Descriptions of Pathogenic Fungi and Bacteria" periodically published by Commonwealth Mycological Institute are very useful for the identification of fungi (paticularly the following numbers: 162, 163, 256, 331, 406, 431, 460, 512, 513, 536). At the 7th Meeting of the Working Group of IOBC/WPRS (International Organisation for Biological Control of Noxious Animals and Plants West Palaearctic Regional Section) held in Zürich-Reckenholz 24-25 February 1994, the importance of seed-transmitted pathogens of rape was emphasized. For this reason we decided to present here some methods for pathogen detection together with comments and suggestions arising from our personal experience and point of view in the seed pathology of rape. We hope that this contribution will be useful to those who have the intention to carrying out some work in this very important and interesting field.

General Rules for all Seed Health Methods of Rape

Instructions for sampling

- Prepare one untreated seed sample per plot (seed-lot), 100 g each
- Mark each sample with a number and include the design and the cultivar
- Clean the sample from impurity
- Dry the samples to less than 9 % moisture in an oven at 30 °C for 24 h
- Pack the samples in paper bags

The working sample must be obtained from the seed sample by progressive sub-division into smaller samples by repeated halving or by combination of small portions of seeds taken at random. Other working samples (replicates) must be drawn after the first working sample; in this case the remainder of the seed sample must be remixed before the second sampling. During each step of the preparation of the working samples, mixing of the seed must be carried out.

1 - Specific Methods

For Cruciferous species, the ISTA's Plant Disease Committee (PDC), after international comparative studies carried out by both Seed Testing Stations and scientists, has standardized specific routine seed health methods (4, 7, 8, 9, 10).

A - Detection of *Phoma lingam* (TODE *EX* SCHW.) DESM. anamorph of *Leptosphaeria maculans* (DESM.) CES. and de Not.

Working sample: 1,000 seeds.

Location of inoculum: Mycelium on and in the seed. Viable conidia can also be found on the seeds after two years of storage (4).

DIRECT INSPECTION

signs of infection: none

METHOD

2,4-D blotter: place 3 pieces of filter paper (Whatman N.1) in each petri dish. To inhibit seed germination, add 5 ml of a 0.2% solution of the salt of 2,4 dichlorophenoxyacetic acid (2,4-D). Pour off the excess of the 2,4-D solution, rinse the seeds in sterile water and place 50 seeds in each dish.

Incubation: 11 days at 20 °C under cycles of 12 hours light and 12 hours darkness.

Examination: after 6 days examine at x25 magnification for loose growing, silver white mycelium and primordia of pycnidia of *P.lingam* on the seed and substrate. After 11 days make a second examination for pycnidia on infected seeds and on the filter paper near the infected seeds. Seeds from which pycnidia of *P.lingam* have developed are recorded as infected.

NOTES (our considerations):

1 - Pycnidia of *P.herbarum* are located deep within the rape seeds and they produce a grey exudate (Fig. 1). Often the pycnidia of *P.lingam* develop on the surface of the filter paper. Virulent and avirulent strains of *P.lingam* occur on seed and can be distinguished by the colour of pycnidium exudates produced in culture of isolates: red-pinkish (virulent - strain A), amethyst (avirulent - strain B) (6). However, recent natural epidemics caused by the two groups were similar: B-group lesions occurred slightly later, but the incidence increased more rapidly and reached a maximum slightly earlier than that of group A (11).

2 - Although the use of 1,000 seeds is recommended for detecting the presence/absence of the fungus, it is possible to save space and time by testing 500 seeds. In this case the lowest level of detectable infection is 0.2 %.

3 - The presence of the fast developing fungus *Rhizopus* spp. can mask the growth of *P.lingam*, resulting in an underestimation of infection percentage. To avoid this problem it is important to disinfect the seed sample with 0.1-0.5% NaOCl; the disinfection slows the development of *P.lingam*, but does not reduce the final pathogen count (5, 12, 13).

4 - If the salt 2,4 dichlorophenoxyacetic acid (2,4-D) is not available, commercial preparations can also be used. However it is important to remember that those preparations

give a reduction in the number of infected seeds (13). 2,4-D is not easily removed from the laboratory glassware.
5 - Field inspection methods are not suitable in those areas where siliquae infection does not develop. Lack of external symptoms on siliquae is not indicative of lack of *P.lingam* seed infection (2).
6 - To avoid overestimation of the percentage of infected seeds, it is important to use a spacing of 10 mm or more between seeds.

B - Detection of *Alternaria brassicae* (BERK.) SACC.
Working sample: 1,000 seeds.
Location of inoculum: mycelium on and in the seed (14).
DIRECT INSPECTION
signs of infection: none.
METHODS
1 - Blotter
pre-treatment: none.
Incubation: 6 days at 18-20 °C under near UV light under 12 h light/12h dark cycles.
Examination: brown spots and streaks on cotyledons and hypocotyl, not so dark as in *A.brassicicola*. Light to dark brown conidia with longitudinal and transverse septa, characterized by long beaks. Conidia 147 µ x 9-33 µ, with beaks 9-148 µ long. Total length of conidia from 39-350 µ (Fig. 2).
2 - Agar plate.
pre-treatment: seeds are immersed in a solution of NaOCl containing 1% available chlorine for 5 minutes followed by draining off the surplus liquid.
medium: malt agar.
Incubation: 5 days at 18-20 °C under near UV light in 12 h light/12h dark cycles.
Examination: the colonies with loose cottony, white or pink aerial mycelium, with 'tea-green' to deep olive rings of conidial patches are recorded as *A.brassicae*. Submerged mycelium shows radiate to curled growth, colourless to dark olive. Profuse sporulation.
NOTES:
1 - The 2,4-D blotter method proposed for *P.lingam* can also be used for the detection of *A.brassicae*.
2 - it is important distinguish the colonies of *A.brassicae* from those of other *Alternaria* spp. (especially *A.alternata*).
3 - 400 seeds/working sample can be used for routine assays.
4 - The fungus may be isolated from apparently healthy seeds.

C - Detection of *Alternaria brassicicola* (SCHW.) WILTS.
Working sample: 1,000 seeds.
Location of inoculum: Mycelium on and in the seed (14).
DIRECT INSPECTION
signs of infection: none.
METHODS
1 - Blotter
pre-treatment: none.
Incubation: 6 days at 18-20 °C under near UV light in 12 h light/12h dark cycles.

Fig. 1 - ***Phoma lingam***

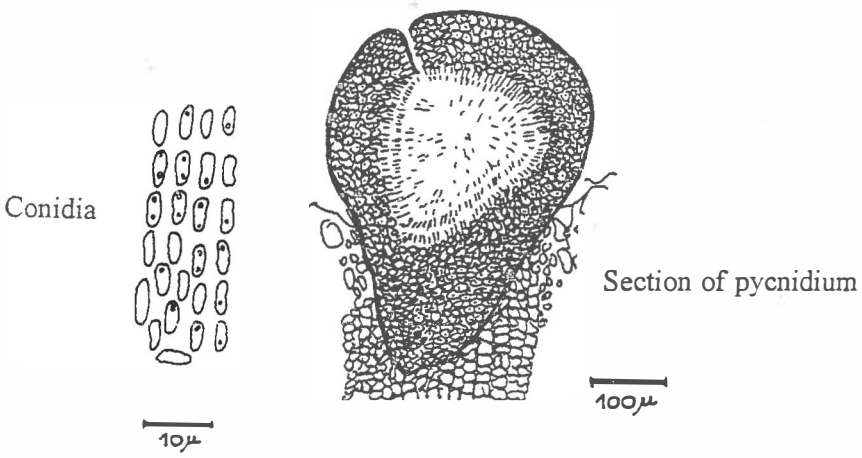
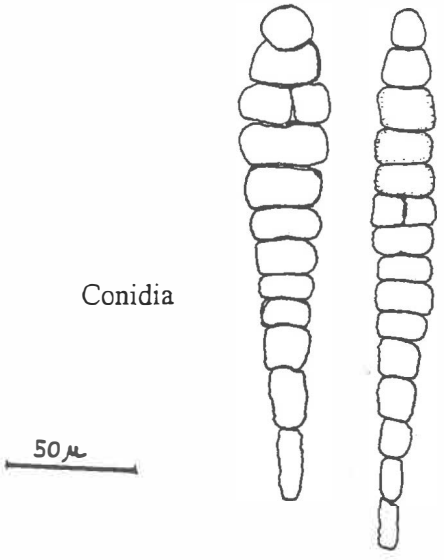


Fig. 2 - ***Alternaria brassicae***



Examination: black or dark brown streaks on cotyledons and hypocotyl. Conidia olive green to dark brown. Short beaks not visible in conidia chains. Conidia 18-120 µ x 8-30 µ. Fewer vertical septa than in *A.brassicae* and darker coloured conidia (Fig. 3).

2 - Agar plate

pre-treatment: seeds are immersed in a solution of NaOCl containing 1% available chlorine for 5 minutes followed by draining off the surplus liquid.

medium: malt agar

Incubation: 5 days at 18-20 °C under near UV light in 12 h light/12h dark cycles.

Examination: are recorded as for *A.brassicicola* . The fungus grows in culture as dark colonies with light-grey-olive webby, aerial mycelium and velvety concentric rings of conidial development. Conidia are numerous, especially in the light.

NOTES:

1 - The 2,4-D blotter method proposed for *P.lingam* can be used for the detection of *A. brassicicola* .

2 - 400 seeds/working sample can be used for routine assays.

3 - It is important to distinguish the colonies of *A.brassicae* from those of other *Alternaria* spp. (especially *A.alternata*).

4 - The fungus may be isolated from apparently healthy seeds.

5 - Surface disinfection before the 2,4-D blotter test reduces fast growing contaminants, but also reduces the pathogen count (1).

2 - Methods for General Use

Different methods of testing are available for some pathogens that frequently are associated with seeds of a number of species (3, 17).

D - Detection of *Sclerotinia sclerotiorum* (LIB.) DE BARY

Location of inoculum: sclerotia occur mixed with seed, mycelium are also present either on the surface or inside the seed coat (15).

METHODS

1 - Direct inspection by the naked eye

signs of infection: none.

Working sample: 500 g of seeds.

Examination: Number of sclerotia/500 g of seed.

2 - Agar plate

Working sample: 400 seeds.

pre-treatment: seeds are immersed in a solution of NaOCl containing 1 % available chlorine for 5 minutes followed by draining off the surplus liquid.

medium: malt agar or potato dextrose agar. About 15 ml of agar per 90 mm petri dishes, 10 seeds per dish.

Incubation: 7 days at 18-22 °C in darkness.

Examination: percentage of seeds producing colonies of profuse, white cottony mycelium of the fungus in the agar plate (Fig. 4).

NOTES:

1 - The 2-4 D blotter method proposed for *P.lingam* can be used for the detection of *S.sclerotiorum*.

2 - Usually seed cleaning removes sclerotia, except those which resemble the seed in shape and size.

3 - Superficial seed disinfection facilitates infected seed detection.

Fig. 3 - ***Alternaria brassicicola***

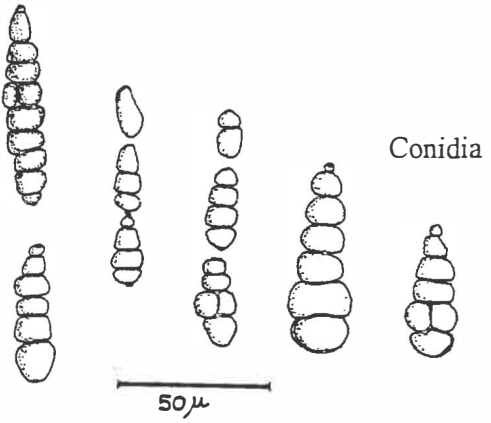
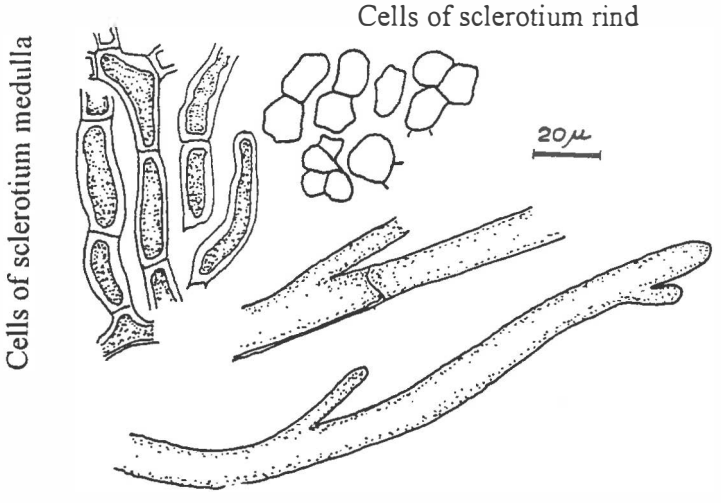


Fig. 4 - ***Sclerotinia sclerotiorum***



E - Detection of *Botrytis cinerea* PERS. *EX* PERS.
Working sample: 400 seeds.
Location of inoculum: mycelium and conidia on the surface of the seed coat, mycelium may be present under the seed surface (20).
DIRECT INSPECTION
signs of infection: none.
METHODS
1 - Blotter
pre-treatment: none. 10 seeds per petri dish.
Incubation: 7-9 days at 20 °C in darkness.
Examination: presence of coarse grey fast-growing mycelium with conidiophores and conidia.
2 - Agar plate.
medium: malt agar or potato dextrose agar. About 15 ml of agar per 90 mm petri dishes, 10 seeds per dish.
Incubation: 7-9 days at 20 °C in darkness.
Examination: after 5-7 days it is possible to observe soft mycelium of tape-like hyphae producing tufts of branching conidiophores with ovoid-hyaline one-celled conidia 8x11-6x19µ (Fig. 5). When analysts are familiar with the fungus, naked eye examination is sufficient for identification.
NOTES:
1 - The 2,4-D blotter method proposed for *P.lingam* can be used for the detection of *B.cinerea*.
2 - The infection is not important after the seedling stage.

F - Detection of *Verticillium dahliae* KLEB.
Working sample: 400 seeds.
Location of inoculum: mycelium on the surface of the seed coat and under the seed surface (22).
DIRECT INSPECTION
signs of infection: none.
METHODS
pre-treatment: washing seeds lot 3 h in running tapwater, disinfection for 2 min in 1% sodium hypochlorite, washing with sterile water for 1 min and dry between two sterile filter paper. Place 10 seeds per petri per dish
1 - 2,4-D blotter: place 3 pieces of filter paper (Whatman N.1) in each petri dish. To inhibit seed germination, add 4.5 ml of a 0.15% solution of the salt of 2,4 dichlorophenoxyacetic acid (2,4-D). Pour off the excess of the 2,4-D solution.
Incubation: 10 days at 18-23 °C under near UV light in 12 h light/12h dark cycles.
2 - Agar plate.
a - medium: potato dextrose agar plus 0.02 % streptomycine sulphate, pH 5.5.
Incubation: 10 days at 18-23 °C under near UV light in 12 h light/12h dark cycles.
b - medium: ethanol agar plate. 0.6% ethanol, 0.8 % agar 0.01% streptomycin sulphate, pH 5.5. About 15 ml of agar per 90 mm petri dishes.
Incubation: 10 days at 21 °C in darkness.
Examination: presence of mycelium and conidia of the fungus (Fig. 6).

Fig. 5 - ***Botrytis cinerea***

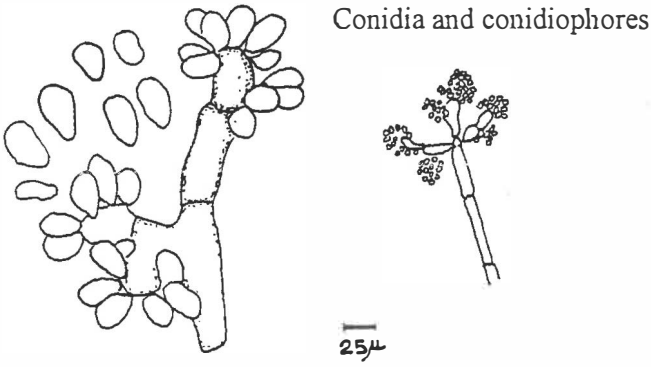
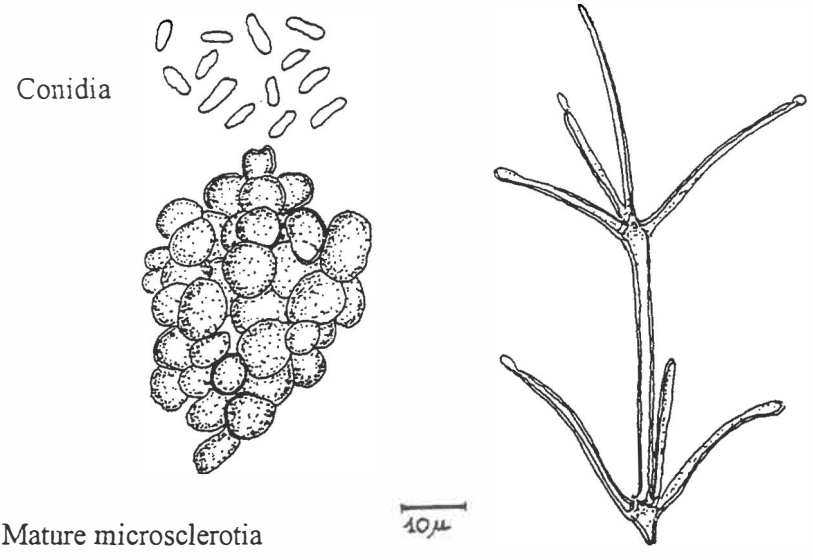


Fig. 6 - ***Verticillium dahliae***



G - Detection of *Rhizoctonia solani* KHŪN
Working sample: 400 seeds.
Location of inoculum: mycelium on and in the seeds (16, 23).
DIRECT INSPECTION
signs of infection: none.
METHOD
Agar plate.
pre-treatment: seeds are immersed in a solution of NaOCl containing 1% available chlorine for 5 minutes followed by draining off the surplus liquid.
medium: malt agar.
Incubation: 5-7 days at 20-22 °C under 12 hours light/12 hours darkness cycles.
Examination: after 7 days the typical mycelium of the fungus can be observed at the microscope for the presence of branching near the distal septum of cells in young vegetative hyphae, some shade of brown hyphal pigmentation, constriction of hyphae and formation of septa a short distance from the point of origin of hyphal branches, dolipore septa and multinucleate cells in young vegetative hyphae (21). Sclerotia can be observed after 2-3 weeks (Fig. 7).
NOTES:
1 - The 2,4-D blotter method proposed for *P.lingam* can be used for the detection of *R.solani*.

Other Diseases of Rape Probably Caused by Seed-Transmitted Pathogens

Downy mildew caused by *Peronospora parasitica* (Pers.) Fr. is one of the most important rape diseases. It occurs wherever the crop is grown and often during the first year of cultivation. The random distribution of diseased plants at early stages of rape development in the fields has been frequently observed (Cappelli, unpublished). This suggests seed transmission of the fungus. In some field experiments, where a number of different seed samples (cultivars) had been used, systemic infection at the cotyledon stage of rape was always observed in different plots of the same cultivar (18).

Systemic infection of the downy mildew fungus at the cotyledon stage also occurs in other *Brassica* spp. especially if daylight exceeds 16 hours (19). Also infection of the seed could occur.

Some researchers have found oospores of *P. parasitica* on the seed coat, but these infected seeds were not used for demonstrating seed transmission (Paul, unpublished).

When infected seeds are available, the examination for disease symptoms of on plants grown from seeds at different combinations of temperature/daylight/moisture should be performed. After this, the choice of the health testing method to be used in routine testing will be important, the number of seeds to be tested in each working sample and some considerations from an epidemiological stand point will be done (e.g. correlation of results of seed testing with field performance of seeds). However it is important to remember that such a method is time and space consuming and it can be used for research purposes only.

Cylindrosporium concentricum Grev. (*Pyrenopeziza brassicae* Sutton and Rawlinson) (Fig. 8) is another fungus where seed-transmission is strongly suspected, but there are no reports on this topic. Usually the fungus survive in the soil on infected plant debris, but in some cases it is possible that these sources of inoculum could be present on seeds in uninfested fields. Also, infection of the seed could occur.

Fig. 7 - ***Rhizoctonia solani***

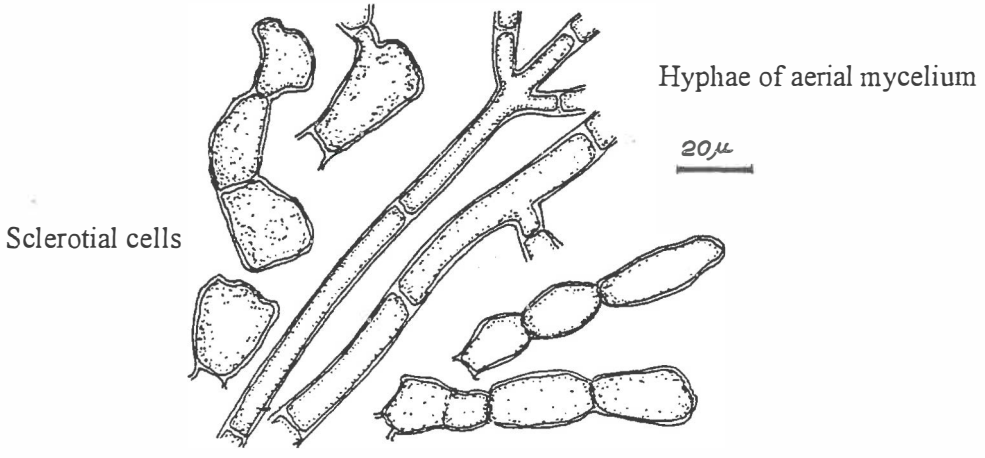
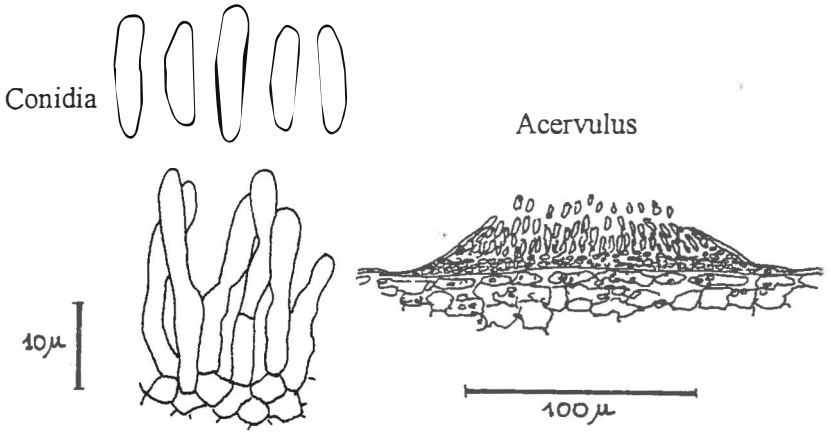


Fig. 8 - ***Cylindrosporium concentricum***



References

Bassey E.O., Gabrielson R.L., 1983. Factors affecting accuracy of 2,4-D assays of crucifer seed for *Alternaria brassicicola* and relation of assays to seedling disease potential. Seed Sci. and Technology, 11(2), 411-420.

Bonman J.M., Gabrielson R.L., 1981. Localized infections of siliques and seed of cabbage by *Phoma lingam*. Plant Disease, 65(11), 868-869.

De Tempe J., Binnerts J., 1979. Introduction to methods of seed health testing. ISTA Handbook on Seed Health Testing. Section 1.2. Seed Sci. and Technology, 7, 601-636.

Gabrielson R.L., 1983. Blackleg disease of Crucifers caused by *Leptosphaeria maculans* (*Phoma lingam*) and its control. Seed Sci. and Technol., 11, 749-780.

Hewett P., 1977. Pre-treatment in seed health testing. Hypochlorite in the 2-4D blotter test for *Leptosphaeria maculans* (*Phoma lingam*). Seed Sci. and Technology, 5, 599- 602.

Humpherson-Jones F.M., 1983. Pathogenicity studies on isolates of *Leptosphaeria maculans* from brassica seed production crops in south-east England. Annals of Applied Biology, 103(1), 37-44.

ISTA, 1966a. International rules for Seed Testing. Proc. int. Seed Test. Ass., 31, 1-152.

ISTA, 1966b. Handbook on Seed Health Testing. *Brassica* spp.- *Alternaria brassicicola* (Schw.) Wilts., S.3, No. 28.

ISTA, 1966c. Handbook on Seed Health Testing. *Brassica* spp.- *Alternaria brassicae* (Berk.) Sacc., S.3, No. 29.

ISTA, 1993. International Rules for Seed Testing. Rules 1993. Seed Sci. and Technology, 21, 213, Supplement.

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

Maguire J.D., Gabrielson R.L., Mulanax M.W., Russel T.S., 1978. Factors affecting the sensibility of 2-4 D assays of crucifer seeds for *Phoma lingam* Seed Sci. and Technol., 6, 915-924.

Maguire J.D., Gabrielson R.L., Mulanax M.W., Russel T.S., 1979. Factors affecting the sensibility of 2-4 D assays of crucifer seeds for *Phoma lingam*. Phytopathology, 69, 1037.

Neergaard P., 1945. Danish species of *Alternaria* and *Stemphylium*. Einar Munksgaard, Copenhagen, 129-148.

Neergaard P., 1958a. Mycelial seed infection of Danish seeds on Certain Crucifers by *Sclerotinia sclerotiorum* (Lib.) D.By. Plant. Dis. Reptr., 42 (10), 1105-1106.

Neergaard P., 1958b. Infection of Danish seeds by *Rhizoctonia solani* Khuen.. Plant. Dis. Reptr., 42(11), 1276-1278.

Neergaard P., 1979. Seed Pathology. Vol I, II. The Mc Millan Press Ltd., London and Basingstoke, 1191 pp.

Paul V.H., Rawlison C.J., 1992. Diseases and pests of rape. Verlag Th. Mann, Gelsenkirchen-Buer, 132 pp.

Polyakov I.M., Vladimirskaya M.E., 1964. The role of light conditions in the resistance of Cabbage to false powdery mildew. Trudy vses. Inst. Zashch. Rast., 21(2), 18-26.

Richardson M.J., 1990. An annotated list of seed-borne diseases. International Seed Testing Association. Zurich, Switzerland, 335 pp.

Sneh B., Burpee L., Ogoshi A., 1991. Identification of *Rhizoctonia* species. APS Press. The American Phytopathological Society, St. Paul, Minnesota, USA, 133 pp.

Heppner C., Heitefuss R., 1995. Untersuchungen zum Auftreten von *Verticillium dahliae* Kleb. und anderen pilzlichen Erregern am Erntegut von Winterraps (*Brassicanapus* L. var. *oleifera* Metzger). Nachrichtenbl. Deut. Pflanzenschutzd., 47(3), S. 57-61.

Tahvonen R., 1979. Seed-borne fungi on cruciferous cultivated plants in Finland and their importance in seedling raising. Journal of the Scientific Agricultural Society of Finland 51(5), 327-379.

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Epidemiology and forecasting of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in the UK

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Abstract Light leaf spot (*Pyrenopeziza brassicae*) is, in the UK, a polycyclic disease of winter oilseed rape (*Brassica napus*), which can affect the leaves, stems and pods. Epidemics may be initiated in the autumn, although characteristic leaf symptoms may not be observed until late winter because the pathogen has a long incubation period. The severity of epidemics differs between seasons, between regions and between crops. Severe epidemics can only be effectively controlled by fungicide spray regimes including both autumn and spring treatments. A provisional scheme for forecasting the severity of epidemics includes a seasonal risk index, an initial crop risk index (both at the start of the season) and improved crop risk indices (during the autumn/winter period). This scheme was used to predict the seasonal risk of light leaf spot in the spring of 1997 (% plants affected in each ADAS region) from the incidence of light leaf spot (% plants with pods affected) in July 1996.

Keywords: Epidemiology, forecasting, fungicide spray regime, light leaf spot, *Pyrenopeziza brassicae*, risk assessment, winter oilseed rape.

1. Epidemiology of light leaf spot

Light leaf spot, caused by *Pyrenopeziza brassicae* Sutton & Rawlinson [anamorph *Cylindrosporium concentricum* (Grev.)] is a widespread damaging disease on winter oilseed rape [*Brassica napus* L. ssp. *oleifera* (Metzger) Sink.] crops in the UK. It is a polycyclic disease which can produce symptoms on leaves, stems, flowers and pods.

1.1 Symptoms

The diagnostic symptoms of light leaf spot are the small white spots produced by masses of spores erupting through surfaces of affected tissues (Hardwick *et al.*, 1991; Paul & Rawlinson, 1992). Sporulation may be observed on both upper and lower leaf surfaces, either before or after development of lesions. Lesions on leaves are initially light green and roughly circular, turning pale, becoming brittle and bleached. They may coalesce to kill leaves entirely and can be confused with frost damage in the absence of sporulation. In crops the disease may be observed as patches (foci) of stunted plants or may be uniformly spread across the field. Severe infections can kill plants and even patches of crop in the winter. On stems lesions are generally superficial, fawn-coloured with black speckling at the edges and may be surrounded by spore masses at high relative humidities. Small light green lesions and the white spots may also be observed on flower

buds, flowers, pedicels and pods. Severe lesions can cause distortion of leaves, pods and even stems, and can kill flower buds. Pod damage may cause premature ripening and pod shattering.

1.2 Life cycle

Epidemics of this disease are often initiated in autumn when spores deposited on leaves germinate to penetrate the cuticle (Rawlinson *et al.*, 1978). Spore germination and infection requires a minimum leaf wetness period of 16 h at the optimum temperature of 15^{0}C (Figueroa *et al.*, 1995) and will require longer wetness periods at lower temperatures common during autumn and winter in the UK. *P. brassicae* is a hemibiotroph; during initial colonisation hyphae ramify between the cuticle and cell walls without penetrating cells (Ashby, 1997). They form a reticulum, from which conidiophores eventually develop to produce acervuli which rupture the cuticle to expose characteristic masses of conidia held in a gelatinous matrix. During the winter there is a long latent period between the initial infection and the subsequent production of new conidia, so that there may be no symptoms or sporulation from infections initiated in autumn until January or February (Rawlinson *et al.*, 1978; Figueroa *et al.*, 1995). The matrix surrounding the spores is dissolved by water and spores are dispersed by rain-splash to initiate new infections. Since the latent period is dependent on accumulated temperature (degree-days), pathogen generation times become shorter as temperatures increase in the spring. The teleomorph of *P. brassicae* has now been observed in the UK; apothecia develop and release ascospores after rainfall in the spring (McCartney & Lacey, 1990) but their role in the epidemiology of light leaf spot is not clear. After harvest, *P. brassicae* survives on crop debris; planting successive winter oilseed rape crops can increase the severity of the disease (Figueroa *et al.*, 1994), although epidemics can also occur in oilseed rape crops after cereals.

2. Control of light leaf spot

Since there is no complete immunity to *P. brassicae* in UK winter oilseed rape cultivars, control of light leaf spot relies on the use of fungicides. Yield responses to control of light leaf spot of >1 t/ha have been obtained in fungicide trials when it has been the main disease observed (Hardwick *et al.*, 1991). Severe epidemics, which start in the autumn, such as that observed on cv. Envol in a trial in Scotland (Fig. 1) can be controlled effectively only if a fungicide is applied in autumn (Table 1). To maintain effective control of this polycyclic disease, a further spray treatment may also be required in spring (Table 1). If the spring spray is delayed until April, it may also provide effective control of the pod phase of the disease, which was not controlled by winter sprays or by summer sprays in the Scottish experiment (Table 1).

Growers should avoid growing successive winter oilseed rape crops to decrease risk of severe light leaf spot and should bury crop debris after harvest, particularly since *P. brassicae* is a poor competitor against saprophytes. Volunteer oilseed rape should be destroyed in case it carries the disease. The basis of the observed differences in resistance between winter oilseed rape cultivars is not clear, but good yield responses to fungicides can be obtained with both more or less resistant cultivars. Some failures in disease control by MBC fungicides associated with the development of MBC resistance in *P. brassicae* have been reported in Scotland (Sutherland *et al.*, 1994), so appropriate products need to be carefully chosen.

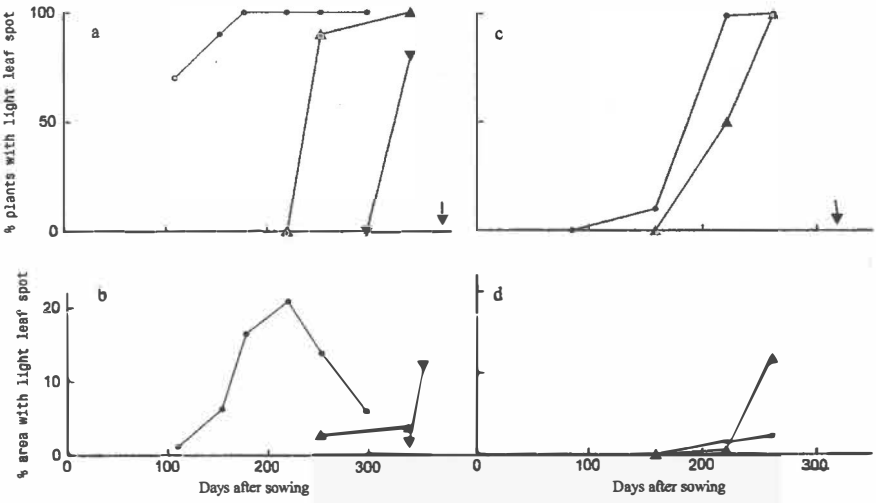

Fig. 1. Progress of light leaf spot (*Pyrenopeziza brassicae*) epidemics (% plants affected (a, c), % area affected (b, d)) on leaves (●) stems (σ) and pods (τ) in unsprayed plots of winter oilseed rape in Scotland (Pennymuick) in 1992/93 (cv. Envol) (a, b) and England (Rothamsted) in 1987/88 (cv. Bienvenu) (c, d). Arrows indicate harvest date in each season.

Table 1. Effects of fungicide spray timing on the development of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (cv. Envol) in Scotland in 1992/93; data for leaves and stems on 1 May and for pods on 8 August 1993.

Spray treatment*	**Incidence / severity of light leaf spot**			
	% plants	**% leaf area**	**% stem area**	**% pod area**
Nil	100	12.8	3.2	
O	100	8.4	2.7	
ONDJ	100	7.7	1.9	
ONDJ FM	60	0.5	0.01	
M	100	7.3	3.00	
JFM	95	3.6	0.6	
DJFM	50	0.6	0.1	
ONDJF				6.5
ONDJFMA				3.5
JJ				8.5
AJJ				2.0
SED (13 df)	18.9	1.81	0.58	2.1 (17 df)

* ONDJFMAJJ, spray treatments in October, November, December, January, February, March, April, June and July, respectively.

3. Forecasting light leaf spot

ADAS/CSL winter oilseed rape disease surveys demonstrate that the severity of epidemics, as indicated by the incidence (%) of plants with infected leaves in March or with infected pods in July, differs greatly between seasons, between ADAS regions and between crops (Fitt *et al.*, 1996; Hardwick & Turner, 1995). Furthermore, they also show that seasonal patterns in use of appropriate fungicides do not relate well to the variation in severity of epidemics so that many crops are sprayed unnecessarily whilst others do not receive the fungicide treatment they require (Hardwick & Turner, 1994). To optimise use of fungicides against light leaf spot, it is necessary to produce a scheme for forecasting the development of severe light leaf spot epidemics at the times when spray decisions need to be made. Therefore, a provisional risk-assessment based forecasting scheme has been produced, with three components:

1. a seasonal risk index to identify high risk seasons;
2. an initial crop risk index to identify high risk crops at the start of the season;
3. an improved crop risk index, based on disease assessments at monthly intervals.

3.1 Seasonal risk index.

Regression analyses on winter oilseed rape survey data for the ADAS Eastern region indicated that it was best to divide light leaf spot epidemics into two stages for the purpose of disease prediction. Firstly, the regional mean % plants with light leaf spot on leaves in March can be predicted from survey data for the previous July (mean % plants with light leaf spot on the pods). Secondly, the regional mean % plants with light leaf spot on pods in July can be predicted from disease incidence in March and rainfall in May. On the basis of these results, predictions for the first stage have been issued in the last few seasons and have proved reasonably accurate for the Eastern region (Gladders, pers. comm.). ADAS/CSL survey data for England and Wales were used to extend the first stage to the whole area. Survey data from July 1996, used to predict the incidence of disease in each ADAS region in the spring of 1997, indicated that the greatest risk of severe epidemics was in the Northern and Midlands & West regions (Table 2). When epidemics risks were classified as slight (< 25% plants affected), moderate (25-50%) or severe (>50%), these predictions could be used to estimate the potential numbers of crops with epidemics in different severity categories in each region (Table 3); thus it was predicted that the proportion of crops at risk of having >50% plants affected was 34% in the Northern region but only 6% in the Eastern region.

3.2 Crop risk index.

Analyses of ADAS/CSL survey data for individual crops suggested that early sowing and cultivar susceptibility both increased incidence of light leaf spot but proximity to previous oilseed rape crops did not. Crop risks were greater in the Northern, Midlands & West and South-west regions than in the East or South-east. Initial crop risk indices (0-100% scale) based on these analyses were used to predict the % plants affected in March for different crops (Fitt *et al.*, 1996). It was not possible to satisfactorily predict % plants affected in July from individual crop data in March using only these factors.

Table 2. Incidence of light leaf spot (*Pyrenopeziza brassicae*) (% plants affected, *s*) in the spring of 1997 in each ADAS region* as predicted from the incidence (% winter oilseed rape plants with pods affected, *p*) in July 1996, using a model of the form $s = a + 0.71\ p$ where *a* takes a different value for each region.

Region	% plants with affected pods in July 1996	Predicted % plants with affected leaves in spring 1997
East	1.6	7.3 ± 2.81
Midlands & West	9.7	23.8 ± 6.96
North	5.7	29.5 ± 7.44
South-west	3.3	18.4 ± 8.15
Wales	13.5	15.7 ± 7.38

* The South-east region has been omitted because the model did not fit the data well.

3.3 Improved crop risk index.

Experiments were done at Rothamsted to provide information for determining how to use monthly samples from crops to improve crop risk indices during the period between October and April. A provisional protocol for use by growers or advisers involved a combination of crop walking and assessment of disease on plants sampled from the crop:

1. inspect crops at monthly intervals between October and April looking for patches of stunted plants with light leaf spot;
2. collect a sample of plants (e.g. 50) in a diagonal transect across the crop;
3. incubate plants (which should be reasonably dry) in polyethylene bags at 10-15^{0}C (for example in a closed barn) for 4-5 days and then assess the incidence of light leaf spot, using the presence of the characteristic white spots to confirm the presence of the disease.

Table 3. Percentage of winter oilseed rape crops in an ADAS region with x% plants with light leaf spot (*Pyrenopeziza brassicae*) in the spring of 1997 as predicted from the incidence of plants with affected pods in July 1996.

Predicted regional mean (% plants affected, *p*)	Predicted % crops in region with x% plants affected in spring			
	x = 0-25	x = 25-50	x = 50-75	x > 75
0 - 25% (slight risk)	89.2	4.4	3.4	3.0
25 - 50% (moderate risk)	55.4	10.8	12.3	21.6
> 50% (severe risk)	29.2	12.5	16.7	41.7

4. Discussion

The provisional scheme for forecasting the development of severe light leaf spot epidemics to optimise use of fungicides against the disease in the UK will require several improvements before a robust, reliable, accurate forecasting scheme is available. The seasonal risk index can be improved by the inclusion of weather factors in the analysis; this will be essential if predictions for the second phase of the epidemic for March to July are to be made. However, if the weather after the time when spray decisions have to be made is shown to be important, a factor for the risk of occurrence of weather favourable for epidemic development will need to be incorporated into the predictions. Further improvements to the seasonal risk indices may be made by analysing disease severity data as well as disease incidence data. Similar analyses may be used to improve the initial crop risk indices; results obtained with analyses of seasonal risks for Eastern region data suggest that it will be important to examine factors such as rainfall in May in order to produce accurate crop risk predictions for the second phase in the epidemic.

Further improvements to the crop risk indices during the period between October and April when control sprays can be applied can be made by including information about occurrence of infection periods (weather favourable for infection by the pathogen) and results of the ADAS/CSL survey in autumn, including information about disease incidence and fungicide use. Development of an accurate, rapid diagnostic test for detection of light leaf spot when symptoms are not visible would help growers to assess the incidence of disease. Further work also needs to be done to optimise sampling strategies and relate observed amounts of disease to actual amounts of disease. It will also be important to relate amounts of disease at different times during the period October to April to ultimate disease severity and yield losses. It should then be possible to determine threshold amounts of light leaf spot associated with risk of specific yield losses (e.g. for x% plants with light leaf spot in November, the risk of a loss in yield of 1 t/ha is y%).

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References

Ashby, A.M. (1997). A molecular view through the looking glass, the *Pyrenopeziza brassicae Brassica* interaction. *Advances in Botanical Research* **24**, 31-70.

Figueroa, L., Shaw, M.W., Fitt, B.D.L, McCartney, H.A. & Welham, S.J. (1994). Effects of previous cropping and fungicide timing on the development of light leaf spot (*Pyrenopeziza brassicae*), seed yield and quality of winter oilseed rape (*Brassica napus*). *Annals of Applied Biology* **124**, 221-239.

Figueroa, L. Fitt, B.D.L., Welham, S.J., Shaw, M.W. & McCartney, H.A. (1995). Early development of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in relation to temperature and leaf wetness. *Plant Pathology* **44**, 641-654.

Fitt, B.D.L., Gladders, P., Turner, J.A., Sutherland, K.G. & Welham, S.J. (1966). Predicting risk of severe light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in the UK. *1996 Brighton Crop Protection Conference - Pests and Diseases*, 239-244.

Hardwick, N.V. & Turner, J.A. (1994). Fungicide use on winter oilseed rape in England and Wales, 1986-1993. *Quatrieme Conference Internationale sur les Maladies des Plantes 1994, Bordeaux, ANPP,* 1163-1170.

Hardwick, N.V. & Turner, J.A. (1995). Winter oilseed rape: survey of pests and diseases, 1995. *ADAS/CSL Research and Development, Harpenden.* 41 pp.

Hardwick, N.V., Fitt, B.D.L., Wale, S.J. & Sweet, J.B. (1991). Oilseed Rape Diseases. *Homes-Grown Cereals Authority Review*, **OS4**.131 pp.

McCartney, H.A., & Lacey, M.E. (1990). The production and release of ascospores of *Pyrenopeziza brassicae* on oilseed rape. *Plant Pathology* **39**, 17-32.

Paul, V.H., & Rawlinson, C.J. (1992). *Diseases and Pests of Rape*. Verlag Th. Mann, Gelsenkirchen-Buer, Germany. 132 pp.

Rawlinson, C.J., Sutton, B.C. & Muthyalu, G. (1978). Taxonomy and biology of *Pyrenopeziza brassicae* sp. nov. (*Cylindrosporium concentricum*), a pathogen of winter oilseed rape (*Brassica napus* ssp. *oleifera*). *Transactions of the British Mycological Society* **71**, 425-439.

Sutherland, K.G., Griffin-Walker, V. & Oxley, S.J.P. (1994). Resistance of light leaf spot (*Pyrenopeziza brassicae*) of winter oilseed rape to MBC fungicides. *1994 Brighton Crop Protection Conference - Pests and Diseases*, 463-468.

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Estimating yield loss from light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK

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Summary: A model for predicting yield loss from light leaf spot on winter oilseed rape was constructed using the data from experiments at different locations in Scotland in 1991/92, 1992/93 and 1993/94. Disease incidence (% plants affected) at GS 3.3 (flower buds visible) was used as the explanatory variable : $y_r = 0.37x - 0.78$ (y_r is percentage yield loss and x is disease incidence at GS 3.3). Data sets from Rosemaund, Thurloxton and Rothamsted in England, which had not been used in developing the model, were used to test the model.

Key words : light leaf spot, model, oilseed rape, *Pyrenopeziza brassicae*, yield loss.

Introduction

Light leaf spot (*Pyrenopeziza brassicae*) is a widespread disease on winter oilseed rape in the UK (Gladders *et al.*, 1995) and has resulted in economic losses estimated at > £30 M annually in recent years (Fitt *et al.*, 1997). Research has been done on a forecasting scheme (Fitt *et al.*, 1996) to optimise use of fungicide sprays against light leaf spot. To obtain a maximum economic benefit from use of fungicides, it is necessary to estimate the yield loss in individual crops. This paper describes work to derive a model for predicting yield loss caused by light leaf spot.

Material and Methods

Field experiments. Data sets used to develop the model were from the field experiments in Scotland at Foveran in 1991/92, Pettymuick in 1992/93 and Udny Station in 1993/94 (Sansford *et al.*, 1996); the cultivar used was Envol with a low National Institute of Agricultural Botany (NIAB) light leaf spot resistance rating of 3. Data sets for testing the model were from experiments at Rothamsted in 1987/88, and Rosemaund and Thurloxton in 1993/94 (Sansford *et al.*, 1996); the experiments were with cvs. Ariana (NIAB resistance rating 5), Bienvenu (6) and Cobra (4) and breeding lines NRPB2, PBI1 and PBI3 in 1987/88, and cv. Envol in 1993/94.

Disease development through the season. In Scotland, the incidence of light leaf spot on leaves in unsprayed plots increased earlier and the severity was greater in 1992/93 than in 1991/92 and 1993/94. The incidence of light leaf spot on stems and pods increased earlier in 1991/92 than in the other two seasons. Early defoliation caused by the disease decreased recorded disease incidence after 26 April in 1991/92 (GS 4.0). Light leaf spot was more serious in unsprayed plots at Rothamsted in 1987/88 than at Rosemaund and at Thurloxton in 1993/94. Other diseases, such as stem canker, which can also affect yield, were absent or at a low incidence in experiments in Scotland and at Rothamsted in 1987/88; the maximum incidence of canker reached 80 % at Rosemaund in July and 50 % at Thurloxton in June in unsprayed plots; the maximum canker severity on a 0 - 4 scale was less than 1.5 at Rosemaund and less than 1 at Thurloxton (Sansford *et al.*, 1996).

Data analyses. Linear regression analysis was used to derive yield loss models. Single point models were used to analyse the relationship between yield loss and disease development. Yield loss for each plot in each year was calculated as the estimated yield at zero disease incidence minus the actual yield at different disease incidences. Percentage yield loss was calculated as the yield loss divided by the estimated yield, expressed as a percentage.

Results

Relating yield to light leaf spot assessments by single point models. Using the data from all sprayed and unsprayed plots, disease intensity (incidence, severity or index) at each sampling date / growth stage was regressed separately against final yield to show the relationships between disease intensity and yield variation for each of the three seasons (Fig. 1).

The % variance accounted for by disease incidence, severity or index showed a similar trend for the three experiments in Scotland but had decreased by day 253 in 1992/93 (GS 3.5/4.0) because disease incidence in most plots was near to 100 % by then.

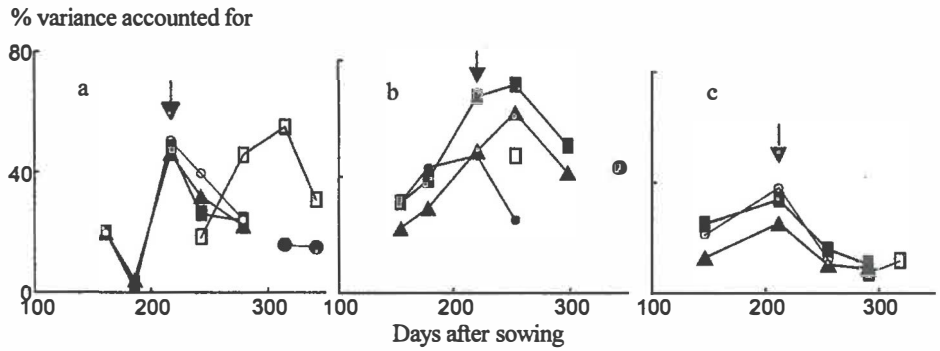

Figure 1. Relationship between yield of winter oilseed rape (cv. Envol) and light leaf spot assessed at different times in the season on leaves, as incidence (○, % plants affected), severity (σ , % leaf area affected) or index (□, incidence x severity), or as incidence on stems (□) or on pods (λ). Relationships were assessed by linear regression of yield on disease incidence or severity for plots with different spray treatments and by estimating the % variance accounted for (r^2) for experiments in (a) 1991/92, (b) 1992/93 and (c) 1993/94. Arrows indicate GS 3.3 in each season.

Single point model for predicting yield loss. When the data from the experiments in Scotland were compared by multiple comparison, no significant differences were detected between the three seasons. Disease incidences at GS 3.3, which accounted for the greatest % variance, were treated as non-replicates and regressed against percentage yield loss to derive the model for forecasting percentage yield loss:

$$y_r = 0.37\,x - 0.78 \quad (r^2 = 68.4\,;\ \text{se} = 10.4;\ \text{df} = 85)$$

where y_r = percentage yield loss and x = disease incidence at GS 3.3.

Testing the yield loss model. Three data sets were used to test the performance of the model (Fig. 2). There were no significant differences between the values predicted by the model and the observed measurements from Rothamsted (P = 0.567) and Rosemaund (P = 0.087) but

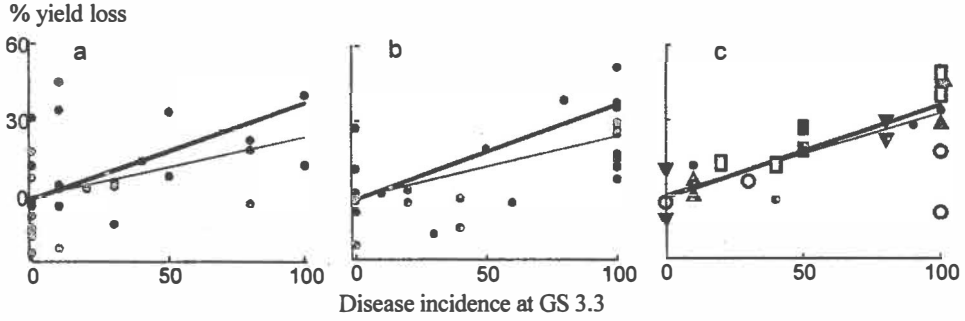

Figure 2. Comparison of the percentage yield losses predicted by the model (thick lines) with fitted values (thin lines) for observed percentage yield losses in (a) 1993/94 at Rosemaund (cv. Envol ⊛), (b) 1993/94 at Thurloxton (cv. Envol ●) and (c) 1987/88 at Rothamsted (cvs. Ariana ●, Bienvenu O, Cobra □, breeding lines NRBP2 τ, PBI1 ν and PBI3 σ).

there was a significance difference for Thurloxton (P = 0.004). Since the predictions of the model were derived from data without considering the effect of disease on stems or pods, the model based on disease on leaves might have overestimated the yield loss when disease did not become severe on stems or pods.

Discussion

This work shows that it is possible to predict yield losses from light leaf spot from disease incidence on leaves several months before harvest (at GS 3.3). The yield loss prediction model developed with data from three experiments in Scotland fitted well to three independent data sets from experiments in England. This yield loss prediction model is easier to use than that based on Area Under Disease Progress Curve (AUDPC) developed by Sutherland *et al.* (1995) since it requires only one disease assessment rather than a series of disease assessments over a period of time. Furthermore, this model allows the grower to predict yield loss at a time when a final spray decision can be made, even though the optimum time for application of sprays to control light leaf

spot is generally between November and February (Sansford *et al.*, 1996). By contrast, the model of Sutherland *et al.* (1995) and the model of Sansford *et al.* (1996), based on % stems with light leaf spot symptoms in July, can only be used to assess yield loss retrospectively at the end of the growing season. This simple model also appears to be more robust since it could be applied in different seasons to crops in different locations whereas the other models required different parameter values for each crop or season. Nevertheless, there is an evidence that it does not predict the yield loss as accurately when severe epidemics occur and light leaf spot incidence reaches 100% early in the season (as at Pettymuick in 1992/93) or when epidemics are slight (as in some experiments at Rothamsted), nor does it give any information about the effects of light leaf spot on yield components (Doughty *et al.*, 1995). It can be applied over different locations only when yield loss is expressed in percentage terms, because many factors other than disease affect the maximum potential yield. Furthermore it has not yet been tested in situations where diseases other than light leaf spot (e.g. stem canker) are also affecting yield.

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References

Doughty, K. J.; Petinon S.; McCartney, H. A. 1995: A comparison of the effects of fungal disease on the growth and field yield of two winter oilseed rape (*Brassica napus* L.) cultivars. Aspects of Applied Biology 42, 43 - 51.

Fitt, B. D. L.; Gladders, P.; Turner, J. A.; Sutherland,K. G.; Welham, S. J. 1996: Predicting risk of severe light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in the UK. Brighton Crop Protection Conference 1996 Pests & Diseases, British Crop Protection Council, 239 - 244.

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

Gladders, P.; Fitt, B. D. L.; Welham, S. J. 1995: Forecasting development of light leaf spot (*Pyrenopeziza brassicae*) epidemics on winter oilseed rape. Proceedings of 9th International Rapeseed Congress, 1001 - 1003.

Sansford, C. E.; Fitt, B. D. L; Gladders, P.; Lockley, K. D.; Sutherland, K. G. 1996: Oilseed rape : disease development , forecasting and yield loss relationships. Home - Grown Cereals Authority Project Report OS17. 185pp.

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

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Host range of *Phoma lingam* (Tode ex Fr.) Desm. isolates from the Region of Poznañ

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Abstract: Greenhouse and field experiments were run in the years 1995 and 1996. In the greenhouse only Tox ^{O} *P. lingam* isolate number 2 and in the field a population of the pathogen from oilseed rape plant debris were used. The Pathogen was introduced with seed into soil in the greenhouse and on the surface of the soil in the field.

Results from the greenhouse were not corelated with those in the field. Of nine species only *Camelina sativa* plants were not infected in the greenhouse. Numbers of infected plants of remaining species varied from 7 (*Sinapsis alba* 'Borowska') to 86.5 % (*Brassica oleracea var. gemmifera* 'Maczuga'). Mean degree of infection was the lowest (1.3) in *Sinapsis alba* 'Nakielska' and the highest (4.7) in *B. oleracea var. gongylodes*.

Plants of all species were infected in field experiments. Numbers of infected plants varied from 2 (*B. oleracea var. gongylodes* 'Wiedeñska' and *B. oleracea var. botrytis* 'Pionier') to 25 % (*B. campestris* 'Przybrodzka'). The lowest mean degree of infection was 1.5 and the highest 2.2 (*B. campestris* 'Przybrodzka').

Key words: *Phoma lingam, Leptosphaeria maculans*, host range

Introduction

Phoma lingam is a commonly occurring pathogen of cultivated and wild cruciferous plants. Among the pathogen population are two groups of strains Tox ^{O} and Tox $^{+}$. A dominant group of the strains in Poland is Tox ^{O} with colorless medium under colonies. Tox ^{O} strains produce only a trace of sirodesmins (Kachlicki, Jêdryczka 1994).

The aim of this work was to evaluate cruciferous seedlings susceptibility to isolate number two of Tox ^{O} *P. lingam* as well as plant susceptibility of the species to the pathogen population occurring in the field.

Methods

One or two cultivars of the following plant species: *Brassica napus* ssp. *oleifera*, *B. campestris*, *Sinapis alba*, *B. juncea*, *B. oleracea var. gongylodes*, *B. oleracea var. capitata*, *B. oleracea var. botrytis*, *B. oleracea var. gemmifera* and *Camelina sativa* were med throughout (Tab. 3).

In greenhouse experiments inoculation was carried out during sowing into sterilised soil. Seed was planted together with 5 mm disk of PDA medium overgrown by *P. lingam* which were introduced into the soil. In each cultivar of all plant species 30 seeds were inoculated (6 pots x 5 seeds). The same number of seeds without pathogen were sown as controls. Numbers of infected plants and mean degree (1-6) of infection were evaluated at the 3-5 leaf stage (Weber, Karolewski 1993).

In the field experiment a randomized, complete block design with four replicates and 4 m^2 plot sizes was used. Sowing was carried out on April 10th, 1995 and April 9th, 1996. Three pieces of stems infected by *P. lingam* the previous year were placed on the field surface of each plot just after sowing. Disease evaluation was done for the whole vegetative period at one week intervals.

Conditions during experiment

Mean temperatures varied from 14 to 22^{O}C in successive weeks of greenhouse experiments (Tab. 1). Weather conditions at the beginning of field experiments in both years were similar (Tab. 2). July, August and September in 1996 were more cold and rainy than in 1995.

Table 1 Greenhouse temperatures during experiment time in March and beginning of April

Weeks	Years	Temperature (°C)		
		mean	minimal	maximal
first	1995	20	11	29
	1996	15	6	24
second	1995	16	6	25
	1996	18	6	31
third	1995	15	5	28
	1996	14	5	25
fourth	1995	22	15	30
	1996	17	3	30

Table 2 Wheather conditions during vegetative growth of cruciferous plants in the field

Months	Temperature °C			Precipitation (mm)		
	perennial	deviations		perennial	percentages	
	means	1995	1996	means	1995	1996
April	7.7	0.8	0.9	39	39	44
May	13.2	- 0.4	- 0.4	53	121	151
June	16.6	- 0.5	- 0.3	49	147	50
July	17.9	2.9	- 2.1	76	37	290
August	17.4	1.6	0.9	56	105	108
September	8.9	- 0.4	- 2.6	46	130	165

Results

Infection of plants was higher in greenhouse than in field experiments. *Camelina sativa* plants were not infected in greenhouse (Tab. 3). The number of *Sinapsis alba* infected plants varied from 7 (cv. Borowska) to 15 % (cv. Nakielska).

Table 3 Infection of cruciferous seedlings by *P. lingam* isolate number two (greenhouse, mean for years 1995 and 1996)

	Cultivars	Percentage of infected plants x)	Mean degree of infection x)
Brassica napus ssp. *oleifera*	Bolko	61 e	2.8 bc
	Mar	72 e	2.9 bc
	Bronowski	84 e	3.2 bcde
	Mazowiecki	62 e	3.0 bcde
B. campestris	Brachina	53 de	3.2 bcde
	Ludowy	42 cde	3.1 bcde
	Przybrodzka	72 e	2.9 bc
Sinapis alba	Borowska	7 ab	2.7 bc
	Nakielska	15 bc	1.3 a
B. juncea	Malopolska	49 de	2.5 b
B. oleracea var gongylodes	Wiedeńska Biala	69 e	4.2 ef
	Dvorskyego	76 e	4.7 f
B. oleracea var. capitata	Slawa z Enkhuizen	27 bcd	2.2 ab
	Kamienna G³owa	53 de	3.7 cdef
B. oleracea var. botrytis	Pionier	77 e	2.5 b
B. oleracea var. gemmifera	Maczuga	86 e	4.1 def
Camelina sativa	Przybrodzka II	0	-

x) - means followed by the same letters in columns are not significantly different;

Numbers of infected plants of the remaining species varied from 27 (*B. oleracea var. capitata* 'Slawa z Enkhuizen') to 86.5 % (*B. oleracea var. gemmifera* 'Maczuga'). Mean degree of infection was the lowest (1.3) in *S. alba* 'Nakielska' and the highest (4.7) in *B. oleracea var. gongylodes* 'Dvorskyego'.

In field experiments three different species groups could be distinguished (Tab. 4). Numbers of infected plants varied from 2 to 3.5 % in the first group, 7.5 to 16.5 % in the second and from 20.5 to 25 % (*B. campestris* 'Przybrodzka') in the third one. Mean degree of infection varied from 1.5 to 2.2 (*B. campestris* 'Przybrodzka').

Discussion

The wide host range of *P. lingam* is very well known (Johnson, Lewis 1994, Juraszek 1958, Maciejowska 1959). In this work *P. lingam* isolate number two of Tox ^{O} group did not infect of *C. sativa* in the greenhouse experiments. In remaining species the number of infected plants varied from 7 to 86.5 % (*B. oleracea var. gemmifera* 'Maczuga'). In field experiments the population of *P. lingam* present infected 2 to 25 % plants (*B. campestris* 'Przybrodzka').

Table 4 Infection of cruciferous plants by *Phoma lingam* (field, mean for years 1995 and 1996)

	Cultivars	Percentage of infected plants [x)]	Mean degree of infection [y)]
Brassica napus ssp. *oleifera*	Bolko	12.5 ^{abcd}	1.7
	Mar	3.5 ^{ab}	1.5
	Bronowski	3.5 ^{ab}	1.5
	Mazowiecki	12.0 ^{abcd}	1.6
B. campestris	Brachina	20.5 ^{cd}	2.1
	Ludowy	16.5 ^{bcd}	1.5
	Przybrodzka	25.0 ^{d}	2.2
Sinapis alba	Borowska	14.5 ^{abcd}	1.6
	Nakielska	8.0 ^{abcd}	1.5
B. juncea	Malopolska	10.5 ^{abcd}	1.5
B. oleracea var gongylodes	Wiedeńska Biala	2.0 ^{a}	1.5
	Dvorskyego	3.5 ^{ab}	2.0
B. oleracea var. capitata	Slawa z Enkhuizen	7.5 ^{abc}	2.0
	Kamienna Glowa	9.5 ^{abcd}	2.0
B. oleracea var. botrytis	Pionier	2.0 ^{a}	2.0
B. oleracea var. gemmifera	Maczuga	3.5 ^{ab}	1.5
Camelina sativa	Przybrodzka II	2.5 ^{ab}	2.0

x) - means followed by the same letters in columns are not significantly different;
y) - no significant differences.

References

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

Juraszek, H. 1958: Sucha zgnilizna roslin kapustnych *Phoma lingam* (Tode) Desm. i jej zwalczanie. Przeglad Ogrodniczy 3.

Kachlicki, P.; Jedryczka, M. 1994: Propertices of *Phoma lingam* (Tode ex Fr.) isolates from Poland. II Secondary metabolites production. Phytopath. Polonica 7 (XIX): 81-86.

Maciejowska, Z. 1959: Z badañ nad biologia *Phoma lingam* (Tode) Desm. grzyba powodujacego sucha zgnilizne korzeni roslin krzyzowych. Praca magisterska w Katedrze Fitopatologii SGGW w Warszawie.

Weber, Z.; Karolewski, Z. 1993: Podatnosc odmian i rodów rzepaku ozimego na *Phoma lingam* w szklarni i wplyw niektórych czynników na wystepowanie suchej zgnilizny rzepaku w polu. Postepy Nauk Rolniczych 245, 5: 133-140.

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The influence of fungicides on *Phoma Lingam* (Tode ex Fr.) Desm. mycelium growth

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Abstract The influence of fungicides on *Phoma lingam* Tox ^{0} and Tox $^{+}$ isolates was estimated in four experiments at 10, 15, 20 and 25°C. Twelve fungicides (Alert 375 SC, Alto 320 SC, Baytan Universal 19,5, Benlate 50 WP, Brawo 500 SC, Ferrax, Oxafun T, Raxil 02 WS, Siarkol K, Sibutol, Vincit, Zaprawa Nasienna T) were added to PDA at 1; 0,1; 0,01; and 0,001 % concentrations. The growth rate of fungi was measured for 21 days. The fungicides Alert 375 SC., Alto 320 SC., Benlate 50 WP, Oxafun T inhibited mycelial growth of *P. Lingam*, most strongly.

Key Words: *Phoma lingam*, mycelium, growth, fungicides

Phoma lingam (sexual stage *Leptosphaeria maculans* [Desm.] Ces. et de Not.) is one of the most severe fungal pathogens of winter oilseed rape in Poland (Frencel et al. 1991). The disease causes economically important yield losses of rape, so chemical control has been often necessary (Paul et al. 1991, Sadowski and Klepin 1991, Sansford 1995).

The aim of present work was to examine the inhibiting effect of fungicides on mycelial growth of *P. lingam* in vitro conditions.

Material And Methods

Two isolates of *P. lingam* were used: N° 2 (Tox °) and N° 54 (Tox $^{+}$). Five mm diameter disks of PDA overgrown by fungi were placed in the middle of 90 mm Petri dishes supplemented with fungicides under test: (Alert 375 SC, Alto 320 SC, Baytan Universal 19,5, Benlate 50 WP, Bravo 500 SC, Ferrax 440 FS, Oxafun T, Raxil 02 WS, Siarkol K, Sibutol 35,9 FS, Vincit 050 FS, Zaprawa nasienna T (Tab. 1).

Table 1. Fungicides used in experiments

Fungicide	Active ingredient	Content of active ingredient
Alert 375 S.C.	flusilazole carbendazin	125,0 g 250,0 g
Alto 320 SC	cyproconazole	320,0 g
Baytan Universal 19,5	triadimenol imazalil fuberidazole	15,0 % 2,5 % 2,0 %
Benlate 50 WP	benomyl	50,0 %
Bravo 500 SC	chlorothalonil	500,0 g
Ferrax 440 FS	flutriafol thiabendazole ethirimol	30,0 g 10,0 g 400,0 g
Oxafun T	carboxin thiram	37,5 % 37,5 %
Raxil 02 WS	tebuconazole	2,0 %
Siarkol K	sulphur carbendazin	80,0 % 5,0 %
Sibutol 35,9 FS	bitertanol fuberidazole	375,0 g 23,0 g
Vincit 050 FS	flutriafol thiabendazole	25,0 % 25,0 %
Zaprawa Nasienna T	thiram	75,0 %

The fungicides were added to PDA at 1; 0,1; 0,01 and 0,001 % concentrations. Each fungicide was evaluated at four temperatures 10, 15, 20 and 25°C. Every combination was performed in five replications. The diameter of fungal colonies were measured for 21 days in 1 week intervals.

Results And Discussion

There was no growth in either isolate of *P. lingam* at any temperature on PDA supplemented with 1 % of Alert 375 SC, Alto 320 SC, Benlate 50 WP, Oxafun T and isolate N° 54 on PDA with 1 % of Siarkol K (Tab. 2, 3, 4, 5). One percentage concentration of Baytan Universal 19,5 and Sibutol strongly inhibited mycelial growth. The inhibiting influence of fungicides on mycelial growth at 0,001 % concentration was slight. There was no influence of temperatures on the efficacy of tested fungicides on *P. lingam* fungal growth inhibition.

These laboratory results are not always in agreement with results of field experiments. Ballinger et al. (1988) showed that flutriafol applied at sowing time reduced disease severity. Wnêkowski and Przy³êcka (1994) noted high efficacy of Siarkol K for control of *P. lingam* on oilseed rape in stage EC-65. Oilseed rape treatment with Benlate didn't influence the percentage of stems infected by *P. lingam* (Bonin and Mota³a 1986). The high efficacy of

fungicides including flusilazole and carbendazim was recorded by Penaud (1995), Wnêkowski and Przy³êcka (1994).

Table 2. The influence of fungicides on *Phoma lingam* mycelium growth at 10°C

Fungicide	Izolate	Linear mycelium growth (mm/24h) *				
		Concentration of fungicides in PDA medium				mean
		1 %	0,1 %	0,01 %	0,001 %	
Alert 375 SC	2	0	0	0.64	1.00	0.41 [h]
	54	0	0	0.06	0.27	0.08 [b]
Alto 320 SC	2	0	0.18	0.93	0.93	0.51 [i]
	54	0	0	0.06	0.06	0.03 [a]
Baytan Universal 19,5	2	0.14	0.42	0.99	1.09	0.66 [k]
	54	0	0.05	0.34	0.46	0.21 [d]
Benlate 50 WP	2	0	0.09	1.09	1.12	0.58 [i]
	54	0	0	0.34	0.37	0.18 [c]
Bravo 500 SC	2	0.95	0.99	1.08	1.10	1.03 [o]
	54	0.30	0.39	0.32	0.38	0.35 [h]
Ferrax 440 FS	2	0.23	1.08	1.15	1.16	0.91 [n]
	54	0	0.14	0.32	0.35	0.17 [d]
Oxafun T	2	0	0	0.80	1.15	0.49 [h]
	54	0	0.02	0.27	0.35	0.16 [c]
Raxil 02 WS	2	0.36	1.04	1.03	1.11	0.89 [n]
	54	0.04	0.28	0.09	0.37	0.20 [f]
Siarkol K	2	0.12	0.88	1.03	1.09	0.78 [l]
	54	0	0.15	0.45	0.48	0.27 [f]
Sibutol 35,9 FS	2	0	0.30	0.98	1.30	0.65 [j]
	54	0	0.04	0.16	0.44	0.15 [c]
Vincit 050 FS	2	0.22	0.88	1.14	1.14	0.92 [n]
	54	0.01	0.29	0.37	0.45	0.28 [f]
Zaprawa Nasienna T	2	0.31	1.07	1.14	1.12	0.91 [n]
	54	0.05	0.35	0.34	0.43	0.29 [f]
Check	2					1.14 [p]
	54					0.38 [h]

* mean followed by the same letter are not significantly different at 5%;

Table 3. The influence of fungicides on *Phoma lingam* mycelium growth at 15°C

Fungicide	Izolate	Linear mycelium growth (mm/24h) *				
		Concentration of fungicides in PDA medium				mean
		1 %	0,1 %	0,01 %	0,001 %	
Alert 375 SC	2	0.06	0.68	1.47	1.68	0.97 [g]
	54	0	0.20	0.72	0.94	0.47 [c]
Alto 320 SC	2	0.04	0.53	1,53	1.60	0.93 [fg]
	54	0	0.04	0.58	0.92	0.39 [b]
Baytan Universal 19,5	2	0.17	0.81	1.34	1.65	0.99 [h]
	54	0	0.07	0.59	0.74	0.35 [b]
Benlate 50 WP	2	0	0.24	1.59	1.60	0.86 [e]
	54	0	0	0.61	0.74	0.34 [a]
Bravo 500 SC	2	1.55	1.45	1.68	1.62	1.58 [m]
	54	0.72	0.67	0.99	1.08	0.87 [ef]
Ferrax 440 FS	2	0.52	1.53	1.70	1.77	1.38 [l]
	54	0.05	0.67	0.67	0.90	0.57 [d]
Oxafun T	2	0	0.11	0.46	1.61	0.55 [c]
	54	0	0.12	0.46	0.65	0.31 [a]
Raxil 02 WS	2	1.27	1.66	1.71	1.72	1.59 [m]
	54	0.69	0.95	0.80	0.82	0.82 [e]
Siarkol K	2	0.24	1.65	1.76	1.76	1.35 [kl]
	54	0	0.83	1.05	1.02	0.73 [d]
Sibutol 35,9 FS	2	0.47	0.72	1.55	1.73	1.12 [i]
	54	0.09	0.08	0.68	0.93	0.45 [c]
Vincit 050 FS	2	0.73	1.25	1.62	1.73	1.33 [kl]
	54	0.18	0.51	0.88	0.85	0.61 [d]
Zaprawa Nasienna T	2	0.17	1.72	1.63	1.68	1.30 [i]
	54	0.05	0.93	0.95	0.97	0.73 [d]
Check	2					1.79 [n]
	54					0.93 [fg]

* mean followed by the same letter are not significantly different at 5%;

Table 4. The influence of fungicides on *Phoma lingam* mycelium growth at 20°C

Fungicide	Izolate	Linear mycelium growth (mm/24h) *				
		Concentration of fungicides in PDA medium				mean
		1 %	0,1 %	0,01 %	0,001 %	
Alert 375 SC	2	0	0.20	1.43	1.55	0.80 ^{e}
	54	0	0	0.80	0.98	0.45 ^{a}
Alto 320 SC	2	0	0.48	1.62	1.69	0.95 ^{gh}
	54	0	0.08	0.78	0.80	0.42 ^{a}
Baytan Universal 19,5	2	0.30	1.40	1.69	2.00	1.35 ^{l}
	54	0.33	0.30	0.91	1.09	0.66 ^{cd}
Benlate 50 WP	2	0	0.29	1.73	1.75	0.94 ^{g}
	54	0	0	0.76	0.89	0.41 ^{a}
Bravo 500 SC	2	1.62	1.73	1.78	1.90	1.76 ^{op}
	54	0.75	0.77	1.05	1.16	0.93 ^{fg}
Ferrax 440 FS	2	0.67	1.61	1.88	2.00	1.54 ^{n}
	54	0.06	0.91	0.95	0.95	0.72 ^{de}
Oxafun T	2	0	0.13	1.50	1.77	0.85 ^{ef}
	54	0	0.17	0.67	0.87	0.43 ^{a}
Raxil 02 WS	2	1.30	1.77	1.83	1.99	1.72 ^{o}
	54	0.70	1.05	1.09	1.04	0.97 ^{h}
Siarkol K	2	0.29	1.70	1.92	2.03	1.49 ^{m}
	54	0	1.00	1.20	1.12	0.83 ^{e}
Sibutol 35,9 FS	2	0.50	0.59	1.71	1.81	1.15 ^{k}
	54	0.08	0.12	0.72	0.97	0.47 ^{ab}
Vincit 050 FS	2	0.43	1.20	1.79	1.98	1.35 ^{l}
	54	0.17	0.60	0.75	0.99	0.63 ^{c}
Zaprawa Nasienna T	2	0.60	1.89	1.95	2.02	1.62 ^{h}
	54	0.27	1.02	1.04	1.08	0.85 ^{ef}
Check	2					2.05 ^{q}
	54					0.99 ^{h}

* mean followed by the same letter are not significantly different at 5%;

Table 5. The influence of fungicides on *Phoma lingam* mycelium growth at 25°C

Fungicide	Izolate	Linear mycelium growth (mm/24h) *				
		Concentration of fungicides in PDA medium				mean
		1 %	0,1 %	0,01 %	0,001 %	
Alert 375 SC	2	0	0.17	1.50	1.51	0.80 ^{d}
	54	0	0	0.94	1.18	0.53 ^{a}
Alto 320 SC	2	0	0.54	1.70	1.70	0.99 ^{fg}
	54	0	0.17	0.85	0.58	0.40 ^{a}
Baytan Universal 19,5	2	0.51	1.47	1.87	1.98	1.46 ^{lm}
	54	0.12	0.55	1.20	1.36	0.81 ^{e}
Benlate 50 WP	2	0	0.38	1.99	1.82	1.05 ^{hi}
	54	0	0	0.90	1.04	0.49 ^{a}
Bravo 500 S.C.	2	1.76	1.81	1.99	2.01	1.89 ^{p}
	54	0.83	0.84	1.14	1.17	1.00 ^{gh}
Ferrax 440 FS	2	0.76	1.66	1.97	1.98	1.59 ^{n}
	54	0.20	1.22	1.27	1.28	0.99 ^{fg}
Oxafun T	2	0	0.15	1.92	1.92	1.00 ^{gh}
	54	0	0.25	0.85	1.09	0.55 ^{b}
Raxil 02 WS	2	1.28	1.91	2.00	2.07	1.82 ^{op}
	54	0.72	1.30	1.13	1.23	1.10 ^{jk}
Siarkol K	2	0.38	1.79	2.05	2.05	1.57 ^{mn}
	54	0	1.14	1.37	1.38	1.00 ^{gh}
Sibutol 35,9 FS	2	0.44	0.61	1.82	1.87	1.19 ^{k}
	54	0.09	0.18	0.89	1.20	0.59 ^{c}
Vincit 050 FS	2	0.56	1.29	1.84	2.01	1.43 ^{l}
	54	0.20	0.62	0.69	1.44	0.74 ^{e}
Zaprawa Nasienna T	2	0.97	2.02	2.08	2.01	1.77 ^{o}
	54	0.63	1.21	1.13	1.15	1.03 ^{hi}
Check	2					2.13 ^{q}
	54					1.39 ^{lm}

* mean followed by the same letter are not significantly different at 5%;

Literature

Ballinger, D.J.; Salisbury, P.A.; Dennis, J.I.; Kollmorgen J.F.; Potter, T.D. 1988: Evaluation of fungicides, applied at sowing, for control of blackleg in rapeseed. Australian Journal of Experimental Agriculture 28: 511-515.

Bonin, K.; Mota³a, G. 1986: Wyniki badañ nad zwalczaniem chorób grzybowych rzepaku ozimego w rejonie pó³nocno-zachodnim Polski. Zeszyty Problemowe IHAR, Wyniki badañ nad rzepakiem ozimym: 234-242.

Frencel, I.M,; Lewartowska, E.; Jêdryczka, M. 1991: The spectrum and severity of fungal diseases in field infections of winter oilseed rape in Poland. IOBC/WPRS Bulletin XIV/6: 137-140.

Paul, V.H.; Gerdiken, K.J.; Günzelmann, A. 1991: Resistance of double low winter rape cultivars to stem canker and their yield response to fungicide treatment. IOBC/WPRS Bulletin XIV/6: 74-88.

Penaud, A. 1995: Protection fungicide contre le phoma du colza. Rapeseed today and tomorrow, 9th International Rapeseed Congress: 995-997.

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

Sansford, C. 1995: Oilseed rape: development of stem canker (*Phoma lingam*) and its effect on yield. Rapeseed today and tomorrow, 9th International Rapeseed Congress: 577-579.

Wnêkowski, S.; Przy³êcka, E. 1994: Porównanie skutecznoœci zwalczania suchej zgnilizny krzy¿owych na rzepaku w zale¿noœci od terminu stosowania zabiegu. Materia³y XXXIV Sesji IOR, cz.II: 163-167.

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Effect of temperature on infestation and development of *Verticillium dahliae* Kleb. on winter oilseed rape

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Abstract In the period 1993-1996 studies on an induced *Verticillium dahliae* epidemic under field conditions were carried out. They included determination of temperature range in which the development of mycelium growing from the pathogen microsclerotia is possible and effects of below 0°C temperatures on over-wintering of the fungus microsclerotia. Rape verticiliosis is a typical epidemic of a „chronic" pattern, even at conditions favourable for its occurrence, i.e. at a high soil infection potential. Inhibition of infection in Poland from the end of October up to the beginning of April, or up to mid-April, seems to be conditioned by soil temperature, under which infections of more plants can occur. Temperature good for germination of microsclerotia into mycelium ranges from 6°C to about 34°C, while the optimum temperature range for this process is much more narrow - 15°C - 28°C. The pathogen microsclerotia are very resistant to temperatures below 0°C lasting for a long time. After an average winter, typical for Poland, the majority remains viable and capable of germination and plant infection.

Keywords: oilseed rape, disease, fungus, *V. dahliae*, epidemiology, temperature

Introduction

Premature rape ripening caused by *Verticillium dahliae* Kleb. has been observed with differing degrees in the main part of Western Europe, especially in the regions of traditional rape cultivation, such as Sweden, France and a large part of Germany (Kroeker 1970). Losses can reach 30 - 70% of the crop (Makowski 1990, Svenson et al. 1987). Up to now infestation of this fungus has not been a problem in Poland (Zieliñski and Sadowski 1994), but because of difficulties with controlling it, resulting from its specific properties, investigations of its biology and epidemiology were carried out.

The *V. dahliae* microsclerotia, the main source of infection, can survive for twelve years, sometimes even fourteen years and more (Perry and Evert 1984). Therefore it can be assumed that at the moment of seeding a pathogen inoculation potential is present in soil, ready for germination and infection. Under favourable conditions a microsclerotium germinates into mycelium hyphae, which can infect injured plant roots or hairy roots of plants growing nearby.

Determination of the temperature range in which microsclerotia germinate and develop into mycelium would answer the question of under what thermal conditions new plant infections can occur. In connection with observations of the growth of infected plants on the plots it should give an idea how an epidemic under rape cultivation can develop. To reach

these objectives a series of laboratory and field experiments was carried out to determine the course of an induced rape verticiliosis epidemic under field conditions, the temperature range in which the development of mycelium growing from pathogen microsclerotia is possible, and effects of below zero temperatures on over-wintering of fungal microsclerotia.

Methods

Field experiments

Investigations of the course of the induced rape verticiliosis epidemic were carried out during cropping periods 1993-1994 and 1994-1995 and 1995-1996 under conducive conditions on the plots located at the Bydgoszcz branch of the Institute of Plant Breeding and Acclimatisation. Seeds of double low improved rape varieties 'Bolko' and 'Mar' were sown in four replications in 5 m^2.plots. The sowing time depended only on weather conditions and it never exceed the second week of August. Immediately after sowing the soil was inoculated with a mixture of two *V. dahliae* isolates from naturally infected rape plants. Inoculation was achieved by pouring diluted homogenate of fungus cultures into soil. Its inoculum concentration was 3 x 10^6 spores and 2 x 10^5 microsclerotia. Observations of epidemic development were performed in particular years four weeks after sowing, i.e. in the second decade of September. Every other ten days 4 x 50 plants of each of the varieties studies were sampled for analysis. A cross-section segment of conducting tissues was taken from the crown of every plant. The sections were washed for half an hour under running tap water, surface disinfected in 0.1% mercury chloride and after a triple washing in sterile water put onto Petri dishes containing an alcohol medium with streptomycin (Nadakavukaren and Horner 1959). There were five replications. After two weeks of incubation at 25°C, the per cent of sections infested with *V. dahliae* corresponding with per cent of plants infected with pathogen in the day of sampling was assessed. During the whole period of the experiment meteorological data indicating average weekly soil decade temperature at 5 cm were followed to assess the relationship between the increase of infection (expressed in per cent) and soil temperature in the time studied. The development of epidemic plotted in an arithmetic form. The idea was to make a graph of epidemic course in absolute values of plant damage increase in relation to time. Infection was the y-axis, while dates of observations was the x-axis.

Laboratory experiments

A.Effects of temperature on the development of mycelium growing from microsclerotia

Well developed microsclerotia from four-week-old rape *V. dahliae* F-2 isolate were inoculated in five replications at nine temperatures studied onto the agar-glucose-potato medium. Then the dishes with microsclerotia were kept at constant temperatures ranging 0°C-40°C (±1°C, interval 5°C). After one, two, three, four and five weeks of incubation linear growth of the cultures was evaluated. The results were plotted.

B. Effects of low temperatures on over-wintering of microsclerotia

Severely infected rape shoots having visible symptoms of infection as well developed microsclerotia were kept at constant temperatures ranging from 0°C - 25°C (±1°C, interval 5°C). Then every other 7 days from the beginning of the experiment shoots were defrosted for isolation. Small fragments with fungal sclerotia were taken, surface disinfected in 0.1% mercury chloride and after washing with sterile water, finally put onto Petri dishes with acidified agar-water medium (pH 5.5) with streptomycin sulphate (100 ppm) and ethanol

(0.5%) (Nadakavukaren and Horner 1959). After fourteen days of incubation at 25°C per cent microsclerotia which survived unfavourable conditions and germinated giving a white aerial mycelium was determined. The results were evaluated by analysis of variance. Tukey's test was used to compare the means. The data were plotted presenting percent of viable microsclerotia capable of germination in the period studied.

Results

Results of field experiments

The First symptoms of plant infection were observed near the second week of September, more or less five weeks after the sowing. After that infection (expressed in per cent) gradually increased, reaching similar values somewhat different in particular years and for varieties studied. For instance in 1993: 'Bolko' - 20.5%, 'Mar' - 24.5%.

By the third week of October or the first one of November a rapid drop of the number of infected plants was observed, and then remained steady until spring. At the very end of March a re-increase was noted. The growing tendency was maintained up to the end of plant growth, reaching a maximum at harvest. Inhibition of new infections in the period mentioned earlier can be conditioned by soil temperature (red line on the figure). Analysis of the graphs picturing mean decade soil temperatures at 5 cm and the course of infection indicated that the infection stops when soil temperature drops below 5 - 6°C and this is maintained up to the moment when it passes again this border line (Fig. 1A, 1B and 1C).

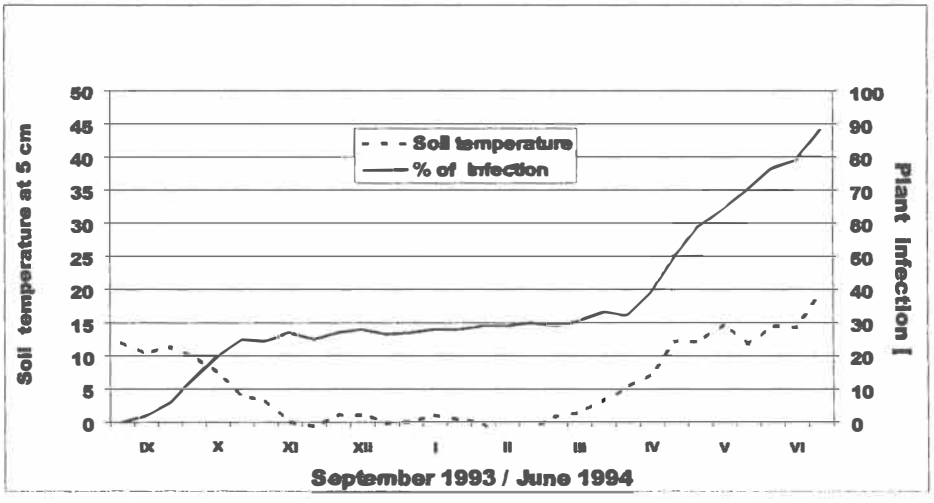

Figure 1. The course of the induced *V. dahliae* epidemic in the whole growth and development periode, Bydgoszcz 1993/1994.

Results of laboratory experiments

Results from field experiments were confirmed by the laboratory experiments on the effect of temperature on germination of microsclerotia and development of pathogen mycelium.

The results of effect of temperature on development of mycelium growing from microsclerotia were given on Fig. 2, showing graphs of linear growth of pathogen mycelium within the range 0°C - 40°C during five weeks. Temperature range in which fungal microsclerotia can develop into mycelium hyphae ranges from about 6°C to 34°C. It explains why no increase of per cent of infected plants was observed in the field experiment from the end of October up to the end of march. Soil temperature was too low, limiting germination of microsclerotia, what is a necessary factor for new infections of plants. However, optimum range in which the growth of mycelia was the quickest was much more narrow (15°C - 28°C).

The *V. dahliae* microsclerotia are very resistant to low temperatures lasting for longer periods. After almost six-month-long period of their storage at temperatures ranking 0°C - 25°C a majority remained still alive and capable for plant infection (Fig. 3). The results suggest that a large part of microsclerotia can maintain their germination capability after many months of relatively severe winter conditions.

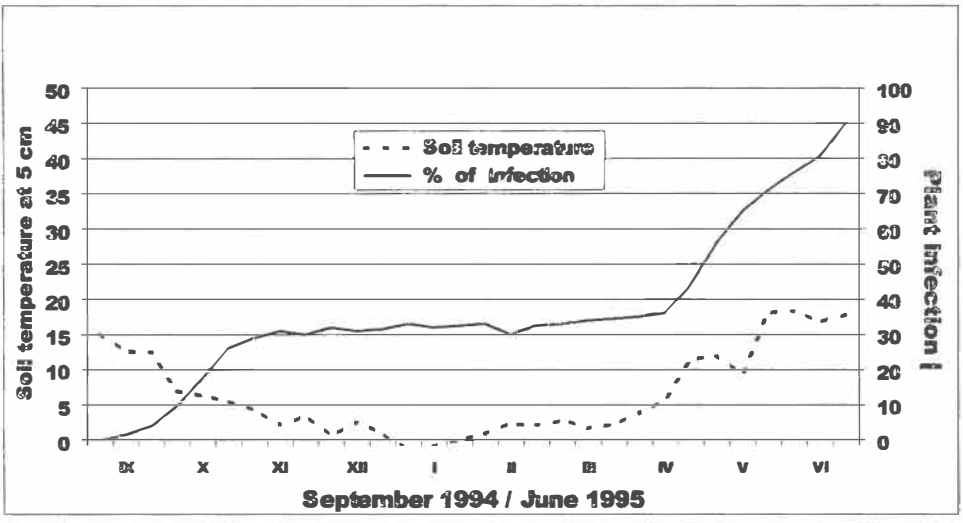

Figure 2. The course of the induced *V. dahliae* epidemic in the whole growth and development periode, Bydgoszcz 1994/1995.

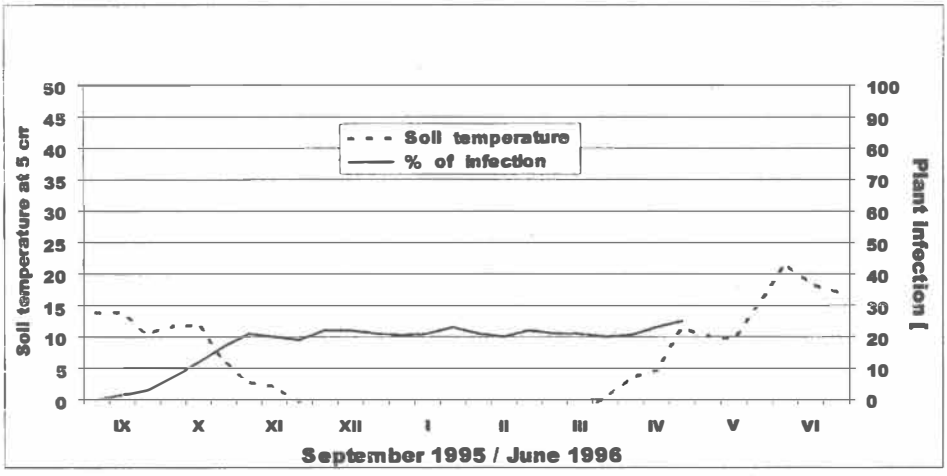

Figure 3. The course of the induced *V. dahliae* epidemic in the whole growth and development periode, Bydgoszcz 1995/1996.

Discussion

The fungus *V. dahliae* does not produce spores on aerial parts of plants and it does not follow the saprophytic way of growth in soil (Jordan and Eddy 1972), while it is closely related to post-harvest residues of the plants infected (Martinson and Horner 1964). Microsclerotia germinate into mycelium using supplies collected earlier and they infect plant roots, most strongly those being very near to them. That is why the main external factor affecting the course of infection seems to be soil temperature, what has been proved in this work. If the temperature declines below the critical point when microsclerotia cannot germinate into mycelium hyphae any more, infection stops. According to some authors this value ranges from 6°C (Devaux and Sackston 1966) to 8°C (Wittman 1971). As was indicated earlier infection stops below about 6°C. These observations were confirmed in laboratory experiments on the effects of temperature on germination of pathogen microsclerotia. As results for literature, *V. dahliae* is not very resistant to higher temperatures and high soil moisture level. Survival rate of microsclerotia decreased rapidly at temperatures above 25°C and soil moisture level higher than 75% maximum water capacity (Fig. 4) (Nadakavukaren and Horner 1961).

Microsclerotia are resistant to low temperatures lasting for longer periods. It can be assumed that after a winter average at our climatic conditions (mean soil temperature at 5 cm during coldest months ranging 5°C - 10°C) a larger part of soil infection potential can be capable of germinating and infecting plants. Fung of microsclerotia kept under such conditions for almost six months maintained their germination ability at the level 86-94% (Fig. 5). They germinated into mycelium capable under natural conditions for infection and infestation of roots and hairy roots of plants near to it.

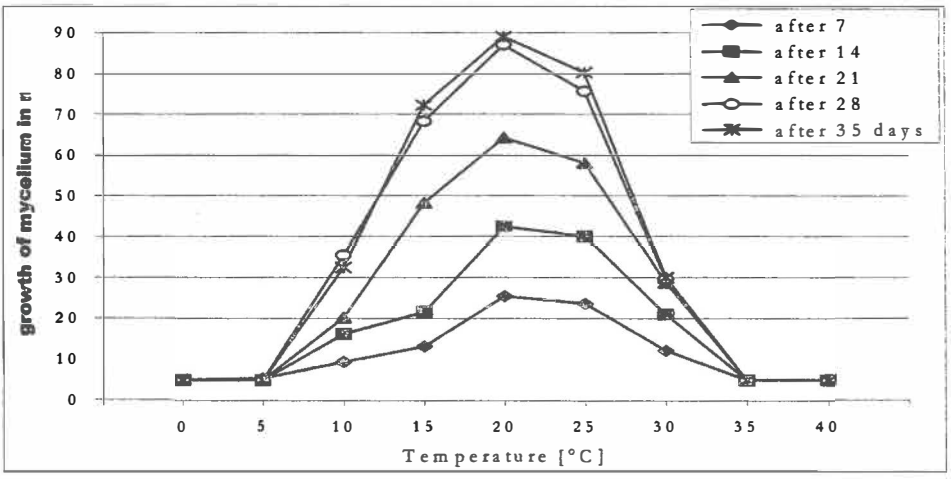

Figure 4. Effect of temperature on mycelium growth from microsclerots.

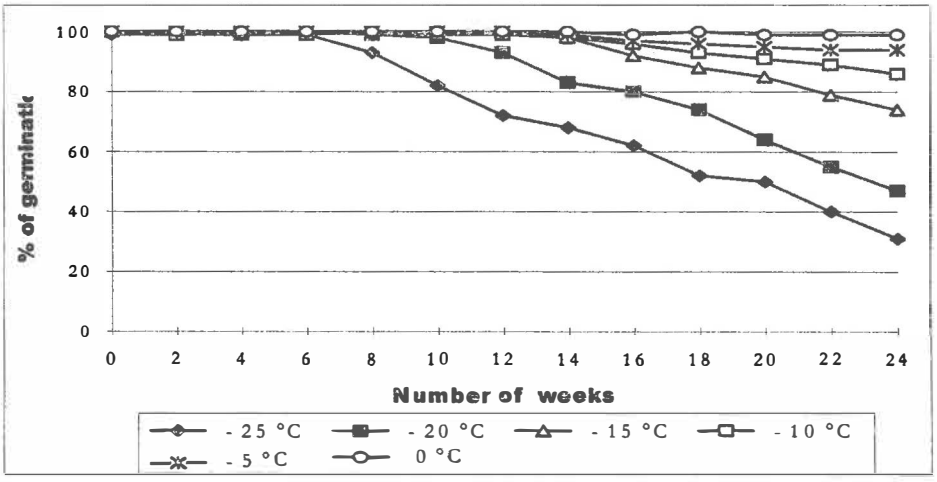

Figure 5. Effect of freezing on germination of *V. dahliae* microsclerots from infected rape stems.

References

Brun, H.; Jacques, M. 1991: Premature ripening in oilseed rape in France: first report on associated fungi. Working group Integrated control in oilseed rape. IOBC- WPRS Bull.: 120-127.

Devaux, A.L.; Sackston, W.E. 1966: Taxonomy of *Verticillium* species causing wilt of horticultural crops in Quebec. Can. J. of Botany 44: 803-811.

Gröntoft, M. 1987: *Verticillium* wilt on rape seed. Proceed. of the 7-th International Rapeseed Congress. Vol. 5: 1228.

Günzelmann, A.; Paul V.H.; Kettrup, A. 1991a: Occurrence, symptomatology, significance and early diagnosis of oilseed wilt caused by *Verticillium dahliae* Kleb. in the Federal Republic of Germany. GCIRC Congress, Proceed., Vol. 1: 262-268.

Jordan, V.W.L.; Eddy, B.P.; 1972: Long Ashton Res. Stn. Rept., 140 - 141. 6. Kroeker G.,1970: Wilt of rape and turnip rape in Sweden caused by *Verticillium*. Abstract. Rev. Plant Pathol. 49: 262.

Krüger, W. 1989: Untersuchungen zur Verbreitung von *Verticillium dahliae* Kleb. und anderen Krankheits und Schaderregern bei Raps in der Bundesrepublik Deusthland. Nachrichtenbl. Deut. Pflanzenschutz. 41: 49-56.

Makowski, N. 1990: Die Winter Raps Produktion in der Deustche Demokratische Republik. Raps 2: 1-79.

Martinson, C.A.; Horner, C.E. 1964: Colonization of plant debris in soil by *Verticillium dahliae*. Phytopath. 54: 900.

Nadakavukaren, M.J.; Horner, C.E. 1959: An alcohol agar medium selective for determining *Verticillium* mikrosklerotia in soil. Phytopath. 49: 527-528.

Nadakavukaren, M.J.; Horner, C.E. 1961: Influence of soil moisture and temperature on survival of *Verticillium* microsclerotia. Phytopath. 51: 66.

Perry, J.W.; Evert, R.F. 1984: Structure of microsclerotia of *Verticillium dahliae* in roots of "Russet Burbank" potatoes. Canad. J. Botany, 62: 396-401.

Svenson, C.; Lerenius, C. 1987: An investigation on the effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) on oilseed rape. Working group Integrated control in oilseed rape. IOBC - WPRS Bulletin: 30-34.

Wittmann, F. 1971: Boden und *Verticillium* - Welke des Hopfens. Bayer Landw. Jb., 48: 206-236.

Zieliński, D.; Sadowski, Cz. 1994: The occurrence of Verticillium dahliae Kleb. on some winter rapeseed crops in Wielkopolska, Pomorze and Kujawy. Rosliny Oleiste - Oilseed Crops, XV, 2: 93-96.

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Results on preservation, epidemiology, and aggressiveness of *Peronospora parasitica* and results with regard to the disease resistance of the pathogen on *Brassica napus*

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Abstract Winter oilseed rape was more or less affected by Downy mildew (*Peronospora parasitica*) in autumn 1996 depending on sowing date and weather conditions. At some locations it occurred randomly so that large areas were totally destroyed at the cotyledon stage. This situation was similar to autumn 1987 when research at our Institute on *P. parasitica* started.

Research was focused on conditions of conidiospore germinability, epidemiological conditions in the field and long-term storage of conidiospores.

Optimal germination require temperatures of 5 to 15 °C and relative humidity of 90 to 98 %. This is also the optimal infection temperature whereas sporulation only occurred at a relative humidity of 98 %. To determine the pracise relation of the sporulation-level to the concentration of conidiospores in the air a daily periodic cycle was recorded using a Burkard-spore trap. The main occurrence of conidiospores was found at 09.00 and 12.00 hours whereas the lowest incidence of conidiospores in the air was recorded between 21.00 and 06.00 hours.

Laboratory tests showed that a long-term preservation of *Peronospora parasitica*-conidia in a freezer at -21 °C for a period of 1 year was possible using glycerine, polyethyleneglycol (PEG) 400 or PEG 1000 as cryoprotective agents. Of these appeared a 20 % (v/v) glycerine / water solution to be most suitable resulting in germination rates of the stored conidia of 21 % after one year.

Differences in virulence of different isolates on the basis of the sporulation-level could be determined using a cotyledon test. In contrast to the field-isolates examined only the generative heterothallic isolate P003 was able to overcome the resistance of the winter oilseed rape cultivar Cresor. This cotyledon test proved to be reliable in determining resistances in oilseed rape cultivars. Compared to greenhouse and field studies to differentiate oilseed rape cultivars according to their susceptibility to *P. parasitica* these results perfectly fitted the results obtained from the cotyledon test.

Introduction

In comparison to other fungal diseases on oilseed rape, Downy Mildew caused by *Peronospora parasitica* (Pers: Ex Fr:) Fr:, has been studied very little despite the fact that this disease is very widespread in many European countries like France, Poland, Germany and the United Kingdom (HARDWICK et al., 1989). This may be partially due to the fact that the disease symptoms of *P. parasitica* in certain stages can easily be mistaken for those of for

example *Phoma lingam*, *Cylindrosporium concentricum* or *Alternaria* spp.. According to SADOWSKI (1987) severe *P. parasitica* infections can cause yield losses of up to 25 %.

Our own research was started in 1987 when severe attacks of oilseed rape by *P. parasitica* occurred due to the mild and humid preceding winter. A similar situation occurred in 1988.

Based upon the data and experiences gathered in these years a parallel programme research in field and greenhouse trials was began in 1989 to determine the response and susceptibility of double-low winter oilseed rape cultivars to *P. parasitica*.

The purpose of this article is to give a short overview concerning the current situation in research on *P. parasitica* and to give some perspectives on basic research with regard to resistance breeding.

Results

In the first preliminary research leaves and cotyledons of nine winter oilseed rape cultivars were used in an in vitro-test to check whether the observed field differences in the susceptibility of these cultivars to P. parasitica could be confirmed. The results obtained seemed to confirm this preliminary suggestion as it was indeed possible to differentiate between cultivars.

These data were used to develop an easy and reliable method to test winter oilseed rape cultivars for their susceptibility to *P. parasitica*. In this tests cotyledons and leaves of different rape cultivars were used in vitro as well as whole plants. The resulting disease symptoms were scaled and based upon these scaling values it was possible to divide rape cultivars into different groups of susceptibility to *P. parasitica*. In these tests, significant differences in the aggressiveness of *P. parasitica*-isolates were found.

Along with the research on aggressiveness of *P. parasitica*-isolates and the susceptibility of rape cultivars it was necessary to determine the life cycle of *P. parasitica* in order be able to transfer to the greenhouse results to open field conditions and to try to predict disease development in the field.

During greenhouse tests a significant influence of temperature on the sporulation of *P. parasitica* on winter oilseed rape was found.

Another important result was the fact that along with temperature the inoculum density and leaf wetness played a major role in the infection process and also on sporulation of *P. parasitica* on infected plants. This sporulation expressed in number of conidia / cotyledon did not rise as inoculum density and leaf wetness, increated.

Using different methods of conidia-capturing in the field by means of microscope slides, Petri-dishes and a Burkard-spore-trap it was possible to measure the quantity of conidia in the field per day (Fig. 1). Based on these data a specific daily schedule of maximum conidia capture could be estimated (Fig. 2a-c). This was done to try to determine specific daytime periods in which, due to high conidiospore-occurrence, a high infection risk and thus a high risk for spread of the fungus could be expected.

On the other hand oospores of *P. parasitica* were found already in the cotyledon stage of winter oilseed rape indicating that these inactive forms of the fungus might be involved in the early stages of epidemiology e.g. early spring when weather conditions are not suitable for sporulation. Unfortunately this hypothesis could not be verified due to the fact that it was not possible to germinate isolated oospores to inoculate winter oilseed rape plants or detached cotyledons or leaves.

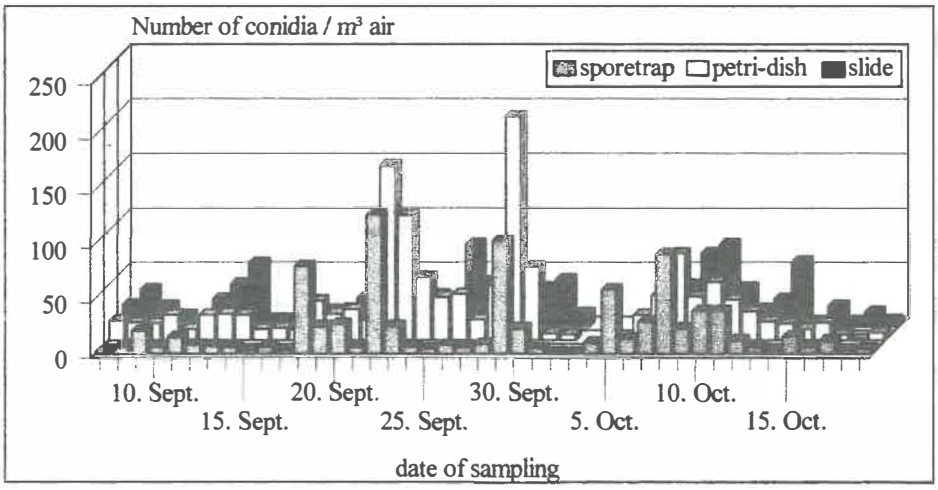
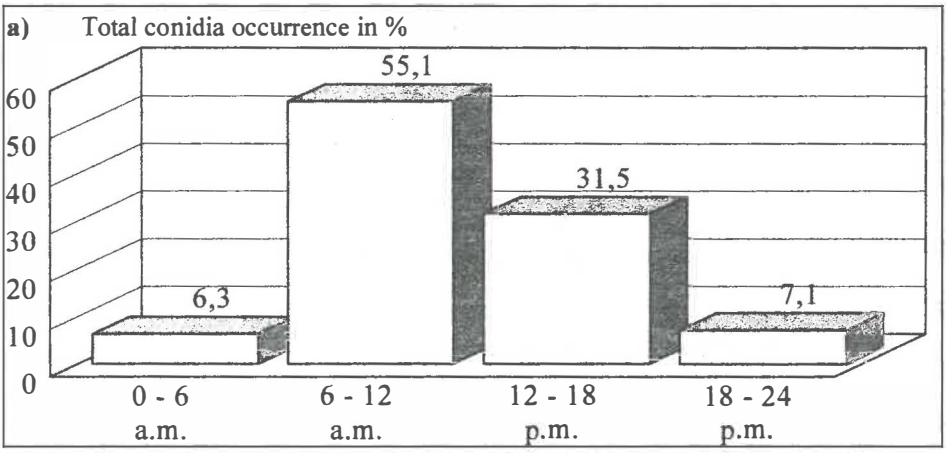
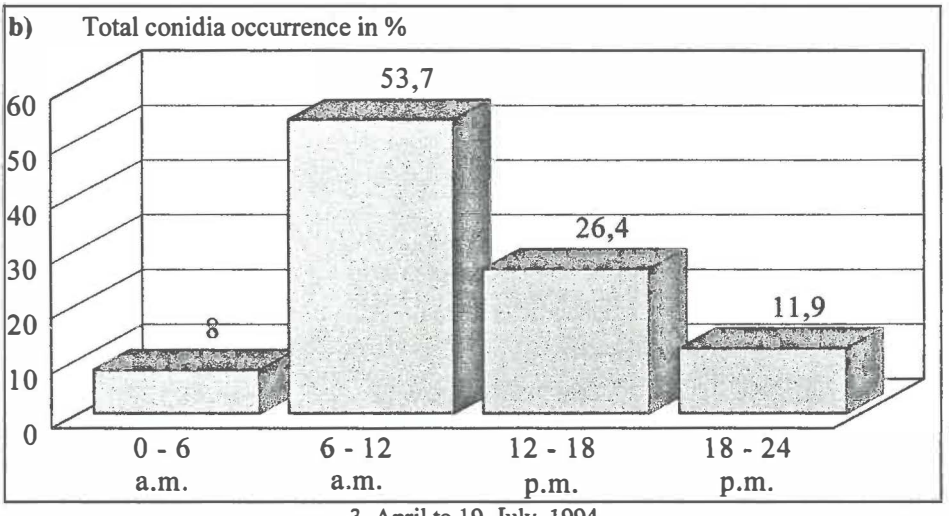


Figure 1. *Peronospora parasitica*-conidia captured by means of a Burkard-sporetrap, petri-dishes and microscope-slides from 7. September, 1993 to 19. December, 1993, in Merklingsen, Versuchsgut der Universität-Gesamthochschule Paderborn, Nordrhein-Westfalen, Germany



7. September to 17. November, 1993



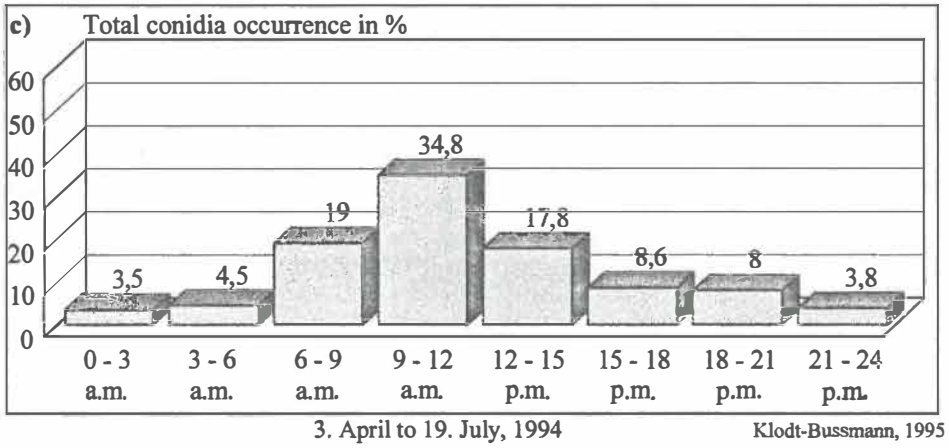


Figure 2a-c. Occurrence of ***Peronospora parasitica***-conidia during the day

Along with the research in the field the method of inoculating cotyledons of oilseed rape was used, as mentioned earlier, to scale the susceptibility or resistance of different winter oilseed rape cultivars to *P. parasitica*.

First the response of nine double-low oilseed rape cultivars to *P. par*asitica was measured finally to result in a ranking of the rape cultivars used (Tab.1).

Table 1. Response of nine double-low oilseed rape cultivars to *Peronospora parasitica* and its sporulation on cotyledons and leaves in a detached-leaf-test

cultivar	sporulation on cotyledons	infected leaf area (in % of the 1. true leaf*)
Cobra	very low	85,7
Liporta	very low	50,0
Liborius	low	46,7
Lirabon	very low	29,4
Ceres	good	35,0
Libravo	good	57,9
Arabella	low	21,4

* no sporulation visible

Klodt-Bussmann, 1995

Simultaneously, field trials were performed with different seed densities to gather data of *P. parasitica*-susceptibility of the rape cultivars under natural conditions.

Nevertheless with all this research mentioned it was of major importance to get to know the most suitable conditions for germination and thus infectiousness of *P. parasitica* conidia. This we found for fresh conidia with a relative humidity of 98 % and a temperature of 25 / 10 °C (day/night) after 24 hours. To ensure quality and aggressiveness of the *P. parasitica*-inoculum used a long-term preservation method for *P. parasitica* conidia was developed. This included deepfreezing of fresh conidia at -21 °C in water with the addition of 20 % of glycerine (KLODT-BUSSMANN, 1995) (Tab. 3).

Table 2. Intensity of Downy Mildew (*Peronospora parasitica*) infestation (%) of six untreated winter oilseed rape cultivars (three different seed densities) in a field trial in 1992/93 (I) and 1993/4 (II)

	Intensity of infestation (%)							
Cultivar	1. Assessment		2. Assessment		3. Assessment		Ø	
	I	II	I	II	I	II	I	II
2 kg / ha seed density								
Envol	3,3 a	3,3 a	0,0 a	0,0	17,5 ab	2,5 a	6,9	1,9
Falcon	31,7 b	26,7 b	47,5 c	10,0	57,5 b	37,5 bc	[illegible]	[illegible]
Liberator	1,7 a	15,0 ab	32,5 bc	2,5	32,5 ab	20,0 abc	22,3	12,5
Lirajet	8,3 a	5,0 a	17,5 abc	0,0	50,0 ab	55,0 b	25,3	20,0

Maxol	3,3 a	13,3 ab	30,0 abc	2,5	12,5 a	5,0 a	15,3	6,9
Samourai	6,7 a	13,3 ab	5,0 ab	0,0	25,0 ab	12,5 ab	12,2	8,6
4 kg / ha seed density								
Envol	8,3 a	13,3 a	7,5 a	0,0	17,5 ab	5,0 a	11,1	6,1
Falcon	45,0 b	41,7 b	57,5 b	12,5	60,0 b	55,0 bc	[illegible]	[illegible]
Liberator	10,0 a	36,7 ab	52,5 b	10,0	10,0 a	22,5 abc	24,2	23,1
Lirajet	13,3 a	15,0 ab	22,5 ab	2,5	35,0 ab	62,5 c	23,6	26,7
Maxol	11,7 a	21,7 a	17,5 ab	5,0	10,0 a	5,0 a	13,1	10,6
Samourai	20,0 ab	11,7 a	10,0 a	0,0	15,0 ab	15,0 ab	15,0	8,9
6 kg / ha seed density								
Envol	13,3 ab	8,3 a	20,0 ab	0,0 a	15,0 ab	7,5 a	16,1	5,3
Falcon	33,3 b	46,7 b	57,5 bc	10,0 b	42,5 b	70,0 b	[illegible]	[illegible]
Liberator	10,0 ab	38,3 b	70,0 c	20,0 b	15,0 ab	20,0 a	31,7	26,1
Lirajet	8,3 ab	20,0 ab	35,0 abc	20,0 b	45,0 b	42,5 ab	29,4	27,5
Maxol	5,0 a	33,3 ab	40,0 abc	17,5 b	2,5 a	5,0 a	15,8	18,6
Samourai	16,7 ab	10,0 a	17,5 a	0,0 a	12,5 ab	7,5 a	15,6	5,8

Corresponding letters do not differ significantly (significance niveau 95 %)

Klodt-Bussmann, 1995

Table 3 Germination [%] of *Peronospora parasitica*-conidia after 12 month of storage in water with cryoprotective substances in concentration of 5 up to 25 % at -21 °C, n = 6

		Germination in %			
cryoprotective	**conc. in %**	**Experiment 1**	**Experiment 2**	**Experiment 3**	**mean value**
control		0,0 a	0,0 a	0,0 a	0,0 a
Glycerine	5	0,0 a	0,5 ab	0,0 a	0,2 ab
	10	0,5 ab	2,5 ab	0,0 a	1,0 ab
	15	20,5 ef	19,5 c	4,5 abc	14,8 de
	20	23,5 f	18,0 c	21,0 c	20,8 e
	25	16,0 def	9,5 abc	14,5 bc	13,3 cde

PEG 400*	5	2,0	abc	9,0	abc	2,5	ab	4,5	abcd
	10	6,0	bcde	5,0	abc	3,5	abc	4, 8	abcd
	15	2,5	abc	5,0	abc	2, 5	ab	3,3	abcd
	20	3,5	abcd	9,5	bc	4,0	abc	5,7	bcd
	25	9,0	cdef	8,0	abc	20,0	c	12,3	cde
PEG 1000**	5	11,0	cdef	3,0	abc	0,0	a	4,7	abcd
	10	7,5	bcdef	0,0	a	1,0	a	2,8	ab
	15	12,5	cdef	0,5	ab	0,5	a	4,5	abc
	20	10,0	cdef	2,5	abc	0,5	a	4,3	abcd
	25	8,5	bcdef	4,5	abc	0,0	a	4,3	abcd

* Polyethylenglycol 400 Klodt-Bussmann, 1995

** Polyethylenglycol 1000

Corresponding letters do not differ significantly (significance niveau 95 %)

Conclusions

Using the detached cotyledon test it is possible to reliably differentiate winter oilseed rape cultivars according to their susceptibility to *P. parasitica*. The results obtained correlate well with field results. Relative humidity and temperature are of major importance for the sporulation of *P. parasitica*. The same is true for the conditions of germination of *P. parasitica* and infection of winter oilseed rape. This means that leaf wetness is not important if the relative humidity needed (98 %) is present.

According to the spore-trap experiments the density of *P. parasitica*-conidia in the air in the field is highest between 9.00 a.m. and 12.00 a.m. and lowest between 9.00 p.m. and 6.00 a.m.. Greenhouse and in vitro studies have indicated that primary infections arise from active mycelium in host plants. Although the pathogen might form oospores to sustain adverse conditions already at the cotyledon stage these oospores are not involved in the epidemiology of the disease in the field.

The conidiospores of *P. parasitica* can be stored in a 20 % (v/v) solution of glycerine in water for at least 12 months in a commercial deepfreezer at -21 °C, with reduction of their germinability of up to 79 %.

P. parasitica exhibits differences in aggressiveness in various isolates depending on the location where they were collected and depending on the winter oilseed rape cultivar. These cultivars show differences in resistance to *P. parasitica*. In practice this resistance may be influenced by the seed density in the field.

Résumé

With regard to differences in aggressiveness of *P. parasitica* and differences in susceptibility of winter oilseed rape cultivars to this pathogen, selection of resistant cultivars is the most suitable way to avoid infections by downy mildew and thus to avoid yield losses. Seed density also is of major importance in practice to avoid losses. A mild and humid winter enhances the threat of *P. parasitica* infection and also the epidemiological spread of this pathogen.

To avoid yield losses due to *P. parasitica* rape cultivars resistant to the most aggressive and virulent *P. parasitica*-isolates known are urgently needed. This goal can only be reached by intensive research on the epidemiology of *P. parasitica* and its most suitable proliferation and infection conditions on the one hand and by developing and applying methods of resistance screening in modern plant breeding. This goal has to be purchased by thorough and intensive co-operation between research institutes and practical plant breeders.

References

Evans E.J., Gladders P., Davies J.M., Ellerton D.R., Hardwick N.V., Hawkins J.N., Jones J.R., and Simkin M.B., 1984: Current status of disease control of winter oilseed rape in England. Aspects of Applied Biology, **6**: 323-324

Frencel I.M., Lewartowska E., and Jedryczka m., 1991: The spectrum and severity of fungal diseases in field infections in winter oilseed rape in Poland. Bulletin SROP **14**(6): 137-140

Hardwick N.V., Culshaw F.A., Davies J.M.L., Gladders P., Hawkins J.H., and Slawson D.D., 1989: Incidence and severity of fungal diseases in winter oilseed rape in England and Wales 1986-1988. Aspects of applied Biology **23**: 383-392

Klodt-Bussmann E., 1995: Untersuchungen zur Epiudemiologie und sortenspezifischen Pathogenität von *Peronospora parasitica* an Winterraps (*Brassica napus* L.). Dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn, Landwirtschaftliche Fakultät, pp. 160

Sadowski Cz., 1987: An investigation on the occurrence and control of downy mildew on winter oilseed rape. Proceedings of the 7th Intern. Rapeseed Congress, Poznan, Poland, **5**: 1079-1103

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Effects of *Verticillium dahliae* on linseed (*Linum usitatissimum*) crops

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Abstract In linseed crops at Rothamsted characteristic symptoms of *Verticillium dahliae* infection (dark brown stripes on the stem) first appeared between late July and early September in 1990 - 1996. By the time that symptoms appeared the pathogen had spread up to the top of the stem and severity of symptoms gradually increased with time. When symptoms appeared early, *V. dahliae* spread from the stems to the capsules and seeds. Seed infection with *V. dahliae* was decreased by surface sterilization and declined with time in storage at 4^{0}C. There were no consistent effects of fungicide seed treatments or sprays during flowering on the development of *V. dahliae* symptoms in crops or the incidence of seed infection. In a field experiment the previous crop had no effect on the severity of *V. dahliae* symptoms, although there was evidence for differences in pathogenicity between isolates from different hosts in pot experiments.

Keywords: *Verticillium dahliae*, linseed, symptoms, epidemiology, seed infection

1. Introduction

Symptoms of infection by *Verticillium dahliae* Kleb were first observed in crops of linseed cultivars of *Linum usitatissimum* L. in the UK in 1990 (Fitt *et al.*, 1992) although the disease had been known for many years on fibre flax cultivars of *L. usitatissimum* in continental Europe. *V. dahliae* is known to be a damaging soil-borne pathogen with a wide host range which is favoured by high soil temperatures (optimum 24^{0}C) (Schnathorst, 1981) but there is no information on the seasonal frequency of its occurrence on linseed in the UK. Infection of linseed seed by *V. dahliae* has been demonstrated (Fitt *et al.*, 1992) but its significance is not clear. Soil-borne inoculum is considered to be the most important source of disease (Schnathorst, 1981) but the influence of previous cropping on the development of disease symptoms in linseed has not been studied. This paper reports observations on the development of symptoms of *V. dahliae* on linseed from 1990 to 1996 and work on the significance of seed-borne inoculum and effects of previous cropping on disease development.

2. Symptom development

The first symptoms of infection by *V. dahliae* observed in linseed crops at Rothamsted were dark brown stripes on the stems and branches of maturing plants, which were most frequently near the tops. With time these symptoms spread round and down the stems until they were completely brown, increasing the severity score (proportion of stem with browning, 0-5 scale) (Fig. 1). Affected stems were brittle and broke easily, and white mycelium was observed at the base of plants with these symptoms when they were pulled up (Fitt *et al.*, 1992). These symptoms were

first observed on some plants in late July/early August in 1990, 1992, 1994 and 1996 but not until early September in 1991 and 1993. The severity score increased rapidly from 1.2 to 4.5 between 8 and 14 August 1990, when the weather was hot and dry. The mean soil temperatures (at 10-20 cm deep) at Rothamsted were 17.3 and 18.2^{0}C, respectively, in July and August 1990. Only 1.3mm of rain fell between 7 July and 14 August and the maximum air temperature was >30^{0}C each day from 1 to 4 August, just before symptoms were first observed.

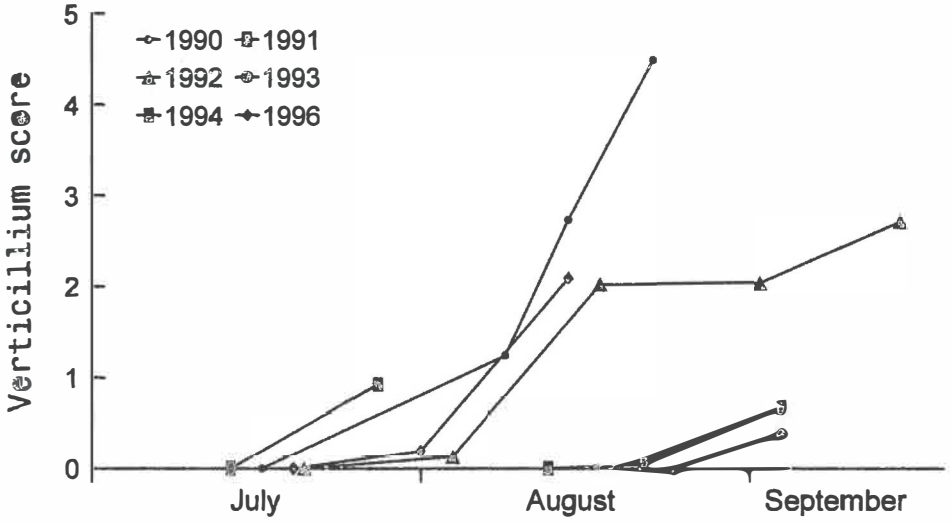

Figure 1. Development of disease caused by *Verticillium dahliae* (severity on a 0 - 5 scale) on stems of linseed (cv. Antares) in unsprayed plots at Rothamsted in 1990 - 94 and 1996.

When pieces of linseed stems or branches with these symptoms were surface sterilized in sodium hypochlorite (1% available chlorine) and placed on V8-juice agar at 20^{0}C, colonies of *V. dahliae* with typical conidiophores and conidia (Fitt *et al.*, 1992) developed. Subsequently large numbers of black microsclerotia developed in these colonies. By isolation, it was shown that *V. dahliae* had already spread up plants to the tops of the stems when symptoms were first observed; the results suggested that the pathogen can spread rapidly up the plant in less than 2 weeks (Table 1). *V. dahliae* was also isolated from linseed plants before symptoms were observed. In the linseed crops the colour of affected stems gradually changed from brown to grey, as large numbers of microsclerotia formed and lifted the surface layers of cells.

Table 1. Percentages of linseed plants (cv. Antares) sampled on three dates in 1996 from which *Verticillium dahliae* was isolated from different plant parts.

	% plants from which *Verticillium dahliae* was isolated		
Plant part	19 July	31 July	14 August
root	0	100	100
stem base	0	100	100
lower stem	0	100	100
upper stem	0	100	100
top of stem	0	100	100
sepals	0	63	100
capsule wall	0	63	100
seed	0	0	0

3. Seed infection

Isolation from capsules sampled from the linseed crops suggested that *V. dahliae* can spread from stems to capsule sepals and capsule walls (Table 1) and ultimately to the developing seeds (Fig. 2). The observed incidence of seed infection was greatest in 1992, when symptoms on the stems had developed early in the season (Fig. 1). No seed infection was observed before mid-August and the incidence of seed infection increased as crops matured. In 1993, no seed infection was detected before harvest but a small incidence of infection was observed on harvested seed (Table 2). *V. dahliae* was isolated from harvested seed when it was stored at -14^{0}C before testing in 1992 and 1993, but not when it was stored at 4^{0}C in 1990 and 1991, even though it had been isolated from seed before harvest in those years. This suggested that the incidence of seed infection may decline rapidly after harvest under normal storage conditions, presumably because *V. dahliae* is present as hyphae or conidia rather than as microsclerotia.

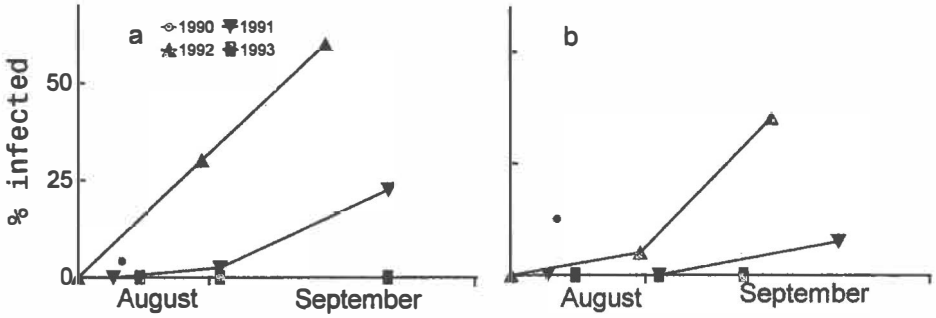

Fig. 2. Incidence (%) of *Verticillium dahliae* in capsule walls (a) and seed (b) of linseed (cv. Antares) in unsprayed plots at Rothamsted in 1990, 1991, 1992 and 1993, assessed by isolation on V8 - juice agar after surface sterilization and dissection of capsules sampled from the crop.

Table 2. Incidence of *Verticillium dahliae* on linseed seed (cv. Antares) harvested from plots with (+) or without (-) fungicide spray treatment in 1990, 1991, 1992 and 1993 on V8-juice agar; seed either unsterilised (US) or surface sterilised (SS) before testing.

Harvest date	Date of testing	Incidence (%)					
		US			SS		
		-	+	SED(df)	-	+	SED(df)
23 Aug 1990	Jan 1991*	0	0		0	0	
10 Oct 1991	Nov 1991*	0	0		0	0	
29 Sept 1992	July 1993†	28.3	45.9	8.05 (27)	7.5	5.8	1.65 (27)
23 Oct 1993	Dec 1993†	4.2	7.5	1.71 (27)	0	0	

* Stored at 4^{0}C after harvest
† Stored at -18^{0}C after harvest

Table 3. Severity of symptoms of *Verticillium dahliae* (score on a 0 - 5 scale) on stems of linseed (cv. Antares) at growth stage 11 in plots with (+) or without (-) fungicide seed treatments and sprays in 1990-1994 and 1996.

	Verticillium score		
	-	+	SED(df)
1990	2.7	2.6	0.33 (15)
1991	0.7	1.2	0.57 (21)
1992	2.7	2.0	0.56 (27)
1993+	0.4	0.4	0.19 (27)
1994+	0.9	0.5	0.25 (13)
1996+	2.1	2.4	0.40 (10)

+ In 1993, 1994 and 1996 seed for all plots received a fungicide treatment

4.Effects of fungicides and previous cropping

There was no evidence that either fungicide seed treatment applied to seed before sowing in 1990, 1991 and 1992 or fungicide spray treatment applied during flowering (for control of grey mould caused by *Botrytis cinerea* Pers. and leaf blight caused by *Alternaria linicola* Groves & Skolko) in 1990 - 1996 affected the development of symptoms of *V. dahliae* in linseed crops (Table 3). This was not surprising since *V. dahliae* is a soil-borne pathogen, initial infection of roots probably occurs after seed treatments have lost their activity and the pathogen then spreads within the plant. Since inoculum of *V. dahliae*, in the form of microsclerotia on infected crop debris, is known to survive for several years (Schnathorst, 1981), crop rotation seems the most appropriate strategy for controlling the disease. However, in an experiment to examine the effects of previous cropping on

the severity of symptoms on linseed, a one-year break with a non-susceptible crop (spring oilseed rape) did not decrease the severity of symptoms (Table 4). There were no differences in symptom development between plots cropped previously with lupin, linseed or sunflowers, providing no evidence for physiological specialization of *V. dahliae* isolates to specific hosts. This conclusion is supported by results of a controlled environment experiment comparing pathogenicity to linseed of isolates of *V. dahliae* from different hosts; there were differences between isolates in the severity of symptoms produced and in the growth of plants but the isolate from potato was more pathogenic to linseed than the isolate from linseed (Table 5).

Table 4. Effects of previous cropping on severity of symptoms of *Verticillium dahliae* (0-5 scale) on stems of linseed (cv. Antares) in 1996 after different crops in 1995*.

Previous Crop	*Verticillium* score		
	25 July	12 August	22 August
Linseed	0	3.1	3.9
Lupin	0	3.2	4.0
Spring rape	0	2.9	3.7
Sunflower	0	3.2	4.0
SED (9 df)		0.26	0.27

* Previous crops: 1994, sunflowers; 1993, spring barley; 1991, winter beans; 1990 & 1992, winter wheat; 1987, 1988 and 1989, linseed.

Table 5. Effects of *Verticillium dahliae* isolates from different hosts on the growth of linseed (cv. Antares) in pot experiment in 1996.

Original host	Verticillium score (0-5 scale)		Plant height (cm)		Plant dry weight (g)
	27 Aug	3 Sept	27 Aug	3 Sept	9 Sept
Control	0	0.3	24.9	29.1	0.97
Linseed	1.1	3.0	24.0	27.8	0.51
Potato	2.0	3.9	18.0	19.9	0.15
Strawberry	1.6	3.3	23.6	26.9	0.48
Sunflower	1.2	3.3	23.6	26.9	0.48
Tomato	1.7	3.4	20.8	22.9	0.23
SED (35 df)	0.16	0.24	0.63	0.83	0.064

5.Discussion

The occurrence of disease caused by *Verticillium dahliae* in linseed crops at Rothamsted in all recent seasons (Fig. 1), together with its occurrence on commercial crops in both England and Germany (Fitt *et al.*, 1992), suggests that this disease may be a widespread, unrecognized problem in linseed crops. Symptoms may not be observed in crops because they occur in the period before harvest when the crop is already turning brown. However, a survey is needed to confirm the extent of the problem in commercial crops. Pot experiments, both at Rothamsted (Table 5) and in Germany (Fitt *et al.*, 1992), demonstrate that *V. dahliae* can greatly decrease the growth, and consequently yield, of linseed. However, it is difficult to confirm these results in field experiments because it is difficult to find a treatment which will eliminate soil-borne *V. dahliae* whilst leaving other factors unchanged. The disease also greatly decreases the quality of stem fibres from linseed or fibre flax crops, rendering them commercially useless, so will need to be considered if the area of *L. usitatissimum* grown for fibre increases in the UK.

Field observations suggest that the effects of *V. dahliae* on the growth and yield of linseed are likely to be greatest in seasons when symptoms appear early in August, rather than just before harvest, although this needs to be confirmed in experiments with plants inoculated at different times. It seems likely that hot, dry seasons will favour the early development of the disease since the optimum temperature for *V. dahliae* is 25^0C (Schnathorst, 1981) but a detailed examination of the relationship between thermal time and symptom development has not yet been done. Furthermore, if such a relationship could be established, it might be possible to identify climatic zones in the UK where the risk of the disease is greatest and to quantify risks associated with any future climate change.

The evidence suggests that strategies for control of disease caused by *V. dahliae* should aim at decreasing the amount of soil-borne inoculum. It seems unlikely that seed-borne inoculum has sufficient longevity to contribute to epidemics the following season (Table 2), unless microsclerotia are present, and fungicide seed treatments and spray treatments at flowering are also ineffective (Table 3). Fungicides have been combined with solarization to control soil-borne *V. dahliae* in high value horticultural crops like strawberry (Hartz *et al.*, 1993) but this strategy would be uneconomic for linseed. The only available option appears to be the use of crop rotations including non-susceptible crops, but results of experiments on previous cropping (Table 4), together with the known longevity of *V. dahliae* microsclerotia (up to 14 years in experiments and the observation of symptoms in a linseed crop after a 6 year break (Fitt *et al.*, 1992)), suggest that long intervals between susceptible crops will be needed to decrease amounts of soil-borne inoculum appreciably.

It is not clear why *V. dahliae* has only recently been recognized as a potentially important problem on linseed. A succession of favourable summers might have meant that symptoms have been recognized more easily but similar summer temperatures were observed before 1990. Changes in cropping patterns, such as the increase in the area of linseed grown, may have increased the concentration of soil-borne inoculum available. Furthermore, populations of *V. dahliae* may have changed to include greater proportions of isolates virulent to linseed. Molecular studies have shown that there is considerable variation in populations of the haploid *V. dahliae* which infects linseed in the UK, and is distinct from the related diploid *V. longisporum* which causes disease on winter oilseed rape in other countries (Karapapa *et al.*, 1997) but work is required to investigate *V. dahliae* populations from different seasons and from sites with different cropping histories and to relate molecular differences between isolates to differences in pathogenicity to different hosts.

Whilst the risks posed by soil-borne inoculum of *V. dahliae* to horticultural crops such as strawberry (Harris & Yang, 1990) have been recognized for many years, it is only recently that the risks to arable crops are becoming apparent. Besides linseed, crops such as potato (Isaac & Harrison 1968), sunflower (Church & McCartney, 1995) and lupins (Bateman *et al.*, 1991) are known to be susceptible to the pathogen. The risks to these arable crops, and to horticultural crops in the same rotation, need to be assessed, especially if these crops are to be grown more widely.

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References

Bateman, G.L., Jenkyn, J.F., Johnson, S.A. & Da Silva, L. (1991). Fungal pathogens of grain lupins, 1986-1991. *Aspects of Applied Biology* **27**, 129-132.

Church, V.J. & McCartney, H.A. (1995). Occurrence of *Verticillium dahliae* on sunflowers (*Helianthus annus*) in the UK. *Annals of Applied Biology* **127**, 49-56.

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

Harris, D.C. & Yang, J. (1990). Preplanting prediction of strawberry wilt (*Verticillium dahliae*) risk as an aid in disease management. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases* **1991**, 117-122

Hartz, T.K., Devary, J.E. & Elmore, C.L. (1993). Solarization is an effective soil disinfection technique for strawberry products. *Horticultural Science* **28**, 104-106.

Isaac, I. & Harrison, J.A.C. (1968). The symptoms and causal agents of early dying disease (Verticillium wilt) of potatoes. *Annals of Applied Biology* **69**, 231-244.

Karapapa, V.K., Bainbridge, B.W. & Heale, J.B. (1997). Morphological and molecular characterization of the new species *Verticillium longisporum* pathogenic to oilseed rape. *Mycological Research* (In Press).

Schnathorst, W.C. (1981). Life cycle and epidemiology of Verticillium. In: *Fungal Wilt Diseases on Plants* (Ed. by Mace, M.E., Bell, A.A. & Beckman, C.H.), pp. 81-111, Academic Press, New York.

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Occurrence of False Flax Diseases (*Camelina sativa* (L.) CRTZ.) in Field Trials in Germany in 1995 and 1996

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Abstract In field experiments in 1995 and 1996 assessments of disease incidence on 10 cultivar/breeding lines of false flax (*Camelina sativa* (L.) CRTZ.) were carried out. The assessments were made at the emergence, leaf production, stem extension, flowering and maturity growth stages. The diseases observed were: white rust (*Albugo candida*), grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphe* spp.), downy mildew (*Peronospora parasitica* [Syn.: *P. camelina*]), sore shin and damping (off *Rhizoctonia solani*), stem rot (*Sclerotinia sclerotiorum*), verticillium wilt (*Verticillium dahliae*) and a bacterial anthracnosis identified as *Pseudomonas syringae* pv. spec.

In 1995 and 1996 low disease occurrence at all locations and on all cultivars/breeding lines of *C. sativa* was observed. Anthracnosis caused by *Pseudomonas syringae* pv. spec. appeared most often at all locations was. In one location (Thüringen) up to 75% of the plants were affected.

The yields ranged between 10 dt/ha (Kleinmachnow/ Brandenburg) and 23 dt/ha (Thüle/Nordrhein-Westfalen) in 1995 with an average of about 15 dt/ha and between 7 dt/ha (Merklingsen/Nordrhein-Westfalen) and 33 dt/ha (Thüle/Nordrhein-Westfalen) in 1996 with an average of about 20 dt/ha.

Introduction

False flax or gold of pleasure (*Camelina sativa* (L.) CRTZ.) is a very old oilseed crop. It was first used in the late Neolithikum 4000-1750 B.C. The earliest findings of false flax being used by humans is from 2000 B.C. Findings have determined that it was cultivated in the lower Rhein region (Germany), the Netherlands and the north coast of Germany around 500 B.C. Other crops grown around that same period until 300 A.D. were barley, oats, linseed, and field beans. The oldest known evidence of larger agricultural cultivation of false flax is from the 15th. Century. After that, the cultivation of false flax was reduced for no known reason. In the 16th. Century it was referred to as a weed. The cultivation of false flax continued only in areas not suitable for other crops such as the Schwäbische Alps (Germany), where it was planted in small amounts for the farmers own use. This continued up until the early 1950's (SCHUSTER, 1992). In parts of Russia and Poland this planting practice is still being followed.

In the search for regenerable energy resources, false flax has been re-discovered to be a possible, viable alternative. At present, the oil from false flax can not be used alone. However, in combination with other oils, such as linseed, it can be used in soaps, laquers and paints.

Advantages of false flax are e.g. its ability to grow in poor soils, its comparatively short growing period (100- 120 days), its low demand on water resources and its health.

False flax belongs to the same family as oilseed rape (Cruciferae). It has small pale yellow flowers which are arranged in a racemous inflorescence. The fruit is divided into two chambers with 4-5 very small gold-yellow seeds each, and a thousand grain weight (tgw) between 0,7 to 1,6 g.

The main goals of these studies were to evaluate the occurence of *C. sativa* diseases in the field in different regions of Germany with special regard to different varieties and to develop infection methods for the main pathogens under laboratory conditions as a tool for resistance breeding.

Material and Methods

In field plot trials 10 cultivars/breeding lines (Table 1) of false flax (*Camelina sativa* (L.) CRTZ.) were grown at different locations in Germany and assessed for disease susceptibility in the years 1995 and 1996. In 1995, eight locations and in 1996, seven locations (Table 2), were used.

Table 1: Cultivars/breeding lines of false flax (*Camelina sativa* (L.) CRTZ.) used in field plot trials at different locations in Germany in 1995 and 1996.

Cultivar- / Breeding line-No.	Descent
1	Lindo (DSV)
2	Bavaria (DSV)
3	Soledo (DSV)
4	Licalla (DSV)
5	Limaga (DSV)
6	Ligena (DSV)
7	095/1 (breeding line)
8	095/2 (breeding line)
9	095/3 (breeding line)
10	095/4 (breeding line)

Table 2: German locations used for field trials with false flax (*Camelina sativa* (L.) CRTZ.) in 1995 and '96

Location No.	sites and states in 1995	sites and states in 1996
1	Merklingsen (Nordrhein-Westfalen)	Merklingsen (Nordrhein-Westfalen)
2	Thüle (Nordrhein-Westfalen)	Thüle (Nordrhein-Westfalen)
3	Groß-Gerau (Hessen)	Groß-Gerau (Hessen)
4	Rauischholzhausen (Hessen)	Rauischholzhausen (Hessen)
5	Dornburg/Rohrbach (Thüringen)	-
6	Thyrow (Mecklenburg-Vorpommern)	-
7	Kleinmachnow (Brandenburg)	Kleinmachnow (Brandenburg)
8	Kritzkow (Mecklenburg-Vorpommern)	Kritzkow (Mecklenburg-Vorpommern)
9	-	Lübeck (Schleswig-Holstein)

Two methods of assessments were used. In the first year 10 Plants were taken in a W-like pattern from each plot and then assessed for disease symptoms. Since false flax has not been cultivated for some time it was not known what symptoms and diseaes to expect. Therefore, in addition to the field assessments laboratory methods were used for the identification of unknown pathogens (surface sterilisation of diseased plant parts, isolation of the pathogens, cultivation on different culture media and identiffication by means of microscope). In the second year the percentage of plants with specific diseases and symptoms was estimated for each plot. The assessments for disease susceptibility were carried out at the growth stages shown in Table 3.

Table 3: Assessment stages for false flax in field trials 1995 and '96

assessment No.	growth stages in 1995		growth stages in 1996	
1	EC 53 to EC 70	pod development	EC 10 to 19	emergence
2	EC 76 to EC 89	leaf to stem senescence	EC 20 to 27	leaf production
3	EC 92	stubbles	EC 60 to 69	flowering
4	EC 01	seeds	EC 80 to 89	senescence

Results

In the two years of this study false flax showed comparatively little degree of disease symptoms. Nevertheless it was possible to find most of the symptoms and pathogens described by SPAR et al. (1990). The main fungal pathogens (diserases) found were: *Sclerotinia sclerotiorum, Rhizoctonia solani, Albugo candida, Peronospora parasitica* (Syn.: *P. camelinae* GÄUM.), *Erysiphe* spp., and *Botrytis cinerea.* Furthermore a bacterium

identified as *Pseudomonas syringae* pv. spec. by Prof. Rudolph (University of Göttingen) was found.

In 1995 more than 30 symptoms were found, but only 7 diseases could be identified (s.a.). The occurrence varied widely between the different locations (Table 4), except for *Pseudomonas syringae* pv. spec. which was found on all sites. No difference in disease susceptibility could be found between the cultivars/breeding lines when all locations were compared.

In 1996 the same 7 pathogens were found. But compared to 1995 the overall disease occurrence was lower and not as many symptoms were found as in 1995. An exception was powdery mildew which appeared in EC 85 and infected close to 100% of the plants at some sites (e.g. Merklingsen) (Tab. 5).

The one pathogen that was found most often in both years was *Pseudomonas syringae* pv. spec.. There were great differences in the occurrence of this pathogen between the different locations but no difference of susceptibility was found between the 10 cultivars/breeding lines under field conditions. (Table 4 + 5). In 1995 up to 70% (Groß Gerau) and in 1996 up to 80% (Merklingsen) of the plants were affected.

Table 4: Summary of the most important disease assessment results in false flax field trial experiments in 1995 (occurence of diseases/pathogens per site in %, n= 10)

Location	growth stages	anthracnosis (*Pseudomonas syringae* pv. spec.)	sore shin /damping off (*Rhizoctonia solani*)	stem rot (*Sclerotinia sclerotiorum*)	powdery mildew (*Erysiphe* spp.)	white rust (*Albugo candida*)
Groß-Lüsewitz	EC 57	10	-	-	-	-
	EC 75-80	45	-	-	-	-
Groß-Gerau	EC 64-67	24	-	-	-	-
	EC 88	37	-	-	-	-
Merklingsen	EC 53-61	10	-	-	-	-
	EC 80	59	10	-	10	10
Kleinmachnow	EC 80	65	-	-	10	-
Kritzkow	EC 67	10	-	-	10	-
	EC 80	44	-	-	-	-
Rauischholzhausen	EC 57	10	-	-	-	-
	EC 82	30	-	-	-	-
Rohrbach	EC 75	10	-	10	-	-
	EC 80	75	-	10	59	-
Thüle	EC 70	10	-	10	-	-
	EC 80	42	10	10	56	15
Thyrow	EC 60-65	10	-	-	-	10
	EC 76	47	-	-	10	-

- = not found

Table. 5: Summary of the most important disease assessment results in false flax field trial experiments in 1996 (occurence of diseases/pathogens per site in %)

Location	growth stages	anthracnosis (*Pseudomonas syringae* pv. spec.)	sore shin /damping off (*Rhizoctonia solani*)	stem rot (*Sclerotinia sclerotiorum*)	downy mildew (*Peronospora parasitica*)	powdery mildew (*Erysiphe* spp.)	white rust (*Albugo candida*)
Groß-Gerau	EC 63	-	<1	-	vz	-	-
	EC 79	<1	<1	1	vz	-	-
	EC 89-92	<1	-	-	-	54	-
Kleinmachnow	EC 33-51	-	7,2	-	-	-	-
	EC 69-71	<1	-	-	-	-	vz
	EC 85	1	-	-	-	33,25	-
Kritzkow	EC 21-25	-	7,25	-	-	-	-
	EC 64	<1	-	-	-	-	-
	EC 75	37	-	<1	-	-	vz
Lübeck	EC 25-51	-	<1	-	-	-	-
	EC 64-69	vz	-	-	-	-	-
	EC 83	<1	-	vz	-	1	-
Merklingsen	EC 69-71	vz	-	-	-	-	-
	EC 79	68,62	-	vz	-	99,7	<1
Rauischholzhausen	EC 53	-	-	-	vz	-	-
	EC 64-69	vz	-	-	<1	-	-
	EC 79	1	-	<1	<1	1	-
Thüle	EC 79	1,6	-	-	-	-	-
	EC 85	3,4	-	<1	-	59,25	<1

- = not found

vz. = sporadic appearance (less than 5 Plants/plot)

The yields ranged between 10 dt/ha (Kleinmachnow) and 23 dt/ha (Thüle) in 1995 (Table 6) with a means of 15 dt/ha and between 7 dt/ha (Merklingsen) and 33 dt/ha (Thüle) in 1996 (Table 7) with a means of 20 dt/ha. The average yield from all ten cultivars/breeding lines per location for 1995 are shown in Fig. 1 and for 1996 in Fig. 2.

Table 6: Average yield results of false flax in field trials in 1995 (in dt/ha; converted to 91% dry matter)

Cultivar- / Breeding line-No.	Merklingsen (n = 5)	Rohrbach (n = 1)	Thyrow (n = 1,5)	Rauischholzhausen (n = 1)	Groß Gerau (n = 1)	Kleinmachnow (n = 1)	Thüle (n = 2)
1	15,5*	18,2	13,0	13,3	12,8	12,9	20,4
2	19,9	20,4	12,2	14,9	15,3	10,0	20,6
3	22,5	24,9	13,8	15,7	11,5	12,7	18,7
4	18,8	19,5	9,6	16,7	12,7	10,1	19,2
5	24,5	19,8	11,7	15,4	11,7	7,9	26,4
6	23,0*	21,3	11,7	15,1	13,5	8,9	29,0
7	20,9	18,5	14,7	14,5	15,5	7,0	20,2
8	19,6	15,8	11,4	12,7	15,3	6,4	19,6
9	20,6	20,1	15,5	14,7	11,2	7,1	22,2
10	21,4	18,8	16,2	16,1	14,8	6,7	23,3

* Average calculated from n=4

Table 7: Average yield results of false flax in field trials in 1996 (in dt/ha; converted to 91% dry matter)

Cultivar- / Breeding line-No.	Merklingsen (n=6)	Thüle (n=2)	Kritzkow (n=2)	Groß-Gerau (n=1)	Rauischholzhausen (n=1)	Kleinmachnow (n = 2)
1	8,6	29,3	22,2	15,6	25,8	13,31
2	11,6	34,6	18,4	17,4	22,8	19,12
3	11,4	32,8	20,0	16,2	22,2	17,59
4	9,9	31,8	15,8	14,1	27,6	11,40
5	9,6	34,5	20,5	16,9	28,3	15,04
6	7,1	34,5	17,9	18,0	26,9	20,29
7	9,0	31,4	15,7	16,7	27,7	16,64
8	7,3	34,1	18,5	17,5	19,8	20,43
9	8,5	28,1	18,3	15,5	25,1	18,54
10	7,3	28,3	17,4	15,8	19,8	13,34

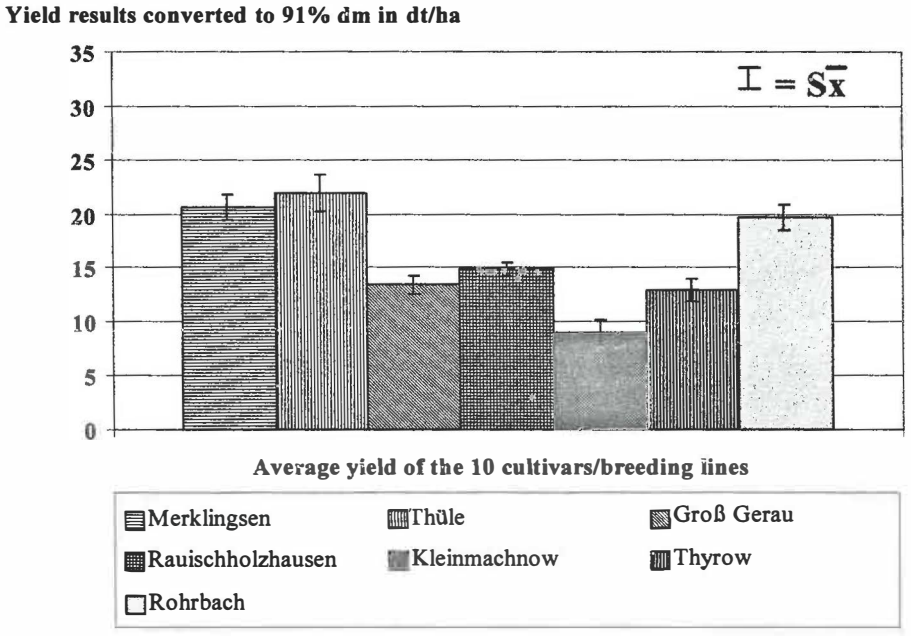

Figure 1: Yield results of a field trial on 7 locations with 10 cultivars/breeding lines of summer false flax in 1995

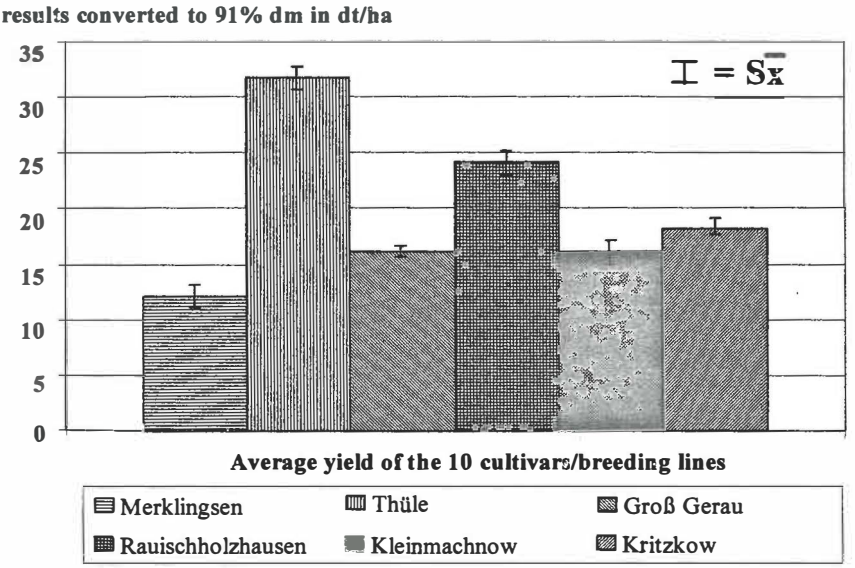

Figure 2: Yield results of a field trial on 6 locations with 10 cultivars/breeding lines of summer false flax in 1996

In the two years of our study most diseases appeared after the stem extension stage (EC 20-25) except for *Rhizoctonia solani* which was found as early as the four leaf stage (EC 14). Downy mildew (*P. parasitica*) appeared when false flax showed the first flower buds, *Ps. syringae* pv. spec., powdery mildew (*Erysiphe* spp.), stem rot (*Sclerotinia sclerotiorum*) and white rust (*Albugo candida*) appeared at an early ripening stage, grey mold (*Botrytis cinerea*) appeared also at an early ripening stage, but apparently only on weakened plants.

Discussion

Altogether, the first results obtained indicate that false flax is not as disease resistant as expected. Most pathogens described in the literature (SPAR et al., 1990) were found and an additional pathogen, *Ps. syringae* pv. spec., has been identified. The main pathogens described for false flax are: white rust (*Albugo candida*), downy mildew (*Peronospora parasitica*) and sore shin/damping off (*Rhizoctonia solani*) (RÜTHER, 1957; BECKER-DILLINGER, 1928) other authors have found stem canker (*Phoma lingam*), light leaf spot (*Cylindrosprium concentricum*), clubroot (*Plasmodiophora brassicae*) and verticillium wilt (*Verticillium dahliae*) (SPAR et al., 1990). So far, with exception of powdery mildew and *Ps. syringae* pv. spec., the degree of infection was not very high and *Phoma lingam*, *Plasmodiophora brassicae* and *Verticillium dahliae* have not been found under field conditions so far. Since it has been established in laboratory trials that *Plasmodiophora brassicae* and *Verticillium dahliae* can infect false flax they have to be expected to appear under field conditions as well. Furthermore it has to be expected that the occurrence of pathogens will increase if false flax is cultivated on a regular basis as an oilseed crop.

It has not been possible to show a difference in disease susceptibility between the cultivars/breeding lines under field conditions in the studies, mainly for the reason of low incidence. There might be another probable reason for this effect. False flax has not been genetically improved for some time and the material currently cultivated is still genetically quite homogenous.

The yield results were higher in 1995 than in 1996 except for Meklingsen. Here the yield results were reduced due to a caterpillar infestation. The yield results of cultivars/breeding lines were similar in each location but there were great differences between the different locations because of the many different soil types used in this study. A further effect of the low disease incidence is, that it was not possible to show a correlation between disease and yields.

Summary

The yields of false flax from field experiments in Germany ranged between 15 dt/ha (average 1995) and 20 dt/ha (average 1996) with a maximum of 29 dt/ha in 1995 and 34,6 dt/ha in 1996.

The most commonly found fungal diseases were: stem rot (*Sclerotinia sclerotiorum*), sore shin and damping off (*Rhizoctonia solani*), white rust (*Albugo candida*), downy mildew (*Peronospora parasitica* [Syn.: *P. camelina*]), powdery mildew (*Erysiphe* spp.), and grey mould (*Botrytis cinerea*).

Furthermore an up to now unknown bacterium identified as *Pseudomonas syringae* pv. spec. was found to seriously infect false flax at all locations.

Disease appearance was low in both years, except for *Ps. syringae* pv. spec. This bacterium affected up to 75% (Rohrbach) of the plants in 1995 and up to 68% (Merklingsen) in 1996.

Prospects

1. Field trials with false flax will be continued at different locations in Germany. The main attention is directed on disease occurrence under field conditions with special regard to host changes of pathogens occurring on other cruciferae and the yields.
2. Furthermore a field trial is planned to evaluate crop rotation diseases of false flax. For this purpose false flax will be grown on the same location two years in a row.
3. Further cultivars/breeding lines will be added to the program.

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References

Rüther, H., 1957: Untersuchunngen über den Anbauwert von Leindotter als Ölfrücht.

Spar, D.; Kleinhempel, H.; Fritsche, R.; 1990: Öl- und Faserpflanzen. Springer Verlag

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

Schuster, W.; 1992: Ölpflanzen Europas. DLG-Verlag

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First laboratority investigations on the reaction of cultivars and breeding lines of *Camelina sativa* (L.) CRTZ. to downy mildew (*Peronospora parasitica* (syn.: *P. camelina*) clubroot (*Plasmodiophora brassicae)* and Verticillium wilt (*Verticillium dahliae*)

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Abstract Different cultivars/breeding lines of false flax (*Camelina sativa* (L.) CRTZ.) shown in Table 1, were inoculated with *Peronospora parasitica* (syn.: *P. camelina*), *Plasmodiophora brassicae* and *Verticillium dahliae* under laboratory conditions and evaluations for disease susceptibility were carried out in the field.The work represents the first variety/cultivar screenings with *P. parasitica* (syn.: *P. camelina*), *P. brassicae* and *V. dahliae* were accomplished.

In Rauischholzhausen *P. parasitica* was observed before false flax started flowering. Spores were collected from heavily sporulating plants, preserved, and used for inoculation experiments. In growth chamber experiments it was established that false flax could be infected with *P. parasitica* at the cotyledon stage and that spores germinated best at temperatures of 12/19°C at a dark/light period of 10/14 hours. All 10 cultivars/breeding lines were inoculated under these conditions and differences in the susceptibility of the cultivars/breeding lines were observed.

Since false flax belongs to the same family (*Crucifereae*) as oilseed rape (*Brassica napus*), it was expected that false flax could be infected by winter oilseed rape pathogens. To answer this question false flax was inoculated with the following important oilseed rape pathogens: *Pl. brassicae* from a winter oilseed rape field near Lübeck [Schleswig-Holstein] and *V. dahliae* isolated from winter oilseed rape. The results indicated that the isolates of *V. dahliae* used were able to infect false flax.

Introduction

While searching for new crop plants especially with regard to renewable natural resources in the furnish of culture plants, *Camelina sativa,* an old crop plant was re-discovered. The oil of *C. sativa* can be used to produce pigments, cosmetics and varnishes. Yet, the cultivation of false flax is not, at the moment significant in Germany.

To make *C. sativa* a reasonable crop for industrial purposes, it is necessary to reduce the heterogeneous composition of the oleic acids and to limit the contents of glycosinolate. Therefore breeding programs with *C. sativa* should combine this main goal on the one hand with breeding for disease resistance on the other. For such a breeding program it is necessary to first determine all possible diseases affecting *C. sativa.* Secondarly, effective and reliable inoculation methods as well as reliable assessment- and scaling-methods for the various diseases need to be established.

Since both false flax and oilseed rape belong to the family of *Cruciferae* and the culture of winter oilseed rape is already established, it has to be determined whether or not pathogens of winter oilseed rape can also affect *C. sativa*.

Table 1: Cultivars/breeding lines of false flax (*Camelina sativa* (L.) CRTZ.) used in laboratory research in 1995 and 1996.

cultivar- / breeding line-No.	descent
1	Lindo (DSV)
2	Bavaria (DSV)
3	Soledo (DSV)
4	Licella (DSV)
5	Ligena (DSV)
6	Limaga (DSV)
7	095/1 (breedingline)
8	095/2 (breedingline)
9	095/3 (breedingline)
10	095/4 (breedingline)

Material and Methods

Downy Mildew (*Peronospora parasitica*)

In Rauischholzhausen *P. parasitica* was observed before false flax started flowering (EC 50-57). From heavily infected plants conidia were collected, preserved and used for inoculation experiments. In growth chamber experiments it was established that false flax could be infected with *P. parasitica* at the cotyledon stage and that spores germinated best at temperatures of 12/19°C and 14 hours light.

For genotype screening, plants were grown in multi-plant-plates up to the 2 or 4 leaf stage (EC 20-25), and inoculated by spraying with a conidia suspension of 10^4 conidia/ml. Each plate was treated with 10 ml of this conidia suspension. After inoculation the plates were covered with a plastic lid to reach 98% humidity and incubated at 12/19°C and 14 hours light in a climatic chamber for seven days. Subsequently the plants were assessed according to the scale shown in Table 2. Winter oilseed rape (Diadem) was treated similarly.

Table 2 : Assessment schema for *Peronospora parasitica (syn.P. camelinae)* on *Camelina sativa*

Assessment value	Sporulation area on the first two leaves in %
1	no sporulation
2	1 - 25 %
3	26 - 50 %
4	51 - 75 %
5	76 - 100 %

Clubroot (*Plasmodiophora brassicae*)

Pl. brassicae has not been found on false flax under field conditions so fare. For inoculation studies with *Pl. brassicae*, contaminated soil from a winter oilseed rape field near Lübeck was used. The soil was mixed 1:1 with peat and this substrate was used to grow *C. sativa*. As a control, the winter oilseed rape cultivar Diadem (Ko = winter oilseed rape) was used and for false flax the cultivar one (Ko_1). The disease were incubated under greenhouse conditions. After 40 days the plants were inspected for the presence and size of galls.

Verticillium Wilt (*Verticillium dahliae*)

To examine the possible transmission of *V. dahliae* from winter oilseed rape to *C. sativa* two isolates of *V. dahliae* from winter oilseed rape were used. The isolates (V10, V33) were obtained from the laboratory of „Universität Gesamthochschule Paderborn". An inoculation method has already been established for winter oilseed rape (Moser and Sackston, 1973) and was used to inoculate *C. sativa*. The isolates were incubated 14 days at room temperature on an orbital shaker (120 rpm) in a nutrient growth medium (Czapex Dox Broth, Difco) and afterwards filtered through gauze (100 µm diameter) to separates spores from the mycelium. Spores were adjusted to a density of 10^6 spores/ml in water. The plant material was grown in a special substrate (Seramis) in a climatic chamber (12/19°C and 14 h light, 60 % humidity) up to the 4 leave stage (EC 20-25). The plants were then taken out of the substrate, washed and the roots trimmed with a razor blade to a total length of 4 cm and placed in the spore solution in the climatic chamber under the conditions described above. After 24 h the plants were replanted in sterilised soil (sand: peat 1:3) and cultured in a climatic chamber under the conditions mentioned above. To obtain a wilt-index the plants were assessed as described in Table 3.

Table 3: Assessment schema for the assessment of Verticillium Wilt severity on *Camelina sativa* (according to HOLTSCHULTE, 1992).

Assessment value	Symptom severity
1	no symptom
2	less than 25 % of the total leaf area is chlorotic
3	25-50 % of the total leaf area is chlorotic
4	50-75 % of the total leaf area is chlorotic
5	more than 75 % of the total leaf area is chlorotic
6	plant dead

Results

Downy Mildew (*Peronospora parasitica*)

The results of the spray inoculation of *C. sativa* are shown in Fig.1. All false flax plants showed symptoms, however the control (Diadem) showed no effect. Furthermore differences in the susceptibility of the 10 cultivars/breeding lines of false flax were observed. The cultivars 3, 5 and 8 showed a higher susceptibility than the other cultivars.

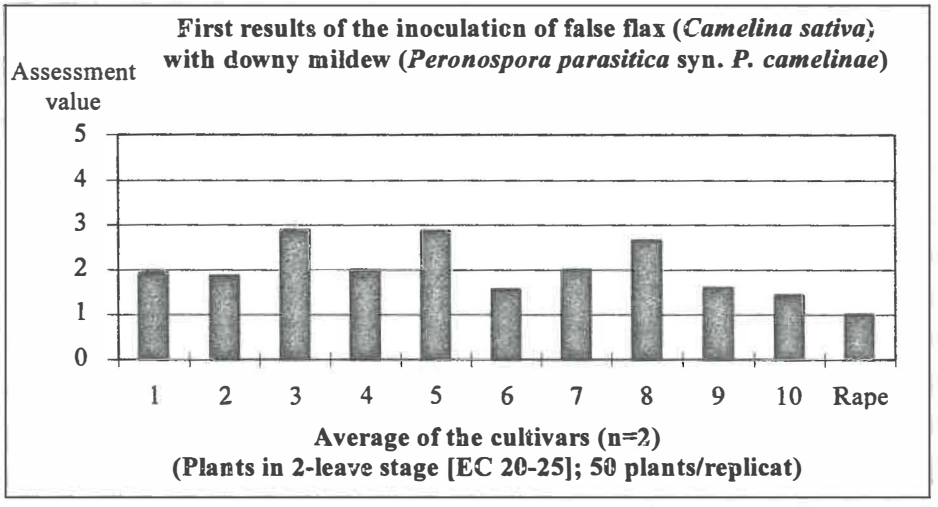

Figure 1: Results of spray inoculation with *Peronospora parasitica* in the 2 leave stage (EC 20-25). The assessment was done as shown in Table 3.

Clubroot (*Plasmodiophora brassicae*)

The results of this study showed that it was possible to transfer *Pl. brassicae* from winter oilseed rape to *C. sativa*. All plants showed typical galls on their roots and the resting spores were found. No differences in the susceptibility of the 10 cultivars/breeding lines of false flax used were observed in the severity of developed galls (Fig.2). However there is a difference in the length of *C. sativa* plants between the control and the inoculated plants (Fig.3). The size of the galls of the cultivar/breeding line 2 was larger than that of the other *C. sativa* cultivars/ breeding lines (Fig.4).

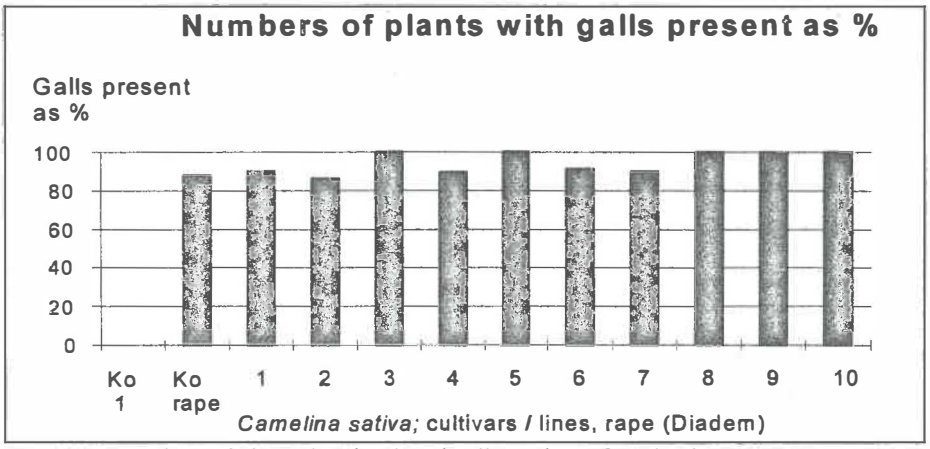

Figure 2: Numbers of plants that developed galls 49 days after planting, n=20.

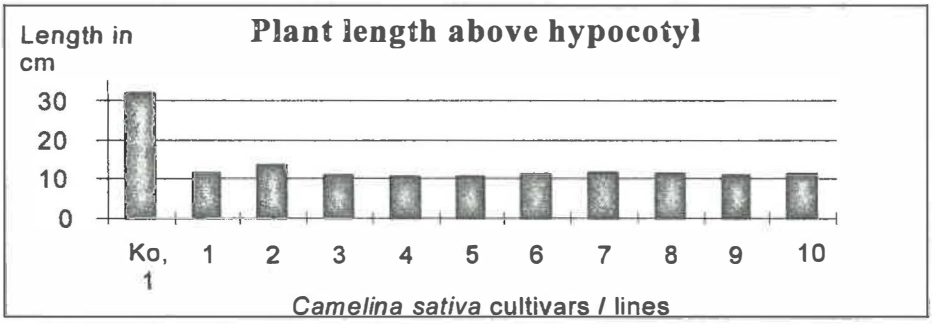

Figure 3: Length of plants measured above hypocotyl 49 days after inoculation, n=20.

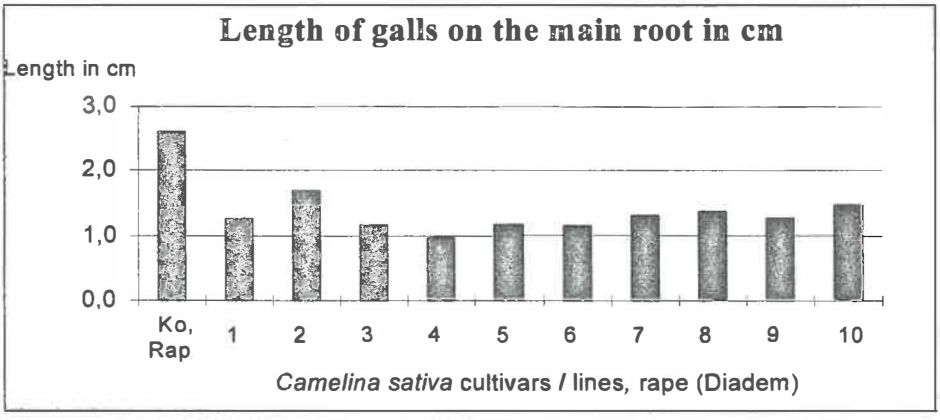

Figure 4: Length of the galls on the main root 49 days after plant inoculation, (n=20).

Verticillium Wilt (*Verticillium dahliae*)

The results obtained showed that the two *V. dahliae* isolates, V33 and V10 from winter oilseed rape were transferable to *C. sativa*. Ten days after inoculation first symptoms were observed. The oldest leaves became chlorotic. The chlorotic leaf withered rapidly and abscised. The symptoms described from winter oilseed rape (Zeise, 1992) could not be observed. Furthermore some plants showed depressions of growth. Some of the stunted plants died before the end of the experiment. In Table 4 the differences between the *C. sativa* cultivars/breeding lines to the two *V. dahliae* isolates used are shown.

The wilt-index of the infested *C. sativa* cultivars/breeding lines ranged from 2.8 to 4.5 with a GD 5% of 0.46. There were some differences between V33 and V10. For example the isolate V10 caused a higher wilt-index on the cultivare Soledo than the isolate V33. In another case the isolate V33 caused a higher wilt-index in the cultivare Licella than V10. Further investigations are in progress.

Table 4: Wilt-index for *Camelina sativa* cultivars/breeding lines (according to HOLTSCHULTE, 1992) 28 days after inoculation with two different isolates of *Verticilium dahliae* in 1995.

	***Verticillium dahliae*-isolates**		
cultivars/breeding lines	V 10	V 33	Conrol
Licalla	3,3 a	3,9 b	2,2
Soledo	3,6 ab	2,8 a	1,8
095/1	3,6 ab	3,4 a	2,7
Limaga	3,9 b	4,1 b	2,7
Lindo	4,0 b	3,7 ab	1,8
095/3	4,0 b	3,9 b	2,7
Ligena	4,0 b	4,3 b	2,2
Bavaria	4,3 bc	4,6 bc	2,1
095/2	4,3 bc	4,3 b	2,1
095/4	4,5 c	3,8 ab	2,1
mean value	3,9	3,8	2,1
$GD_{5\%}$	0,46		

Discussion

The first results of this investigation indicate that it is possible to develop inoculation methods for *C. sativa* and use them to differentiate between the 10 cultivars/breeding lines used for susceptibility to specific pathogens.

The investigations with clubroot (*Pl. brassicae*) and Verticillium wilt (*V. dahliae*)indicate that pathogens exist on winter oilseed rape that can also affect false flax in greenhouse and growth chamber experiments. In this study only two pathogens from winter oilseed rape were successfully tested. It is urgently necessary to test more winter oilseed rape pathogens in order

to determine their ability to affect *C. sativa.* It is not known yet how far the results obtained are transferable to field conditions and what their relevance in crop rotations may be.

Furthermore, weeds occurring in *C. sativa* crops may potentially be used as a host by pathogens that affect false flax. For example *Capsella bursa-pastoris* also belongs to the family *Cruciferaceae.*

The studies with *P. parasitica* from false flax show that it was not possible to inoculate winter oilseed rape with the inoculation methods used. Furthermore, there are differences in the susceptibility to *P. parasitica* between the 10 cultuvars/breeding lines used. It seems possible that the inoculation methods developed could be used for screening resistance breeding in false flax.

The two studies with *P. parasitica* and *Pl. plasmodiophora* were carry out with only one isolate at this time. It is likely that differences exist in aggressivness and virulence of other pathogen isolates.

Summary

In this study it has been proven that the winter oilseed rape pathogens *V. dahliae* (Verticillium wilt) and *Pl. brassicae* (Clubroot) can affect both false flax and winter oilseed rape. Downy mildew from false flax could not be transferred to winter oilseed rape with the methods used.

Additionally no differences in the susceptibility of the 10 cultivars/breeding lines of false flax used were observed when inoculated with *V. dahliae* (Verticillium wilt) or *P. parasitica* (downy mildew).

Prospects

In this study it has been proved that the oilseed pathogens *V. dahliae* (verticillium wilt) and P. brassicae (Clubroot) were transferable to false flax under laboratory and greenhouse conditions. How far the results obtained are transferable to field conditions is not known, at present. Likewise, their relevance in crop rotation is still unknown. The research on *P. parasitica* and *P. brassicae* was carried out with one isolate of each pathogen. It will be necessary to repeat the studies with different isolates of these pathogens.

The inoculation method used to transfer *P. brassicae* to *C. sativa* has to be improved especially with regard to the quantity of the conidia used. This is necessary to look for a correlation between the inoculum of Clubroot and the response of *C. sativa.*

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References

Holtschulte, B. 1992: Untersuchungen zur Biologie und Bedeutung von *Verticillium dahliae* Kleb. und *Leptosphaeria maculans* (Desm) Ces et de Not. (Anamorph: *Phoma lingam* [Tode ex Fr]) innerhalb des Erregerkomplexes der Krankhaften Abreife von Raps. Diss. Göttingren

Moser, P.E.; Sackston, W.E. 1973: Effect of concentration of inoculum and method of inoculum on development of Verticillium-wilt of sunflowers in: Basic Plant Pathology Methods (DHINGRA, O.).ORC Press USA

Zeise, K. 1992: Gewächshaustest zur Resistenzprüfung von Winterraps (*Brassica napus* L. var. *oleifera* Metzger) gegen den Erreger der Rapswelke *Verticillium dahliae* Kleb. Nachrichtenbl. Deutscher Pflanzenschutzb. 44 (6): 125-128

Disease Resistance and Integrated Control of Diseases

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Techniques for the evaluation of cultivar resistance to light leaf spot (*Pyrenopeziza brassicae*) in winter oilseed rape and interpretation of results

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Abstract Resistance to light leaf spot *(Pyrenopeziza brassicae)* has been assessed in the UK National List system by inoculating small plot trials with infected stem debris. Recent naturally occurring epidemics of light leaf spot indicated that differences in resistance were much greater than those previously observed in the inoculated trials. Four different inoculation techniques comprising infected stem debris, conidia derived from washings of infected leaves, conidia derived from *in vitro* cultures, and infected transplants were investigated to determine whether the accuracy of the inoculated test could be improved. Spore suspensions derived from leaf washings produced more severe epidemics than other techniques, and these were more closely related to records from severe natural infections. Area under the disease progress curve was used to calculate cultivar resistance ratings, in comparison to calculations from individual assessments or means of selected assessments. These results will be used to improve the predictive value of the resistance ratings derived from inoculated tests.

Keywords: cultivar resistance, inoculation techniques, *Pyrenopeziza brassicae*

Introduction

Light leaf spot (*Pyrenopeziza brassicae*) is one of the most damaging diseases of winter oilseed rape in the UK. There are marked regional differences in its occurrence, with northern regions suffering widespread and severe infections in most years, and southern areas being prone to more sporadic, but still severe, attacks. Cultivars which are submitted for statutory National List trials must be evaluated for resistance to the disease. Resistance ratings on a linear 1 to 9 scale, with 9 being very resistant, are produced. Once cultivars become commercialised, farmers may use the resistance ratings as an aid to their disease control strategies.

Resistance is assessed by inoculating small plot trials with infected stem debris, recording % plant area showing symptoms, and then converting mean % scores over trials and years to ratings by scaling according to the level of disease on control cultivars with fixed ratings (Thomas and Wright, 1995) Records from cultivar performance trials which become naturally infected are also included in the mean. Recently, during 1994 and 1995, light leaf spot became much more widespread and severe in the south of the UK than it had been for the preceding six or seven years (Thomas and Walker, 1994). Scores from naturally infected trials indicated that cultivar differences were much greater than those predicted from the inoculated tests. Cultivars are only trialled for two years prior to National Listing, and thus may not always be exposed to high levels of natural infection. Inoculated tests may therefore be the only source

of information for evaluating resistance, and it is thus critical that they should reflect the level of resistance which a cultivar is likely to exhibit under high, naturally occurring, disease pressure.

This work was undertaken to investigate inoculation techniques which would be capable of generating epidemics sufficiently severe to allow differentiation between cultivars even under unfavourable conditions. Some different approaches to the calculation of relative cultivar resistance ratings are also discussed.

Methods

Inoculation techniques

Four cultivars (cvs Bristol, Apex, Express and Nickel) were drilled on 13.09.95 in 4 m long three row plots. Stem debris collected from crops of cv Bristol and Envol severely infected with light leaf spot in June 1995 was scattered over the plots on 15.10.95 at the rate of 5 stems/m^2 Conidia were collected from infected leaves of cvs Bristol and Envol and frozen in water in June 1995, before thawing and spraying onto plots at 1 x 10^6 spores/ml, 100 ml/plot, on 01.11.95. Cultures of light leaf spot collected from cv Bristol were grown on malt extract agar, and a spore suspension in water of 1 x 10^5 spores/ml applied to the plots on 01.11.95, 100 ml/plot. Finally, plants of cv Bristol were inoculated with light leaf spot in a growth room and incubated until acervuli appeared before transplanting into the trial area (3 plants/plot) on 01.11.95.

The trial was separated into four blocks for each of the inoculation treatments. 1.5 m strips of a light leaf spot resistant cultivar were sown between treatments. Cultivars were randomised within the treatment areas with three replicates. The area was irrigated three times daily for a total of 6 minutes in periods of dry weather until mid November, then again from early March onwards. Disease was assessed by recording the % leaf area infected on a whole plot basis at intervals from late January onwards.

Data from cultivar performance trials

Disease scores from naturally infected cultivar performance trials were made as % leaf area infected according to the method described in the UK National List/Recommended List trials protocol (Anon, 1996). Paired yield trials treated or untreated with fungicide which were used to derive yield response data in the presence of light leaf spot were also carried out according to the methods described in the protocol. Fungicides to control light leaf spot were applied in the autumn and at stem extension, and consisted of either 1.85 l/ha of Sportak, or 0.4 l/ha of Punch C at each timing.

Results

Spore suspensions derived from leaf washings induced a much more severe epidemic than other inoculation techniques (Table 1 for cv Bristol)

Table 1 Severity (% leaf area infected) of light leaf spot on cv Bristol with four inoculation techniques

Technique	22.01.96	27.02.96	25.03.96	24.04.96	03.06.96
stem debris	0.05	1.87	5.30	10.00	6.70
leaf washings	11.7	15.00	19.30	18.30	12.30
in vitro spores	0.44	6.00	7.30	14.30	1.20
transplants	0.00	0.40	3.30	4.70	0.80
lsd (p=0.05)	0.870	3.608	3.216	2.124	1.349

Differentiation between cultivars known to be susceptible (cv Bristol) and resistant (cv Express) was greater with the leaf washings technique than with the standard stem debris, particularly at the early stages of the epidemic. There was very little difference between a cultivar thought to have moderate resistance (cv Apex), and the resistant cultivar using the stem debris technique (Table 2)

Table 2 Severity (% leaf area infected) of light leaf spot on cvs Bristol, Apex and Express inoculated with stem debris or leaf washings

Technique/variety	22.01.96	27.02.96	25.03.96	24.04.96	03.06.96
stem debris - Bristol	0.05	1.87	5.33	10.00	6.67
stem debris - Apex	0.00	1.20	0.70	7.00	3.67
stem debris - Express	0.00	0.17	0.23	5.00	0.53
leaf washings - Bristol	11.70	15.00	19.33	18.33	12.33
leaf washings - Apex	3.00	17.00	18.33	17.00	5.33
leaf washings - Express	6.00	7.00	9.33	11.70	3.67

lsd (p=0.05) within treatments = 3.889

Area under the disease progress curve (AUDPC) for the leaf washings technique gave a very similar degree of differentiation to the mean of all five scores (Table 3). However, differentiation was less between Bristol and Apex if the mean of scores taken on 25.03.96 and 24.04.96 was calculated. Cultivar % infection levels from a much larger database comprising

natural infection and stem debris inoculated test records from 1990-94 showed a further difference in relative separation.

Table 3 Comparison of cultivar resistance rankings using AUDPC, mean and selected mean data

Cultivar	AUDPC	mean % (5 scores)	mean % (2 scores)	1990-94 % mean
Bristol	71.75	15.34	18.80	12.13
Apex	54.50	12.13	17.66	4.00
Express	35.41	7.54	10.51	2.78
Nickel	35.30	7.36	10.0	1.00

Relationships between AUDPC, the various mean scores and responsiveness of the four cultivars to fungicide in trials severely affected by light leaf spot are shown in Figs 1 and 2. The mean yield response (treated yield less untreated yield) over a set of nine paired trials where infection on cv Bristol exceeded 20% at any time was used. The relationships all appeared to be linear, but were almost completely linear for AUDPC and the five assessment mean.

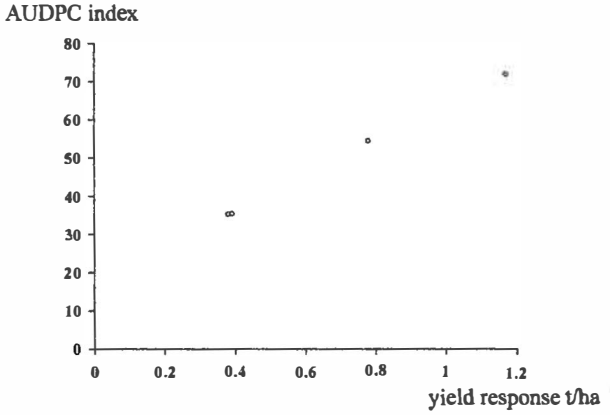

Figure 1 Relationship between AUDPC for four cultivars inoculated with light leaf spot and yield responses in fungicide trials

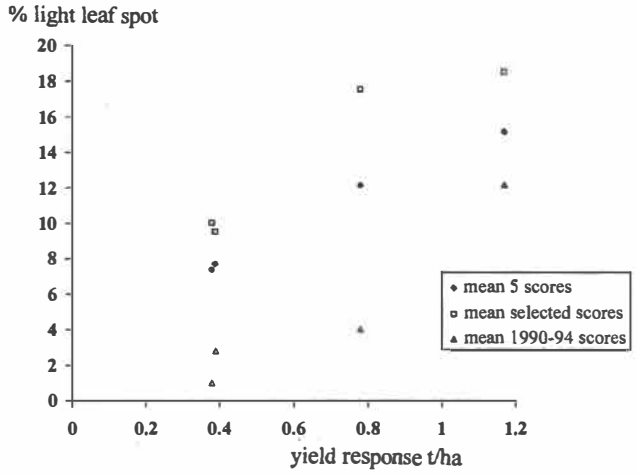

Figure 2 Relationship between mean scores for four cultivars inoculated or naturally infected with light leaf spot and yield responses in fungicide trials

Discussion

Approximately 800 plots are sown each year for the assessment of light leaf spot resistance in UK National List trials, covering an area of about 3000 m^2 . Inoculation techniques are thus required to be rapid and manageable. Growth of *in vitro* cultures is slow, and large numbers of plates would be needed to inoculate the area required. While spores produced from cultures gave a moderately intense epidemic, concentrations would probably need to be higher than the 10^5 spores/ml achieved. Washing spores from infected leaves, providing these can be reliably generated in any one season, was rapid, and the resulting suspension was easy to store in sealed plastic bags in a domestic freezer, with no apparent decline in infection potential over the five month storage period used here. Transplanting infected plants was labour intensive and while collecting and distributing stem debris was rapid, neither of these methods produced a severe epidemic.

Inoculated tests for the determination of cultivar resistance may be criticised for using techniques which are unrealistic in terms of the ways in which the pathogen increases in nature. However, it is probably reasonable to assume that the use of an overall conidial spray inoculum will reflect the type of epidemic which is initiated early in the autumn from high levels of ascospores and/or conidia moving from infections on volunteers to emerging crops. It was difficult to differentiate between cultivars with moderate and higher levels of resistance using stem debris inoculum, and to judge degree of susceptibility. Since resistance ratings should reflect the risk to which cultivars are exposed when disease occurs, it is preferable that they should be derived from high infection pressure conditions. Over estimations of resistance are likely to result from low infection pressure trials.

Multiple records of disease over the season from inoculated or naturally infected trials are not usually available. If they are, the record, or possibly mean of two records, which shows the

best discrimination between susceptible and resistant control cultivars, is selected. However, when this was carried out with the data from the leaf washings inoculation, a different pattern of cultivar separation was seen compared to the mean of all records or AUDPC, and it was clear that relative separation of cultivars changed over time. One or two assessment dates are probably not sufficient to describe cultivar resistance, and this is supported by the fact that AUDPC or all assessment means had a closer linear relationship to yield responses from fungicide trials than a selected mean. The mean from 1990-94 data was also less closely related to yield responses. Data within this period was mostly derived from single assessments of individual trials, and though a minimum of 17 records for a cultivar was available, it would appear that resistance levels were not adequately described based on the criterion of relation to yield responses in fungicide trials.

It is not known whether disease resistance rankings will show a completely linear relationship with yield responses to fungicide over a larger range of material. Other factors such as leaf area and canopy structure (Figueroa *et al.*, 1994) are likely to play a part in the responsiveness of a cultivar. However, the relative resistances of the cultivars investigated here were markedly different using the AUDPC method from those currently published, with Apex in particular being more susceptible, and this agreed well with its level of responsiveness. The predictive value of resistance ratings would be enhanced if certain points were likely to be reliably associated with certain yield loss risks.

AUDPCs were calculated from the day of sowing, though the first score was not taken until late January. Further work has been undertaken to determine the progress of early season disease, using additional cultivars of different growth habits. Results will be analysed to try and identify key assessment times and whether means derived from these can be used to calculate ratings with improved ability to predict yield losses.

Acknowledgement

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References

Anon. 1996. United Kingdom National List Trials Protocol for oilseed rape, 1995/6. NationalInstitute of Agricultural Botany, Cambridge

Figueroa, L., Shaw, M. W., Fitt, B. D. L., Mc Cartney H.A., and Welham, S.J. 1994. Effect of previous cropping and fungicide timing on the development of light leaf spot (*Pyrenopeziza brassicae*), seed yield and quality of winter oilseed rape (*Brassica napus*). *Annals of Applied Biology*, **124**, 221-239.

Thomas, J. E. and Walker, K. C. 1994. Exploitation of disease resistance in oilseed rape cultivars. *Aspects of Applied Biology* **40** (1) 237-243.

Thomas, J. E. and Wright, I. R. 1995. Evaluation of disease resistance in UK performance trials of oilseed rape. *Proceedings of the 9th International Rapeseed Congress*, vol 4. 1234-1236.

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Potential for exploiting resistance to stem canker *(Leptosphaeria maculans)* in cultivars of winter oilseed rape

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Abstract Selection for resistance to stem canker *(Leptosphaeria maculans)* is part of most winter oilseed rape breeding programmes, and resistance is one of the characters evaluated in the UK statutory National List oilseed rape trials system. Though the number of very susceptible cultivars entering commercial use in the UK has declined, there is still a large number of susceptible cultivars in use, and very few have resistance which exceeds the best level found in older types. Cultivars with good resistance to stem canker have generally been lower yielding, and have not proved popular with growers though there have been some exceptions. In a fungicide trial designed to give optimum control of canker with currently available agrochemicals, the cultivar with the best canker resistance gave only a very small yield response. While this result indicates that there is potential for exploiting canker resistance by reducing fungicide inputs, a much wider range of cultivar material with good resistance and good agronomic features is needed.

Keywords: control with cultivar resistance, *Leptosphaeria maculans*

Introduction

Stem canker (*Leptosphaeria maculans*) can cause large yield losses throughout many rape growing areas. Though fungicides are available to control the disease, they may not be completely effective, and correct timing of application may be difficult to achieve. Oilseed rape breeding programmes thus frequently include selection for improved levels of canker resistance. Adequate resistance integrated with fungicide use and disease forecasting is likely to offer the most effective method of reducing the effects of stem canker.

In the UK, oilseed rape cultivars undergo two years of statutory, or National List, performance trials after which they may be considered for inclusion on the Recommended List, which seeks to identify the best overall cultivars available. Approximately 80% of the UK area of rape is sown with cultivars included on this list. As part of the trials programme, cultivars are tested for canker resistance. Results from these tests can thus provide an indication of the success of plant breeding in improving resistance, and of the availability of resistance in backgrounds which are likely to be acceptable to growers. Though information on comparative cultivar resistance levels is published for the use of growers in the form of resistance ratings, there is little advice available on how resistance can be exploited within an integrated disease control approach.

The results presented here review canker resistance in cultivars submitted for trials over the last six years. In addition, results from a trial using fungicide to control canker on cultivars with different resistance levels are described.

Methods

Small plot (4 m x 0.9 m) trials inoculated with canker infected stubble (five stems/m^2) collected from different regions were used to evaluate resistance. External and internal crown canker symptoms were assessed on 30 stems per plot in mid to late June, using the index system devised by Newman and Bailey (1987). Final disease index (DI) on a 0-100 scale was calculated as a mean of external and internal scores. Two or three trials were carried out in each of two years.

The fungicide trial was carried out with large (24 m x 2 m) plots. Inoculation proved unnecessary following early and severe leaf spot development. Three cultivars, Nickel Synergy and Express, were drilled on 01.09.95. Trial management followed standard commercial practice with the exception of the fungicides which were flusilazole applied as Punch C at 0.4 l/ha on 18.10. 95 and 06.02.96 (treament 1) and on 14.12.95 and 06.02.96 (treatment 2). There were three replicates per treatment, with blocks of cultivars comprising separate trials. Canker was scored on 24.06.96, and the trials swathed on 19.07.96 and combined on 04.08.96. Yields were detemined at 9% moisture.

Yield potential of canker resistant cultivars compared to other cultivars was derived from routine performance trials treated with a full fungicide programme, and carried out according to a standar protocol (Anon, 1996).

Results

Cv Cobra is widely recognised as being susceptible to stem canker, and typically 100% of stems were infected, with about 50% of these being in the higher scoring categories (3 or more on the 1-6 scale) for stem base circumference and cross sectional area affected. cv Cobra was included in canker trials from 1991 - 1996, and cultivar disease indices were calculated as varying percentages of the cv Cobra figure for the years when they were trialled (Table 1).

Table 1 Distribution of canker disease indices in relation to cv Cobra (susceptible) for cultivars completing UK National List trials from 1991 to 1996

Year	DI cv Cobra	Number of cultivars with DIs at varying %s of Cobra					Total
		>100	80-99	60-79	40-59	<40	tested
1991	49.5	2	7	5	2	1	17
1992	51.5	0	6	14	5	0	25
1993	44.3	1	6	8	3	0	18
1994	41.6	0	4	6	3	0	13
1995	35.6	0	13	7	1	0	21
1996	42.6	0	11	11	2	0	24

Of the few cultivars with higher levels of resistance such as cv Express, yield potential judged by the seed yield from a large number of fungicide treated trials has not been as good as popular cultivars such as cv Apex (Table 2). However, the newer resistant cultivar Licrown may have a higher potential and could prove more attractive to growers. No cultivar has shown better canker resistance than cv Capricorn to date, and though cv Capricorn was more popular in the early 1990s, its relative yield position has declined and very little is now grown.

Table 2 Canker indices, treated seed yields, and estimated UK area sown for some susceptible and resistant cultivars compared to the most popular.

Cultivar	Canker indices		Treated seed yields t/ha	Estimated %
	1993	1996	1994 -1996 mean	area sown 1996
Capricorn	18.5	*	(3.80)	2
Corniche	*	22.4	4.24	3
Express	21.8	27.7	4.15	3
Licrown	*	27.7	4.53	**
Apex	26.6	31.8	4.24	56
Synergy	*	37.5	4.79	**
Cobra	44.3	42.6	4.32	<1
lsd (p=0.05)	11.50	7.06		

* = cultivar not in trial, ** = no figures available, () = mean derived from 1988-89 trials

The resistant cv Express had lower canker indices than either cv Synergy (moderately susceptible) or cv Nickel (equivalent susceptibility to cv Cobra) in untreated plots in the fungicide trials (Table 3). The combination of October and February fungicide gave better canker control than the December and February timings. Differences in yield (Table 4) were not significant, but nevertheless there was a clear pattern of responses to fungicide, with the more susceptible cultivars showing a larger response than the resistant one, particularly at the earlier October timing.

Table 3 Canker indices for cultivars of oilseed rape treated with fungicide at early or late timings

Treatment	Nickel	Synergy	Express
flusilazole Oct & Feb	19.8	11.4	17.6
flusilazole Dec& Feb	27.9	26.7	21.7
untreated	40.9	36.5	28.8
lsd (p=0.05)	8.01	10.65	4.88

Table 4 Seed yield (9% moisture) of oilseed rape cultivars treated with fungicide to control stem canker

Treatment	Nickel	Synergy	Express
	yield t/ha		
flusilazole Oct & Feb	4.83	4.67	4.78
flusilazole Dec& Feb	4.72	4.38	4.52
untreated	4.20	4.14	4.58
lsd (p=0.05)	0.720	0.921	0.974

Discussion

There were relatively few cultivars with high levels of resistance to canker, and no indication that numbers were increasing over the six year period examined. Many cultivars were only slightly more resistant than cv Cobra, which was first grown commercially in 1988. However, only three cultivars out of 118 were more susceptible. While this suggest that selection for resistance has prevented the occurrence of major problems due to stem canker susceptibility, there does not appear to be much potential for reducing the disease effects further using cultivar resistance unless more effective sources are found (Gerdemann-Knorck *et al.*, 1995).

Of the most resistant cultivars, only cv Capricorn has been widely grown commercially in the past. Maximum yield potential of other cultivars such as cv Express as judged by extensive fungicide treated trials data was slightly less than that of the popular cv Apex, and much less than new hybrid types such as Synergy. However, the new cv Licrown offers better yield potential combined with a relatively high canker resistance. Factors other than yield play an important part in cultivar choice, and though the treated yield of the canker resistant cultivar Corniche was relatively high, it is weak stemmed and this may prove a problem in many areas.

Flusilazole proved to be effective in reducing canker symproms in all three cultivars tested, but large yield benefits were only seen in cv Nickel and cv Synergy and not in the resistant cv Express The trials also illustrated the importance of early season disease control on susceptible cultivars when the leaf spot phase increases in the early autumn However, timing was of much less significance for the yield response on cv Express. While the best levels of resistance would appear to be sufficient to reduce or eliminate the need for fungicide, there are very few available cultivars particularly if yield potential remains a dominant factor in cultivar choice. In addition, the level of susceptibility to light leaf spot must be taken into account. The majority of cultivars at present are likely to benefit from at least one fungicide application to control canker.

Acknowledgment

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References

Anon, 1996. United Kingdom National List and Recommended List Trials Protocols for oilseed rape, 1995/6. National Institute of Agricultural Botany, Cambridge

Gerdemann-Knorck, M., Stenschke, C., Penkert, M. and Schieder, O. 1995. Disease resistant *Brassica* plants obtained by somatic hybridisation. *Proceedings of the 9th International Rapeseed Conference*, vol 4 1263-1265.

Newman, P. L. and Bailey, D.J. 1987. Screening for resistance to canker (*Leptosphaeria maculans*) in winter oilseed rape (*Brassica napus* spp *oleifera*) *Plant Pathology* **38**, 346-354

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Possibilities for early resistance screening for *Phoma lingam* in winter oilseed rape with biochemical methods

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Abstract The acceleration of the conventional resistance test procedure is a common demand. The use of ELISA as a tool for screening doubled haploid lines of winter oilseed rape as to their resistance against *Phoma lingam* in early growth stages of plants following greenhouse inoculation is described. For the purpose of disease diagnosis we developed Phoma-specific polyclonal antibodies based on soluble mycelial proteins.

In order to find inoculation methods suitable for a standard screening procedure based on ELISA and scoring assessment systematic investigations of preferred inoculation methods were carried out. Some experimental variants have shown the expected resistance differentiation of cultivars and will be tested in large-scale screening. A possible screening result from doubled haploid lines using leaf (cotyledons and true leaves) and stem inoculation methods is presented.

Keywords: ELISA (enzyme linked immunosorbent assay), ascomycetes, *Leptosphaeria maculans*, polyclonal antibodies, disease diagnosis, quantification of fungal biomass

Introduction

Different possibilities of plant protection against *Phoma lingam* the causative agent of blackleg disease in winter oilseed rape are known, but the most important way to minimise the damage is still the use of resistant cultivars. However testing of plant material for *Phoma*-susceptibility in resistance breeding with classical methods of visual symptom assessment under natural growing conditions is time and work expensive. Therefore there is a demand for acceleration of the resistance test procedure.

Within a three-year research project we are working on the development of a standard test procedure for early resistance screening against *P. lingam* in greenhouse and laboratory. The main topics of this work are methods for disease diagnosis in early growth stages of plants together with methods for artificial inoculation techniques.

For detection of disease infestation we use conventional visual scoring of different symptom expressions in addition to the serological ELISA method. An early evaluation of the fungus/plant interaction and reliable quantitative data of fungal growth inside plant tissues are needed to supplement the visual assessment of symptoms. For this purpose we developed a quantitative ELISA using rabbit polyclonal antisera directed against soluble mycelial proteins of aggressive/Tox^+ isolates. In another major part of our work various techniques of artificial inoculation of different plant parts were applied in order to find methods suitable for a standard screening procedure with ELISA analysis.

Methods

Fungal cultures

Phoma lingam strains used in this project belong to the culture collection of the BBA (Biologische Bundesanstalt)-institute. Single ascospore lines of *Leptosphaeria maculans* isolated from winter oilseed rape were obtained as described by KOCH et al. (1989) and characterised in aggressive and non-aggressive ones according to KOCH et al. (1989). All isolates were maintained on V8 agar medium (100 ml vegetable juice (ALBI), 20 g agar, 2 g $CaCO_3$, 900 ml water) or on HF medium (30 g oat flakes (KÖLLN), 20 g agar, 1 l water) for long term maintenance.

After ten days of growth at 20 °C with day-light the cultures had developed pycnidia. For inoculum production the petri-dishes were flooded with sterile distilled water and the surface was rubbed. The resulting pycnidia suspension was plated on fresh V8 agar media and incubated for approximately 10 days under special light conditions for the release of pycnidiospores. These pycnidiospores were harvested in the same way as the pycnidia and can be stored at -20°C. For artificial greenhouse inoculations pycnidiospore suspensions were thawed and the inoculum concentration was adjusted to 1 mio. pycnidiospores/ml with the aid of a Fuchs-Rosenthal counting chamber under microscope.

For mycelium mass production plugs of approximately 1 cm diameter were cut from the growing mycelium edge of V8-agar plate cultures and transferred to Erlenmeyer flasks containing 30 ml of Czapek-Dox Liquid Medium (OXOID) supplemented with 2 % (w/v) yeast extract (OXOID). The inoculated flasks were incubated at room temperature for 28 days in the dark without shaking.

Antigen preparation

It is known that severe stem canker and dry rot is caused by virulent strains of L. maculans, whereas avirulent pathotypes produce only mild symptoms (KOCH et al., 1989). Therefore we used a mixture of six different aggressive or virulent isolates for antigen preparation. Mycelium of *P. lingam* was separated from the culture medium by filtration (Schleicher and Schuell filter paper 595 ½ No.311 645) and washed three times with deionisised distilled water. The pellet was taken up in PBS- (phosphate-buffered saline) buffer (CASPER & MEYER, 1981), homogenised with mortar and pestle on an ice bath for protein extraction and then centrifuged twice at 15000 x g for 10 min at 4 °C. The supernatants were analysed for protein content by the method of BRADFORD (1976) and used for immunisation.

Antisera production

For immunisation of rabbits the prepared protein extracts were adjusted to 2 mg/ml with PBS buffer. Three intramuscular injections were given weekly. Each injection consisted of 3 ml of a mixture of 1,5 ml protein extract and 1,5 ml Freund's adjuvent complete (first injection) or Freund's adjuvent incomplete (second and third injection). The blood was collected at the marginal vein of the ear before immunisation (präimmunserum) and at 14-day intervals after the last injection until the antibody titre decreased. The quality of different blood collecting dates was first checked with OUCHTERLONY' s agar-diffusion-test (1962) for the best antibody titre, later on this can be done with the optimised ELISA test. Antisera were separated from the coagulated blood, centrifuged at 6000 x g for 30 min at 10 °C and stored as raw sera at 4 °C after the addition of 0,05% (v/v) NaN_3 (25%). Before use in ELISA the sera were purified through adsorption with kaolin according to the methods of SHILLITOE (1982) and conserved for long time storage at 4 °C with addition of 0,2% (v/v) NaN_3 (25%).

Plant material and inoculations

Standard cultivars and doubled haploid lines provided by associated breeder companies were planted as seedlings in plastic pots (13 cm x 13 cm) filled with a soil mixture and maintained in greenhouse- or growth-chamber-conditions. The adjusted temperature and photoperiod depend on the inoculation technique and on the season. In most cases pycnidiospore suspensions of 1 mio. conidia/ml were used for each inoculation.

Inoculation of cotyledons was carried out as described by KOCH et al. (1989) and KUTCHER et al. (1993). About 5-6 days after seeding cotyledon halves of oilseed rape seedlings were punctured with a needle. On each wound (i. e. 3 per cotyledon) a drop (5 µl) of pycnidiospore suspension was placed. After inoculation plants were covered with polyethylene for 72 h to provide 100% relative humidity and cultivated at approximately 20 °C in a growth chamber. Twelve days after inoculation evaluation of resistance reaction is possible. Spraying inoculation of true leaves was carried out after wounding of definite areas. Dip inoculation of stem bases and roots of seedlings were conducted in pycnidiospore suspension for 2 hours with constant shaking. Then the inoculated seedlings were planted in plastic pots with a soil mixture.

After symptom scoring the plant material (cotyledons, leaves, stems) was harvested for the preparation of plant extracts following ELISA analysis. The test samples were homogenized in H-buffer (Quelle oder Rezept) at a 1:30 (w/v) ratio for leaf material and at a 1:3 (w/v) ratio for stems.

ELISA protocol

The ELISA-test adapted to KNAPOVA (1995) was carried out in microtitre plates (GREINER). For each step the reagent volumes were 200 µl per well with the exception of the blocking step with 250 µl. The plates were washed three times with PBS-T (0,5 % Tween-20 in PBS) according to CLARK & ADAMS (1977) between each step.

I. coating

The plates were coated with test sample extracts diluted in H-buffer (10 mM $Na_2S_2O_3$ x 5 H_2O, 2,5% (w/v) PVP in 1 l carbonate buffer, pH 9,6 (CLARK & ADAMS, 1981). Each sample was repeated in triples (three wells per plate). Standard antigens consisting of *Phoma lingam* proteins (positive controls) and healthy plant extracts (negative controls) were included on each plate. After coating the plates were maintained overnight at 4 °C.

II. blocking

Blocking solution (0,2% BSA (w/v) in carbonate buffer, pH 9,6) was added and the plates were incubated for 30 min. at 37 °C.

III. addition of antiserum

Addition of antiserum, diluted 1:1000 (v/v) in PBS-T-0,2% (w/v) BSA and incubation for 30 min. at 37 °C.

IV. conjugate

Addition of goat anti-rabbit IgG conjugated to alkaline phosphatase (CALTAG laboratories, San Francisco) diluted 1:6000 (v/v) in 0,2% (w/v) BSA in PBS-T. Incubation: 30 min. at 37 °C.

V. substrate

Addition of substrate (1 mg/ml p-nitrophenyl phosphate) in diethanolamine buffer, pH 9,8 (CLARK & ADAMS, 1977). The plates were now maintained at room temperature. The optical densities (OD) were read at 405 nm after 1 h and 2 h using an ELISA-reader (SLT, San Francisco)

With the developed polyclonal antiserum based on soluble mycelial proteins only a genus specific detection is possible. Except for *Alternaria brassicae* no cross reactions were observed with mycelial extracts of saprophytes or pathogens of *Brassica naspus* following the developed indirect ELISA-Test. With an antibody dilution of 1:1000 a detection of up to 40 µg of fungal proteins per ml plant extract is possible.

Results

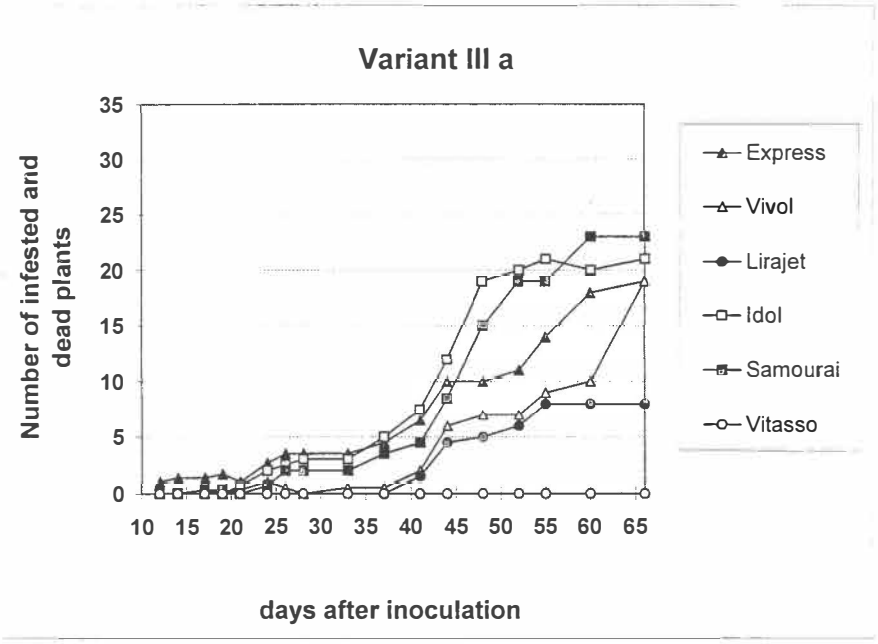
In order to find inoculation methods suitable for a standard screening procedure based on ELISA in addition to symptom assessments systematic investigations of preferred inoculation methods were necessary. For this purpose of we carried out an experiments with three different inoculation techniques (I. spraying after wounding of leaf tissues, II. dip inoculation of root and stem base, III. spraying of root and stem base (modified dip inoculation)). The dates of inoculation were staggered in plant ages of 8, 18, 28 and 38 days (a, b, c, d) and the dates of harvest were conducted in intervals of 25, 45 and 65 days after inoculation (H1, H2, H3) for each experimental variant. Six standard cultivars of different resistance reaction were investigated, each variant including 32 plants. Figure 1 shows the disease development following variants III a and c. Cultivar differentiation occurred 40 days after inoculation (III a) and immediately after beginning of scoring. Based on ELISA and scoring results the expected resistance differentiation of cultivar occurred following the experimental variants III a H3 and III c H3 (Fig. 2). These experimental combinations will be tested in large-scale screening of doubled-haploid lines of winter oilseed rape for resistance against *Phoma lingam*.

Depending on the applied inoculation method the first stages of plant fungus interaction take place in different plant parts, leaf or stem tissues. It is possible that different mechanism of plant defence are localised in different plant tissues. Therefore for the rating of doubled-haploid lines the results of two or more inoculation techniques are necessary to get a final assessment of resistance. A possible screening result of doubled haploid lines based on ELISA assessment and conventional scoring is presented. Variation in resistance was investigated on a range of Brassica genotypes (doubled haploid lines and standard cultivars) based on the disease reaction elicited on the cotyledons, true leaves and in the stem. The screening results following the three different artificial inoculations were compared for each plant genotype for the purpose of a final assessment of plant resistance (Tab. 1). With our resistance test procedure a more detailed ranking of resistant, moderately resistant and susceptible lines was obtained.

Discussion

Resistance behaviour of winter oilseed rape under natural growing conditions is influenced by many environmental factors. Greenhouse testing in comparison with "range" screening include stronger selection conditions, because the resistance reaction is examined under infection pressure of one pathogen. For this reason "range" resistance screening is not

Figure 1: Disease development following experimental variants III (spraying inoculation of stem bases) inoculated at two different plant ages (a - 8 days upper graph, c - 28 days lower graph)



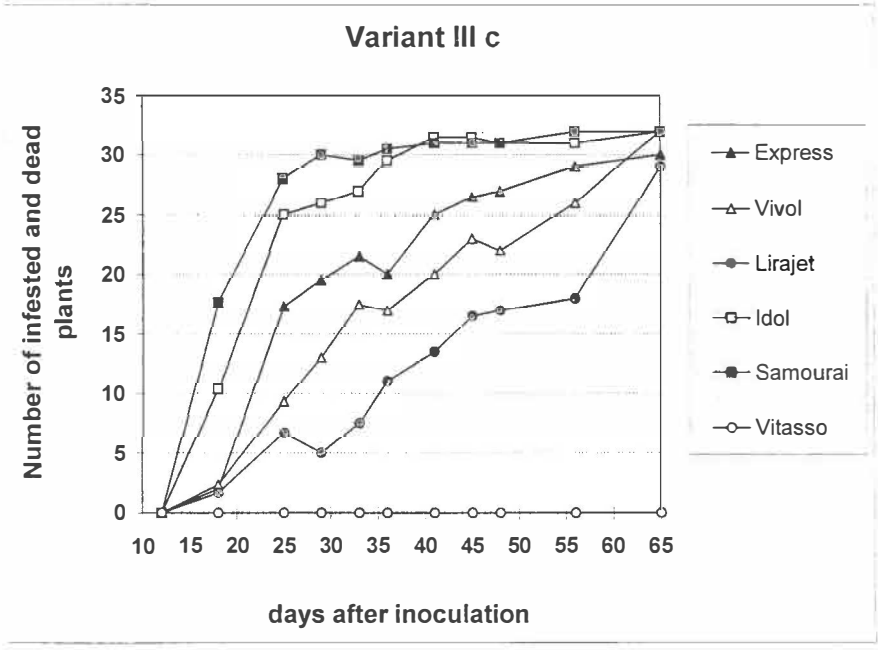
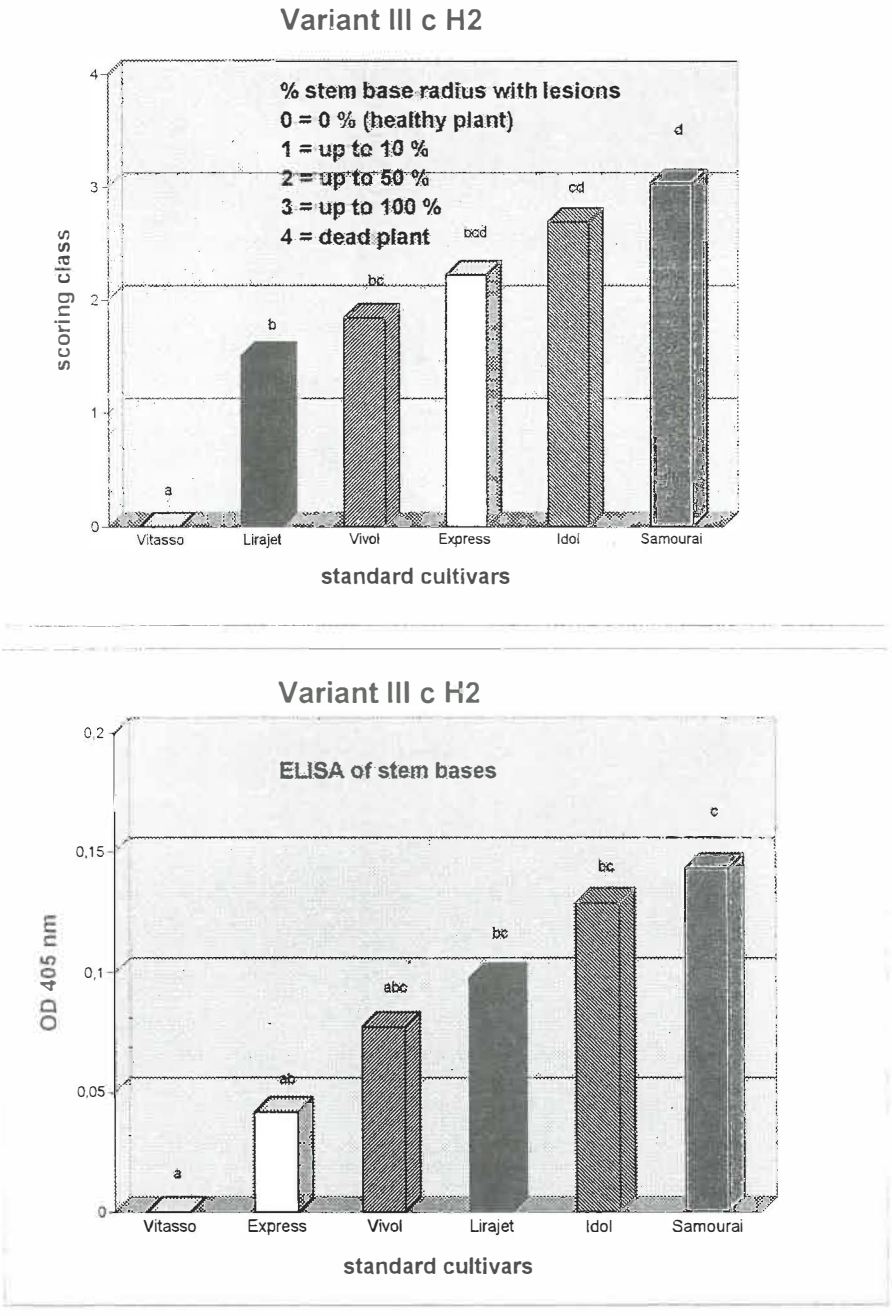


Figure 2: Cultivar differentiation of experimental variant III a H3 (III - spraying inoculation of stem bases, a - plant age of 8 days, H2 - harvested 45 days after inoculation) based on the results of scoring and ELISA.



Classification of doubled haploid lines as to their resistance against *Phoma lingam*

breeder's information and evaluation following greenhouse testing

DH-linie cultivar	breeder's appraisal	inoculation of true leaf		inoculation of cotyledons		dip inokulation	final assessment following greenhouse testing
		ELISA[a]	scoring*	ELISA[a]	scoring**	scoring***	
1	good	0,054	33	0,006	6,12	16,7	good
2	good	0,136	53	0,010	3,18	25,0	good
3	good	0,439	75	0,105	13,56	8,3	middle
4	good	0,228	82	0,039	1,50	8,3	good
5	good	0,218	90	0,129	12,56	4,3	middle
6	good	0,373	76	0,062	5,87	8,7	middle
7	good	0,198	39	0,067	4,25	12,5	good
8	good	0,244	66	0,125	6,46	21,7	middle
9	good			0,065	7,68	4,2	good
10	weak	0,306	85	0,130	16,43	54,2	weak
11	weak	0,483	93	0,100	8,66	29,2	weak
12	weak	0,213	93	0,355	15,75	33,3	weak
13	weak	0,212	80	0,031	1,40	33,3	middle
14	weak	0,297	100	0,012	4,00	29,2	middle
15	weak	0,384	88	0,021	2,06	29,2	middle
16	weak	0,273	59	0,254	13,00		middle
17	weak			0,242	15,56	37,5	weak
18	weak			0,660	18,93	29,2	weak
Falcon	good			0,064	6,93	4,2	good
Idol	middle			0,097	10,25	25,0	middle
Samourai	weak	0,301	93	1,135	19,18	41,7	weak
correlation ELISA/scoring			0,585		0,729		

* % of infested stem bases ** lesions [mm^2] *** % of infested plants a OD values at 405 nm

Table 1: A possible screening result based on three different inoculation methods

dispensable. Further more it has to be checked whether the genotype differentiation following greenhouse testing is in agreement with the differentiation that occurred under natural growing conditions.

The investigations of KUTSCHER et al. (1993) under greenhouseconditions concerring variation in pathogenicity of *Leptosphaeria maculans* on *Brassica* ssp. based on cotyledon and stem reactions showed that a resistant cotyledon reaction was associated with a resistant stem reaction in virtually all combination of isolate-origin and cultivar. A susceptible cotyledon reaction was generally associated with a susceptible or moderately resistant stem reaction. Exceptions with resistant stem reactions could indicate the presence of adult plant resistance. These results are based on symptom scoring only. BALESDENT et al. (1995) employed the DAS-ELISA-technique to quantify *Leptosphaeria maculans* growth in cotyledons of *Brassica napus*. Time-course studies for a given isolate showed that fungal growth in cotyledons assessed with ELISA was correlated with symptom scoring. Using ELISA quantification intermediate symptoms could be differentiated as late resistance response or susceptibility.

However a standard test procedure in the greenhouse as a first step could be helpful in order to reduce large-scale testing. With ELISA a more specific detection of the pathogen, quantification and a more objective assessment of disease intensity in contrast to visual scoring in the early growth stages of plants is possible. In the next step of resistance screening, a reduced number of genotypes could be tested in "range".

References

BRADFORD, M. M.(1976): A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

BALESDENT, M.-H.; DESTHIEUX, I.; GALL, C.; ROBIN, P. and ROUXEL, T. (1995): Quantification of *Leptosphaeria maculans* growth in cotyledons of *Brassica napus* using ELISA. J. Phytopathology 143, 65-73.

CASPER, R.; MEYER K. (1981): Die Anwendung des ELISA-Verfahrens zum Nachweis pflanzenpathogener Viren. Nachrichtenblatt Deut. Pflanzenschutzd. 33 (2), 49-54.

CLARK, M. F. and ADAMS, A. N. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34, 475-483.

KNAPOVA, G. (1995): Entwicklung eines ELISA zum Nachweis von *Phytophthora infestans*. Dissertation, Universität Göttingen.

KOCH E., BADAWY H. M. A. & HOPPE H. H. (1989): Differences between aggressive and non-aggressive single spore lines of *Leptosphaeria maculans* in cultural characteristics and phytotoxin production. J. Phytopathology. 124, 52-62.

KUTSCHER, H. R.; VAN DEN BERG, C. G. J. and RIMMER, S. R. (1993): Variation in pathogenicity of *Leptosphaeria maculans* on *Brassica* ssp. based on cotyledon and stem reactions. Canadian Journal of Plant Pathology 15, 253-258.

OUCHTERLONY, Ö. (1962): Diffusions in gel methods for immunological analysis II. - Prog. Allergy 6, 30-154.

SHILLITOE, E. J. (1982): Decline in specificity of the ELISA due to storage of serum, and its recovery by adsorption with kaolin. Journal of Virol. Methods 4, 241-248.

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Control of canker (*Phoma lingam*) in winter oilseed rape and possibilities of integrated pest management

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Abstract Canker (*Leptosphaeria maculans, Phoma lingam*) is one of the most important diseases of winter oilseed rape in Germany. Effective control is possible by growing less susceptible varieties and spraying fungicides. Growing less susceptible varieties disease index was reduced from 4,7 to 3,8 (scoring 1-9). Using tebuconazole as fungicide in autumn and spring, disease index could be reduced from 4,8 to 4,2 in average of varieties with different susceptibility. Fungicide treatment lead to a positive yield response, depending on the susceptibility of the variety. The yield increase was caused by the reduction of the disease index but also by side effects of the fungicide e. g. the reduction of lodging, increase of winter hardiness and extension of the ripening. Starting-points for the development of disease thresholds or forecasting methods were examined. Only poor information could be reached by using spore traps or weather data. A correlation was found between the incidence of plants with leaf symptoms in late autumn and root neck symptoms before harvest. Further possibilities of criteria for thresholds or forecasting methods are discussed.

Keywords: Canker, *Phoma lingam, Leptosphaeria maculans*, variety, susceptibility, fungicide, integrated pest management, threshold, forecasting system.

Introduction

Canker (*Leptosphaeria maculans* anamorph: *Phoma lingam*) is one of the most important diseases of oilseed rape in Germany. Yield losses appear in all regions with oilseed rape production, they depend on the intensity of oilseed rape production, on rotation, the susceptibility of the varieties and very much on weather conditions during the vegetation period. Controlling canker, the growing of less susceptible varieties is effective, especially under low or medium infestation conditions. Fungicides for the control of canker in Germany are sprayed in situations of higher infestation, it is assessed at 30% of the rape growing area with increasing tendency. Points of discussion for the application of fungicides are the need of the application, the choice of the product, respectively the active ingredient as well as the termination of the application. In Germany the spraying of fungicides for the control of canker is carried out undirected, therefore there is a strong need for the development of thresholds and forecasting systems.

Methods

Field trials with four replications in winter oilseed rape were carried out in Germany in the region of Braunschweig between 1993 and 1995. Different susceptible varieties were grown, which were MAXOL, LIRAJET, ENVOL, IDOL, FALCON, ZEUS and SAMOURAI. Different fungicide treatments were carried out with 1,2 l/ha FOLICUR® (tebuconazol 250 g/l) for the autumn and spring applications and with 1,5 l/ha RONILAN FL® (vinclozolin 500 g/l) for the flowering applications. The plot size was 25 m². During the vegetation period the disease attack was assessed 7 times, 3 times in autumn and 4 times during spring and early summer. The severity of the disease was scored as well as the incidence. Depending on the disease and the appearance of symptoms the root, the root neck, the stem, the leaves and the pods were taken for the assessment. Regarding the symptoms of canker at the root neck the scheme of KRÜGER (1983) was used for the assessment. This include a scoring system from 1 to 9 (1 - healthy plant, 5 - root neck shows a damage of 50%, 9 - plant is dead).

Results

6 year results investigating the effects of fungicides in the region of Braunschweig, Germany. The trials showed a yield response between 0,4 dt/ha and 11,4 dt/ha by controlling canker (*Phoma lingam*) in autumn and spring with tebuconazole. The investigations show, that the most effective control of canker was reached in autumn when the fungicides where sprayed in the beginning and the middle of autumn (BBCH 23-27). Spring applications were most effective when the plant high was between 20 to 30 cm. The flowering applications with vinchlozolin showed only small effects on canker.

Table 1: Root neck symptoms caused by canker (*Phoma lingam*) and the effect of fungicide treatments with tebuconazole (autumn and spring treatments) and vinclozolin (flowering treatments), Braunschweig 1993-95.

VARIETY	FUNGICIDE TREATMENT / DISEASE INDEX			
	UNTREATED	AUTUMN	SPRING	FLOWERING
MAXOL	3,8	-0,20	-0,10	-0,10
LIRAJET	4,1	-0,37	-0,23	-0,03
ENVOL	4,2	-0,43	-0,30	+0,03
IDOL	4,2	-0,47	-0,23	-0,20
FALCON	4,3	-0,40	-0,13	-0,20
ZEUS	4,3	-0,33	-0,13	-0,20
SAMOURAI	4,7	-0,57	-0,50	-0,33
AVERAGE	4,2	-0,40	-0,23	-0,14

Three year experiments (Tab. 1) show that in the average of the years the disease index of canker could be reduced from 4,7 (SAMOURAI) to 3,8 (MAXOL) by growing a less susceptible variety. Spraying fungicides in autumn the disease index was reduced in average by 0,4, depending on the susceptibility of the variety. In SAMOURAI the disease index was reduced by 0,57, in MAXOL by 0,20. This show a stronger need of using fungicides in more susceptible varieties and the advantages of less susceptible varieties. Spring applications of fungicides reduce the disease index less than autumn applications, in average of the varieties by 0,23. Again the reduction depends on the susceptibility of the variety, e. g. in SAMOURAI the reduction was 0,50 and in MAXOL 0,1. As expected, flowering applications were of low effect on the index of the root neck disease, in average the reduction was only 0,14. Over all it could be stated that tebuconazole was of limited effect on the root neck disease.

Table 2: Yields of winter oilseed rape and the effect of fungicide treatments with tebuconazole (autumn and spring treatments) and vinclozolin (flowering treatments), Braunschweig 1993-95.

VARIETY	FUNGICIDE TREATMENT / YIELD RESPONSE YIELD IN DT/HA			
	UNTREATED	AUTUMN	SPRING	FLOWERING
MAXOL	32,46	+1,46	+3,13	+1,22
LIRAJET	32,67	+1,30	+2,81	+1,42
ENVOL	30,82	+1,29	+5,05	+1,19
IDOL	31,64	+0,94	+4,60	+2,33
FALCON	29,86	+0,79	+3,50	+0,81
ZEUS	32,81	+1,56	+2,17	+1,52
SAMOURAI	28,80	+1,64	+4,90	+2,04
AVERAGE	31,30	+1,28	+3,73	+1,50

The yield response after fungicide treatment was higher in susceptible varieties (e. g. SAMOURAI) than in lower susceptible varieties (e. g. MAXOL). In average the yield was increased with autumn application by 1,28 dt/ha, with spring application by 3,73 dt/ha and with flowering application by 1,50 dt/ha. So spring application lead to higher yield response, although autumn application lead to a stronger reduction of root neck disease. Therefore it could be assumed, that beneath the reduction of the canker other factors influenced the yield increase.

During the experiment other diseases were of low or no effect on the yield. Only *Botrytis cinerea* occurred in some extent, but had no effect on the yield. Further, phenotypic factors of the plants were scored. It could be shown (figure 1), that the fungicide treatments lead to an decrease of lodging. After spring application there was a strong decrease of lodging, which was not improved by autumn or spring application. Fungicide treatments had a greening

effect on the plants, the ripening of oilseed rape was delayed. The greening effect was higher with applications in autumn, spring and flowering than with spring application alone, so this effect increased with the intensity of the fungicide treatments.

Figure 1 shows, that the number of plants by harvest was increased by fungicide treatments. This was caused by two effects. First, the number of premature died plants caused by fungal disease was increased. Second the winter hardiness of oilseed rape was improved, in the end of winter higher plant density after the autumn application were scored. Before winter the plant densities of treated and untreated variants were the same.

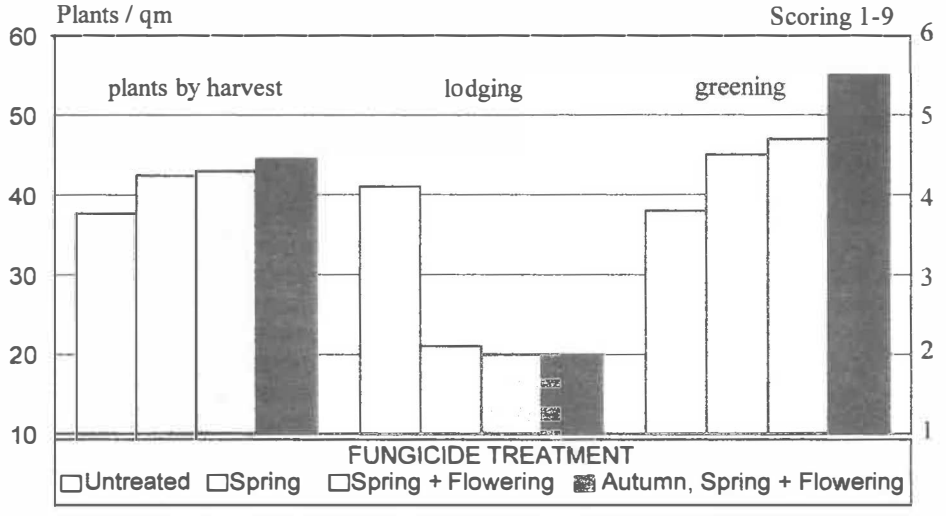

Figure 1: Effects of fungicide treatments on the plant density, lodging and the ripening of oilseed rape (Braunschweig 1994).

The aim of the experiments was further, to look for thresholds or possibilities of aimed fungicide application against canker. Scoring of leaf symptoms and root neck symptoms during October, November, March and April showed no correlation between the incidence and severity of the symptoms and the severity of canker before harvest (BBCH 81). Figure 2 shows that there is a correlation between the incidence of leaf symptoms in December and the severity of canker before harvest (BBCH 81). It could be described by $y=0{,}1335x + 2{,}76$ with $r= 0{,}8275$ (sign. $p< 0{,}05$). The results indicate, that the elaborated correlation could be used as a base for a threshold model.

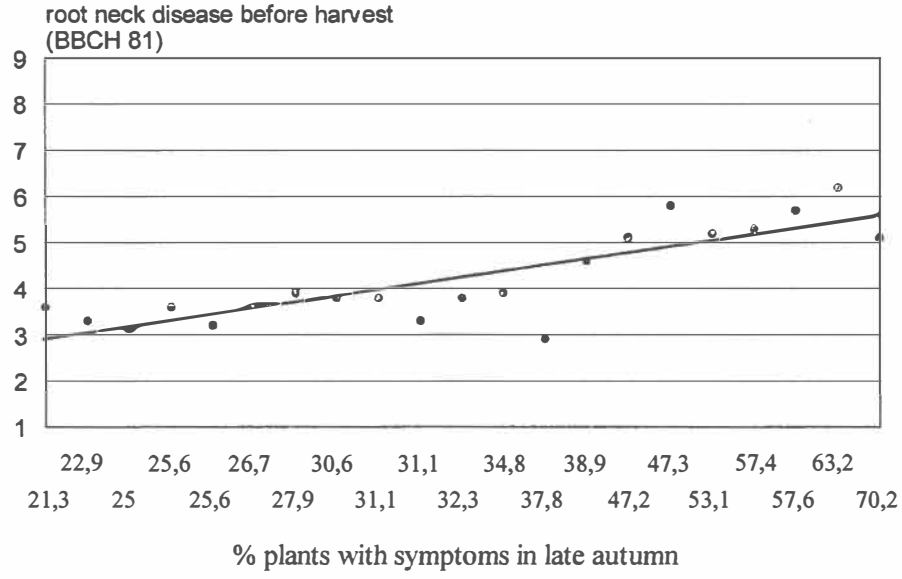

Figure 2: Correlation between the percentage of plants with leaf symptoms in December and the severity of the root neck disease before harvest (Braunschweig 1993-95).

Discussion

Results of GARBE (1993,1994,1995,1996) and GARBE et al. (1993) were confirmed, which show the importance of resistant varieties controlling canker or reducing the infestation level of canker. Low susceptible varieties showed under conditions with high infestation smaller yield losses than high susceptible varieties.

Fungicides which were applied for the control of canker showed lower effects on the disease symptoms in low susceptible varieties and higher effects in susceptible varieties. Under high infestation conditions, the effect of the fungicide was not sufficient. The period of effect of the fungicide is limited to some weeks whereas the infection by asco- or pycnospores is possible during a period between September and December and March and June (THÜRWÄCHTER et al. 1994, THÜRWÄCHTER et al. 1995). For the control of canker by fungicides therefore new substances with a longer period of effect are needed.

The effects on the yields were not only caused by reducing the disease but also by side-effects of the fungicides (azoles) which are a greening effect and a delay of ripening, a reduction of water consumption of the plants, an increase of stress tolerance, a reduction of fast-growth, an increase of winter hardiness and an increase of root/shoot relation (SIEFERT & GROSSMANN 1996). These side effects are very often a reason for growers for applying fungicides although the risk of an infestation by canker is not known. Therefore thresholds for the control of canker are necessary. The correlation between the incidence of plants with symptoms in December and the canker symptoms in July shows, that there are possibilities of estimating the severity of the disease in advance which are useful for a threshold model. The

problem seems to be, that a useful estimation of the later disease by visible symptoms is first possible in December. Fungicides which are today available have only a limited curative effect, therefore in autumn an application is done already in October during a period with strong infection. To use the correlation between the incidence of plants with symptoms in December and the canker symptoms in July for autumn application fungicides with a more curative effect are necessary. Nevertheless the correlation is useful for applications in springtime.

References

GARBE, V. 1993: Effects of fungicide treatments in different varieties of winter rape. Bulletin OILB/SROP **16** (9), 116-134.

GARBE, V. 1994: Auswirkungen von Intensitätsminderungen im Winterrapsanbau auf Krankheiten und Ertragsparameter. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem **301**, 92.

GARBE, V. 1995: Effects of pesticide and fertilizer input reduction on plant diseases and yield in oilseed rape. Proceedings of the 9th International Rapeseed Congress, Cambridge, U.K. Vol. **III**, 983-985.

GARBE, V. 1996: Untersuchungen zur Kontrolle von Pilzkrankheiten im Winterraps. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem **321**, 174.

GARBE, V., E. KLODT-BUSSMANN, D. KNOLL, M. BEINECKE & V. H. PAUL 1993: Europäischer Ringversuch zur Optimierung des Rapsanbaus. Raps **11**(2); 76-80.

SIEFERT, F. & K. GROSSMANN 1996: Nicht fungizide Nebenwirkungen von Triazolverbindungen am Beispiel des Triazolfungizids Epoxiconazol. Gesunde Pflanzen **48**(6);224-231.

THÜRWÄCHTER, F., V. GARBE & H. H. HOPPE 1995: Studies on decision models of the control of blackleg (*Leptosphaeria maculans*). Proceedings of the 9th International Rapeseed Congress, Cambridge, U.K. Vol. **III**, 992-994.

THÜRWÄCHTER, F., V. GARBE & H. H. HOPPE 1994: Erarbeitung von Bekämpfungsentscheidungen der Krankheiten an Winterraps unter besonderer Berücksichtigung der Wurzelhals- und Stengelfäule (*Leptosphaeria maculans*). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem **301**, 88.

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Opportunities to control canker (*Leptosphaeria maculans*) in winter oilseed rape by improved spray timing

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Abstract Canker remains one of the most important diseases of winter oilseed rape in the UK. In eastern England, canker affected 84% and 77% plants pre-harvest in 1995 and 1996 respectively. Control of canker was demonstrated using a mixture of prochloraz and iprodione + thiophanate-methyl used in a series of programmes applied between October and June. Canker incidence was most closely correlated with the incidence of phoma leaf spot at the early stem extension stage (March). Dose rate and timing studies with a range of fungicides showed that commercial programmes gave limited control of canker when applied to a crop where 100% plants had phoma leaf spot in early November. Good control of canker was achieved with high doses of difenoconazole and carbendazim + flusilazole. In commercial crops during 1994-1996, poor control of canker, despite the widespread use of fungicides, was attributed to poor timing of treatments.

Keywords: Canker, fungicides, disease control, spray timing, dose rate, phoma leaf spot, *Leptosphaeria maculans*.

Introduction

Canker caused by *Leptosphaeria maculans* has been one of the major diseases of oilseed rape in the UK for over 20 years (Gladders and Symonds, 1995: Hardwick *et al.*, 1991).

The most serious attacks have been associated with a high incidence of the phoma leaf spot stage in the autumn (Gladders and Musa, 1980). Detailed studies of the development of the pathogen within the host plant has revealed a biotrophic phase as it grows down the leaf petiole to the stem (Hammond *et al.*,1985). Canker lesions developed after a long latent period, provided that the leaves remained attached to the stem long enough for the pathogen to reach the stem (Hammond and Lewis, 1986). The fungicide prochloraz has been used successfully to control canker (Gladders, 1988) but, despite an increase in fungicide use and the recent introduction of new active ingredients in the UK, canker incidence remains high (Fitt *et al.*, 1997). This paper presents preliminary data from canker control experiments on fungicide timing and dose rate and explores why current farm practice is achieving poor control of stem canker.

Methods

Disease surveys

Samples of 25 plants were collected along a transect in commercial crops of winter oilseed rape representative of eastern England in December (GS 1,08 - 1, 12), March (GS 2,1 - 2,3) and July (GS 6,3 - 6,4) from 31 crops in 1994/95 and 27 crops in 1995/96. The incidence and severity (% leaf area affected or 0 - 4 index for canker) of all diseases was assessed (Gladders and Symonds, 1995) and examined in relation to cultivar, and fungicide use.

Disease development experiment

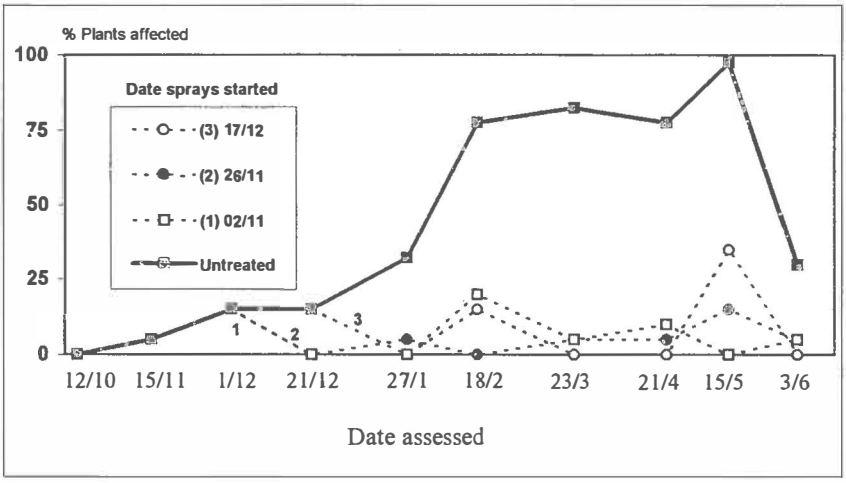
In 1993/94, one of a series of experiments with a systematic design (Sansford *et al.*, 1996) was carried out on winter oilseed rape cv. Envol sown on 7 September 1993 at ADAS Boxworth on clay loam soil. A mixture of fungicides iprodione + thiophanate- methyl (250 + 250 g a.i./ha as Compass) and prochloraz (250 g.a.i/ha as Sportak) was used in a 'wave' design where programmes of treatments were initiated on 15 October and completed progressively later at 3-4 weeks intervals until late June (2 November, 26 November, 17 December, 24 January, 17 February, 21 March, 18 April, 12 May, 6 June and 27 June). The second component of the wave design utilised spray programmes which started progressively later at 3-4 week intervals (as listed above) from 15 October up to 27 June and were maintained up to 27 June. This provided 21 different spray programmes. Treatments were randomised with two replicates which were compared with a double untreated control. Sprays were applied by tractor-mounted sprayer in 200 litres water/ha to plots (plot size 108m^2). Disease assessments were made on 10 plants/plots (after incubation for 1-2 days at ambient temperatures for leaf disease assessments) at or close to each spray date in previously treated and control plots and just prior to harvest (actual dates are shown in Figs 1- 3).

Appropriate fungicide dose experiment

The effect of dose and timing of fungicides was investigated at ADAS Boxworth on winter oilseed rape cv. Rocket sown on 23 August 1994 under high disease pressure in a field previously cropped with seven successive winter oilseed rape crops. The fungicides carbendazim + flusilazole (100 + 200 g a.i./ha as Punch C), carbendazim + prochloraz (185 + 494 g a.i./ha as Sportak Alpha) difenoconazole (125 g a.i./ha as Plover) and tebuconzole (250 g a.i./ha as Folicur) were applied at full rate (as indicated above) and half commercial rates as two spray (11 November and 13 March) or four spray (11 & 29 November, 2 February, 13 March) programmes. Sprays were applied by Oxford Precision Sprayer in 225 litres water/ha. Results are confined to final canker assessment which was carried out just prior to harvest (GS 6,4) on 19 July 1995 on 25 stems/plot.

Figure 1. Incidence of phoma leaf spot on cv. Envol at Boxworth during 1993/94 in untreated control and following treatment with fungicide programmes up to 27 June : a) treatments first applied 2 November to 17 December, b) treatments first applied from 24 January to 12 May.

(a)



(b)

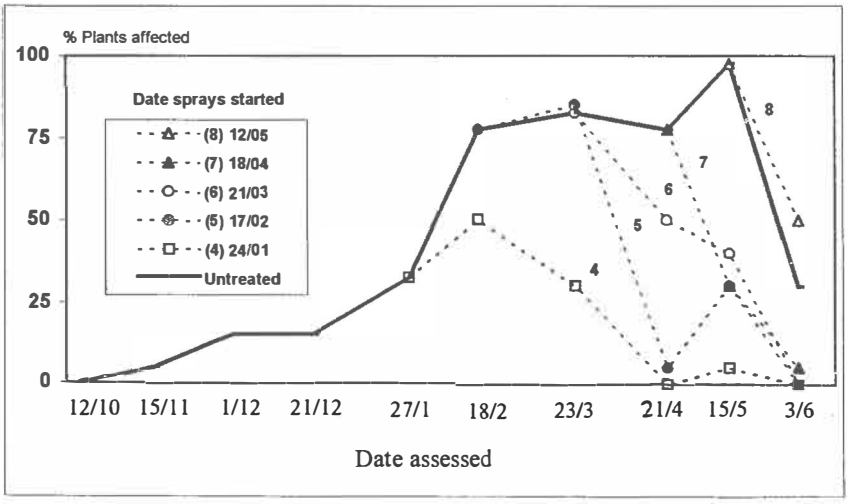
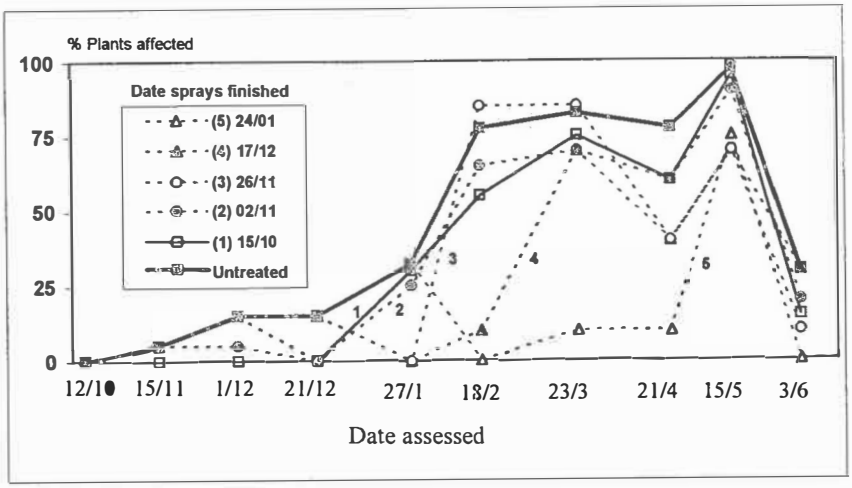


Figure 2. Incidence of phoma leaf spot on cv. Envol at Boxworth during 1993/94 in untreated control and following treatment from 15 October with fungicide programes: a) treatments completed from 15 October to 24 January, b) treatments completed from 17 February to 27 June first applied from 24 January to 12 May.

(a)



(b)

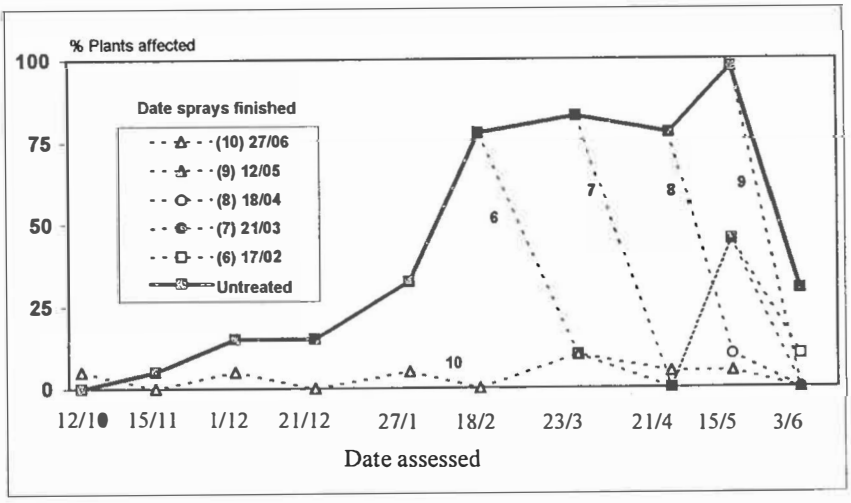
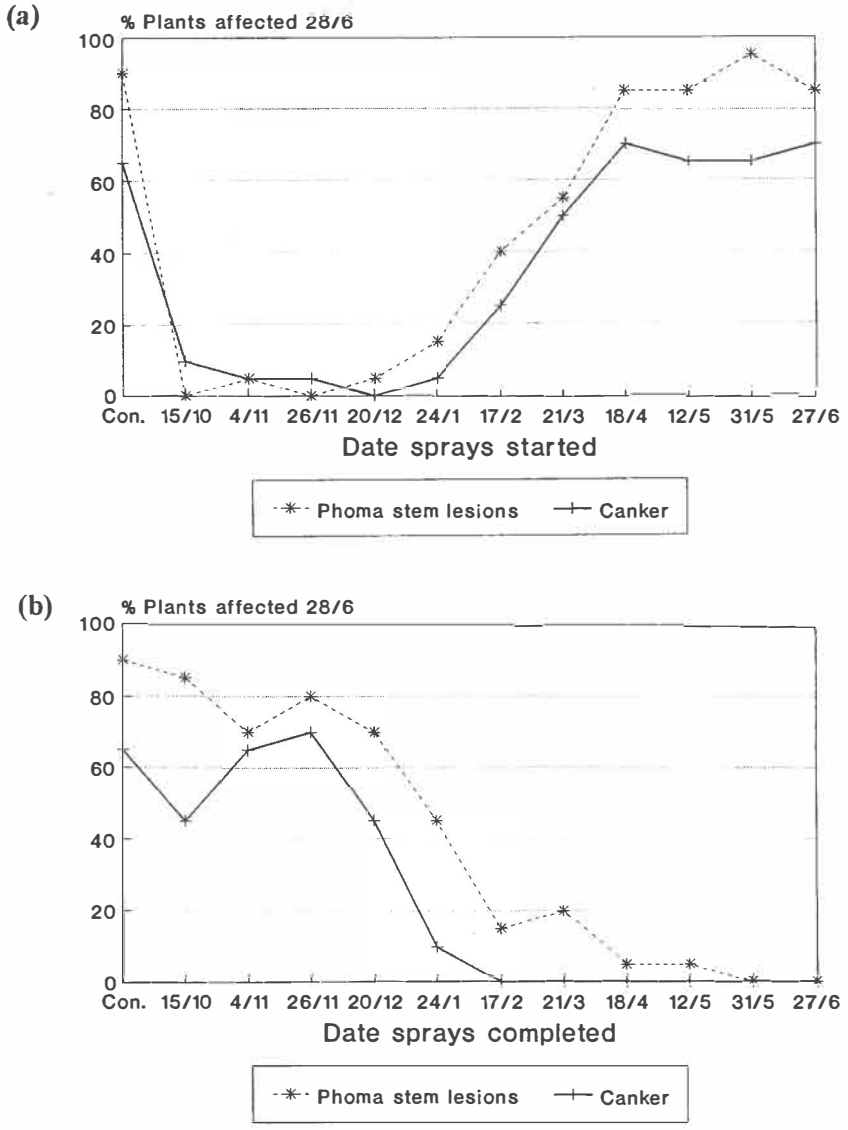


Figure 3. Incidence of canker and phoma stem lesions pre-harvest on cv. Envol at Boxworth in untreated control and following various fungicide programmes : a) fungicide programmes started 15 October to 27 June, b) fungicide programmes completed from 15 October to 27 June.



Results

Disease development experiment

The incidence of phoma leaf spot increased throughout autumn and winter and spray programmes successfully generated a series of different epidemics (Figs 1 & 2). The full programme reduced disease incidence to 5% or less, apart from 23 March assessment when 10% plants were affected compared with 82% in the untreated. Following spray treatment, phoma leaf spot incidence declined sharply 4 - 8 weeks later (Figs 1a & 1b). Re-infection of leaves took place at least 7 weeks after spray programmes had finished (Figs 2a & 2b). Canker affected 65% plants and stem lesions affected 90% plants in untreated plots at the pod ripening stage. Spray timing was very critical for canker control and only programmes continuing up to 24 January or initiated by 24 January gave >85% control (Figs. 3a and 3b). For similar control of stem lesions, programmes initiated by 17 December or completed from 11 April were effective (Figs. 3a &3b).

Appropriate fungicide dose experiment

The first sprays were applied as soon as 100% plants showed leaf spotting symptoms on 11 November. The leaf spot stage was well controlled for 6 - 8 weeks after each treatment (data not presented). Severe canker developed prior to harvest on 69% untreated plants and their mean canker index was 3.1. Canker was most effectively controlled (calculated from reduction in disease index) by both carbendazim + flusilazole and difenoconazole applied as four-spray programmes at either full or half dose (Fig. 4) Limited control was provided by all the two spray programmes and by the four half dose programmes of carbendazim + prochloraz and tebuconazole.

Disease surveys

There were early and severe epidemics of phoma leaf spot in the autumn of 1994 and 1995 which resulted in a high incidence of canker in July (Table 1) despite the widespread use of azole fungicides (Table 2) in 2- or 3-spray programmes (Table 3). Most applications during autumn and winter comprised a single full dose split between two timings. Apex, which is moderately resistant to canker, was the most widely grown cultivar and represented 44% and 48% of crops in 1994/95 and 1995/96 respectively. Fungicides were applied mainly in November and March for control of canker and light leaf spot (*Pyrenopeziza brassicae*) and in April/May for sclerotinia control.

Figure 4. Control of canker (%) with various fungicides applied as four- or two-spray programmes at full or half dose, Boxworth 1994/95 (SED (61df) 8.04). Untreated canker index = 3.1.

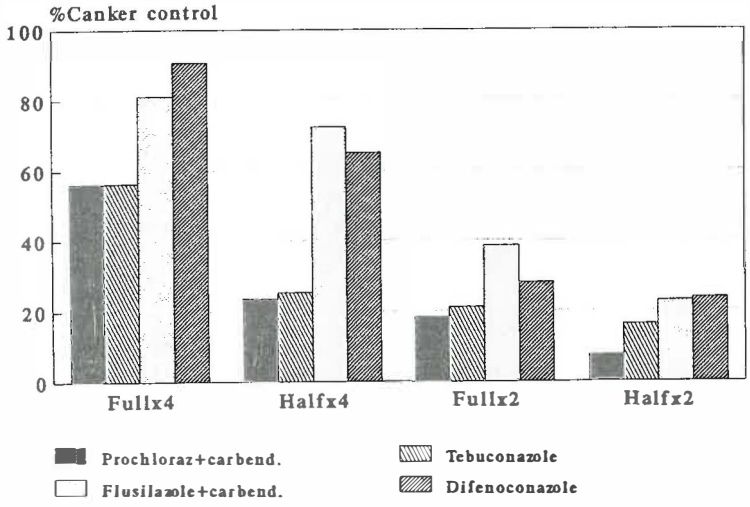


Table 1. Incidence and severity of phoma leaf spot, stem lesions and canker, eastern England 1994-1996

	1994/95 % plants affected (severity)*	1995/96 % plants affected (severity)*
Phoma leaf spot (Dec)	86 (0.5)	61 (0.7)
Phoma leaf spot (Mar)	54 (0.2)	53 (0.1)
Phoma stem lesion (Jul)	47 (0.6)	39 (0.4)
Phoma canker (Jul)	84 (1.4)	77 (1.1)

* % leaf area affected or stem index

Table 2. Percentage of crops sprayed each month and fungicide type used, eastern England 1994-1996

Fungicide applied	1994/95			1995/96		
	Azole ± MBC	*Dicarb ± MBC	MBC	Azole ± MBC	*Dicarb ± MBC	MBC
October	3	0	0	4	0	0
November	26	0	3	41	0	0
December	6	0	0	4	0	0
January	3	0	0	15	0	0
February	3	0	0	7	0	0
March	61	0	0	52	0	0
April	13	19	16	26	0	4
May	0	13	16	4	7	33
June	0	3	3	0	4	7

* Dicarb = dicarboximide group

Table 3. Number of fungicide spray application to winter oilseed rape, eastern England 1994-1996.

Number of spray applications/crop	% crops receiving fungicide	
	1994/95	1995/96
0	3	0
1	26	22
2	45	48
3	23	26
4	3	4

Discussion

The 'wave' experiments, as illustrated by the site at Boxworth, demonstrated that fungicides could provide good control of both phoma leaf spot and canker. There was a close correlation between the incidence of phoma leaf spot on 23 March and canker incidence pre-harvest defined by the equation: % canker = 0.75(% plants with phoma leaf spot on 23 March) - 3.68, (r^2 = 86.2%, P <0.001). This analysis also identified a high residual value for the treatment programme initiated on 17 February, which had 85% plants with leaf spot in March but only developed 25% canker. It is probable that this treatment provided control of canker by

preventing the pathogen reaching or developing in the stem base. The regression model also implies that only 75% of plants with leaf spots develop canker. This may be due to failure of the pathogen to reach the stem before leaf dehiscence (Hammond and Lewis, 1986) and/or fungicidal control after appearance of the leaf spots.

The critical period for canker control was found to be the period from November to February for the whole of this series of experiments (Sansford *et al.*, 1996). At the Boxworth site, however, spray programmes starting or completed in January were effective. Whilst the disease development experiments apparently defined the critical period for canker control, this appears to require careful interpretation in relation to epidemic development. During 1991-1994, epidemics of phoma leaf spot developed slowly from autumn onwards and reached a peak in the spring (ADAS unpublished data). In autumn 1994 and 1995, phoma leaf spot was much more prevalent in October than in previous years and in many crops its incidence was higher in autumn than in spring. The appropriate dose experiment at Boxworth clearly demonstrated for the first time that current commercial programmes based on two treatments at either full or half dose rate have limited activity against canker if leaf spotting is already present. It appears that carbendazim + flusilazole and difenoconazole have some curative activity against phoma leaf spot at high dose rates. These dosages are generally higher than currently recommended and are not necessarily cost-effective. Further analyses are in progress to define economic optima in relation to disease development. For effective control of canker, however, it appears that fungicides need to be applied as protectants prior to widespread development of leaf spotting.

Examination of commercial treatments over the last two years (when phoma leaf spot symptoms were seen earlier than usual and developed rapidly during October) suggests that most treatments were applied too late to achieve good control. Treatments applied in October and February would be optimal. Our observations in unreplicated plots in 1995/96 suggested that delaying sprays from October to November could reduce yield responses by half. Timing of the first spray was critical and relatively few farmers made applications in October and none made applications in October and February. Hammond and Lewis (1986) showed that leaves with phoma leaf spot attached to the plant at the rosette stage produce cankers. However, these basal leaves senescence during stem extension growth (*c.* GS 3,3) and fungicides should be applied before this stage if cankers are to be controlled. This would appear to explain why February sprays were the latest stage at which canker could be controlled in 1993/94 (Fig. 2). Most spring sprays were applied by farmers in March (Table 2) and this would therefore generally be rather late to control canker. Canker affected 100% plants in 13 out of 57 surveyed crops (mean data from all crops is given in Tables 1-3), clearly demonstrating the limited efficacy of fungicide treatments.

Recent progress in understanding the development of canker and its control with fungicides has highlighted the potential to improve canker control in commercial crops. More detailed information on forecasting epidemic progress in the autumn is needed as the timing of the first spray is critically important. There is also potential to improve control by making spring applications earlier and by careful selection of product and dose. In addition, the value of maintaining control during the winter with repeated reduced dose treatments merits wider investigation. There are financial benefits for the farmer by reducing yield loss of up to 1 t/ha from canker (Sansford, 1995) and environmental benefits from improved targeting of treatments at reduced dose rates.

Acknowledgement

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References

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

Gladders, P. 1988: The contribution and value of pesticides to disease control in combinable break crops. pp 29-50 in : Control of Plant Diseases : Cost and Benefits, Clifford, B.C.; Lester, E. ed. Oxford, Blackwell Scientific Publications.

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

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

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

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

Hardwick, N.V.; Fitt, B.D.L.; Wale, S.J.; Sweet, J.B. 1991: *Oilseed rape diseases*. HGCA Oilseed Rape Research Review No. OS4, 131pp.

Sansford, C.E. 1995: Oilseed rape: development of canker *Leptosphaeria maculans* and its effect on yield. Proceedings of 9th International Rapeseed Congress 2: 577-579.

Sansford, C.E.; Fitt, B.D.L.; Gladders, P.; Lockley, K.D.; Sutherland, K.G. 1996: *Oilseed rape: disease development, forecasting and yield loss relationships*. Home-Grown Cereals Authority Project Report OS17. 18pp.

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Development and orientation of research programmes on winter rape protection in the plant protection institute in Poznań

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Oilseed rape in Poland is attacked by a large number of diseases and pests and needs proper plant protection in order to assure sufficient yield. In the researches carried out by Plant Protection Institute oilseed rape protection has always played an important role.
Taking into consideration an increasing number of results as well as world trends the orientations of research has changed with the time. The aim of research carried out in 1950-1970 was mainly to elaborate the biology and noxiousness of separate disease and pests species as well as the programmes of their control. Further, more attention was paid to the safety of control treatments to the environment and humans as well as to the need of treatments and the role of environmental factors in decreasing disease and pest problems.

Research was developed on the documentation of appearance and incidence of the main pest species, on the economical thresholds, on the selectivity of applied plant protection products for beneficial insects, on the occurrence and importance of natural enemies of rape pests, on the importance of agrotechnical treatments, on varietal resistance, on the use of plant protection products in tank-mixes, on the role of glucosinolates, on plant protection techniques and other problems.

The research was accompanied by the implementation of good plant protection practice.

An important role was played by new Plant Protection Law, which is the background of proper development of plant protection in Poland.

The results of research as well as their implementation and dissemination made possible the change of recommended programmes of oilseed rape protection, taking into consideration an increasing number of factors related to integrated pest management.

The scientific staff of Plant Protection Institute published up to 1995 225 scientific publications connected with rape protection (Pruszyński at al.1996b).

In the Table 1 (Pruszyński at al. 1996a) the scientific achievements of Plant Protection Institute staff concerning rape protection is presented together with the development of associated scientific programmes. Several programmes and the results obtained will be discussed below.

Main risks and present state of winter rape protection

In the Figure 1, the main pests and diseases damaging winter rape in Poland are presented. This list should be supplemented with pest species occurring less frequently, but being from time to time the cause of considerable losses, such as: turnip gall weevil, turnip sawfly, cabbage fly, and lately, after the implementation of „oo" rape varieties - also rodents and slugs.

Table 1. Winter oilseed rape-research programmes carried out in the Plant Protection Institute in Poznañ in the years 1955 - 1995 (Pruszyñski et al., 1996)

Research subjects	1955 - 1960	1961 - 1965	1966 - 1970	1971 - 1975	1976 - 1980	1981 - 1985	1986 - 1990	1991 - 1995	Number of publications
Pests	—	—	—	—	—	—	—	—	49
Diseases	—			—		—	—	—	19
Weeds					—	—	—	—	16
Protection programmes		—	—	—	—	—	—	—	12
Tank-Mix						—	—	—	18
Growth regulators						—	—	—	14
Cultivation	—	—	—	—	—	—	—	—	8
Forecast and pest regist.	—	—	—	—	—	—	—	—	31
Pesticide residues				—	—	—	—	—	7
Pest resistance			—	—	—				3
Varieties resistance				—		—	—	—	15
Biological methods							—	—	3
Nonchemical methods	—	—	—	—	—	—	—	—	8
IPM								—	3
Ecology and Environment							—	—	13
Biochemistry						—	—	—	7
Economy					—	—	—	—	17

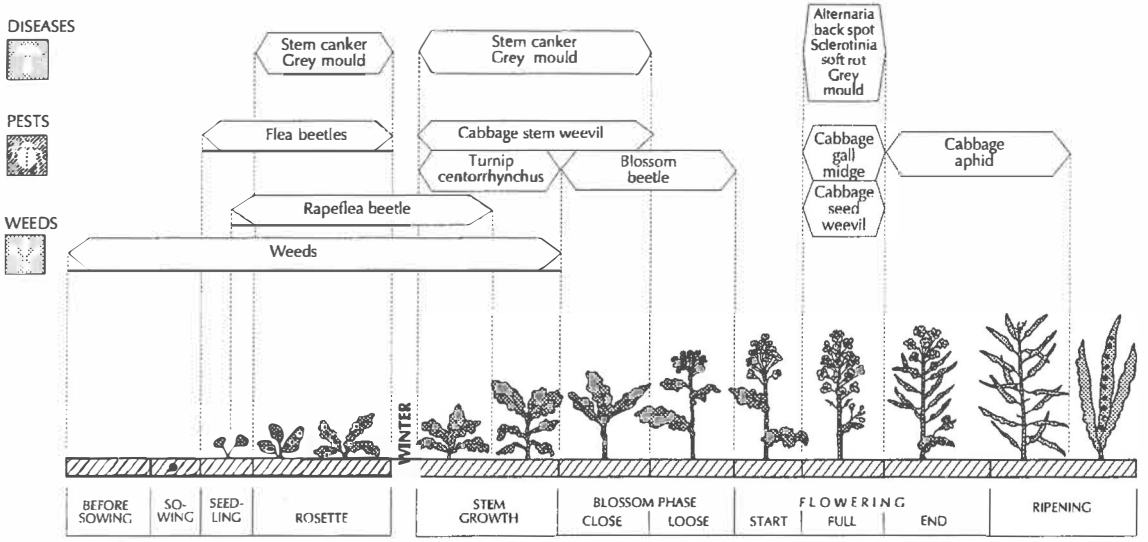


Fig 1 **The occurrence of the main pests and diseases on winter rape plants in Poland**

Taking into consideration, the high risk caused by pests and diseases, as well as frequent appearances of diseases and pests - the state of winter rape protection in Poland should be estimated as unsatisfactory. According to Mrówczyñski et al. (1995) during 1993/1994 season one treatment of pest control was performed on 95.9% of winter rape fields, and two treatments on 72.1% of fields. In 1994/1995 season one treatment was performed on 93.4% of fields, and two treatments on 30.5% of fields. As the result of unsatisfactory winter rape protection and apart from the reduction of fertilization and unfavourable atmospheric conditions, a considerable decrease of rape yield has been stated, amounting from 25.4 q/ha (average from 1986-1990) to 16.6 q/ha in 1996 or 17.1 - 22.4 q/ha in 1992-1995.

It should be emphasised that the state of winter rape protection is the result of general, very low use of plant protection products in Poland, which now amounts to approximately 0.5 kg of active ingredient/ha.

Previous scientific programmes

Registration of appearance and numerousness of pests and diseases and forecasts of their occurrence

The Plant Protection Institute since the beginning of its history has paid special attention to the research connected with this problem, and in the consequence of that already at the and of the fifties the system of collection of information on the occurrence of main diseases and pests of agricultural crops on the territory of the whole country has been achieved.

This system, performed presently using computer techniques, takes also into account 11 main pest and disease species of winter rape.

As the result of observations and interpretation of obtained data the informations on present occurrence of separate species on the territory of the whole country (Fig. 2), as well as the averages of their occurrence within trends of development (Fig.3) have been collected. The materials mentioned above are every year published by Plant Protection Institute and they are the background of planning and preparing the actions of control treatments of separate crops (Walczak at al. 1997).

In presenting the backgrounds of the decisions on the need for control treatment the economic thresholds should be taken into account. The values of the thresholds have been published by the Institute in 1986 (Piekarczyk, WoŸny 1986), nevertheless they should be currently verified with the consideration of biological and climatical factors. It is a very important scientific task to be performed and the participation of several scientific centers should be foreseen in the realisation of this task.

Figure 2. The occurrence of *Turnip ceutorrhynchus* (*Ceutorhynchus napi* Gyll.) on winter rape plantations in Poland in 1996 (Walczak et al. 1997)

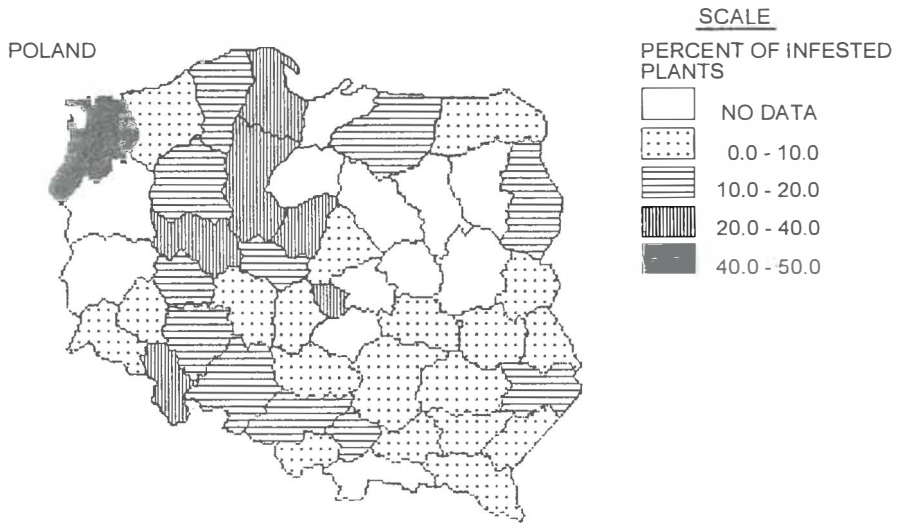
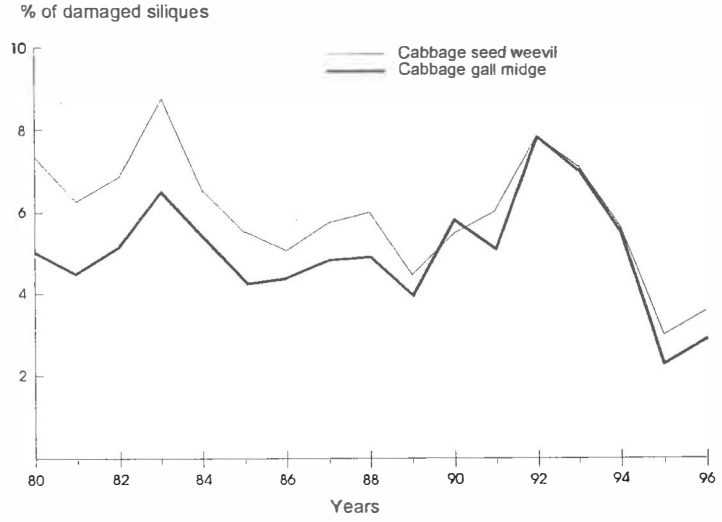


Figure 3. The occurence of cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) and cabbage gall midge (*Dasyneura brassicae* Winn.) on winter rape in Poland in the years 1980-1996 (Walczak et al. 1997)



Chemical control

The research carried out concern systematic improvement of the programmes of winter rape control through the introduction of new plant protection products. In Poland the same plant protection products as in the countries of western Europe are allowed to be used, nevertheless their use is not optimal.

For example pest control is weighted towards use of synthetic pyrethroids (Table 2), and the need for rotation of plant protection products as well as the protection of beneficial species are not taken into account (Mrówczy□ski at al. 1995). This situation is in a great part caused by economical factors (low price of synthetic pyrethroids), nevertheless it can be the reason of the development of pest resistance (there is lack of research on this problem in rape pests in Poland) and is unfavourable for the environment.

Table 2. Insecticides applied in winter rape protection in Poland (Mrówczyñski et al., 1995)

active ingredient	% of treated area	
	1993 / 1994	1994 / 1995
alfa - cypermethrin	32.2	30.4
deltamethrin	30.2	32.8
Lambda - cyhalotrin	15.1	14.0
zeta - cypermethrin	6.9	9.1
esfenvalerat	5.9	1.7
bensultap	2.8	2.6
cypermethrin	2.8	6.8
others	4.1	2.6

The new trend of research, now well developed by the Plant Protection Institute, was the elaboration of the possibility of use plant protection products and fertilizers in tank-mixes in the protection and fertilization of winter rape. This research were started as the result of farmer`s requests, who could save time and money applying tank-mix techniques. The results obtained in these research programmes were in a short time implemented in practice (Pruszyñski edit. 1996).

Application of biological and other non-chemical methods

In the research carried out by Plant Protection Institute special attention was always paid to as large as possible implementation in practice of such the methods which could permit reduced chemical control. Very early (Szulc 1959) the researches on the role of proper cultural techniques in winter rape protection were started and they are continued up to now (Palosz, Sieñkowski 1992). One of the questions taken into account in research was the evaluation of the effectiveness of rang treatments (Palosz 1980) as well as the use of trapping plants in the protection of winter rape against pollen beetle.

In research on the role of biological factors in the reduction of incidence of winter rape pests, studies were developed on the parasites of pollen beetle and cabbage seed weevil larvae, on the effect of chemical plant protection products use on the incidence and species composition of Carabidae (Palosz 1995), on the selectivity of insecticides to beneficial insects (Palosz at al. 1994), as well as studies on the pollen beetle populations in several European countries (Lipa, Hokkanen 1992).

The problem of resistance

Unfortunately, on pest resistance to plant protection products were only carried out in Plant Protection Institute for a short time. Because of staff and financial reasons development of this trend in scientific research did not succeed.

Considerably mole extensive research has been undertaken on the susceptibility of new cultivars and varieties of winter rape to the main pests (Mrówczyñski 1993a,b,c).

These researches have been supplemented with the explanation of the role of glucosinolates in rape resistance to the pests (Waligóra at al. 1991), and in other researches (Ro¿ej 1974) the problem of rape varieties resistance to diseases has been undertaken.

Integrated Pest Management

Using the results of the above and other research carried out in other scientific centers, activities have been undertaken aimed at the elaboration and implementation in practice of integrated pest management in the protection of winter rape (Palosz at al. 1994, Pruszyñski at al. 1995).

In integrated pest management of winter rape the following elements have been taken into account: recommendations concerning proper agrotechnique (four years break in rape culture on the same field, space isolation, proper timing of sowing and quality of seeds, use of trapping plants, proper fertilization, proper and complete cultivation preceding the sowing), natural elements of the ecosystem (observations on the occurrence and role of natural enemies of pests), as well as proper use of plant protection products (decision on chemical treatment basing on present pest numerousness, use of selective products, if possible - introduction of rang treatments, use of tank-mix techniques, complying with regulations for the protection of honey-bees).

Through training as well as other methods of dissemination of achievements (demonstrations, articles in the press) numerous elements of integrated pest management are presently taken into consideration in agricultural practice.

Good plant protection practice

Good plant protection practice requires compliance with several conditions, such as: proper decision on the treatment, proper choice of plant protection product, proper application rate and proper timing of the treatment, efficient plant protection equipment etc.

Good plant protection practice is not directly connected with scientific research, nevertheless the Plant Protection Institute pays special attention to the dissemination of its rules to the farmers. Good plant protection practice should be treated as the „introduction" to

the integrated pest management, and without complying with good plant protection practice requirements - integrated pest management is out of the question.

Thanks to the regulations of Plant Protection Law the implementation of good plant protection practice to agricultural production became much easier (Pruszyñski 1996). According to Plant Protection Law the performes of plant protection treatments should be trained, they should record the treatments using plant protection products of Ist and 2nd class of toxicity, plant protection equipment should be inspected and checked (this regulation comes into force January 1st, 1999), as well as plant protection products should be used only according to their authorization presented in the label. To supervise the regulations of Plant Protection Law State Inspectorate of Plant Protection has been appointed.

As it has been writen earlier, the Plant Protection Institute through the training, demonstrations, lectures, publications and articles in the press (Lipa, Bartkowski 1996, Germaziak, Podgórska 1996) implements and disseminates the rules of good plant protection practice, which are one of the most important elements in making control treatments in winter rape more safe and effective. It is especially important in Poland, where the number of farmers is very high, and their professional level is highly differentiated.

Conclusion

The changes taking place in world and Polish plant protection seem to be good evidence of systematic progress in the research and improvement of the programmes of protection, including of winter rape.

The pressure of world public opinion, and first of all good understanding of science and agriculture of the necessity of develompent and implementation of environmentally safe integrated approach to the protection of crops and technology of production, has caused numerous favourable changes.

In Poland a very important moment, mentioned earlier, was the enforcement of new Plant Protection Law, where, in issue 29 we can read, that „the priority should be given to biological, agrotechnical, cultural and other methods, and plant protection products should be used only in justified cases".

From the other side, each limitation of the use of chemical methods requires suitable presentation of alternative methods based on many-years research and observations and assuring profitable production to the farmer.

New trends in plant, change of varieties, husbandry, as well as world trade can be the reason of the necessity of new research trends., in Poland the example of such new trends was the development of rodents feeding on „00" rape varieties or the appearance of slugs.

That is why there is need for further intensive continuation and development of research on winter rape protection, because only by basing the programmes of protection on the results of scientific research can we assure their effectiveness and safety.

References

Germaziak N., Podgórska B. 1996. Dobra praktyka ochrony roslin w dokumentach Unii Europejskiej. Ochrona Roslin 5: 508

Lipa J.J., Bartkowski J. 1996. Dobra praktyka ochrony Roslin: rekomendacje EPPO. Progress in Plant Protection (Postêpy w ochronie roslin) 36(1): 81-87

Lipa J.J., Hokkanen H. 1992. Nosema meligethi I and R (*Microsporidia*) in populations of Meligethes spp. in Europe. Biocontrol Sciences and Tewennol, 2: 119-125

Mrówczyñski M. 1993a. Uszkadzanie typów i odmian rzepaku ozimego przez szkodniki. Cz. I. Szkodniki jesienne. Prace Naukowe Instytutu Ochrony Roslin 34, 1/2: 5-31

Mrówczyñski M. 1993b. Uszkadzanie typów i odmian rzepaku ozimego przez szkodniki. Cz. II. Szkodniki lodygowe. Prace Naukowe Instytutu Ochrony Roslin 34, 1/2: 32-44

Mrówczyñski M. 1993c. Uszkadzanie typów i odmian rzepaku ozimego przez szkodniki. Cz. III. Szkodniki luszczynowe. Prace Naukowe Instytutu Ochrony Roslin, 34 1/2: 45-62

Mrówczyñski M., Widerski K., Wachowiak H., Krasiñski T. 1995. Stan ochrony rzepaku ozimego przed szkodnikami w Polsce w badaniach ankietowych. Materialy 35 Sesji Naukowej Instytutu Ochrony Roslin. Cz. II. Postery: 104-106

Palosz T. 1988. Ekonomiczne, ekologiczne i agrotechniczne elementy doskonalenia programów zwalczania szkodników rzepaku ozimego. Instytut Ochrony Roslin. Poznañ. Rozprawa habilitacyjna: 1-79

Palosz T. 1995. Ground beetles (*Carabidae*) on winter rape crops with different plant protection, rotation and soil characteristics. Proceeding 9th International Rape Seed Congres. Cambridge 4: 1321-1323

Palosz T., Sieñkowski A. 1992. Wyniki zastosowania roslin pulapkowych w ochronie rzepaku przed szkodnikami. Zeszyty Problemowe Instytutu Hodowli i Aklimatyzacji Roslin. Rosliny Oleiste 14 (2): 304-309

Palosz T., Mrówczyñski M., Musnicki Cz. 1994. Podstawy Integrowanej ochrony rzepaku ozimego przed agrofagami. Materialy 34 Sesji Naukowej Instytutu Ochrony Roslin. Cz. I. Referaty: 111-116

Palosz T., Sieñkowski A., Kord I. 1994. Wyniki badañ skutecznosci selektywnych insektycydów (Bancol, Bitoksybacilin) w ochronie rzepaku przed slodyszkiem i szkodnikami luszczynowymi. Materialy 34 Sesji Naukowej Instytutu Ochrony Roslin. Cz. II. Postery: 27-31

Piekarczyk K., WoŸny J. 1986. Progi ekonomicznej szkodliwosci chorób i szkodników roslin uprawnych. Instytut Ochrony Roslin. Poznañ 37 str.

Pruszyñski S. (red.). 1996. £¹czne stosowanie agrochemikaliów w uprawach rolniczych. Wydanie II uzupelnione. Instytut Ochrony Roslin. Poznañ 89 str.

Pruszyñski S., Palosz T., Mrówczyñski M. 1995. Niekotoryje elementy sistemy integrovannej zašèity ozimogo rapsa ot vreditelej, bolezni i somiahov. Information Bulletin EPRS IOBC, 31: 133-139

Pruszyñski S., Palosz T., Mrówczyñski M. 1996a. Badania Instytutu Ochrony Roslin nad ochron¹ rzepaku przeciwko szkodnikom, chorobom i chwastom. Rosliny Oleiste. Instytut Hodowli i Aklimatyzacji Roslin. T. XVII(1): 11-19

Pruszyñski S., Palosz T., Mrówczyñski M. 1996b. Publikacje naukowe pracowników Instytutu Ochrony Roslin w Poznaniu dotycz¹ce ochrony rzepaku przed agrofagami w latach 1957-1996. Instytut Ochrony Roslin. Poznañ. 21 str.

Pruszyñski S. 1996. Ochrona srodowiska w zapisach Ustawy o ochronie roslin uprawnych. Progress in Plant Protection (Postêpy w ochronie roslin) 36(1): 35-42

Ro¿ej A. 1974. Podatnosa ró¿nych odmian rzepaku ozimego na pora¿enie przez *Alternaria brassicae* (Berk.) Sacc. Roczniki Nauk Rolniczych. A.100, 2: 67-71

Szulc P. 1959. Badania nad wplywem zabiegów uprawowych na straty w plonie rzepaku ozimego wywolane przez szkodniki. Prace Naukowe Instytutu Ochrony Roslin 1(1): 231-276

Walczak F., Grendowicz L., Manys P., Piekarczyk J., Piekarczyk K., Skorupska A., Wójtowicz A. 1977. Szkodliwosa wybranych agrofagów roslin uprawnych w Polsce w 1996 roku oraz wstêpne prognozy na rok 1997. Poster zaprezentowany na 37 Sesji Naukowej Instytutu Ochrony Roslin

Waligóra D., Krzymañska J., Lisiecka E. 1991. Zawartosa glukozynolanów w lisciach rzepaku, a rozwój mszycy kapuscianej (*Brevicoryne brassicae* L.). Materialy 31 Sesji Naukowej Instytutu Ochrony Roslin. Cz. II. Postery: 228-231

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Resistance of linseed cultivars to *Fusarium* Wilt

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Abstract In the years 1980-1990 the studies on the resistance of liseed cultivars to *Fusarium* wilt were carried out. Resistance of 56 cultivars under natural infection conditions was estimated. in the environment of these studies it was found that 24 cultivars were resistant and 20 susceptible to *Fusarium* wilt.

AC Linora, Mikael, Summit, M-3266,Taragui and others were very resistant. Barbara, Liflora, Istru, Buchara yellow, Abisynian and others were very susceptible.

Keywords: *Fusarium* wilt, linseed cultivars, resistance

Introduction

At the turn of the century *Fusarium* wilt was the limiting factor in flax production. Different species of genus Fusarium are the most frequently occurring pathogens of flax in all regions of the world. The major agent of Fusarium wilt of flax is the fungus *Fusarium oxysporum* f. sp. *lini*. The pathogenicity of these species in relation to flax has been studied by many authors under polish conditions. (Zarzycka 1973), (Laciowa et al. 1978), (Sadowski et al. 1980).

Studies of all flax varieties from our collection for resistance to Fusarium wilt have been carried out in the Institute of Natural Fibres in Poznan since 1977.

The aim of the studies presented here was to estimate the resistance of many linseed cultivars to *Fusarium* wilt. This paper presents the results of the completed testing of some varieties.

Methods

The experiments were carried out in conditions conducive to infection in the field in which the soil was infested with species of genus *Fusarium*. The soil was also inoculated with mixture of six species of *Fusarium*, isolated from infected flax.

The fields were devided into several areas so that varieties of flax were rotaded and were not grown in the same area more often than once every 4 years.

Experiments were performed using the random block method in 4 replications. Disease resistance of each species was evaluated on the basis of the health of 1000 plants (4 x 250). Healthy seeds were used in the tests. The control variety was Klein 18 – very resistant to *Fusarium* wilt.

During the experiments herbicides were not used and weeding was done by hand.

The health of the tested varieties was evaluated during the following stages of flax grows: seedling, the "fir tree" stage (6 to 12 high), pre-flowering and the green seed capsule stage.

The main indicator of varietal resistance utilised in this method was the number of healthy plants in the green seed capsule stage expressed as the per cent of the total number of seedlings.

The criteria of the final evaluation of varietal resistance was the everage per cent of healthy plants after completion of the testing cycle (4 – 6 tests) in comparison with the average per cent of healthy plants in the control variety Klein 18.

Varieties have been divided in groups of resistance according to the following scale:

Very resistant	> 95%	healthy plants in relation to the control
Resistant	> 80 – 95%	healthy plants in relation to the control
Moderately resistant	> 50 – 80%	healthy plants in relation to the control
Moderately susceptible	> 30 – 50%	healthy plants in relation to the control
Very susceptlible	< 30%	healthy plants in relation to the control

Results

Between 1980-1990 90 linseed cultivars were tested. The experiments were performed under natural conditions where non-race specific or field (horizontal) resistance was evaluated.

The first of flax wilt occurredsporadically duriong the seedling stage.

The next disease attack was observed in the "fir tree" and the rapid growth stage. This was a typical *Fusarium* wilt of flax beginning at the top of thew plant where necrosis also began. Reisolated species of *Fusarium* from these plants were mainly: *Fusarium oxysporum* f. sp. *lini*, *Fusarium culmorum* and *Fusarium gibbosum*.

Fusarium wilt of flax occurred often also in the pre-flowering stage – drying of the plant apex and flower bud. The couse of wilt was the infection which was mainly from *Fusarium oxysporum* f. sp. *lini*, *Fusarium culmorum*, *Fusarium avenaceum* and *Fusarium sambunicum*.

The drying of older plants after the seed capsules stage was also sporadically observed.

After detailed analysis of results it was found that most of the tested varieties were very or moderately resistant to *Fusarium* wilt. (Table 1) These varieties clearly showed resistance in all phases of plant development.

Among these varieties, of particular interest are those which showed in all tests greater resistance to fungal infection than the control, Klein 18. There are: Areco, Atalante, Charrua MA, M-3266, Summit, Mikael and AC Linora.

The other varieties studied were susceptible to fusariosis (Table 2). In tests there was observed a lack of resistance in all stages of development in most plants of these varieties. This was most evident in the "fir tree", rapid growth and pre-flowering stages.

Reisolated fungi confirmed that the cause was fusariosis and mainly fusariol wilt of flax. In total quantity (20) of susceptible varieties 10 are considered to be vera susceptible. There are: Barbara, Liflora, Istru, Buchara yellow, Abisynion, Ecotipo Camporeale, Ocean, Mc Gregor, Wilden and Azur.

Varieties in which more than 31 per cent of the plants wilt would be regarded as very susceptible and would not ordinarily be considered further in a program of breeding for wilt resistance.

Discussion

Fusarium species are soil-borne fungal pathogens of flax, which can cause serious plants losses under intesive cultivation of these crops.

Results of many studies showed that these fungi are very common in cultivated soil in Poland and in other countries. (Ondrej 1977), (Krylova 1978), (Zarzycka et al. 1993).

The effectineness of testing for *Fusarium* wilt resistance has been illustrated in past decades by the selection and release of wilt resistant varieties.

Since flax varieties always have been selected and tested in the field for resistance, the resistance obtained is not specific but horizontal-field resistance. This well-known fact is the basis for selection wilt-resistant lines (Kommendahl 1970).

Analysis of our studies results showed variation in resistance of linseed cultivars to *Fusarium* wilt.

The response of susceptible and less susceptible varieties to the pathogenicity of the fungi and to the process of infection was different in all phases of plant development.

Varieties: AC Linora, Mikael, Summit, M-3266, Taragui, Viking and Redwood would be considered further in a program of breeding in wilt resistant linseed varieties.

Very susceptible varieties such as the following: Azur, Wilden, Mc Gregor, Ocean, Ecotipo Camporeale and Barbara, Liflora, Istru, would not be cosidered further.

Table 1. Resistance of varieties of flax to *Fusarium* wilt

No	Varieties	Average per cent of healthy plants		
		varieties	control (Klein 18)	relation to control
Varieties very resistant				
1	AC Linora	66,6	37,7	176,7
2	Mikael	68,7	39,6	173,5
3	Summit	70,5	51,2	137,7
4	M 3266	67,0	51,2	130,9
5	Taragui	69,0	54,9	125,7
6	Charrua MA	61,0	48,6	125,5
7	Atalante	77,4	62,6	123,6
8	Areco	45,4	37,7	120,4
9	Victory A	66,1	55,4	119,3
10	Koto	79,0	69,5	113,7
11	Viking	51,5	48,6	106,6
12	Redwood	57,2	53,9	106,1
Varienties resistant				
1	Cass	45,7	48,6	94,0
2	Bison	51,8	55,4	93,5
3	Buck 114	47,0	50,8	92,5
4	Repetible 117	65,7	71,8	91,5
5	Tabare	45,6	50,8	89,8
6	Argentine 407 - 6	67,5	75,5	89,4
7	Hindukush	55,0	62,6	87,9
8	Stewart	49,4	56,3	87,7
9	Birio	41,4	48,6	85,2
10	Williston golden	37,9	46,6	81,3
11	Williston brown	50,8	62,6	81,2
12	LCSD 200	46,9	57,8	81,1
Varienties moderatelly resistant				
1	Polk	48,3	62,6	77,2
2	Malabrigo	50,7	71,8	70,6
3	Dakota CI 1071	38,8	56,3	68,9
4	Record	46,8	69,5	67,3
5	Bolley golden	37,4	56,3	66,4
6	Kenya CI 709	35,0	55,1	63,5
7	Albufeira	37,6	61,8	60,8
8	Bowman	38,8	56,8	59,5
9	Leona	42,7	71,8	59,5
10	Rocket	32,4	55,1	58,8
11	Bionda	34,5	59,8	57,7
12	Vitagold	36,9	69,5	53,1

Table 2. Resistance of varieties of flax to *Fusarium* wilt

No	Varieties	Average per cent of healthy plants		
		varieties	control (Klein 18)	relation to control
Varieties moderately susceptible				
1	Isikulskij	36,5	71,8	50,8
2	Rota II	30,9	62,6	49,4
3	Ottawa 770 B	21,2	44,9	47,2
4	Pole blue crimped	26,4	56,2	47,0
5	Sofia	29,1	67,0	43,4
6	Newland	20,6	51,2	40,2
7	Antares	25,4	63,4	40,1
8	Akmolinsk	28,1	75,5	37,2
9	Maxi gold	13,8	39,6	34,9
10	Ile de Crête	21,6	62,6	34,5
Varieties very susceptible				
1	Azur	10,0	53,9	18,6
2	Wilden	11,5	62,6	18,4
3	Mc Gregor	5,7	37,7	15,1
4	Ocean	8,2	56,3	14,7
5	Ecotipo Camporeale	8,3	58,7	14,2
6	Abisynian	8,3	61,8	13,4
7	Buchara yellow	4,9	61,8	7,9
8	Istru	3,6	48,1	7,5
9	Liflora	1,5	37,7	4,0
10	Barbara	0,0	37,7	0,0

References

Kommendahl T., Christensen J.J. 1970: A half century of research in Minesota on flax wilt caused by *Fusarium oxysporum*. Minn. Agr. Sta. Tech. Bull. 273: 1-35.

Krylova T.V. 1978: Ustojcivye k gribnym zabolevanijam obrazcy Ina-dolgunca. Selek. i Semenov. 2: 32-33.

Laicowa B., Kiecana I. 1978: Badania nad chorobami Inu (Linum usitatissimum L.) uprawianego na Lubelszczyznie. Roczniki Nauk Rolniczych. S.E.t.8, z. 2: 95-107.

Ondrej M. 1977: Nové poznatky o fuzariozách Inu. Len a Konop. 15: 33-45

Sadowski S., Sowa A., Zawislak K. 1980: Fuzarioza Inu w uprawie monokulturowej i w zmianowaniu. Rocz. Nauk Rolniczych. SET 10, z. 1-2: 163-173.

Zarzycka H. 1973: Les fusarioses du lin en Pologne. Fibra 18. 3: 30-41

Zarzycka H. Czyzewska S.1993: Przeglad patogenicznych gatunkow Fusarium na roslinach uprawnych w Polsce w latach 1945-1991. II. Wystepowanie gatunkow Fusarium na roslinach przemysloych. Mater. z Symp. nt. „Biotyczne srodowisko uprawne a zagrozenie chorobowe roslin", Olztyn, 19 wrzesnia 1993, 453-461.

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Resistance of some sunflower hybrids to *Sclerotinia* Wilt

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Abstract Wilt caused by *Sclerotinia sclerotiorum* (Lib.) de Bary reduced yield and quality of sunflower seeds. We studied the infection of eight sunflower hybrids and cultivars by two isolates of *S. sclerotiorum* (Lib.) de Bary. The inoculation was carried out at different growth stages of the plants, cultivated in greenhouse and in field conditions. We evaluated the length of lesions, dry weight of plants and thousand seed weight. We found out that the resistance of hybrids is the same in field and greenhouse conditions. The most resistant hybrid was Sunstar 277 and the most susceptible Suntop 268 using both isolates. The isolates were differed in aggressiveness. The greatest damage of sunflowers was observed when inoculation by *S. sclerotiorum* were carried out in the bud stage.

Keywords: *S. sclerotiorum,* sunflower, resistance evaluation

Introduction

Wilt caused by *Sclerotinia sclerotiorum* (Lib.) de Bary reduces yield and quality of sunflower seeds. The plants of sunflower are infected by mycelium from overwintering sclerotia. The mycelium attacks the roots and basal stalks (Huang, Dueck, 1980). The infection can also produce ascospores that infect the capitulum, mid-stem or leaves (Masirevic, Gulya, 1992). The aim of this study was to find out the resistance of our sunflower hybrids inoculated by *S. sclerotiorum* at different stages of plant development.

Material and methods

Inoculations of plants were carried out according to Sedun and Brown (1989) under the greenhouse conditions and in the field at the stages of flower bud, anthesis and maturation. The resistance of hybrids was evaluated after 21 days in the greenhouse and at the end of the vegetative stage in the field plots. This evaluation was done on the base of the analysis of variance and the differences among obtained values were tested by Scheffe`s multiple range test.

Results

The most resistant hybrids to attack by *S. sclerotiorum* were Sunstar 277 and Sandra. The most wilted plants were hybrids Suntop 268 and Sunbred 285 (Fig. 1). The percentage of infected plants correlated with the decrease of dry weight of sunflower plants cultivated in the greenhouse (Tab. 1).

Figure 1. Percentage of wilted sunflower hybrids and cultivars after inoculation by *Sclerotinia sclerotiorum* (Lib.) de Bary

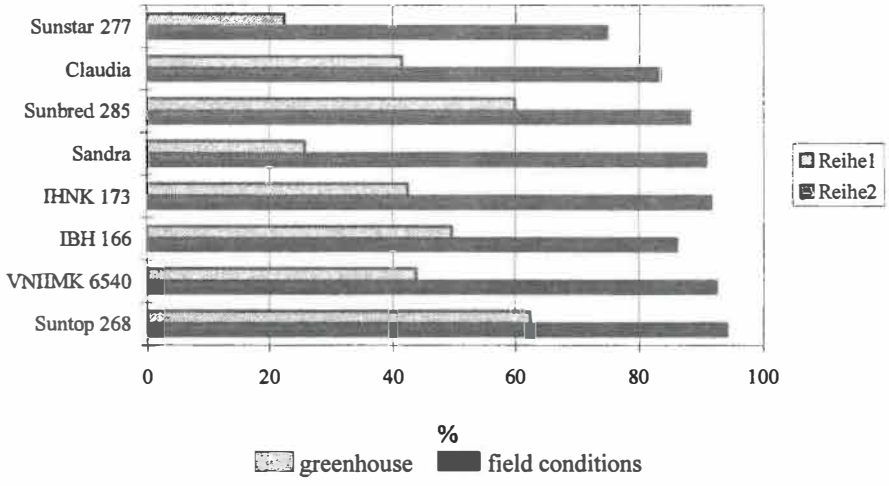

Table 1: The influence of *Sclerotinia sclerotiorum* (Lib.) de Bary on the sunflower hybrids and cultivars

Sunflower hybrids	Lesion length [mm]	Decrease of [%]			Diameter of the head [mm]	TKW [g]
		Dry matter	TKW	Head (5)		
Sunstar 277	104.32^{a}	11.98^{a}	4.16^{abc}	2.82^{a}	138.56^{ab}	59.45^{a}
Claudia	131.71^{ab}	12.35^{a}	4.74^{ab}	13.18^{a}	129.67^{a}	70.16^{ab}
Sunbred 285	141.70^{abc}	24.88^{a}	7.65^{a}	3.10^{a}	142.42^{ab}	62.45^{a}
Sandra	166.87^{abc}	9.20^{a}	26.15^{c}	16.16^{a}	132.89^{ab}	62.38^{a}
IHNK 173	156.03^{abc}	17.40^{a}	8.91^{abc}	10.08^{a}	129.47^{a}	63.78^{ab}
IBH 166	184.82^{cd}	14.92^{a}	15.00^{abc}	8.23^{a}	133.71^{ab}	68.70^{ab}
VNIIMK 6540	215.75^{d}	11.78^{a}	20.37^{bc}	17.15^{a}	137.95^{ab}	68.91^{ab}
Suntop 268	212.97^{d}	29.78^{a}	0.88^{abc}	10.42^{a}	148.14^{b}	78.86^{b}
SX^2	12780.40	1451.84	7177.82	1611.79	267.18	2665.09
$F_{vyp.}$	18.13^{++}	3.32^{+}	7.29^{++}	3.26^{++}	5.18^{++}	5.10^{++}

$^{+}P_{0.05}$ $^{++}P_{0.01}$

TKW - thousand kernel weight
SX^2 - Sum of squares
$F_{vyp.}$ - F calculated (F - test)
a,b,c,d - Values followed by the sane letter in each column are not significantly different from each other at the a= 0,05 level (Scheffe's multiple range test)
P - Significant differences at significant level a= 0.05 and a= 0.01 (F - test)

The percentage of infected plants in the field plots was higher than in the greenhouse (Fig. 1). The cultivar VNIIMK 6540 and hybrid Suntop 268 were mostly infected in contrast to hybrid Sunstar 277.

There were significant differences among hybrids in lesion length. The smallest lesion's length was on the hybrid Sunstar 277 and the largest one on the hybrid Suntop 268 and cultivar VNIIMK 6540.

The highest values of capitulum diameter and 1000-seeds weight (TSW) were found in cultivar VNIIMK 6540 and by hybrids Sandra and Suntop 268 in contrast to hybrid Sunstar 277 with the lowest (Tab. 1). The decrease of these values coused by the pathogen were highest in hybrids that had obtained the highest absolute values of TSW and capitulum diameter. The hybrids with low values of these parameters had lower percentage decreases of them. There were differences in TSW of infected plants and TSW of four infected hybrids had higher weight compared to healthy ones.

The plant inoculation at maturation stage caused the least damage (decrease of lesion length) in contrast to inoculations at earlier stages of plant development (192.3 mm at the flower bud stage and 125.1 at the maturation stage). The plants inoculated at flower bud stage did not create any capitulum and dessicated. There were no significant differences in capitulum diameter among plant infected at later stages (130.1 mm flowering, 136.3 maturation).

Discussion

There were no significant differences in lesion length among sunflower hybrids. The resistance to *S. sclerotiorum* was better expressed by percentage of infected plants in the field conditions. The same relation was evident in evaluation of dry weight and it was the same regardless of isolate of S. sclerotiorum, and appears to be horizontal resistance sensu Vanderplank (Vanderplank, 1984).

A similar increase of TSW of some hybrids infected by S. sclerotiorum has also been observed by Rashid and Dedio (1992). We can assume a negative correlation between heredity of yield potential of hybrids and heredity of resistance to S. sclerotiorum. Tourviele and Vear (1990) observed a similar correlation between resistance and oil content in seed.

The results showed that resistance of hybrids increases with the age of plants. The damage was less on the hybrids inoculated at the later stages of plant development (after flowering). These results correspond with the results obtained by Dorrell and Huang (1978).

References

Dorrell D. G.; Huang H. C. 1978: Influence of sclerotinia wilt on seed yield and quality of sunflower wilted at different stages of development. Crop Science 18: 974 - 976.

Huang H. C.; Dueck J. 1980: Wilt of sunflower from infection by mycelia-germinating sclerotia of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 2: 47 - 52.

Masirevic S.; Gulya T. J. 1992: *Sclerotinia* and *Phomopsis* - two devastating sunflower pathogens. Field Crop Research 30: 271 - 300.

Rashid K. Y.; Dedio W. 1992: Differences in the reaction of sunflower hybrids to sclerotinia wilt. Can. Journal of Plant Science 72: 925 - 930.

Sedun F. S.; Brown J. F. 1989: Comparison of three methods to assess resistance in sunflower to basal stem rot caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor*. Plant Disease 73: 52 - 55.

Tourvieille De Labrouche D.; Vear F. 1990: Heredity of resistance to *Sclerotinia sclerotiorum* in sunflower. III. Study of reactions to artificial infection of root and cotyledons. Agronomie 10: 323 - 330.

Vanderplank J. E. 1984: Disease resistance in plants. Second ed., Orlando, Academic Press.

Monitoring Pests – Biology of Harmful and Beneficial Insects

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The effect of trap design and 2-phenylethyl isothiocyanate on catches of stem weevils (*Ceutorhynchus pallidactylus* Marsh. and *C. napi* Gyll.) in winter oilseed rape.

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Abstract The effect of insect trap design and of the brassica volatile 2-phenylethyl isothiocyanate (NCS) on catches of *Ceutorhynchus pallidactylus* and *C. napi* immigrating into oilseed rape in spring was tested at a crop near Wroclaw, Poland. Three trap types, yellow water traps, yellow sticky traps and cone traps (modified cotton boll weevil traps), each with and without a lure containing 2-phenylethyl NCS, were set out in a Latin square design. Over an 18 day period, 4364 *C. napi* and 3283 *C. pallidactylus* were caught, 70% of these in the first three days. Water traps caught 90% of all *C. pallidactylus* and 73% of all *C. napi* whereas cone traps caught fewest of each species. Over the course of the experiment, lures caused an approximately three-fold increase in trap catches of each species and in the first three days the lures appeared to affect weevil numbers most in water traps and least in sticky traps. The influence of trap design and 2-phenylethyl NCS are discussed in relation to the potential for improved monitoring of stem weevil immigration to crops and better targeting of insecticide applications.

Keywords: *Ceutorhynchus pallidactylus*, *C. quadridens*, *C. napi*, stem weevil, *Brassica napus*, oilseed rape, 2-phenylethyl isothiocyanate, monitoring trap, semiochemical.

Introduction

Ceutorhynchus pallidactylus Marsh. (= *C. quadridens* Panz.) and *C. napi* Gyll. are important pests of winter oilseed rape in Poland. Their larvae bore tunnels within the stem. Both species are univoltine, overwinter as adults outside the crop and migrate to established rape crops in spring. The temperature threshold for their flight is c. 10°C (Lechapt, 1980) and immigration to crops, therefore, occurs in early spring, when weather conditions can be unpredictable.

The importance of the colour and design of traps for monitoring oilseed rape pests has been much studied (Wasmann 1926; Nolte and Fritzsche 1954; Laska and Kocourek 1991; Kostal 1992). However, with present trapping techniques it is still difficult to record the beginning of stem weevil flights into crops and hence to signal the correct timing for their control. The aim of this study was to determine the effect of a lure releasing the host-plant volatile 2-phenylethyl isothiocyanate (NCS) on catches of stem weevils in different types of trap.

Methods

The experiments were conducted in a 6 ha crop of winter oilseed rape, cv. Ceres, at GOSP Oporów, Wroclaw, Poland. Most of the field was sprayed with the pyrethroid insecticide Karate (0.25 EC) against stem weevils on 20 April but 0.5 ha remained untreated. A line of six insect traps was arranged on the ground along the edge of each of the areas with and without insecticide. There were six trap treatments, consisting of three types of trap, each with or without a semiochemical lure.

The trap types were: yellow water traps (235 mm diam), yellow sticky traps (Oecos, UK Ltd.; 100 ´ 200 mm tall) and modified boll weevil cone traps (Glinwood et. al. 1993). Each cone trap consisted of a cylindrical, fluorescent-green plastic base unit with a wire mesh cone and a clear plastic collecting unit fitted above it (overall dimensions 220 mm high ´ 110 mm diam). Four semi-circular holes were cut in the base of each cone trap to allow weevils to walk up inside. The semiochemical lure consisted of a polythene bag dispenser which released 2-phenylethyl NCS at approximately 5mg/day (Smart et.al. 1996). Lures were suspended inside cone traps or just above the water and sticky traps.

Traps were placed in position on 17 April 1996, when an air temperature above 5°C was recorded for the first time that year. A sequential Latin square design was used in which columns were separated in space (18 m between traps) and rows were separated in time (each row occupying a three-day trapping period). Every three days until 5 May, insects were emptied from the traps which were then repositioned to form a new row of the Latin square. Statistical analysis was by analysis of variance using a Log_{10} n+1 transformation to allow for skewed data.

Results

Numbers of *C. pallidactylus* and *C. napi* trapped in the part of the field with insecticide were 32% and 39% fewer, respectively, than in the part without insecticide. As the residual mean squares from analysis of variance of data from each part of the field were similar, all the data were combined for an analysis which ignores the effect of insecticide.

The total numbers of *C.napi* and *C. pallidactylus* trapped in the experiment were 4364 and 3283, respectively, and only two other individuals of the genus *Ceutorhynchus* were caught. The largest numbers of each weevil species were caught in the first three days of the experiment (69% of all *C. napi* and 71% of all *C. pallidactylus*); in that period, water traps, sticky traps and cone traps caught 72%, 66% and 53%, respectively, of their total catches of weevils. Thereafter, the numbers of insects caught in all traps declined markedly (Fig. 1).

There were significant differences in the numbers of both *C. napi* and *C. pallidactylus* that were attracted to different trap types. For each species, most were caught in water traps (73% of *C. napi* and 90% of *C. pallidactylus*) and fewest in cone traps (Table 1).
Traps with 2-phenylethyl NCS lures caught 2.7 times more *C. napi* and 3.2 times more *C. pallidactylus* than traps without lures and this effect was significant for *C. pallidactylus* (Table 1). During the first three days, the lures appeared to influence trap catch most in water traps and least in sticky traps (Fig. 1) but over the course of the experiment there was no statistical evidence of this interaction (Table 1).

Discussion

The brassica volatile, 2-phenylethyl NCS significantly increased catches of *C. pallidactylus* in field traps and there was some evidence of a similar response for *C. napi.* This is the first time that stem weevils infesting oilseed rape have been demonstrated to respond to this isothiocyanate which has already been shown to have potential for use in monitoring two other coleopterous pests of oilseed rape, *Ceutorhynchus assimilis* Payk., and *Meligethes aeneus* Fab. (Smart et al. 1996). Water traps, already in general use for monitoring the arrival of these insects in oilseed rape crops in Poland, were more effective at catching both species of stem weevil than either sticky traps or cone traps and there was some evidence that the lures may have influenced the efficiency of water traps more than that of other traps.

Water traps and sticky traps are primarily designed to catch flying insects. In 1996, conditions were not suitable for stem weevil flight at Wroclaw until mid April, after an exceptionally prolonged winter. Thereafter, immigration flights of stem weevils rapidly reached a peak, the maximum numbers of weevils being caught in the first three days of our experiment. The rate of decline in the numbers of weevils caught (Fig. 1) agrees well with the temporal dynamics of stem weevil immigration flights from overwintering sites as reported by other authors (Laska and Kocourek , 1991; Kuhne, 1977; Palosz and Sienkowski, 1991; Kostal, 1992) and is unlikely to bear any relation to the abundance of stem weevils already in the crop.

There were indications of a decline with time in the influence of the lure on catches of stem weevils in water traps (Fig. 1). The importance of host-finding cues is likely to diminish when the host has been located and colonised and there is evidence for *C. assimilis* that some host-plant volatiles are attractive only during immigration to or emigration from the crop (Smart et al. 1997; Smart and Blight, submitted). Therefore, to be effective as a monitoring tool, flight traps with lures must be positioned at the crop before the start of immigration.

The cone traps were primarily designed to catch insects walking on the ground (Glinwood et al. 1993). They caught fewer stem weevils than either water traps or sticky traps. Although less sensitive indicators of flight activity, cone traps might be more useful for assessing the abundance of weevils at overwintering sites prior to immigration flights.

Water traps with 2-phenylethyl NCS caught more stem weevils of both species than did other traps. However, water traps attract many insect species other than the target species and trap catches can be difficult and time-consuming to sort. We were fortunate to catch few other insects of the genus *Ceutorhynchus*. The ideal monitoring trap is attuned to the behaviour of the target insect and is selectively attractive to it. The use of host-plant volatiles or other semiochemicals offers the potential to develop selectively attractive lures for trapping target species. Furthermore, the increased attractiveness of the trap to the target species may offer more scope for reducing the trap's general attractiveness to non-target insects by altering the physical aspects of trap design (colour and shape).

Further studies are in progress to test the influence of trap design and of plant volatiles presented singly and in combination on catches of stem weevils in oilseed rape.

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References

Glinwood, R.T.; Blight, M.M.; Smart, L.E. 1993 Development of a monitoring trap for the pea and bean weevil, using the aggregation pheromone. Bulletin IOBC/wprs 16: 347-350.

Kostal, V. 1992 Monitoring of activity and abundance of adults of the pollen beetle (*Meligethes aeneus* F.) and cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) in winter rape stands. Rostlina - Vyroba **38**: 3/4: 297-306.

Kuhne, W. 1977 Untersuchungen zur Befallsverteilung der Russelkafer *Ceutorhynchus napi* Gyll., *Ceutorhynchus quadridens* Panz. und *Ceutorhynchus assimilis* Payk. innerhalb großfachiger Winterrapsbestande. Archiv für Phytopathologie und Pflanzenschutz **2**: 109-115.

Laska, P.; Kocourek, F. 1991 Monitoring of flight activity in some crucifer-feeding pests by means of yellow water-traps. Acta Entomologia Bohemoslovacia **88**: 25-32.

Lechapt, G. 1980 Methodes de determination des risques concernant les insectes nuisibles au colza. Bulletin OEPP **10**: 119-128.

Nolte, H.W.; Fritzsche, R. 1954 Untersuchungen zur Bekampfung der Rapsschädlinge. III. Zur Biologie und Bekampfung des Kohlschotenrusslers (*Ceuthorrynchus assimilis* Payk.) und der Kohlschotengallmücke (*Dasyneura brassicae* Winn.). NachrichtenBlatt für Deutschen PflanzenSchutzdienst, n.F. **8**:128-135.

Palosz, T.; Sieńkowski, A. 1991 Występowanie I szkodliwość chowacza brukwiaczka na plantacjach rzepaku ozimego Pomorza Środkowego w 1990 r. Ochrona Roślin **4**: 5-7.

Smart, L.E.; Blight, M.M.; Hick A.J. 1997 Effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis* (Paykull) (Coleoptera: Curculionidae). Journal of Chemical Ecology **23**: 889-902.

Smart, L.E.; Blight, M.M.; Lane, A. 1996 Development of a monitoring trap for spring and summer pests of oilseed rape. Proceedings, 1996 Brighton Crop Protection Conference - Pests and Diseases **1**: 167-172.

Smart, L.E.; Blight, M.M. (Submitted) Field discrimination of oilseed rape, *Brassica napus* (L.) (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Paykull) (Coleoptera: Curculionidae).

Wasmann E., 1926 Versuche über den Farbensinn des Rapsglanzkafers (*Meligethes aeneus* F.). Zeitschrift für Wissensschafliche Insect Biologie **21**: 147.

Figure 1 The temporal dynamics of the numbers of weevils caught in three types of traps with (open squares) and without (closed squares) a 2-phenylethyl NCS lure.

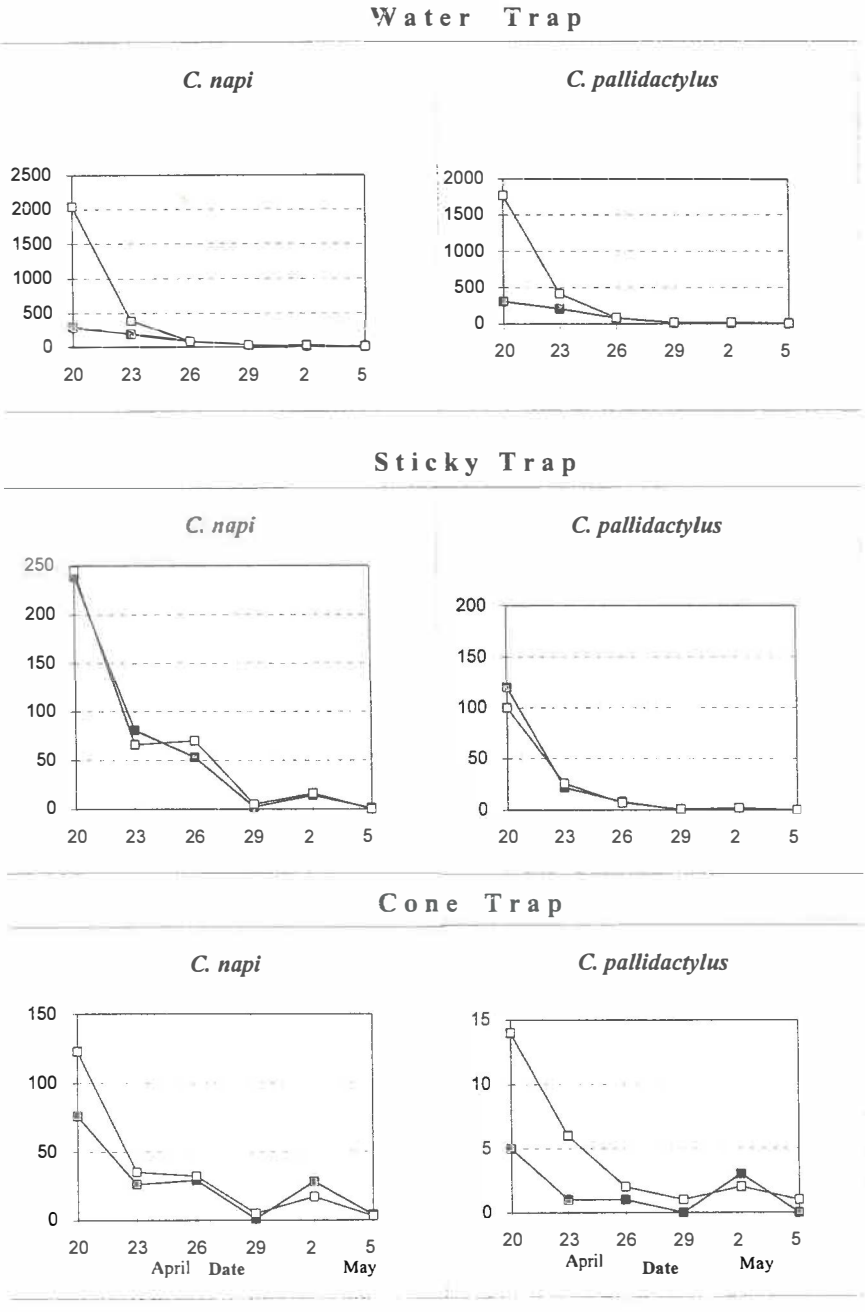

Table 1 Mean numbers and analysis of variance of stem weevils caught in different types of trap with and without 2-phenylethyl NCS lures (Log_{10} n+1 transformation, arithmetic means in parentheses).

	C. napi			*C. pallidactylus*		
Trap type	- lure	+ lure	+ and - lure	- lure	+ lure	+ and - lure
Water	1.43 (51.8)	1.67 (214.3)	1.55 (133.0)	1.36 (53.0)	1.61 (193.6)	1.48 (123.3)
Sticky	1.06 (32.5)	1.05 (33.5)	1.05 (33.0)	0.63 (12.7)	0.64 (11.3)	0.63 (12.01)
Cone	0.94 (13.7)	1.01 (17.9)	0.97 (15.8)	0.19 (0.8)	0.38 (2.2)	0.28 (1.5)
All traps	1.14 (32.6)	1.24 (88.6)	1.19 (60.6)	0.73 (22.2)	0.87 (69.0)	0.78 (45.6)
Analysis of variance						
Comparisons	F value (d.f.)	*P*	S.E.D.	F value (d.f.)	*P*	S.E.D.
Trap type (n=24)	30.01 (2,45)	<0.001	0.081	121.26 (2,45)	<0.001	0.079
+ and - lure (n=36)	2.31 (1,45)	0.14	0.067	5.36 (1,45)	0.025	0.065
Trap type x lure interaction (n=12)	1.11 (2,45)	0.34	0.114	1.25 (2,45)	0.296	0.112

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The relationship between the stem weevil (*Ceutorhynchus pallidactylus* Marsh.) injury and losses of the flower buds

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Abstract The studies were carried out in experimental fields of winter oilseed rape at the Agricultural University Experimental Station in Pawlowice near Wroclaw during 1988-1993. The 1-ha field was divided into three equal parts: UP - without any chemical treatments, SWP - treated against *C. pallidactylus*, and PBP - treated against the pollen beetle.

At the stage of seed ripening, 30 plants were collected randomly from each part of the field. The number of podless stalks and the length of the feeding tunnel in the stem were recorded.

In the years of the study, 8 - 79 % of podless stalks resulted from the *C. pallidactylus* injury.

The significant positive correlation was found between the length of the feeding tunnel and the number of podless stalks on the main as well as side stems.

The results of the present study show that the oilseed rape plants injured by the *C. pallidactylus* larvae may shed more pods than the uninjured plants. In this light, attributing pod shedding only to the pollen beetle injury appears incorrect.

Keywords: oilseed rape, stem weveels, injury, podless stalks,

Introduction

In all previously published studies on oilseed rape protection, the reduction of the flower buds in oilseed rape has been attributed to two main reasons: the so called natural shedding due to water deficit or temperatures unsuitable for the processes of ovule nourishment (Mendham et al. 1981), and the feeding of imagos and larvae of the pollen beetle.

In early spring, the mass flights of *C. pallidactylus* Marsh. to winter oilseed rape occur. After a short period of feeding on plants which begin to grow, the females lay eggs into the main vein of the leaf and the larva moves into the stem where it feeds usually until the end of June (Dmoch 1959). Contrary to *C. napi* which causes tapering and twisting of the stems, *C. pallidactylus* does not generate any visible damage and, in practice, this species is considered of no economic importance.The studies of recent years have shown many *C. pallidactylus* larvae induced plant reactions unfavourable to the yield (Kelm et al. 1994, 1995). For example, the delay in flowering of oilseed rape unprotected against the stem weevils was observed despite the absence of the pollen beetle.

The aim of the present data analysis was to determine the effect of the stem injury on the process of flower bud shedding in oilseed rape, i. e., on the number of podless stalks on the pod bearing racemes.

Methods

The plant material for the study in 1988-1993 derived from the 1 ha field of oilseed rape grown on light clay. The field was divided into three equal parts:
UN - untreated
SWP - protected against stem weevil
PBP - protected against pollen beetle.

The data on oilseed rape variety and time of pesticide treatments are given in the tab.1 .

30 randomly selected plants from each part of the field were collected at the seed maturation stage. For each plant, the number of podless stalks on the main and side racemes was determined and the length of the stem weevil larval tunnels was measured.The mathematical analysis of data, i. e. solving an equation system of three unknown quantities, allowed determination of the the percentage of podless stalks caused by the stem weevil injury (SWI), natural shedding (NS), and pollen beetle injury (PBI).

The data collected in the untreated field during 1991-1993 were analysed statistically. The correlation between the stem weevil larval feeding tunnel length in the stem and the number of short podless stalks was calculated

Results

In the years of study, the average number of short podless stalks on plants from the three parts of the field was 34 - 140. The plants from the untreated part (UN) had the largest number of podless stalks and the plants protected against the pollen beetle (PBP) the smallest. The plants from the stem weevil protected part (SWP) were in the middle position (tab 1).

Table 1. The experimental fields and the average number of podless stalks on oilseed rape plants.

Year	Variety	Insecticide and date of applay	No. of podless stalks/plant	Reason of bud injury
1988	Jantar	SWP-Decis12.04	43	NS+PBI
		PBP- Decis 20.04	34	NS+SWI
		UN-no treatment	61	NS+SWI+PBI
1989	Jantar	SWP- Decis 29.03	38	NS+PBI
		PBP-Decis8.04&25.04	17	NS+SWI
		UN- no treatment	54	NS+SWI+PBI
1991	Ceres	SWP- Decis 18.03	66	NS+PBI
		PBP-Decis 24.03	46	NS+SWI
		UN- no treatment	73	NS+SWI+PBI
1992	Ceres	SWP- Fastac 14.04	130	NS+PBI
		PBP-Fastac 30.04	97	NS+SWI
		UN- no treatment	140	NS+SWI+PBI
1993	Bolko	SWP- Fastac 15.04	29	NS+PBI
		PBP- below treshold	-	-
		UN- no treatment	104	NS+SWI+PBI

C. pallidactylus caused the shedding of 8 - 79% of flower buds of the total number of the lost buds (tab. 2). This reaction was the weakest in 1992. In 1992, the reduction due to natural shedding was the highest. The water deficit occurred from April until June, i. e. during the whole period of flowering and pod formation. A similar situation was observed in 1991 (tab. 3). The losses of the flower buds due to the stem weevil were the highest in 1993. It was a year of the highest rainfall. In 1993, the pollen beetle infestation was the lowest. Its number was below the treshold, i. e. below 1 beetle per plant.

Table 2. Percentage of podless stalks caused by natural shedding (NS), pollen beetle injury (PBI) and stem weevils injury (SWI).

Years	NS	PBI	SWI
1988	26	44	30
1989	4	68	28
1991	53	36	11
1992	62	30	8
1993	21	0	79

Table 3. Average temperatures (T) and rainfall (R) in April - June 1988 - 1993.

	1988		1989		1991		1992		1993	
Month	T	R	T	R	T	R	T	R	T	R
April	8.3	23.8	9.2	67.6	8.5	25.7	7.0	20.3	9.3	18.6
May	14.9	10.4	14.0	24.1	10.2	44.6	13.7	19.5	17.0	99.4
June	16.4	83.0	15.6	83.0	15.1	68.8	19.4	50.6	14.7	188.2

The values of the correlation coefficients calculated for the length of the feeding tunnel and the number of short podless stalks in unprotected plants are given in tab. 4. The correlation was always positive. The longer the feeding tunnel in the stem the larger the number of podless stalks on a plant. The significant correlations were found for dry years 1991 and 1992. In 1993, when high rainfall occurred, the correlation was not significant.

Table 4. The correlation coefficient (r) between the stem larval tunnelling and the losses of flower buds.

Year	Main raceme	Secondary racemes
1991	0.26103	0.4628*
1992	0.4103*	0.3998*
1993	0.3146	0.1561

*- significant correlation

Discussion

The harmfulness of the phytophagous insects which occur on winter oilseed rape can be determined for each species individually and in relation to a specific injury caused by a given species. Hitherto, it has not been considered that the stem weevils which damage plants during the vegetative growth period may influence the noxiousness of the insects which appear later in the season. Only Lerin (1988) compared the noxiousness of *C. napi* alone to its noxiousness in combination with the pollen beetle injury. The author has found that the plants injured only by one species alone easily compensated for the losses. On the contrary, the feeding of the pollen beetle on plants injured by *C. napi* resulted in serious losses of yield. In the cited paper, there is an opinion that firstly, *C. napi* lowers the productivity of main shoot through a slight decrease in pod number and pod productivity, and the plant's ability to compensate for further damage is also impeded.

The results of our study on *C. pallidactylus* confirm the existence of an interaction between the noxiousness of stem weevils which damage stems and the noxiousness of the pollen beetle which damages the flower buds. In 1993, the compensation for the stem weevil injury was found under field conditions. This year, the infestation by the pollen beetle was below the economic treshold (Kelm et al. 1994, 1995).

The results of several year study allow us to speculate that weather, and especially the water deficit which results in a so called natural physiological pod shedding, is the primary factor which decides on the magnitude of losses of the flower buds. The pod shedding due to weakening of plants injured by the stem weevils is, in practice a contribution to the water deficit effect. In the situation of high natural reduction the effect of the stem weevil is diminished and, the other way round, the increased natural reduction weakens the negative reaction of the plant to the feeding of weevils. Nevertheless, the correlations between the length of the stem weevil larval feeding tunnel and the number of short podless stalks become significant in dry years.

In conclusion, a hypothesis can be drawn that attributing the losses of flower buds exclusively to the effect of the pollen beetle feeding is weighed with a serious error especially when high infestation of the stem weevils is recorded (Mrowczynski, 1993). The plants with stems damaged by *C. pallidactylus* may be less productive and this may be manifested by the increased losses of the flower buds.

References

Dmoch J. 1959: Badania nad chowaczem czterozebnym Ceutorrhynchus quadridens Panz. - morfologia, biologia, ekologia oraz znaczenie dla rzepaku. Prace naukowe IOR 1(3):31-73.

Kelm M.,Walczak B., 1994: Reakcje roœlin rzepaku ozimego na uszkodzenia spowodowane przez chowacze lodygowe (Ceutorhynchus sp.). Materialy XXXIV Sesji Naukowej IOR.2:285-289.

Kelm M.,Walczak B., Williams I.H.,1995: Changes in growth and yield parameters of winter rape as result of injury by the stem weevil (Ceutorhynchus quadridens). 9th International Rapeseed Congress 2: 673-675.

Lerin J., 1988: Pertes de rendement associees a ravageurs successifs (Ceuthorrhynchus napi Gyll. et Meligethes aeneus F.) sur colza d'hiver (variete Bienvenu). Agronomie. 8 (3), : 251-256.

Mendham N.J., Shipway P.A., Scott R.K., 1981: The effect of delayed sowing and eather on growth, development and yield of winter oilseed rape (Brassica napus). Journal of Agric. Scien. Cambridge, 96:389-416.

Mrowczynski M., 1993: Uszkadzanie typow i odmian rzepaku ozimego przez szkodniki . Czesc II. Szkodniki lodygowe. Prace Naukowe Instytutu Ochrony Roslin, XXXIV (1/2) : 32-45.

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Studies on pest damage of cultivars and lines of winter oilseed rape

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Abstract In the years 1990-1996 studies on injuries of new winter rapeseed cultivars and lines were carried out. They also included and analysis of the susceptibility to injuries by *Ceutorhynchus napi* Gyll, *Ceutorhynchus quadridens* Panz, *Meligethes aeneus* F., *Ceutorhynchus assimilis* Payk. and *Dasyneura brassicae* Winn., which performed on 13 cultivars and 14 lines.

Keywords: Winter rapeseed cultivars and lines, susceptibility, pests.

Methods

Observations on the intensity of the pests in 1990-1996 were conducted at the Research Centre for Cultivars Testing Slupia Wielka. The studies were carried out on 13 winter rapeseed varieties and 14 lines. The observations were performed in 4 replications (each covering 50 plants).

Results

Tables 1 and 2 present the percentage of injuries to winter rapeseed cultivars and lines by pests.

Conclusions

1. Large differences were found in the degree of injuries to winter rapeseed cultivars and lines coused by:

Ceutorhynchus napi Gyll.,
Ceutorhynchus quadridens Panz.,
Meligethes aeneus F.,
Ceutorhynchus assimilis Payk. and *Dasyneura brassicae* Winn.

2. Large differences were also found in the intensity of pest occurence in different localities, where the studies were conducted.

Table 1. Percentage of injuries to winter rapeseed cultivars by pests in the years 1990-1996

Cultivars	Pests			
	Ceutorhynchus napi	*Ceutorhynchus quadridens*	*Meligethes aeneus*	*Ceutorhynchus assimilis* and *Dasyneura brassicae*
Bermuda	7	27	7	9
Bolko	5	33	18	15
Bor	5	38	18	16
Idol	13	38	14	11
Kana	1	2	3	3
Leo	12	40	16	10
Lirajet	6	40	17	17
Liropa	7	37	18	16
Mar	10	40	18	12
Marita	9	47	21	18
Polo	11	42	17	14
Silvia	8	39	14	14
Wotan	7	35	14	12

Table 2. Percentage of injuries to winter rapeseed lines by pests in the years 1990-1996

Lines	Pests			
	Ceutorhynchus napi	*Ceutorhynchus quadridens*	*Meligethes aeneus*	*Ceutorhynchus assimilis* and *Dasyneura brassicae*
BKH 894	1	3	2	3
BKH 995	2	2	4	3
BKH 1095	1	1	2	3
BKH 1195	2	4	5	5
BOH 1794	2	4	4	4
GOH 1895	2	3	6	3
LAH 494	0	1	2	2
LAH 595	1	3	4	3
LAH 695	4	7	10	5
LAH 795	3	4	6	4
LAH 895	2	5	7	5
POH 495	2	3	5	4
POH 595	3	5	8	6
POH 695	4	7	10	7

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Gas exchange and growth of winter rape as influenced by *Meligethes aeneus* feeding

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Abstract In a field experiment with winter rape cv. Leo dealing with different ways of plant protection against blossom beetle gas exchange parameters and LAI were determined at the beginning of insect feeding and during pod formation. Water use efficiency, intrinsic water use efficiency and photosynthetic activity were calculated. Plants where simultaded damage was carried out during pod formation showed the lowest tranpiration compared to the start of feeding whereas noncontrolled ones where there had been no insect control showed the highest transpiration. Photosynthetic activity was not only higher but also highly differentiated at the start of feeding (the controlled plots showed the highest value, while noncontrolled had an intermediate one) than during pod formation but in the case of simulated treatment it remained at the same level. Compensation was noted when the simulation of inflorescence damages by agrophages was performed and then there was no tendency for internal WUE to reverse.

Keywords: winter rape, *Meligethes aeneus*, photosynthesis, transpiration, WUE, LAI, compensation

Introduction

Very recent development of highly sophisticated, computerized equipment used in crop physiology enables many principal data that characterize, for instance, such processes as gas exchange of plants grown in canopies (Nalborczyk 1997) to be accummulated quickly. Such devices are therefore of great importance for complex investigations of the compensation responses which very often occurring in the rape during insect feeding on the host plant. Compensation may manifest itself in forming an increased number of auxiliary shoots and developed floral buds (Szulc 1959) which in turn can lead to nearly complete cancelling out of detrimental effects caused by feeding insects on the final seed yield. On the canopy scale compensation should be reflected in crop assimilatory surface in term of leaf area index (LAI) and/or in some increase of gas exchange parameters.

Material and methods

Three factorial field experiment with winter rape cv. Leo dealing with different way of plant protection against blossom beetle under various sowing density and fertilization level (Podlaska et al. 1996) were carried out. Within these various parameters characterizing performance of the plant canopies at the beginning of insect feeding and during pod formation

were measured. On fully developed young leaves of the main stem a range of gas exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate, intercellular carbon dioxide concentration) was measured using a Li-6200 (Lambda Instruments Corporation, Li-Cor, Lincoln, Nebraska) portable photosynthetic system. The ratios of photosynthetic rate to transpiration rate (water use efficiency, WUE) and to stomatal conductance (intrinsic water use efficiency, WUE_i, Blum 1988) were calculated. Simultaneously, using a plant canopy analyzer LAI 2000, the leaf area index (LAI) was evaluated. Multiplying photosynthetic rate by LAI a photosynthetic activity was calculated. Data were analyzed statistically using ANOVA.

Results

Photosynthetic rate of winter rape cv. Leo was higher at the start of feeding than at pod formation independently of treatment combination. The only exception was treatment where the inflorescences were removed to cause compensation response. Photosynthetic rate during pod formation was slightly higher in simulated damage than noncontrolled and controlled combinations (Fig. 1).

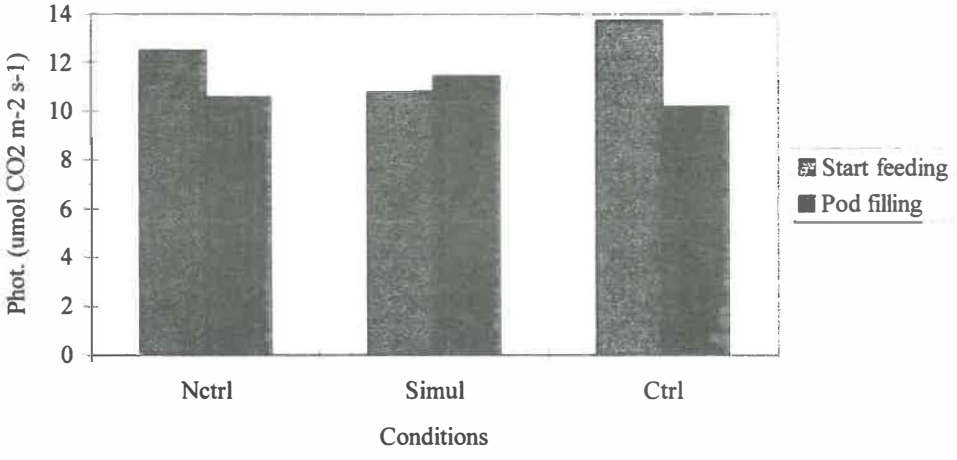

Figure 1. Photosynthesis of winter rape cv. Leo (mmol CO_2 m^{-2} s^{-1}) as influenced by *Meligethes aeneus* feeding.

Transpiration rate during pod formation was greater than that during the start of feeding, the only exception again was the simulated combination and in this case transpiration rate was the same (Fig. 2). The highest increase in transpiration rate was found for noncontrolled combinations. Transpiration rate at the start of feeding was nearly the same for all combinations analyzed. At pod formation transpiration rate was the parameter of gas exchange which displayed the highest differentiation. Plants from treatments where the simulation of

damages was performed showed the lowest value whereas noncontrolled ones showed the highest transpiration rate. Especially the higher density noncontrolled treatment, which had with 8.21 mmol H_2O m^{-2} s^{-1} (data not shown).

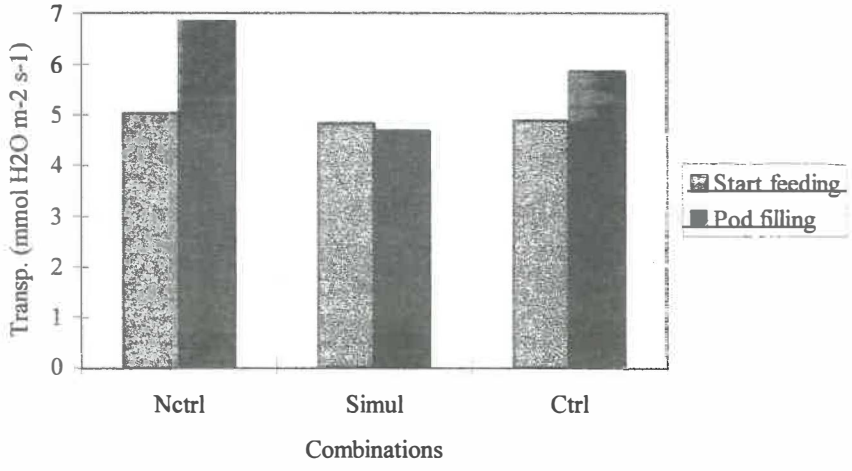

Figure 2. Transpiration of winter rape cv. Leo (mmol H_2O m^{-2} s^{-1}) as influenced by *Meligethes aeneus* feeding.

Water use efficiency (WUE) ranged from 2.2 to 2.8 at the beginning of feeding while that at pod formation period showed values of 1.5 - 2.4 mmol CO_2/mmol H_2O (Fig. 3). It is noteworthy that WUE of simulated combination was the lowest at the start of feeding whereas during pod formation it was the highest did not show any substantial difference in its values.

Stomatal conductance was higher at start of feeding than at pod formation state and ranged from 0.4 to 0.45 mol H_2O m^{-2} s^{-1} During pod formation no significant differences were seen though a slight prevalent of noncontrolled was denoted (Fig. 4).

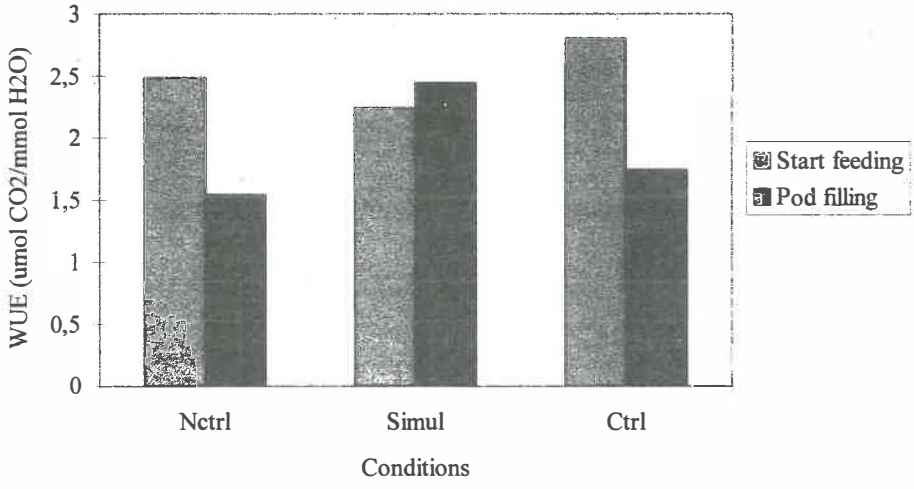

Figure 3. WUE of winter rape cv. Leo (mmol CO_2/mmol H_2O) as influenced by *Meligethes aeneus* feeding.

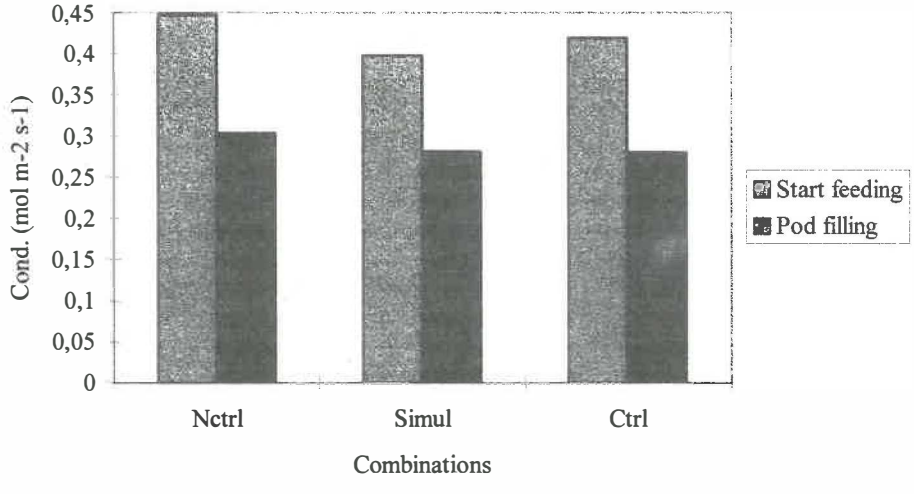

Figure 4. Stomatal conductance of winter rape cv. Leo (mol H_2O m^{-2} s^{-1}) as influenced by *Meligethes aeneus* feeding.

Intrinsic WUE at start feeding was lower than during pod formation (27 to 33 vs. 35 to 41 mmol CO_2/mol H_2O). There was no differences between noncontrolled and simulated combinations in intrinsic WUE, while that for the controlled combination did significantly differ. On the contrary, during pod formation the values for simulated damage significantly exceeded the other combinations (Fig. 5).

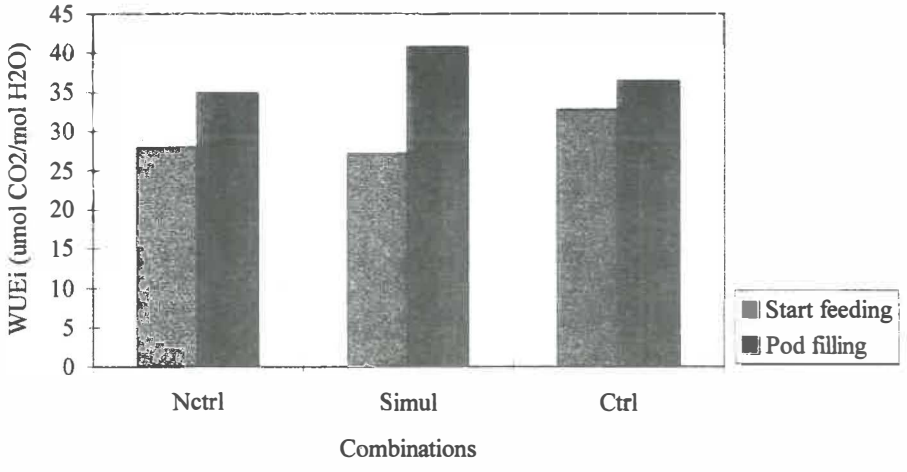

Figure 5. Intrinsic WUE of winter rape cv. Leo (mmol CO_2/mol H_2O) as influenced by *Meligethes aeneus* feeding.

Intercellular CO_2 concentration did not show substantial differences whithin the combination used though it was higher during pod formation and the simulated combination was by then, leading by a slight margin (Fig. 6).

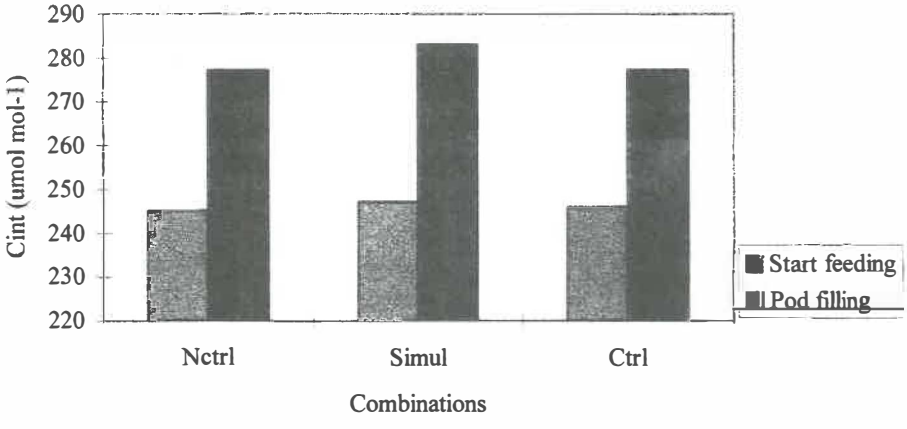

Figure 6. Intercellular CO2 concentration of winter rape cv. Leo (mmol CO_2/mol CO2) as influenced by *Meligethes aeneus* feeding.

Leaf area index was higher at the start of feeding ranging from 3.47 to 3.68 vs 3.17 to 3.39, for two analyzed phases, respectively. Plants in controlled treatment had the highest value at the start of feeding while it was less at pod formation, shown particulary by the decrease of more than 0.5 LAI unit. Simulated damage performed firstly at the same level while later on showed a smaller decrease and thus differed from the others.

Photosynthetic activity being the product of LAI and photosynthetic rate was not only higher but also highly differentiated at the start of feeding (the controlled showed the highest value, while noncontrolled on intermediate level) than during pod formation but in the case of simulated treatment it remained at the same level (Table 1). There was no difference in photosynthetic activity of noncontrolled and controlled plants in this phase.

Table 1. Leaf area index (LAI) and photosynthetic activity of differently protected winter rape cv. Leo in two developmental phases.

Treatment	Start feeding	Pod formation	Start feeding	Pod formation
	LAI	LAI	Photosynthetic activity (μmol CO_2 m^{-2} s^{-2}	Photosynthetic activity (μmol CO_2 m^{-2} s^{-2}
Noncontrolled	3.47	3.22	43.27	33.97
Simulated	3.61	3.39	38.88	38.71
Controlled	3.68	3.17	50.38	32.21

Discussion

Taking into account all investigated parameters of photosynthetic productivity one can observe in experimental data the effect of small number of the insects 1-2 per one inflorescence in 1995 year (data not shown). The plants attacked by the blossom beetle were characterized by a tendency to give the highest transpiration while taking into account low photosynthesis reflected in very low WUE. In the case of noncontrolled plots at a density of 80 plants/m^2 all parameters of gas exchange indicate a serious weakening of the plant condition which might not be able to respond by compensation. Even with a low frequency of agrophages per plant a compensation effect maybe found at least in a canopy of higher plant density (data not shown). A tendency to compensate damage caused by *Meligethes aeneus* was manifested in the plants grown at the density of 160 plants/m^2.

In general, reactions of compensation may be noted when the simulation of inflorescence damages by agrophages was performed (the highest photosynthetic rate and WUE). Indeed, the simulation of inflorescence damage seems to be overestimated compared to the real state of blossom beetle invasion in 1995. Nevertheless it showed the possibility or even the degree of eventual compensation if the number of insects had been at the mean level of previous years . Such dramatic damage produced plant compensation that manifested itself in some increase of many photosynthetic productivity indices and in maintaining various parameters at the same level independent of the developmental phase. There was no effect on transpiration and photosynthetic rate, or WUE, and only slight effect on intrinsic WUE, LAI. In the case of simulation of damages the compensation reaction occurred independently of plant density and higher gas exchange of plant in such a canopy compared to other combinations resulted from

the presence of many small sized leaves that manifested higher photosynthesis (significant difference both for stomatal conductance and resulting photosynthetic rate).

Insect feeding seems to have a strong negative effect on WUE by increasing transpiration rate and it is very distinct plant response in the experiment. To analyze this effect more deeply the authors have attempted to leave aside external factors and used intrinsic WUE (Blum 1988). Intrinsic WUE values were always increased at pod filling. Therefore it may be concluded that not controlling insect feeding or controlling it, affect the trend of intrinsic WUE by changing WUE through lowering it, whereas simulation of damage or probably an effect of high population of insects per inflorescence, does not reflect in WUE the tendency of intrinsic WUE due to the set of compensation responses taking place within the plant.

References

Blum A.1988. Plant Breeding for Stress Environments. CRC Press, Inc. Boca Raton, Florida

Nalborczyk E. 1997. Dobor i wykorzystanie nowoczesnej aparatury kontrolno-pomiarowej w doswiadczalnictwie polowym. Cz.I. Oznaczanie wymiany gazowej, struktury przestrzennej i bilansu energii fotosyntetycznie czynnej radiacji roslin w lanie. Zesz. Probl.Post Nauk Roln. 447: 81-90.

Podlaska J., Markus J., Dmoch J, Lewandowski M. Loboda T., Pietkiewicz S. 1996. Kompensacja uszkodzen powodowanych przez slodyszka (*Meligethes aeneus* F.) na rzepaku ozimym przy roznej obsadzie i nawozeniu. Cz. I. Cechy morfologiczne roslin. Rosl. Oleiste, 17, 311-318.

Szulc P. 1959. Badania nad wlywem zabiegow uprawowych na straty w plonie rzepaku ozimego wywolane przez szkodniki. I. Znaczenie slodyszka rzepakowca jako szkodnika rzepaku. Prace nauk. IOR, 1, 231-276.

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A bibliography of the parasitoids of the cabbage seed weevil (*Ceutorhynchus assimilis* PAYK.)

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Abstract A search of the literature has revealed *c.* 50 publications in which reference is made to parasitoids attacking the cabbage seed weevil [*Ceutorhynchus assimilis* Paykull; syn. *Ceutorhynchus obstrictus* (Marsham)] in Europe and North America. The egg stage is attacked by four species of the family Mymaridae. The larval stage is attacked by up to 28 ectoparasitic species of the Chalcidoidea or Braconidae, although only three species are common, and eight endoparasitic species: three Ichneumonidae and five Braconidae. The adult stage is possibly attacked by 2 species of Braconidae. Reported levels of parasitism are given.

Keywords: bibliography, cabbage seed weevil, *Ceutorhynchus assimilis*, parasitoids

Parasitism of *C. assimilis* eggs

Species of the family Mymaridae (Hymenoptera: Chalcidoidea) are cited as attacking the egg stage of *C. assimilis*: *Patasson declinata* (Soyka) in France (Jourdheuil 1960) and Holland (Ankersmit 1956); *Patasson brachygaster* Debauche in France (Risbec 1953) and the UK (Nasreldin 1973); *Mymar autumnalis* (Förster) in Germany (Godan 1959). In Poland, Miczulski (1968) reared *Patasson* sp. and also recorded *Anaphes fuscipennis* Haliday as a probable parasitoid of *C. assimilis* eggs.

In most instances percentage parasitism was small: Jourdheuil (1960) recorded a maximum of 4%, Nasreldin (1973) recorded 2%, whereas Risbec (1953) states that parasitism by *P. brachygaster* was of negligible importance. In contrast, Godan (1959) found that *M. autumnalis* parasitised up to 90% of *C. assimilis* eggs in 1952 but none were found in subsequent years.

Parasitism of *C. assimilis* larvae

By ectoparasitoids

The most commonly cited parasitoids of *C. assimilis* are ectoparasitoids attacking the larval stage (Tables 1 & 2). The species involved belong either to the Chalcidoidea or the Braconidae, with the most cited ones being *Trichomalus perfectus* (Walker), *Mesopolobus morys* (Walker) and *Stenomalina muscarum* (L.) (Pteromalidae, Pteromalinae) (Table 1).

Table 1. Species of ectoparasitoid of the family Pteromalidae cited as attacking *Ceutorhynchus assimilis* larvae.

Family	Species	Country (Literature source)
Pteromalidae	*Trichomalus perfectus* (Walker)	France (2,20,32); Germany (17,21,35,36,38); Holland (1); Poland (7,8,27,34,37); Sweden (16,33); Switzerland (4); UK (25,28,29); Yugoslavia (23); Canada (24, [c]); USA (5,6,10,14,24,39,)
	Trichomalus sp.	USA (10)
	Mesopolobus morys (Walker)	France (20,32); Poland (7,27); Sweden (16,33) Switzerland (4); UK (25,28,29); Yugoslavia (23); USA (5,10,14,24,39, [c])
	Mesopolobus mayetiolae (Gahan)[a]	USA (5,24, [c])
	Mesopolobus sp.	UK (29); USA (10,14)
	Stenomalina muscarum (L.)	Poland (7,8,27,34); Sweden (16,33); Switzerland (4)
	Stenomalina gracilis (Walker)	UK (29)
	Habrocytus semotus (Walker)[b]	Poland (8); UK (29)
	Habrocytus dispar (Curtis)[b]	Poland (8)
	Habrocytus sp.	Poland (17); Canada (24); USA (14,39)
	Chlorocytus diversus (Walker)	UK (29)
	Chlorocytus sp.	UK (25)
	Anisopteromalus calandrae (Howard)	France (32)
	Trimeromicrus maculatus Gahan*	USA (5,24,39, [c])
	Zatropis sp.*	Canada (24); USA(14)

* Not recorded in the UK (Fitton *et al.*, 1978)
[a] Possibly hyperparasitic (cf. McCleod, 1953)
[b] Possibly hyperparasitic (cf. Graham, 1969; Dmoch & Sulgostowska, 1986)
[c] for further citations in N. America see Peck (1963)

Table 2. Species of ectoparasitoid (other than Pteromalidae) cited as attacking *Ceutorhynchus assimilis* larvae.

Family	Species	Country (Literature source)
Eurytomidae	*Eurytoma curculionum* Mayr [a]	Poland (7)
	Eurytoma sp.	Canada (24); USA (10,14)
Chalcididae	*Spilochalcis side* (Walker)*	USA (5,24,[d])
Eupelmidae	*Macroneura vesicularis* (Retzius) [b]	Poland (7); Canada (24); USA (10,14,[d])
Eulophidae	*Necremnus duplicatus* Gahan*	USA (5,10,11,14,24,39,[d])
	Necremnus tidius (Walker)	Poland (27)
	Necremnus sp.	Germany (21); UK (25,29)
	Eulophus hegemon ?Wek*	France (18)
	Tetrastichus galectobus (= ? *galactopus*) (Ratzeburg) [c]	UK (29)
Braconidae	*Bracon discoideus* Wesmael	France (20)
	Bracon fulvipes Nees	France (18)
	Bracon maculiger Wesmael	France (18)
	Bracon sp.	Canada (24)

* Not recorded in the UK (Fitton *et al.*, 1978)
[a] Possibly hyperparastic (cf. Dmoch, 1975)
[b] Possibly hyperparasitic (cf. Doucette, 1948)
[c] Probably hyperparasitic (cf. Domenichini, 1966)
[d] for further citations in N. America see Peck (1963)

Study of the literature is hampered by synonymy and misidentification, eg. Lerin (1987) found that *T. perfectus* had been named by different authors as: *Trichomalus fasciatus* (Thomson); *Trichomalus herbidus* (Walker); *Trichomalus laevinucha* (Thomson); *Pteromalus decorus* (Walker); *Pteromalus decisus* (Walker). *Trichomalus fasciatus* and *Pteromalus perfectus* Walker are commonly used in older references (see Peck 1963). Likewise, *M. morys* is referred to in older papers as *Xenocrepis pura* Mayr, *Xenocrepis morys*, or *Pteromalus morys* Walker, *Mesopolobus mayetiolae* (Gahan) as *Amblymerus mayetiolae* (Gahan) or *Eutelus mayetiolae* Gahan and *Macroneura vesicularis* (Retzius) as *Eupelmella vesicularis* (Peck 1963). Also, it is sometimes unclear whether the species cited is a primary parasitoid or a hyperparasitoid. The only certain references to hyperparasitism of *C. assimilis* are those of Nasreldin (1973) who recorded *Mesopolobus mediterraneous* (Mayr) as parasitic on *Habrocytus semotus* (Walker) and *Chlorocytus diversus* (Walker), and Dmoch (1975) who found *Eurytoma aciculata* (Retzius) parasitic on a *T. perfectus* larva.

The percentage parasitism of individual *C. assimilis* larvae by ectoparasitoids can be large eg.: Nasreldin (1973) (UK) found 29-34; Murchie (1996) (UK) 31-73; Jourdheuil (1960) (France) 12-65; Laborius (1972) (Germany) 11-43; Dmoch (1975) (Poland) 20-68; Büchi (1993) (Switzerland) 3-70. Herrström (1964) found at one location in Sweden 100% parasitism of *C.*

assimilis larvae. Parasitism can be great even at low host densities, Lerin (1987) reports that with only 0.95 *C. assimilis* larvae per plant, 69% were parasitised by *T. perfectus*.

By endoparasitoids

The most comprehensive study on endoparasitism of *C. assimilis* larvae is that of Jourdheuil (1960) in France who records five species of hymenopteran parasitoid: two ichneumonids - *Tersilochus* sp., *Aneuclis melanarius* (Holmgren) (Ichneumonidae, Tersilochinae); and three braconids - *Sigalphus obscurellus* Nees (Braconidae, Cheloninae), *Diospilus morosus* Reinhard, *Diospilus oleraceus* Haliday (Braconidae, Helconinae). Börner et al.(1921) and Speyer (1925a) also reared *D. oleraceus*. Hoffmann (1951) refers to Speyer as citing *Probles (Euporizon) rufipes* (Holmgren) (= *Thersilochus moderator* (L.)) (Ichneumonidae, Tersilochinae) and to Reinhard as citing *D. oleraceus*, *Diospilus affinis* Wesmael, *Sigalphus obscurellus* Nees and *Sigalphus pallipes* Nees, as endoparasitoids of *C. assimilis* larvae but bibliographic references are not given.

The levels of parasitism were low: Jourdheuil (1960) found that *D. oleraceus* parasitized 1-10% of *C. assimilis* larvae; both Herrstrom (1964) in Sweden and Laborius (1972) in Germany did not find any endoparasitoids.

Parasitism of *C. assimilis* adults

Microctonus melanopus Ruthe (Hymenoptera: Braconidae, Euphorinae) parasitises adult *C. assimilis* (Speyer 1925b; Ankersmit 1956; Bonnemaison 1957; Jourdheuil 1960; Harmon & McCaffrey 1995). Miczulski (1967) also reports *Microctonus* cf. *deceptor* Wesmael as a probable parasitoid of *Ceutorhynchus* spp. in oilseed rape, while Risbec (1953) found larvae of Euphorinae parasitising *C. assimilis* but did not specify the species. Murchie (1996) reared through a species of *Microtonus* from *C. assimilis* but since it was male, identification to species was not possible. Levels of parasitism were variable: Bonnemaison (1957) reported that *M. melanopus* was the most important parasitoid of *C. assimilis* achieving, in some crops, levels of 50-60% parasitism but only 1-3% parasitism on others nearby; Jourdheuil (1960), also in France, found 40-50% parasitism of adult *C. assimilis*; Harmon & McCaffrey (1995), working in northern Idaho (USA), recorded levels of parasitism of 0-70%, and Murchie (1996), in southern England, up to 7% parasitism.

Acknowledgements

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References

Ankersmit, G.W. 1956: De levenswijze en de bestrijding van de koolzaad snuitkever (*Ceutorhynchus assimilis* Payk.) en de koolzadgalmug (*Dasyneura brassicae* Winn.). Verslagen van landbouwkundige onderzoekingen der Rijkslandbouw-proefstations 62.9: 1-59.

Bonnemaison, L. 1957: Le charançon des siliques (*Ceuthorrhynchus assimilis* Payk.). Annales des Épiphyties 4: 387-543.

Börner, C., Blunck, H., Speyer, W. & Dampf, A. 1921: Beiträge zur Kenntnis vom Massenwechsel (Graduation) schädlicher Insekten. Arbeiten aus der Biologischen Reichsanstalt für Land- und Forstwirtschaft, Berlin 10: 405-466.

Büchi, R. 1993: Monitoring of parasitoids of the cabbage seed weevil, *Ceutorhynchus assimilis* during 1990 and 1991 in Switzerland. Bulletin IOBC/WPRS 16: 145-149.

Carlson, E.C., Lange, W.H. & Sciaroni, R.H 1951: Distribution and control of the cabbage seedpod weevil in California. Journal of Economic Entomology 44: 958-965.

Crowell, H.H. 1952: Cabbage Seedpod Weevil Control with Parathion. Journal of Economic Entomology 45: 545-546.

Dmoch, J. 1975: Badania nad paso_ytami chowacza podobnika (*Ceuthorrynchus assimilis* Payk.). I. Sk_ad gatunkowy i znaczenie ektopaso_ytów larwalnych. Roczniki nauk Rolniczych, Seria E 5: 99-112.

Dmoch, J. & Sulgostowska, T. 1986: Parazytoidy chowacza podobnika (*Ceutorhynchus assimilis* PAYK.) (Coleoptera, Curculionidae) na uprawach kapusty nasiennej g_owiastej. Polskie Pismo Entomologiczne 56: 431-435.

Domenichini, G. 1966: Hym. Eulophidae palearctic Tetrastichinae. Index of entomophagous insects, Paris: 13-101.

Doucette, C.F. 1948: Field Parasitization and Larval Mortality of the Cabbage Seedpod Weevil. Journal of Economic Entomology 41: 763-765.

Gahan, A.B. 1941: A revision of the parasitic wasps of the genus *Necremnus* Thomson (Eulophidae; Hymenoptera). Journal of the Washington Academy of Sciences 31: 196-203.

Godan, D. 1959: *Mymar autumnalis* (Foerst.)[*Anaphes autumnalis* Foerst.] *(Chalcidoidea,Mymar.)*, ein bisher unbekannter Eiparasit des Kohlschotenrüsslers. Nachtrichtenblatt des Deutschen Pflanzenschutzdienstes 11: 161-164.

Graham, C.W. 1981: Pests of oilseed rape and their control. In Oilseed Rape Book, a manual for growers, farmers and advisers, pp. 97-105. Cambridge, Cambridge Agricultural Publishing.

Hanson, A.J., Carlson, E.C., Breakey, E.P. & Webster, R.L. 1948. Biology of the cabbage seedpod weevil in northwestern Washington. State College of Washington Agricultural Experimental Station Bulletin 498: 1-15.

Harmon, B.L. & McCaffrey, J.P. 1995: Biology and impact of *Microctonus melanopus*, a parasite of *Ceutorhynchus assimilis* Paykull, in northern Idaho (USA). Proceedings of the Ninth International Rapeseed Congress, Cambridge, 4-7 July 1995 pp. 1304-1305.

Herrström, G. 1964: Untersuchungen über Parasiten von Ölfruchtschädlingen in Schweden. Statens Växtskyddsanstalt. Meddelanden 12: 437-448.

Heymons, R. 1922: Mitteilungen über den Rapsrüssler, *Ceutorrhynchus assimilis* Payk. und Seinen Parasiten, *Trichomalus fasciatus* Thoms.. Zeitschrift für angewandte Entomologie 8: 93-111.

Hoffmann, A. 1951:. Contribution a l'étude de *Ceuthorrhynchus assimilis* Payk. (le Ceuthorrhynque des siliques). Revue de Pathologie Végétale et d'Entomologie Agricole de France 30: 238-252.

Horstmann, K. 1971: Revision der europäischen Tersilochinen (Hymenoptera: Ichneumonidae). Teil I. Veröffenlichungen der Zoologischen Staatssammling München 15: 45-138.

Jourdheuil, P. 1960: Influence de quelques facteurs écologiques sur les fluctuations de population d'une biocénose parasitaire: Étude relative à quelques Hyménoptères (Ophioninae, Diospilinae, Euphorinae) parasites de divers Coléoptères inféodés aux Crucifères. Annales des Épiphyties 11, 445-660.

Laborius, A. 1972: Untersuchungen über die Parasitierung des Kohlschotenrüsslers (*Ceuthorrhynchus assimilis* Payk.) und der Kohlschotengallmücke (*Dasyneura brassicae* Winn.) in Schleswig-Holstein. Zeitschrift für angewandte Entomologie 72: 14-31.

Lerin, J. 1987: A short bibliographical review of *Trichomalus perfectus* Walker, a parasite of the seed pod weevil, *Ceuthorrhynchus assimilis* Payk.. Bulletin IOBC/WPRS 10: 74-78.

Maceljski, M., Balarin, I. & Danon, V. 1980: Rezultati vi_egodi_njih prou_avanja pojave i _tetnosti insekata na uljanoj repici. Za_tita bilja 31: 317-324.

McCleod, J.H. 1953: Notes on the cabbage seedpod weevil, *Ceutorhynchus assimilis* (Payk.) (Coleoptera: Curculionidae), and its parasites. Proceedings of the Entomological Society of British Columbia 49: 11-18.

McKenna, D. 1972: *Study of the effects of insecticide management on the seedpod weevil (*Ceuthorrynchus assimilis Payk.*) and its parasites*. Unpublished MSc thesis, University of London, London.

Miczulski, B. 1967: B_onkówki (Hymenoptera) w biocenozie upraw rzepaku. Cz__× III. M_czelkowate (Braconidae) i mszycarzowate (Aphidiidae). Polskie Pismo Entomologiczne 37: 167-191.

Miczulski, B. 1968: B_onkówki (Hymenoptera) w biocenozie upraw rzepaku. Cz__× VI. Bleskotkowate (Chalcidoidea). Polskie Pismo Entomologiczne 38, 341-385.

Murchie, A.K.1996: Parasitoids of cabbage seed weevil and brassica pod midge in oilseed rape. Unpublished PhD thesis, University of Keele, Keele.

Nasreldin, N. 1973: A study of the weevil complex on mustard with special reference to the seedpod weevil. Unpublished PhD thesis, University of London, London.

Nolte, H.W. 1953: *Trichomalus fasciatus* Först. als Parasit der Mohnstengelgallwespe (*Timaspis papaveris* Kieff.). Mitteilungen - Biologische Bundesanstalt für Land- und Forstwirtschäft, Dahlem, Berlin 75: 56-58.

Peck, O. 1963: A catalogue of the Nearctic Chalcoidea (Insecta: Hymenoptera). The Canadian Entomologist Supplement no. 30.

Risbec, J. 1953: Contribution a l'étude des parasites du charançon du colza *Ceuthorrhynchus assimilis* Payk.. Revue de Pathologie Végétale et d'Entomologie Agricole de France 32: 37-43.

Rosen, H. von 1964: Untersuchungen über die Verbreitung und Biologie von zwei Pteromaliden in Rapsschoten *(Hym., Chalcidoidea)*. Statens Växtskyddsanstalt - Meddelanden 12: 451-465.

Skrocki, C. 1972: Biologia chowacza podobnika (*Ceuthorrynchus assimilis* Payk.). Roczniki nauk Rolniczych, Seria E 2 :7-20.

Speyer, W. 1925a: Kohlschotenrüssler (*Ceutorrhynchus assimilis* Payk.), Kohlschotenmücke (*Dasyneura brassicae* Winn.) und ihre Parasiten. Arbeiten aus der Biologischen Reichsanstalt für Land- und Forstwirtschaft, Berlin 12: 79-108.

Speyer, W. 1925b: *Perilitus melanopus* Ruthe (Hym. Braconidae) als imaginalparasit von *Ceutorrhynchus quadridens* Panz. zugleich eine kurze zusammenfassung unserer bisherigen kenntnisse von schlupfwespen als parasiten der käfer-imagines. Zeitschrift für angewandte Entomologie 11: 132-146.

Szczepanski, H. 1972: Kosmacinek rzepakowy - *Trichomalus perfectus* (WALK.) (*Hymenoptera, Pteromalidae*) w biocenozie le_nej a problem biologicznej ochrony rzepaku. Polskie Pismo Entomologiczne 42: 865-871.

Thiem, E. 1970: Die Bedeutung der Feldrandbehandlung für die Bekämpfung von *Dasyneura brassicae* Winn. (Dipt., Cecidomyiidae) und *Ceuthorrhynchus assimilis* Payk. (Col., Curculionidae) im blühenden Raps und der Einfluss dieser Insektizidmassnahme auf das

Vorkommen von *Trichomalus perfectus* Walker (Hym., Chalcidoidea, Pteromalidae) an den Larven des Kohlschotenrüsslers. Archiv Phytopathologie Pflanzenshutz 6: 83-98.

Walz, A.J. 1957: Observations on the biologies of some hymenopterous parasites of the cabbage seedpod weevil in northern Idaho. Annals of the Entomological Society of America 50: 219-220.

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Kairomones and searching behavior of *Trichomalus perfectus* Walker

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Abstract Host-finding behavior of *Trichomalus perfectus* Walker (Hymenoptera, Pteromalidae) females were investigated in the laboratory. Eighteen different activities were described, eight of them connected with host-finding and eggs laying, 10 other activities are connected with resting and feeding. Host-finding activities are forming logical sequence. All observed females realized the line of the sequence but with individual symptoms. The time of the activities in the sequence was individual except eggs laying time. Female, after the egg laying can start immediately new searching and it cans starts from different points of the sequence.

Cabbage seedpod weevil (*Ceuthorrhynchus asimilis* Payk.) (Curculionidae: Coleoptera) is known as serious pest in Europe and USA. It destroys pods of oilseed rape and seed plantations of cabbage and cauliflower.

In Poland we found eight species of parasitoids (Dmoch 1975,1977,1978; Dmoch and Rozum 1986; Dmoch and Sulgostowska 1986) attacking larvae of the pest. Among them *Trichomalus perfectus* Walker (Hymenoptera, Pteromalidae) is the most important. The mortality rate of the larvae on the oilseed rape plantation was 50% to 90%, (Dmoch 1975). The mortality on cabbage seed plantations (Dmoch and Sulgostowska 1986) was only 9% to 15%. From 80% to 90% of the attacked by parasitoids larvae were killed by *Trichomalus perfectus* (Table 1).

I think that *T. perfectus* is one of the most important factors keeping the population of cabbage seedpod weevil in a low level.

The source of kairomon is the frass host oldest larval instar (Dmoch i Rutkowska-Ostrowska 1978) . Our previous results encouraged us to undertake again experiments to describe the host finding and host-acceptance behavior of *Trichomalus perfectus* Walker females.

Table 1. Parasitization of cabbage seedpod weevil larvae by parasitoids:

Oil seed rape plantations		Cabbage and cauliflower seed plantations	
Trichomalus perfectus **Walker**	**90.2%**	***Trichomalus perfectus*** **Walker**	**82.8%**
Mesopolobus morys **Walker**	**5.5%**	***Stenomalina muscarum*** **(Linnaeus)**	**14.3%**
Eurytoma curculionum **Mayr.**	**1.5%**	***Habrocytus dispar*** **(Curtis)**	**1.3%**
Stenomalina muscarum **(Linaeus)**	**1.1%**	***Habrocytus semotus*** **Walker**	**0.8%**
***Habrocytus* sp.**	**1.1%**	***Pirenae* sp.**	**0.6%**
Eupelmella vesicularis **(Retz.)**	**0,6%**	***Isurgus morionellus*** **(Holmgr)**	**0.4%**
Total	**100,0%**	**Total**	**100.0%**
Average larval parasitization 50%-90%		**Average larval parasitization 9%-15%**	

Materials and Methods

Under the 1 m x 1 m x 2 m high isolator, situated on the plantation of oilseed rape, we have put, during the flowering of the winter rape, 50 males and 50 females of *C. assimilis*. The pods with the larvae were necessary for laboratory experiments.

Females of *T.perfectus* were collected with the sweeping net on the winter rape plantations situated near Warsaw.

We put one female with 10 pods (5 infested and 5 healthy) in the middle of a round observation arena. Petri dishes, 20 cm diameters, were used as arena. Each seance of the observation was 30 minutes long. During that time, the watcher and CCD camera were observing parasitoids female. The camera was connected with video tape and monitor. We repeated this procedure with 40 females. Video tapes were used for analysis of behavior. Video film show the sequence of females activities.(available under the authors address - in polish). Observation was carried out in 1994 and 1995 seasons, from the second decade of June to the end of July.

Results

Based on the video material we have described 18 different activities of the female.

OPENING activities

Cleaning (brushing) different parts of the body:

1.Antennae

2. Head and thorax

3. Legs

4. Abdomen

5. Wings

II. Host location activities

6. Radaring

7.Touching

8.Drumming

9.Stroking

10.Tapping with the abdomen

III. Eggs laying activities

11. Ovipositor's inserting

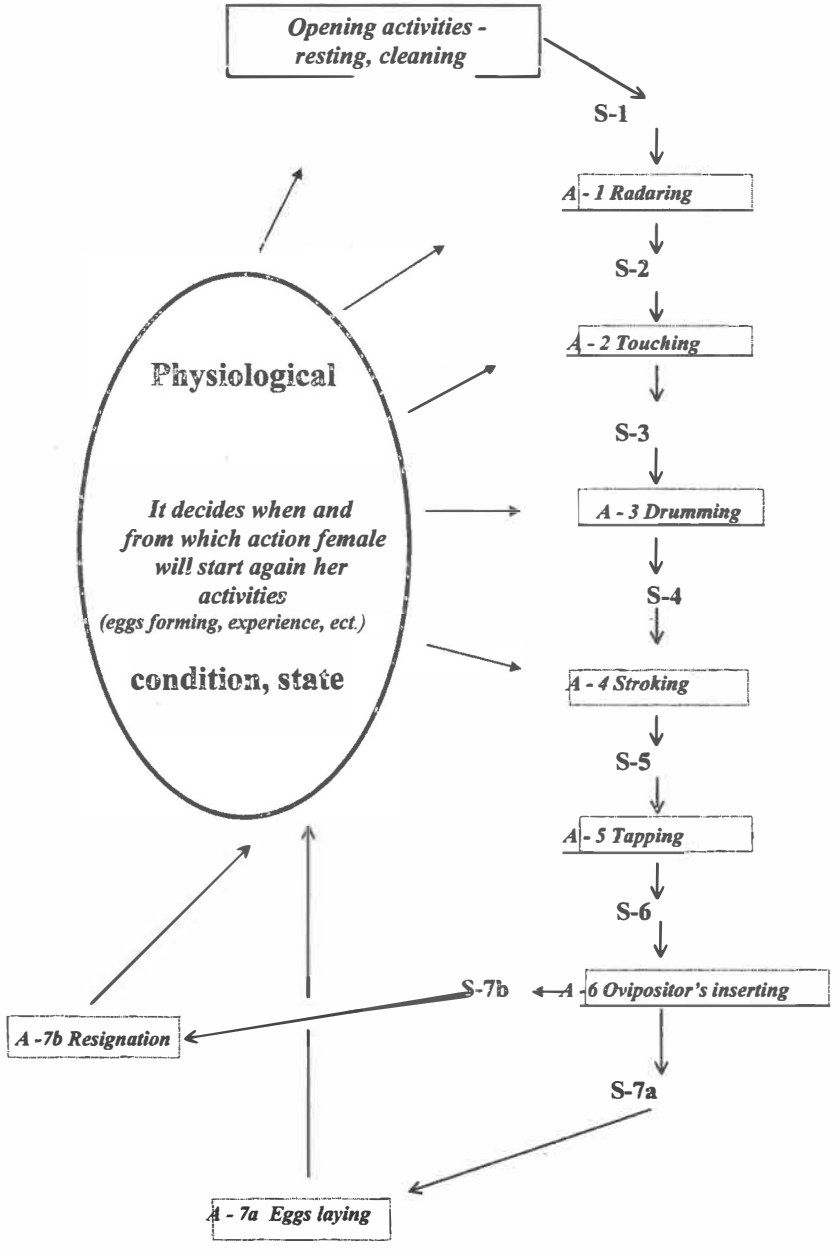
12. Eggs laying

13. Resignation

Five other activities are connected with feeding and resting. It is not obvious, what role play, in host-finding behavior, such activities like jumping and flying. Lewczuk (1994) described all activities in details but the most expressing is video tape. (Dmoch and Lewczuk 1995). The most important activities from the list are presented on fig.2

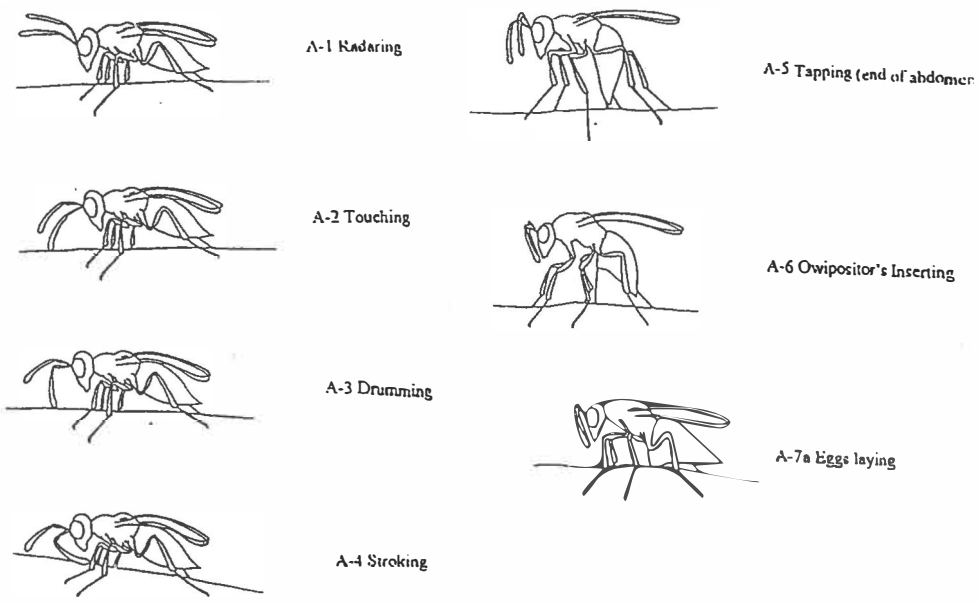
That activities are forming the behavioural sequence (Fig.1) 8 of that activities are connected with host-finding and eggs laying.

Figure 1. Behavior of *Trichomalus perfectus* Walker females during localization of the host and eggs laying



S - Stimulus; A - Action

Figure 2. Selected activities of the female (*Trichomalus perfectus* Walker) - schematic pictures



Drawing: Adam Wieczorek

Discussion

All observed females realized the sequence (fig. 1). The behavior of each female could have individual symptoms.

Real time of various activities, differ from female to female, sometime on e large scale. Eggs laying time it is en exception - it last usually from one to two minutes.

Females can start to search immediately after they finish egg laying. In other cases they rest, shorter or longer time, clean the body and than start to search again.

We have observed that females resigned to lay the egg but we do not know, if *Trichomalus perfectus* can discriminate larvae with the parasitoid eggs or no.

References

Dmoch, J. 1975. Badania nad pasozytami chowacza podobnika (*Ceuthorrhynchus assimilis* Pay.). I. Sklad gatunkowy i znaczenie pasozytów larwalnych. Roczniki Nauk Rol.,Ser.E 5(1):99-112

Dmoch, J. 1975. Badania nad paso¿ytami chowacza podobnika (*Ceuthorrhynchus assimilis* Payk.). II. Czas wystepowania i rozwój chowacza podobnika (*Ceuthorrhynchus assimilis* Payk.) i jego pasozytów larwalnych na rzepaku ozimym Roczniki Nauk Rol.,Ser.E 5(1):113-124

Dmoch, J.Klimek,G. 1975. Badania nad pasozytami chowacza podobnika (*Ceuthorrhynchus assimilis* Payk.). III.Obserwacje nad biologia *Trichomalus perfectus* (Walker). Roczniki Nauk Rol.,Ser.E 5(1):125-136

Dmoch, J. 1977. Biologiczne podstawy ochrony rzepaku ozimego przed szkodnikami. Materialy XVII Sesji Naukowej I.O.R. Poznañ 1977: 333-345

Dmoch, J. 1978. Mozliwosci wykorzystania metod biologicznych w zwalczaniu szkodników rzepaku. w Biologiczne Metody Walki ze Szkodnikami roslin. red. Boczek,J., Lipa,J.J. PWN, Warszawa 1978;431-441

Dmoch,J. Rutkowska-Ostrowska, Z.1978. Host-finding and Host-acceptance Mechanism in *Trichomalus perfectus* Walker (Hymenoptera:Pteromalidae) (Mechanizm odnajdowania i akceptacji zywiciela u *Trichomalus perfectus* (Hymenoptera:Pteromalidae)) Walker. Bull. de L'Acad. Polomaise Sc. Ser. Sc. biol. 26 (5): 317-323.

Dmoch, J.,Rozum B. 1986. Ocena efektywnosci parazytoidów chowacza podobnika. w 1985 r. Zeszyty problemowe.Wyniki badañ nad rzepakiem ozimym 1985: 262-274

Dmoch, J.,Sulgostowska,T. 1986. Parazytoidy chowacza podobnika (Ceuthorrhynchus assimilis Payk.) (Coleoptera: Curculionidae) na uprawach kapusty nasiennej glowiastej.Polskie Pismo Entomologiczne 56:431-435

Lewczuk, P. 1994. Zachowanie samic kosmacinka rzepakowego (*Ttichomalus perfectus* Walker) na etapie umiejscawiania i pora¿ania zywieciela. Praca magistaerska wykonana na Wydziale Rolniczym SGGW :1-47

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Effect of host size on the sex of *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae)

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Abstract *Trichomalus perfectus* (Walker) is the most abundant ectoparasitoid of the larvae of *Ceutorhynchus assimilis* Paykull which feed on seeds within the pods of oilseed rape. Field-collected parasitoid larvae were reared to adults in the laboratory and their sex determined. The number of seeds eaten by the larval host was used as an index of its size at the time of parasitisation. Female *T. perfectus* were reared from larvae that had consumed more seeds than those from which males had been reared and the larger female *T. perfectus* had a longer pupal development time than the smaller male. In addition to killing larvae through parasitisation, *T. perfectus* reduces damage to infested pods by preventing host larvae from eating their full complement of seeds.

Keywords: *Ceuthorhynchus assimilis*, host, oilseed rape, parasitoid, Pteromalidae, seed weevil, *Trichomalus perfectus*

Introduction

Trichomalus perfectus (Walker) is one of the more abundant ectoparasitoids attacking *Ceutorhynchus assimilis* Paykull [syn. *Ceutorhynchus obstrictus* (Marsham)], the cabbage seed weevil, on oilseed rape (Lerin 1987; Murchie 1996; Murchie & Williams 1997). The female *C. assimilis* lays its eggs singly into young pods of oilseed rape. The *C. assimilis* larva feeds on *c.* five developing seeds within the pod for 2-3 weeks and, when mature, chews its way out through the pod wall and drops to the soil to pupate (Dmoch 1965a & b). *Trichomalus perfectus* is a solitary idiobiont; the female seeks out a *C. assimilis* larva, pierces the pod wall with its ovipositor, stings and immobilises the larva and lays a single egg onto it. The parasitoid larva feeds externally from the *C. assimilis* larva eventually consuming all but its head capsule and skin and when mature pupates inside the pod. On emergence from pupation, the adult *T. perfectus* chews its way out through the pod wall. Hymenopteran parasitoids, with their haplodiploid genetic system, can choose the sex of the offspring by controlling the release of sperm from the spermatheca: unfertilised eggs are male whereas fertilised ones are female. This study investigates the sex of *T. perfectus* progeny in relation to the size of their host.

Methods

Collection of ectoparasitoids

Pods were examined from three winter oilseed rape crops (cv. Falcon or Libravo) at Rothamsted, Hertfordshire, during 1993-4. They were collected in late June to mid-July at growth stage (G.S.) 6.4 (most seeds in pods were green-brown mottled; Sylvester-Bradley & Makepeace 1984); at this time most *C. assimilis* larvae had emerged from pods or been parasitised. Ten or 20 pods were taken from each plant at regular intervals along transects through the crops. Equal numbers of pods were collected from the primary and secondary racemes, taking every third pod along the raceme. Pods were dissected under a binocular microscope and each parasitoid found was removed with its host larva and reared.

Rearing of ectoparasitoids

Immature parasitoids were placed with their hosts on moist filter paper within 50 mm diameter single vented Petri dishes and kept at 18°C. They were examined every two days and, in 1993, the developmental stage noted. The filter paper was kept moist during parasitoid larval and early pupal stages but was allowed to dry out as the pupae matured. In 1994, the lengths of parasitoid pupae were measured.

Seeds eaten by *C. assimilis* larvae

Since *C. assimilis* larvae eat their way along the pod and rarely completely consume seeds, the seeds which they have eaten can be counted. Partly consumed seeds were recorded as eaten if more than 50% was damaged. Due to the discrete nature of the data, comparisons between the number of seeds eaten by different groups of *C. assimilis* larvae were made using a proportional odds regression in Genstat 5 (Genstat 5 Committee 1993).

Results

T. perfectus reared

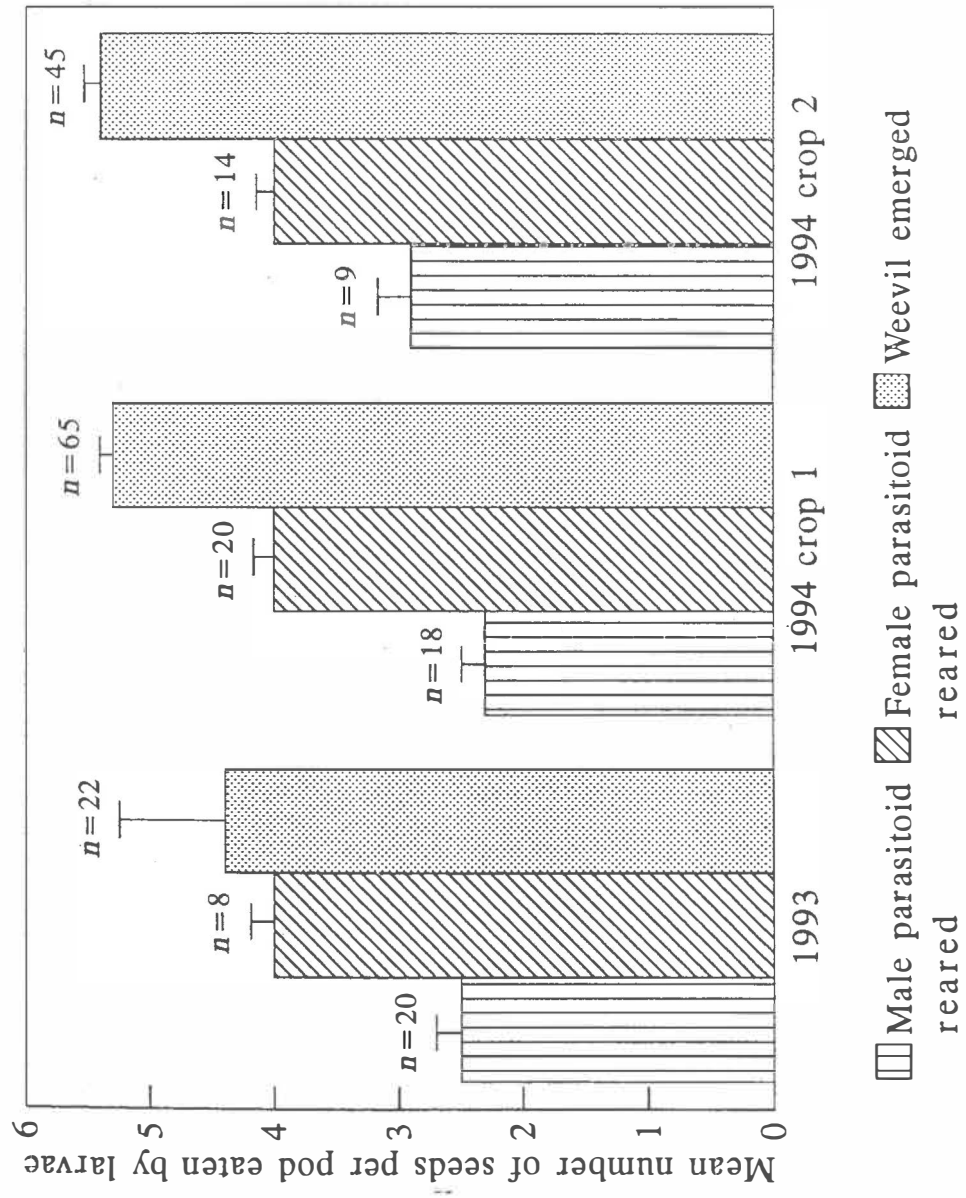
A total of 115 *T. perfectus* out of 235 found were successfully reared to adult. The sex ratio of *T. perfectus* was male biased (1.6 _ :1_, n= 51) in 1993 but female biased (1 _ :1.4 _, n=64) in 1994. The development time for the pupal stage was: male 11.0 ± 0.56 days (n=10); female 12.5 ± 0.26 days (n=15); the female pupal stage took significantly longer to develop than the male (t=2.50, d.f.=12.8, P=0.03). Male pupal length varied between 1.2-2.6 mm (mean=2.0 ± 0.1, n=27) and female 2.1-3.2 mm (mean=2.8 ± 0.1, n=37); female pupae were significantly longer than male pupae (F=83.12, d.f.=1,61, P<0.01).

Seeds eaten by *C. assimilis* larvae

Fewer seeds were consumed by *C. assimilis* larvae that had been parasitised compared with those which had successfully emerged (F=54.05, d.f.=1,210, P<0.01) (Figure 1). Overall, the mean number of seeds eaten by parasitised *C. assimilis* larvae was 3.2 ± 0.11 (n=89) compared with 5.2 ± 0.08 (n=132) when larvae had successfully emerged from pods. On three occasions, early instar *C. assimilis* larvae that had consumed only one seed were found parasitised.

Male *T. perfectus* were reared successfully from *C. assimilis* larvae which had consumed fewer seeds compared with those from which females were reared (F=17.25, d.f.=1,210, P<0.01) (Figure 1). The numbers of seeds per pod consumed by *C. assimilis* larvae (parasitised and healthy) differed between crops (F=4.69, d.f.=2,210, P=0.01).

Figure 1. Mean number of seeds per pod eaten by *Ceutorhynchus assimilis* larvae, that had been parasitised by male or female *Trichomalus perfectus* or had emerged. Error bars are one standard error.



Discussion

Male *T. perfectus* were reared successfully from *C. assimilis* larvae which had eaten fewer seeds than those from which females were reared. Assuming that the number of seeds eaten by a *C. assimilis* larva is indicative of its size, male *T. perfectus* were reared from *C. assimilis* larvae which were smaller than those from which females were reared. There are two possibilities why this may be so. Either there is differential survival of the sexes on different sized hosts, i.e. females survive better on large hosts and males on small hosts, or *T. perfectus* exhibits the ability to allocate the sex of its progeny according to the size of its host, which agrees with the sex allocation theory proposed by Charnov et al. (1981). Hymenopteran parasitoids, with their haplo-diploid genetic system, can choose the sex of their progeny by controlling the release of sperm from the spermatheca (Gould & Bolton 1988): unfertilised eggs are male whereas fertilised ones are female. Charnov et al. (1981) predicts that a mother parasitoid will lay a female egg when the host has greater resources (compared with surrounding hosts) as opposed to a male egg. Given that the size of the host determines the size of its parasitoid and assuming that a female parasitoid gains proportionally more by being larger compared with the normally short-lived male, then the mother, by this process of allocation, is maximising the fitness or number of her offspring. In their original paper, Charnov et al. (1981) also investigated a pteromalid wasp, *Lariophagus distinguendus*, which attacks the larva of the granary weevil (*Sitophilus granarius*) living inside a wheat grain; they demonstrated that the proportion of males reared from hosts of a certain size increased when the parasitoid could choose between them and larger hosts but decreased when they were presented together with smaller hosts.

The cues by which *T. perfectus* may determine the size of the concealed *C. assimilis* larva are unknown. Dmoch & Rutkowska-Ostrowska (1978) studied the host-finding and host-acceptance mechanisms of *T. perfectus* and determined that frass from third (last) instar *C. assimilis* elicited oviposition. In laboratory studies, they found that only third instar *C. assimilis* were parasitised, although, in field studies, second instar larvae were also parasitised, albeit in small numbers (Dmoch 1975). In this study, the larval instar parasitised was not determined, but from variation in size between host larvae found, it is certain that second instars were attacked. *Trichomalus perfectus* may be able to detect qualitative or quantitative differences in chemical cues produced by different sizes of *C. assimilis* larvae.

The development times of the immature stages of *T. perfectus* tended to be slightly longer than those reported by Dmoch & Klimek (1975), who reported that the pupal stage lasted eight days although he did not specify at what temperature. The shorter pupal development time of the male than that of the female, is compatible with males being reared from smaller, and therefore younger, hosts and the finding of Dmoch & Klimek (1975) that male *T. perfectus* emerge before females.

The sex ratio of *T. perfectus* differed between years indicating plasticity. This would be expected from the influence of host size; if the host age stratum differs between crops the sex ratio will also differ.

For the oilseed rape grower, the most important consequence of ectoparasitism is direct mortality of *C. assimilis* larvae leading to a reduction in adults for the following year (Murchie et al. 1997). However, parasitoids also reduce *C. assimilis* damage to the standing crop by preventing larvae from consuming their full complement of seeds. Parasitised larvae stopped feeding and consumed 38% less seeds compared to their healthy counterparts. Dmoch (1975) reports a similar reduction of 28%.

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References

Charnov, E.L. 1982: The theory of sex allocation. Princeton, Princeton University Press.

Charnov, E.L.; Hartogh, R.L. Los-Den; Jones, W.T. & Assem, J. van den 1981: Sex ratio evolution in a variable environment. Nature 289: 27-33.

Dmoch, J. 1965a: The dynamics of a population of the cabbage seedpod weevil (*Ceuthorrhynchus assimilis* Payk.) and the development of winter rape. Ekologia Polska - Seria A 8: 249- 287.

Dmoch, J. 1965b: The development of a population of the cabbage seepod weevil (*Ceuthorrynchus assimilis* Payk.) and the development of winter rape. Part 2. Ekologia Polska - seria A 13: 463-489.

Dmoch, J. 1975: Badania nad paso_ytami chowacza podobnika (*Ceuthorrynchus assimilis* Payk.). I. Sk_ad gatunkowy i znaczenie ektopaso_ytów larwalnych. Roczniki nauk Rolniczych, Seria E 5: 99-112.

Dmoch, J. & Klimek, G. 1975: Badania nad paso_ytami chowacza podobnika (*Ceuthorrynchus assimilis* Payk.). III. Obserwacje nad biologi_ *Trichomalus perfectus* (Walker). Roczniki nauk Rolniczych, Seria E 5: 125-136.

Dmoch, J. & Rutkowska-Ostrowska, Z. 1978: Host-finding and Host-acceptance Mechanism in *Trichomalus perfectus* Walker (*Hymenoptera, Pteromalidae*). Bulletin de l'Academie Polonaise des Sciences. Série des sciences biologiques: 26: 317-323.

Genstat 5 Commitee 1993: Genstat 5 Release 3 Reference Manual. Oxford, Clarendon Press.

Lerin, J. 1987: A short bibliographical review of *Trichomalus perfectus* Walker, a parasite of the seed pod weevil, *Ceuthorrhynchus assimilis* Payk.. Bulletin IOBC/WPRS 10: 74-78.

Murchie, A.K. 1996: Parasitoids of cabbage seed weevil and brassica pod midge in oilseed rape. Unpublished PhD thesis, University of Keele, Keele.

Murchie, A.K.; Williams, I.H. 1997: A bibliography of the parasitoids of the cabbage seed weevil, *Ceutorhynchus assimilis* Paykull. This volume.

Murchie, A.K.; Williams, I.H.; Alford, D.V. 1997: Effects of commercial insecticide treatments to winter oilseed rape on parasitism of *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae) by *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae). Crop Protection (in press).

Syvester-Bradley, R. & Makepeace, R.J. 1984: A code for stages in development in oilseed rape (*Brassica napus* L.). Aspects of Applied Biology 6: 399-419.

Biological and Integrated Control of Insect Pests

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BORIS: an EC-funded concerted action on the bio-control of oilseed rape pests

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Summary A 3-year programme of work, funded by the European Commission, was begun in 1997 to minimize pesticide use and environmental impact by the development and promotion of bio-control strategies for oilseed rape (OSR) pests. The project, which operates under the acronym BORIS, includes a programme of winter workshops and summer meetings on the natural enemies of OSR pests: namely, parasitoids, predators and pathogens. Specialists from eight countries (Austria, Denmark, Finland, France, Germany, Sweden, Switzerland and the UK) are involved. Regular newsletters are to be produced and these will be given wide distribution; a database on natural enemies of OSR pests is also beeing developed.

Keywords: bio-control, insect pathogens, oilseed rape pests, parasitoids, pesticide use, predators.

Introduction

In january 1997, a 3-year programme of work began, with the aim of minimizing pesticide use and environmental impact by the development and promotion of bio-control strategies for oilseed rape (OSR) pests. This Concerted Action is funded by the European Commission as part of the FAIR Programme (project FAIR3–CT96–1314) and involves specialists from Austria, Denmark, Finland, France, Germany, Sweden, Switzerland and the UK. From the outset, the concertation was identified by the acronym **BORIS** (derived from **B**io-control of **O**ilseed **R**ape **I**sect Pest**S**). Participants in the concertation have a wide range of expertise related to pests of oilseed rape, and a particular interest in at least one of three key bio-control areas: parasitoids, predators or insect pathogens.

The main objectives of the concertation are:

- to establish a network amongst European scientists currently working on bio-control of OSR pests, by provising a forum for information exchange;
- through winter workshops and summer meetings, to focus on technological exchange and to co-ordinate current and future research on natural enemies of OSR pests;
- to reduce the environmental impact of pesticides on a major European Union (EU) crop by devising strategies for farmers to exploit natural enemies and thereby minimize and better rationalize pesticide use on OSR;
- to make recommendations for the direction, harmonization and prioritization of future research efforts in the above-mentioned areas.

These objectives seek to maximize developments on natural, non-chemical methods of controlling insect pests on OSR, thereby reducing pesticide use. The adoption of such strategies would provide both environmental and economic benefits.

Two major activities within the concertation will be the production of regular newsletters and the development of a database on natural enemies of OSR pests. The former will be afforded wide distribution and access to the latter, which will continue for at least two years beyond the life of the concertation, will eventually be made available on a world-wide basis via the internet.

Discussion

Developments envisaged arising as a direct consequence of the concertation could significantly reduce the environmental impact of pest control measures by reducing and rationalizing pesticide use. They could also improve crop management strategies and form a significant part of modern integrated crop management (ICM) programmes for OSR. Also, the general principles development from this OSR-based projeckt could form a useful basis or a working example for similar approaches and developments on other arable crops.

The outcome of the concertation should allow opportunities for reducing arable farmers' reliance on pesticides, with consequential environmental benefits. The concertation should also broaden horizons and widen the scope for future work on OSR pests and their control, and should enable a better-targeted approach to ICM to be advocated within OSR crops generally. A final report on the groups's activities and conclusions is expected to be produced towards the end of 1999.

References

Anon. (1997). BORIS Newsletter of the European Network for Research Co-ordinating in Biocontrol of Oilseed Rape Insect Pests. No. 1, March 1977, 5 pp.

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Influence of host density and host distribution on parasitism of *Ceutorhynchus assimilis* by *Trichomalus perfectus*

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Abstract The relationship between host density, host distribution and parasitism by *Trichomalus perfectus* (Walker) (Hym.; Pteromalidae), a larval ectoparasitoid of the cabbage seed weevil *Ceutorhynchus assimilis* Paykull (Col.; Curculionidae), was examined in field experiments on winter oilseed rape in 1993 and 1995. Variation of host densities was established by releasing different numbers of adult seed weevils into cages. After oviposition, cages were removed and weevil larvae were exposed to natural parasitization.

In 1993, only small differences of weevil larvae densities occurred between plots and the number of parasitised larvae was not affected by host density and host distribution analysed on a single-plant scale. On average 36 % of larvae were parasitised. In 1995, the average proportion of infested pods varied between 15 % and 57 % in different plots. Correspondingly, levels of weevil larvae parasitism increased from 39 % to 50 %. However, again parasitism was not related to the position of infested pods on the main and secondary racemes. As in 1993, we could not detect a density-dependent relationship of parasitism of the seed weevil by its main parasitoid *T. perfectus*.

Keywords: *Ceutorhynchus assimilis*, density dependence, distribution, oilseed rape, parasitoid, *Trichomalus perfectus*

Introduction

The cabbage seed weevil *Ceutorhynchus assimilis* Paykull (syn. *C. obstrictus* (Marsham)) (CSW) is one of the most common insect pests of oilseed rape in Europe and North-America (Boyd & Lentz 1994, Ekbom 1995). By feeding on developing seeds, CSW larvae can cause significant damage (Winfield 1992). The larvae are attacked by various hymenopterous parasitoids. Among these the ectoparasitic parasitoid *Trichomalus perfectus* is the most important and widely distributed species (Murchie & Williams 1997). Although high levels of parasitization have been reported from different European countries (Dmoch 1975a, Edner & Daebeler 1984, Lerin 1987, Buechi 1993, Alford et al. 1995), the percentage of parasitism varies considerably between different sites and different years (Laborius 1972, Dmoch 1975a). However, in most studies the factors underlying these variations and the impact of the natural enemies on the population dynamics of the CSW are not addressed in detail and therefore need further investigation.

To be an effective antagonist of pest populations, a parasitoid should particularly meet two prerequisites (Kidd & Jervis 1996): 1. The functional response of the parasitoid should be positively related to increasing densities of the host. 2. The host searching behaviour of the

female parasitoid should be adapted to locate the hosts with respect to their spatial distribution on the host plants.

We used a factorial field experiment to examine the relationship between host density of CSW and the proportion of hosts parasitised by the parasitoids and to determine whether this parasitism is affected by the spatial distribution of host larvae on the rape plant.

Methods

Factorial field experiments were performed in 1993 and 1995 on unsprayed winter oilseed rape fields near Goettingen, Northern Germany. The experiments were set-up on 4 May 1993 and 18 May 1995, respectively, when the crop was in the flowering stage. 20 plots were allocated at random to five treatments with four replicates each. Plots of four treatments were enclosed in gauze cages (1m wide x 1m long x 1.8 m high; mesh size 0.3 mm). The naturally occurring densities of CSWs at time of caging were not counted. To establish a range of various weevil infestation levels, additional numbers of CSWs, collected from an infested crop in the neighbourhood, were introduced into the cages for oviposition: In 1993, 0, 30, 60, 90 CSW/m^2 were released. In 1995, the numbers of added CSWs per cage were doubled in order to produce a wider range of infestation levels. This resulted in 0, 60, 120, 180 added CSWs/m^2. For the assessment of weevil larvae densities and levels of parasitism under conditions of natural infestation four additional plots were left uncaged as controls in each year.

The period of caging CSWs for oviposition varied between years: In 1993 it was restricted to 9 days (May 4 to May 13), in 1995 it was extended to 21 days (May 18 to June 6). When all pods were fully developed, the cages were removed from the plots in order to allow access of parasitoids to CSW larvae. The period of potential CSW parasitization was 36 days in 1993 and 27 days in 1995.

On 18 June 1993 and 5 July 1995, respectively, when the crop was at the beginning of pod maturation, the plants were harvested separately from each plot. In 1993, 30 pods of the main raceme and 10 pods of two secondary racemes were sampled at random from each plant. The position of these pods along the racemes was recorded in a numerical succession, beginning with no.1 at the bottom and increasing numbers towards the top. In 1995, we used a different sampling procedure. Within each plot the pods were removed from all plants and divided into 3 subsamples of approximately the same size, representing different sections along the racemes: The bottom section (pods at a height <90 cm above ground), the medium section (90 - 105 cm) and the top section (>105 cm above ground). From each of these subsamples, 150 pods were selected at random, resulting in a total of 450 pods per plot.

In each year, the rape pods were dissected in the laboratory and examined for infestation of CSW larvae (live or dead) as well as eggs, larvae, pupae and adults of parasitoids. The exit holes of weevil larvae or parasitoids were counted. Parasitoid eggs, larvae and pupae were reared individually in gelatine capsules to the adult stage.

Results

Field experiment 1993

The level of infestation of pods by CSW larvae did not show significant differences between control plots (7.4%) and plots colonised with 30, 60 or 90 additional weevils (6.9% - 7.8%).

Parasitism of CSW larvae ranged from 30.8% to 44.8% and did not differ significantly between treatments either (Table 1). For further analysis, the plants of all treatments were pooled and examined in respect to the effects of specific growth parameters on infestation and distribution of CSW larvae and parasitoids. Parasitization of weevil larvae was not related to the total number of pods per plant as well as the number of pods infested by weevil larvae (Figure 1). Parasitism was exceptionally high at 6 infested pods/main raceme, but this pattern was not significantly different from other infestation levels. In conclusion, analysed on a single plant scale, parasitism obviously was not density dependent in relation to the number of infested pods per main raceme.

Table 1. Effect of different numbers of *Ceutorhynchus assimilis* released into caged plots of oilseed rape on pod infestation and parasitism of weevil larvae (field experiment 1993)

Number of adult weevils released per cage (1 m^2)	Average pod infestation (%)	Average level of parasitism (%)
+ 0	7.3	44.0
+ 30	7.8	35.1
+ 60	7.3	30.8
+ 90	6.9	35.6
natural infestation (uncaged plots)	7.4	35.1

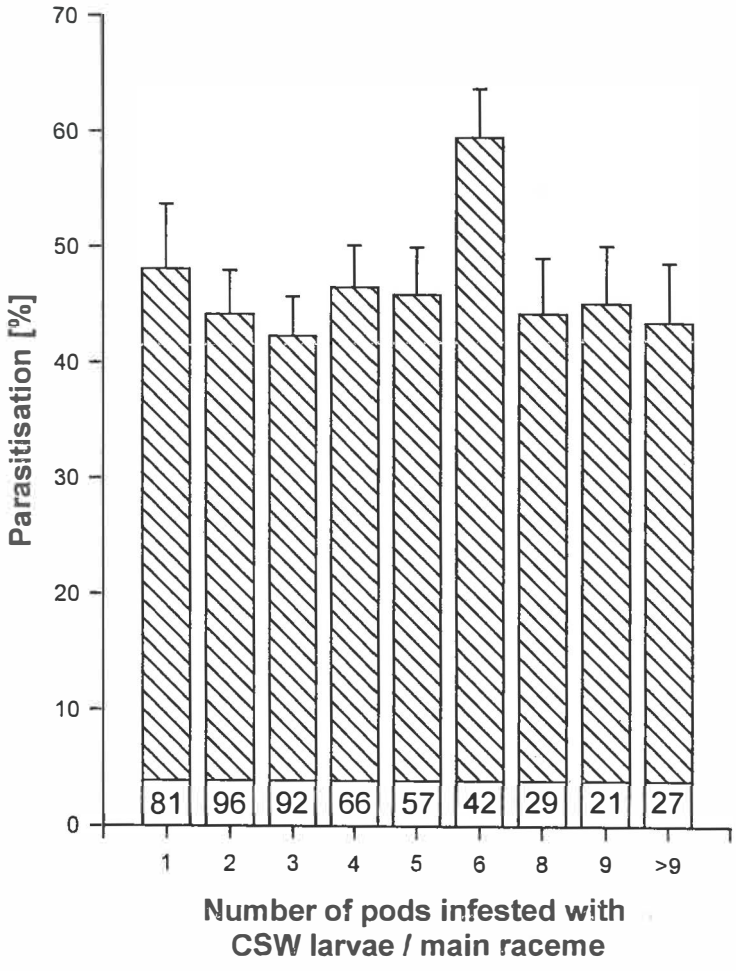

Figure 1. Parasitization of *Ceutorhynchus assimilis* (Ö±S.E.) in oilseed rape plants with different number of pods infested per main raceme. Figures at the base of the columns give the number of plants per group (field experiment 1995)

The distribution of parasitoids at different pod positions of the main raceme was almost uniform (Figure 2). There was a tendency of higher levels of parasitization at the lowest position of pods and this coincided with higher infestations of these pods by weevil larvae. However, the differences between parasitation of weevil larvae at different pod positions were not statistically significant.

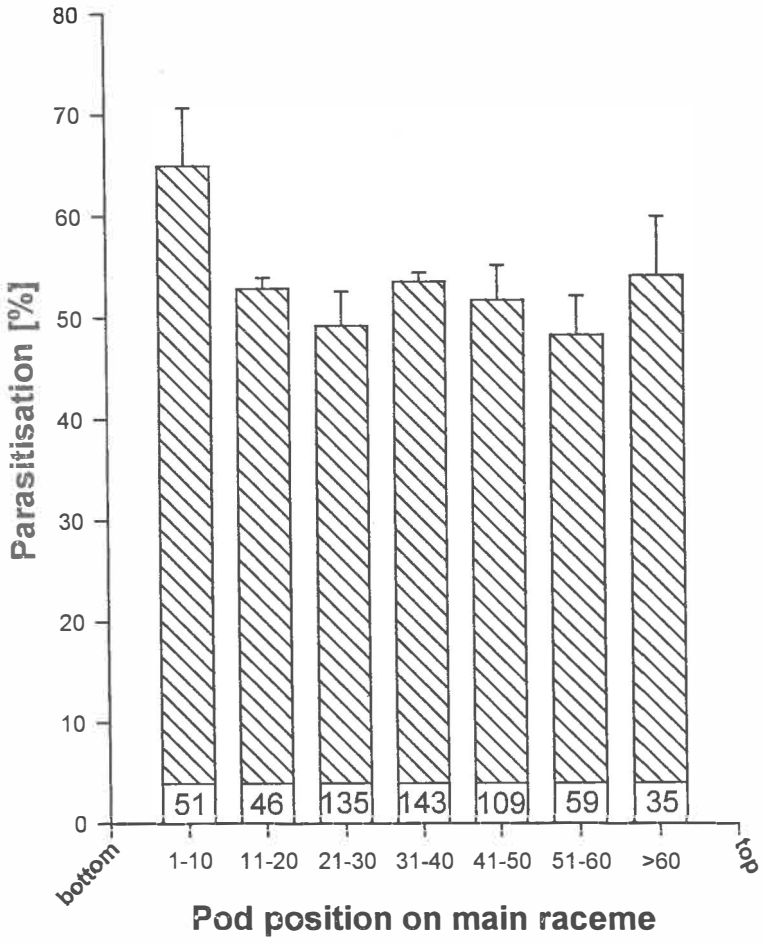

Figure 2. Parasitization of *Ceutorhynchus assimilis* (Ø±S.E.) at different pod positions on main racemes of oilseed rape plants . Figures at the base of the columns give the number of plants per group (field experiment 1995)

In 1993, four ectoparasitic parasitoid species were found on CSW larvae. The most abundant species *Trichomalus perfectus* accounted for 83.7% of the total number of parasitoids. *Mesopolobus morys* was much less abundant (15.2%), and *Eurytoma curculionum* and *Stenomalina gracilis* were only found incidentally on the CSW larvae (<1%).

Field experiment 1995

In 1995, we analysed the data on a total plot basis instead on a single plant scale. The average level of pod infestation increased with the number of weevils added from 15.5% to 56.8% (Figure 3). The infestation of pods in the high, median and low position of the racemes was not significantly different. However, as had been already observed in the experimental data of 1993, lower pod positions were slightly preferred by weevils.

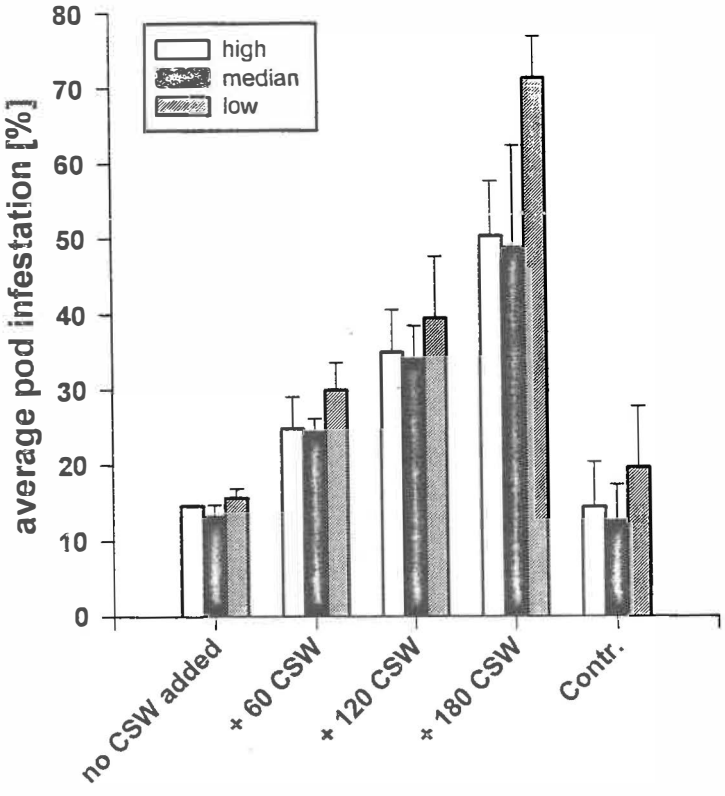

Figure 3. Effect of different numbers of *Ceutorhynchus assimilis* (CSW) released into caged plots of oilseed rape on the level of pod infestation by weevil larvae in low, median and high positions on the racemes (Ö±S.E.). Contr. = naturally infested uncaged plots. (field experiment 1995)

The average level of parasitization of CSW larvae increased in correspondence to the infestation levels from 39.1% to 50.5% (Figure 4). However, large variations occurred between replicated plots and the differences between treatments were statistically significant only between the lowest and highest CSW variants. Parasitism of CSW larvae was not significantly affected by the vertical position of the pods on the plants. These results are in agreement with those of 1993. In the 1995 experiment, *Trichomalus perfectus* was even more dominating, covering more than 95 % of the total number of parasitoids reared from weevil larvae. *Mesopolobus morys* and *Stenomalina gracilis* were only found in low numbers.

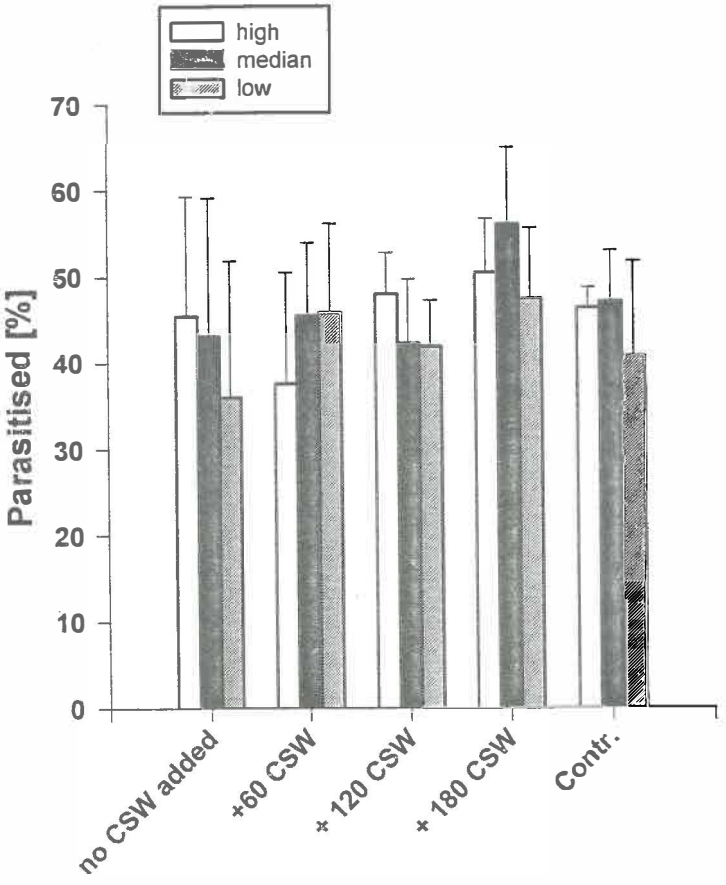

Figure 4. Effect of different numbers of *Ceutorhynchus assimilis* (CSW) released into caged plots of oilseed rape on the percentage of parasitised weevil larvae in low, median and high positions on the racemes (Ö±S.E.). Contr. = naturally infested uncaged plots.(field experiment 1995)

The relationship between the total number of pods infested by CSW larvae and the numbers parasitised by *T. perfectus* showed a significant correlation (Figure 5). As indicated by the slope of the regression line which runs parallel to the bisector of the angle, the number of parasitised weevil larvae increased proportionally to the number of larvae present. This shows that the percentage of parasitised larvae was almost constant over the whole range of larval densities.

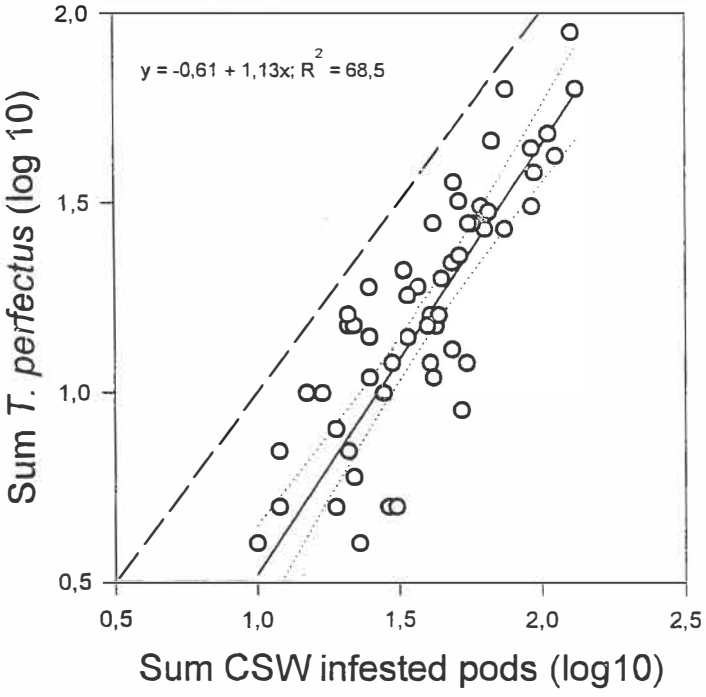

Figure 5. Relationship of *Ceutorhynchus assimilis* density and parasitism by *Trichomalus perfectus* in plots of oilseed rape (field experiment 1995)

Discussion

The failure to increase the numbers of CSW larvae in 1993 by releasing CSWs into the cages may be due to the unsuitable timing and an insufficient short period chosen to allow adults to oviposit on the pods. Oviposition of CSWs usually starts at flowering in May and lasts until July; it is affected by crop stage, plant vigour and weather conditions (Edner & Daebeler 1984, Lerin 1991). In 1995, when the number of CSWs released into cages was doubled and the period of oviposition was extended to 21 days, larval densities increased in relation to the number of CSWs per cages. Similar relationships between adult CSW populations and larval infestation have been found in cage experiments by Free et al. (1983) and Lerin (1991).

The weevil densities used in our experiments did not exceed the densities occurring in commercial fields of oilseed rape. In 1995, the release of 60, 120 and 180 weevils per cage resulted in 1.1, 1.9 and 3.2 adult weevils per plant, respectively, which is in the range of the presently discussed threshold for control of *C. assimilis* (Lane & Walters 1993, Garbe et al. 1996).

In both experiments, the infestations of plants in cages which did not receive extra CSWs were nearly equal to the infestation of naturally infested uncaged plants, indicating that cages had no adverse effect on CSW oviposition. Moreover, the parasitism of weevil larvae in uncaged plots was at the same level as in the caged plots with natural infestations of CSWs. This confirms that parasitization obviously was not hindered by the cages. The main period of activity of newly emerged *T. perfectus* adults takes place from late June to early July (Dmoch 1975b; Alford et al. 1995) in the rape crop. This makes it reasonable that the parasitoids had access to the pods for their main parasitization activity.

The proportion of parasitised larvae did not differ in pods at the low, median and high position of the racemes. Thus, *T. perfectus* does not show a preference for any specific section of the racemes in all treatments. This points out that the host finding behaviour of *T. perfectus* is well adapted to changing spatial and temporal patterns of the host distribution. In the course of the egg-laying period *C. assimilis* progressively oviposits into newly formed pods in distal positions at the racemes. Accordingly, the distribution of the 3rd instar weevil larvae which are preferred by *T. perfectus* for parasitization (Dmoch 1975a) changes with time from lower to upper pod positions.

The percentage of CSW parasitization can vary considerably between different sites and different years, ranging from 10.8% to 43.2% (Laborius 1972) or from 19.5% to 68.1% (Dmoch 1975a). Lerin (1987) found a 69% level of parasitization even at densities of CSW larvae as low as 0.95 larvae/plant. In insecticide trials at different sites in England, Alford et al. (1995) determined average levels of parasitization by *T. perfectus* in untreated plots of 21%, 73% and 54% with infestation levels by *C. assimilis* of 2.4%, 4.9% and 22.5 %, respectively. However, there are no quantitative data on the behavioural response of *T. perfectus* to different host densities in the literature.

When analysing 171 field studies on host abundance and parasitism, Stiling (1987) found evidence of density dependent behaviour of the parasitoids in only 25%, whereas inverse density dependence and independence was detected in 52% and 23%, respectively. The results of our experiments provide evidence that *T. perfectus* does not parasitise CSW larvae in a density-dependent way even when the host densities vary considerably. However, we did not analyse the searching behaviour of the female parasitoids in the field. Thus, using the pods of whole plants or pods pooled from several plants may be a crucial patch size in respect to spatial variations in parasitization patterns, and spatial heterogeneity may obscure the detection of density dependence (see Hassell et al. 1989 or Murdoch & Stewart-Oaten 1989). Moreover, even without density dependence *T. perfectus* may contribute considerably to CSW mortality and might restrict populations of this oilseed rape pest to a level below the economic threshold in many years. In addition, Alford et al. (1996) found evidence that adult females can cause additional mortality of CSW larvae by host feeding. In our study larval mortality due to host feeding by *T. perfectus* could not be analysed exactly because it was impossible to distinguish between larvae killed by host-feeding and by other mortality factors. Generally, around 50 % of non parasitised weevil larvae were found dead within pods.

References

Alford, D.V.; Murchie, A.; Williams, I.H. 1995: Observations on the impact of standard insecticide treatments on *Trichomalus perfectus*, a parasitoid of seed weevil on winter oilseed rape in the UK. IOBC/WPRS Bulletin 18(4): 122-126.

Alford, D.V.; Walters, K.F.A.; Williams, I.H.; Murchie, A.K. 1996: A commercially viable low-cost strategy for the management of seed weevil populations on winter oilseed rape in the UK. Brighton Crop Protection Conference -Pests and Diseases- 6C-4: 609-614.

Boyd, M.L.; Lentz, G.L. 1994: Seasonal incidence of the cabbage seedpod weevil (Coleoptera: Curculionidae) on rapeseed in West Tennessee. Environmental Entomology 23: 900-905.

Buechi, R. 1993: Monitoring of parasitoids of the cabbage seed weevil, *Ceutorhynchus assimilis* during 1990 and 1991 in Switzerland. IOBC/WPRS Bulletin 16(9): 145-149.

Dmoch, J. 1975a: Study on the parasites of the cabbage seed weevil (*C. assimilis* Payk.). I. Species composition and economic importance of the larval ectoparasites. Roczniki Nauk Rolniczych E 5: 99-112. (in polish)

Dmoch, J. 1975b: Study on the parasites of the cabbage seed weevil (*C. assimilis* Payk.). II. Relation between the times of occurrence and development of the cabbage seed weevil and those of its parasites on winter rape. Roczniki Nauk Rolniczych E 5: 113-124. (in polish)

Edner, B.; Daebeler, F. 1984: Zum Schadgeschehen durch den Kohlschotenrüßler (*Ceutorhynchus assimilis* Payk.). Nachrichtenblatt Pflanzenschutzdienst der DDR 38: 115-117.

Ekbom, B. 1995: Insect pests. pp 141-152 in: Brassica Oilseeds. Production and Utilization, Kimber, D.S.; McGregor, D.J. ed. CAB International Wallingford, 141-152.

Free, J.B.; Ferguson, A.W.; Winfield, S. 1983: Effect of various levels of infestation by the seed weevil (*Ceutorhynchus assimilis* Payk.) on the seed yield of oil-seed rape (*Brassica napus* L.). Journal of agricultural Science, Cambridge 101: 589-596.

Garbe, V.; Broschewitz, B.; Erichsen, E.; Hossfeld, R.; Lauenstein, G.; Steinbach, P.; Ulber, B.; Zellner, M. 1996: Schadensschwellen bei Rapsschädlingen- Instrumente einer wirtschaftlichen Winterrapsproduktion. Raps 14: 58-63.

Hassell, M.P; Latto, J.; Mmay, R.M. 1989: Seeing the wood for the trees: dectecting density dependence from existing life table studies. Journal of Animal Ecology 58: 883-892.

Kidd, N.A.C.; Jervis, M.A. 1996: Population dynamics. pp 293-374 in: Insect natural enemies - practical approaches to their study and evaluation, Jervis, M.; Kidd, N. eds. Chapman and Hall, London.

Laborius, A. 1972: Untersuchungen über die Parasitierung des Kohlschotenrüßlers (*Ceutorhynchus assimilis* Payk.) und der Kohlschotengallmücke (*Dasyneura brassicae* Winn.) in Schleswig-Holstein. Journal of applied Entomology 72: 14-34.

Lane, A.; Walters, K.F.A. 1993: Recent incidence and cost effective control of pests of oilseed rape in England and Wales. IOBC/WPRS Bulletin 16(9): 185-192.

Lerin, J. 1987: A short bibliographical review of *Trichomalus perfectus* Walker, a parasite of the seed pod weevil, *Ceutorhynchus assimilis* Payk. IOBC/WPRS Bulletin X/4: 74-78.

Lerin, J. 1991: Influence de la phénologie de la plante hôte sur la reproduction de *Ceutorhynchus assimilis* Payk. Journal of applied Entomology 111: 303-310.

Murchie, A.K.; Williams, I.H. 1997: A bibliography of the parasitoids of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.). This volume.

Murdoch, W.W.; Stewart-Oaten, A. 1989: Aggregation by parasitoids and predators: effects on equilibrium and stability. American Naturalist 134: 288-310

Stiling, P.D. 1987: The frequency of density dependence in insect host - parasitoid systems. Ecology 68: 844-856.

Winfield, A.L. 1992: Management of oilseed rape pests in Europe. Agricultural Zoology Reviews 5: 51-95.

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Reduction of the cabbage aphid, *Brevicoryne brassicae* (L.), population by *Diaeretiella rapae* (McIntosh) on oilseed rape, white mustard, and *Brassica* vegetables.

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Abstract The degree of natural reduction of *B. brassicae* population by the parasitoid *Diaeretiella rapae* (McIntosh) on different cruciferous crops in relation to their growing period and the cabbage aphid infestation period was investigated. The oilseed rape and mustard should be considered the main niches for *Brevicoryne brassicae* in the field. These plants supported the aphid population from the end of April until the second 10 day period of August. The first parasitised aphids were found approximately two weeks after the first aphid colonies appeared and this coincided with maximum aphid number on any of the crops. Initially, the percentage of parasitisation was very low (below 5 %). The maximum parasitisation (below 35 %) occurred two weeks after initial parasitisation and coincided with the decline of aphid population due to migration. The ineffectiveness of *Diaeretiella rapae* in reducing *B. brassicae* population is discussed in terms of its searching and post-emergence behaviour.

Keywords: *Brevicoryne brassicae, Diaeretiella rapae*, natural reduction, parasitoid effectiveness.

Introduction

Cruciferous crops are grown on a large scale in Poland. The oilseed rape fields alone cover over 350 thousand hectares (Burakiewicz et al. 1994). Vegetables of the genus *Brassica* make up 40 % of horticultural land (ca. 35 thousand hectares) and 50 % of all vegetables grown (Orlowski and Kolota 1992). White mustard, *Sinapis alba* L., is grown for seed on only 10 thousand hectares but its actual acreage is hard to estimate. Mustard is also used as a supporting plant, a component of plant mixtures, a forage plant, and as a natural fertilizer (Herse 1986). The cruciferous crops appear in succession in the field and some of them occur almost all the year round (fig. 1). The availability of young and susceptible plants through the year promotes the development of one of the most important pest insects, the cabbage aphid, *Brevicoryne brassicae* (L.). Moreover, some of the plants (oilseed rape, Brussels sprouts) are major sites of overwintering for this aphid (Gadomski 1996).

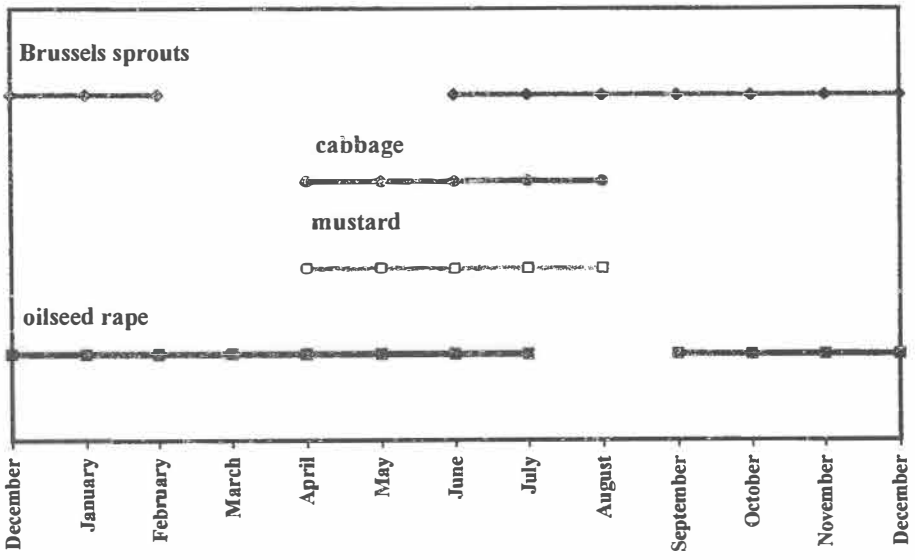

Figure 1. Succession of the cruciferous crops in the field

The aim of the present study was to determine the degree of natural reduction of *B. brassicae* population by the parasitoid *Diaeretiella rapae* (McIntosh) on different cruciferous crops in relation to their growing period and the cabbage aphid infestation period.

Methods

The study was carried out in the fields of the Agricultural University Experimental Station in Pawlowice near Wroclaw, Poland. The aphid number was counted on 25 randomly chosen plants of each crop, i.e., oilseed rape, white mustard, cabbage and Brussels sprouts twice a week from mid April to mid August in 1991-1993. The degree of parasitisation was determined by sectioning the field collected aphids once a week. A sample consisted of ca. 150 aphids.

Results

The winter oilseed rape was the first crop infested by *B. brassicae* in the spring (figs. 2-4). The first aphid colonies were found in the second 10 day period of April, the maximum in aphid numbers occurred in mid June and the population declined in the second 10 day period of July. The high number of aphids (over four thousand per plant) occurred for five to six weeks. The infestation of mustard and Brassica vegetables occurred during the first 10 day period of June, the maximum in aphid numbers - during the first 10 day period of July and the population declined at the end of July. At maximum, the highest number of aphids (over four thousand per plant) was observed on mustard. Usually, the white mustard was the first crop infested in June and the last one to be left by *B. brassicae* population. In 1991, single aphid

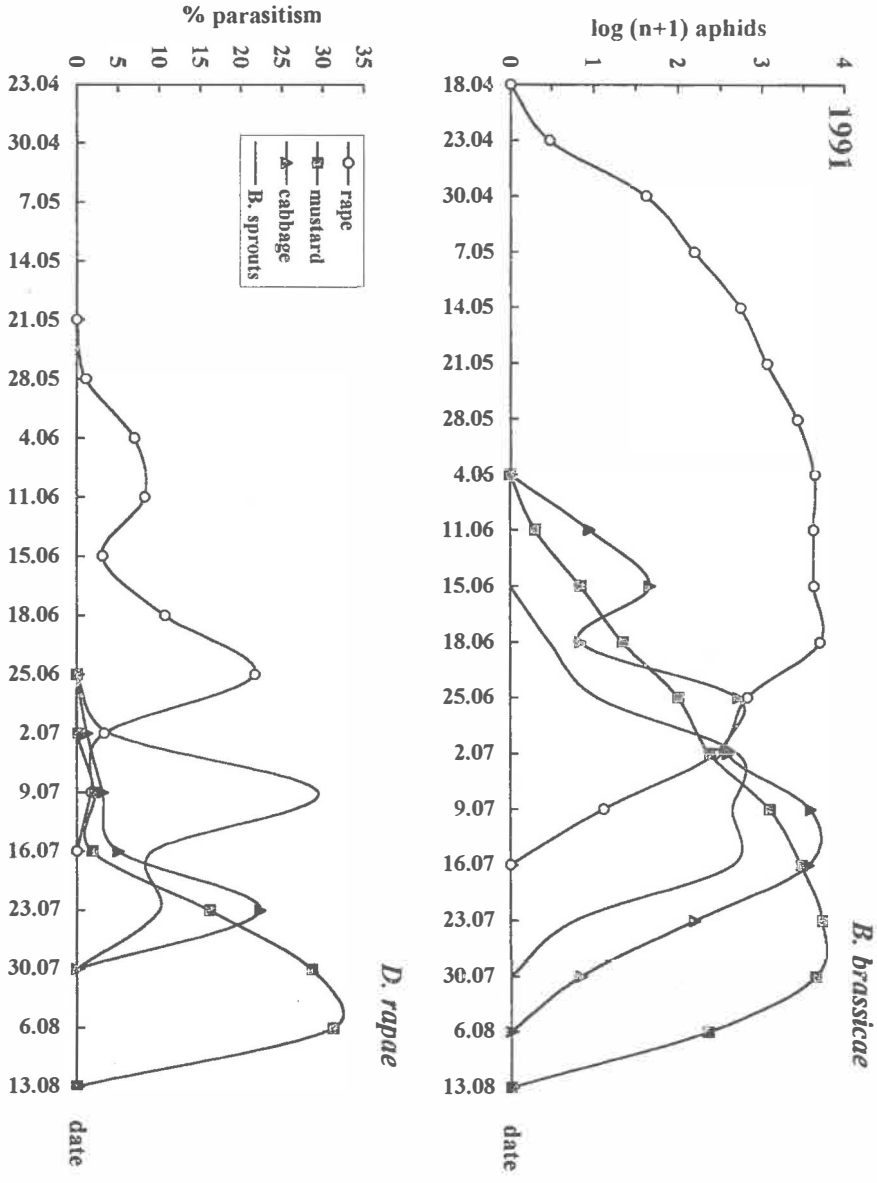

Figure 2. Development of the *B. brassicae* population on cruciferous crops and natural reduction by *D. rapae* in 1991

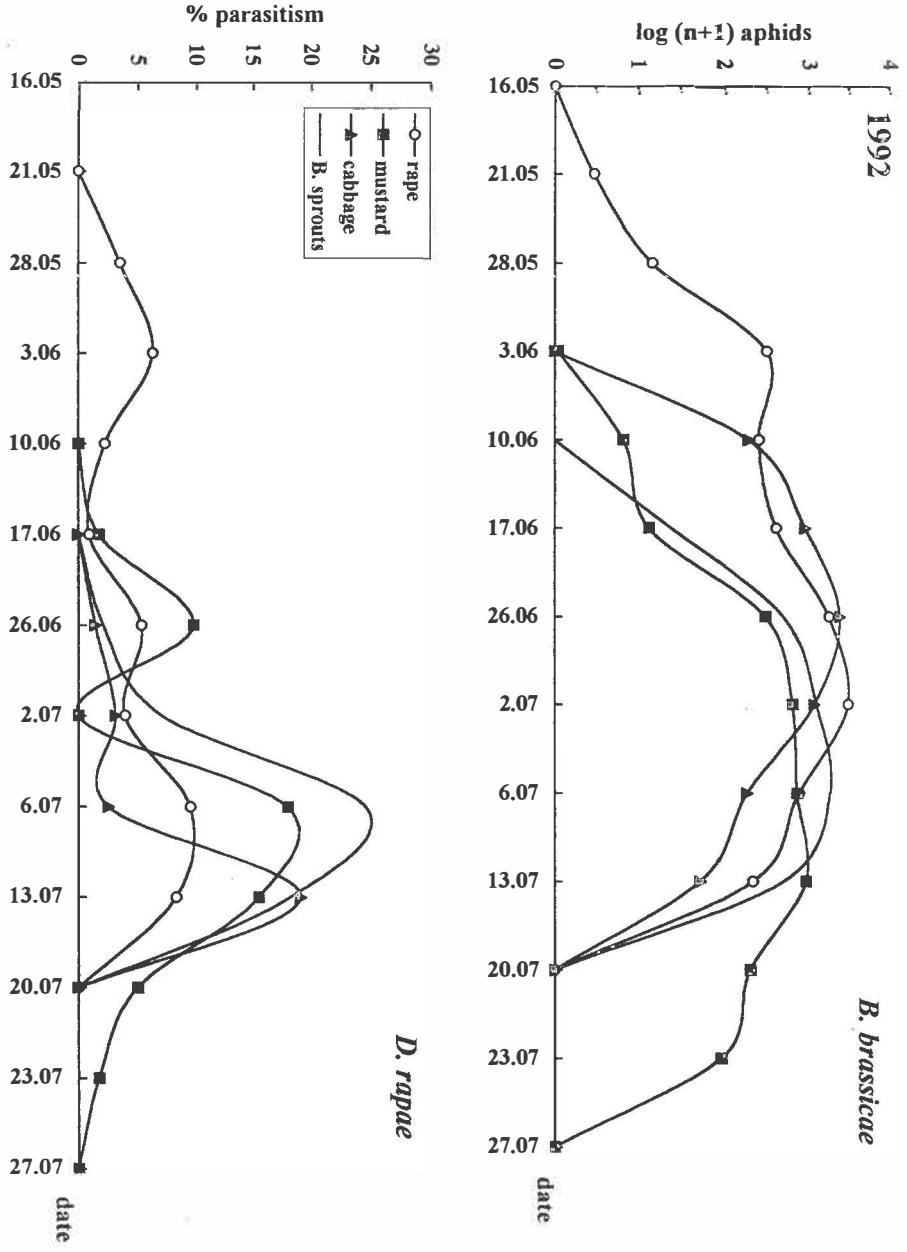

Figure 3. Development of the *B. brassicae* population on cruciferous crops and natural reduction by *D. rapae* in 1992

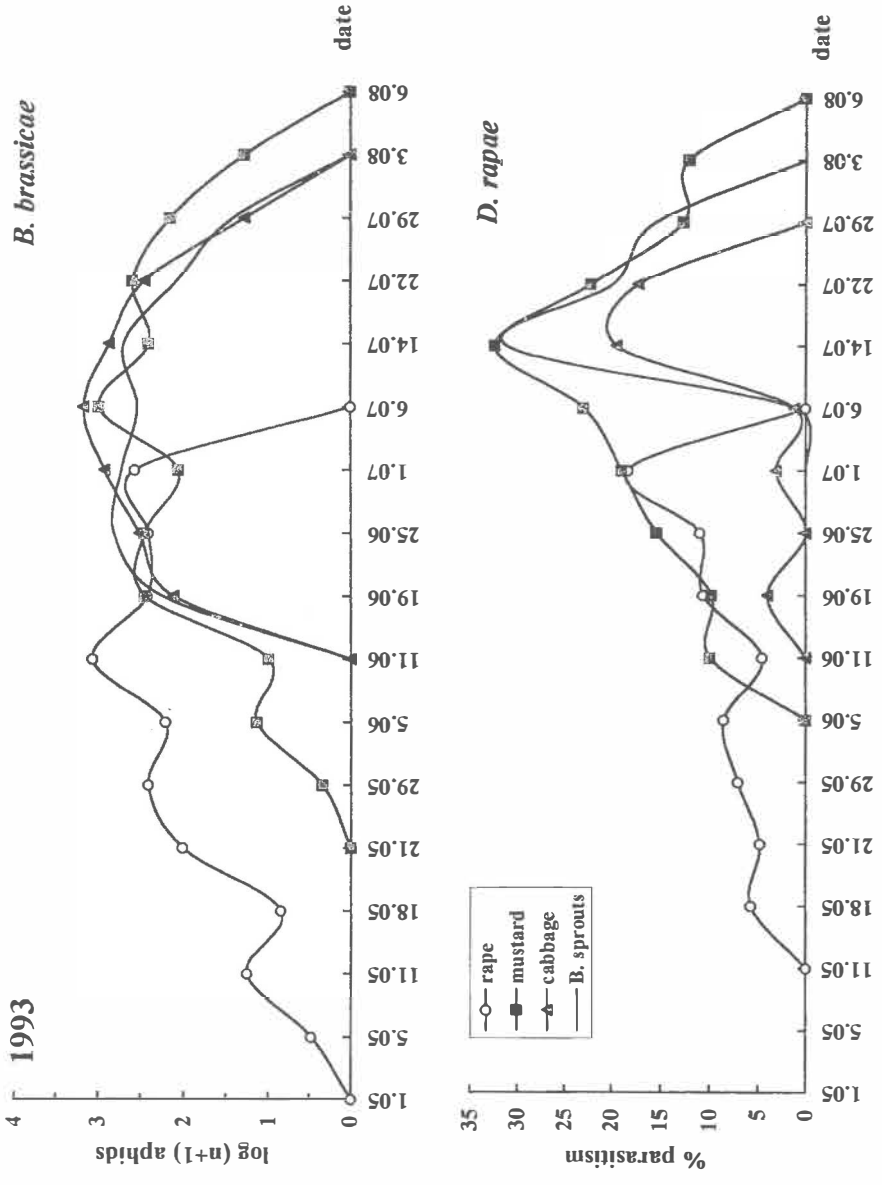

Figure 4. Development of the *B. brassicae* population on cruciferous crops and natural reduction by *D. rapae* in 1993

colonies on mustard were found in mid August. Generally, the production of alatae forms in colonies started at the maximum population number when the plants matured.

The first parasitised aphids were found in colonies on oilseed rape in the second and third 10 day period of May that is one week (1992, 1993) or five weeks (1991) after aphid infestation of plants. On mustard and Brassica vegetables, parasitised aphids were found two to three weeks after plant infestation. The initial parasitisation on all plants was below 5%. The increase in parasitisation (up to 20 - 33 %) occurred generally two to three weeks after initial parasitisation and coincided with aphid population decline on all crops.

Discussion

The parasitoid *D. rapae* appeared ineffective in reducing aphid number during the initial stage of plant infestation and this has already been reported by many authors (Paetzold and Vater 1966, Nawrocka 1972, Chua 1978, Gadomski 1994). The parasitoids appeared late and in low numbers. It has been found in Holland (Hafez 1960) that the spring emergence of *D. rapae* occurs between the second week of April and the second week of May. At the same time, the developmental time a mean temperature of 10°C, which is common in spring, is 43 days while at mean temperature of 22°C the period from oviposition to emergence takes 13 days on average. The increase in percent of parasitsed aphids which occurs two to three weeks after initial parasitisation is probably due to oviposition by emerging females within a given crop. *D. rapae* is able to oviposit immediately after emergence (Hafez 1960). It could be expected however, that high densities of aphids which occur on the early infested crops may serve as a source of parasitoids to limit aphid populations on other crops (Horn 1989). It was not the case in the present study. The initial parasitisation on cabbages and mustard was similar to that on oilseed rape. This means that the migration of *D. rapae* from oilseed rape to other crops was rather limited. It might have resulted from the unwillingness of the partasitoid to leave aphid-abundant colonies on oilseed rape (Sheehan and Shelton 1989). In consequence, the parasitoids which eventually arrived on other crops had reduced fecundity (Hafez 1960). Moreover, *D. rapae* does not discriminate between parasitised and non-parasitised aphids (Hafez 1960).

From the practical point of view, oilseed rape and mustard should be considered the main niches for the cabbage aphid development. The aphid population may be supported by these plants from mid April until mid August. *D. rapae*, despite its low effectiveness in reducing *B. brassicae* numbers, should not be excluded when planning aphid control strategies. It must be kept in mind that its low impact on aphid populations is only partly due to its post-emergence and searching behaviour. The role of hyperparasitoids must not be underestimated (Chua 1978, Gabrys and Sobota 1994, Gadomski 1994). Probably, reducing their effect will be an effective way of increasing the potential of *D. rapae*.

References

Burakiewicz, J.; Bielecki, J.; Dzwonkowski, W.; Krol-Pol, E.; Rosiak, E. 1994: Rynek rzepaku, stan i perspektywy. Ministerstwo Rolnictwa i Gospodarki Zywnosciowej 5: 1-24.

Chua , T. H. 1978: Pattern and influencing factors of emergence in *Diaeretiella rapae* and its parasites. Z. ang. Ent. 85: 436-442.

Gabrys, B.; Sobota, G. 1994: Species spectrum, relative abundance and population dynamics of parasitoids and hyperparasitoids of *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.) (*Homoptera, Aphididae*) on white mustard (Sinapis alba L.). Aphids and other homopterous insects 4: 33-39.

Gadomski, H. 1994: The effectiveness of *Diaeretiella rapae* (McIntosh) in the reduction of the cabbage aphid *Brevicoryne brassicae* (L.) on cruciferous crops. Aphids and other homopterous insects 4: 41-46.

Gadomski, H. 1996: Occurrence and damage caused by the cabbage aphid, *Brevicoryne brassicae* (L.), to cruciferous crops. Aphids and other homopterous insects 5: 47-52.

Hafez, M. 1960: Seasonal fluctuations of population density of the cabbage aphid, *Brevicoryne brassicae* (L.)in the Netherlands, and the role of its parasite, *Aphidius (Diaeretiella) rapae* Curtis. Tijdschr. Plantenziekten 67: 445-449.

Herse, J. 1986: Szczegolowa uprawa roslin. Warszawa, PWN.

Horn, D. 1989: Secondary parasitism and population dynamics of aphid parasitoids (*Hymenoptera: Aphidiidae*). Journal of the Kansas Entomological Society 62(2): 203-210.

Nawrocka, B. 1972: Metoda integracji walki biologicznej z chemiczna przeciwko mszycy kapuscianej (*Brevicoryne brassicae /L./*) na kapuscie glowiastej bialej. Biul. IOR 52: 379-387.

Orlowski, M.; Kolota, E. 1992: Uprawa warzyw. Szczecin, PWN.

Paetzold, D.; Vater, G. 1966: Population dynamics of the parasites and hyperparasites of *Brevicoryne brassicae* (L.). pp 279-281 in: Ecology of aphidophagous insects, Hodek, I. ed. Prague, Academia.

Sheehan, W.; Shelton, A. M. 1989: Parasitoid response to concentration of herbivore food plants: finding and leaving plants. Ecology 70(4): 993-998.

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Strategies to control the cabbage stem weevil (*Ceutorhynchus pallidactylus* [Mrsh.]) and the oilseed rape stem weevil (*Ceutorhynchus napi* Gyll.) by a reduced input of insecticides

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Abstract In seven years of field experiments for *Ceutorhynchus pallidactylus* (Mrsh.) peaks of flight activity were regularly recorded in the early spring, whereas increasing abundances in emergence traps occurred several weeks later. From this knowledge a control strategy has been developed which needs only one insecticide application, independent of whether and how often the action thershold was crossed. In experiments the timing of this pyrethroid application has been correlated with the increase of the abundance recorded by the emergence traps. The comparison of yield levels showed the feasibility of this strategy for *C. pallidactylus*. The results presented here give some background information on which biological or ecological characteristics these effects were based upon.

For *C. pallidactylus* the separation of sexes from yellow trap samples showed that in all years and locations the males leave their hibernation sites earlier than the females and predominate to a high extent at the beginning of the immigration period, whereas the percentage of females increases gradually with time. This limits the possibilities of copulation and egg-laying. Emergence trap samples of the new generation (in June, July) however demonstrate that in principle that the sex ratio is equalized. Egg-carrying females were recorded on average in the yellow traps only 15 days after the first flight activity. After about 28 days 50% of the females carried eggs. Further studies indicate that also egg-maturity is reached to a higher extent only several weeks after the immigration in the fields. Immediately after the eggs are laid the females produce new eggs. The time when in yellow trap samples a higher percentage of females carry eggs and egg-maturity is reached, coincides with the time of increasing abundances recorded by emergence traps. In these emergence trap samples the females predominate and all of them are carrying mature eggs, so that this time is assumed to indicate the main egg-laying period and to be the best moment for an insecticide application. At that time also flight activity peaks of *Meligethes aeneus* were also recorded regularly in the yellow traps.

The knowledge on biology and ecology of *C. pallidactylus* can not be transferred to *C. napi*: The Oil Seed Rape Stem weevil shows generally a greater variability regarding the phenology of sex ratio and the occurrence of egg-carrying females. In contrast to *C. pallidactylus* for *C. napi* a high percentage of female is frequently recorded right after the beginning of immigration into the fields. The time when 50% of the females carry eggs is on average reached earlier by *C. napi* (9-11 days) in comparison to *C. pallidactylus* (28 days). From these findings recommendations for practical farming are derived.

Keywords: action thresholds, *Ceutorhynchus pallidactylus, Ceutorhynchus napi*, ecology/ biology, egg production, emergence traps, Integrated Pest Manegement, oil seed rape; sex ratio, yellow traps

Introduction

To protect natural resources, one aim of agroentomology is the development of control strategies taking advantage of the knowledge of population dynamics, the biology and the ecology of the pest organisms. The consequent convertion of this knowledge makes a remarkable reduction of the input of pesticides possible, so that a pollution of agro-ecosystems can be avoided from the outset.

In approaching these aims this paper tries as well as former publications have done to contribute to the development of environmental friendly control strategies already elaborated for the cabbage stem weevil (*C. pallidactylus*) and Oil Seed Rape Stem Weevil (*C. napi*) (Büchs 1992, 1993, 1994, 1996, 1997a). These weevils are one of the most important pests of the oil seed rape stems in Central Europe. Their larvae damage the oil seed rape plants in regard to stability, nutrient supply and, last but not least, the building of pods (Broschewitz 1985, Büchi 1988, Büchs 1993, 1997b, Günthart 1949, Kelm & Walczak 1997, Lerin 1995, Oppermann 1990). A function of these pests as vectors of diseases such as *Phoma lingam* is also discussed (Broschewitz et al. 1993, Ulber 1994).

Materials and Methods

The experiments have been carried out by means of yellow traps (fig. 1a), which record the immigration of the beetles from hibernating sites, and emergence traps (fig 1b), the location of which was changed weekly. As emergence traps they mainly measure the actual (weekly) population density of the weevils in the oil seed rape fields. But, to reach the upper vessel of an emergence trap the weevils have also to be active to a certain extent. Therefore, beside the population density of the weevils emergence traps record also the „motion activity" of the weevils within the oil seed rape stands (Büchs 1993). Further information regarding the experimental conditions is given in table 1.

The average time to reach a certain percentage of females carrying eggs or egg-maturity has been calculated on weekly yellow trap- or emergence trap-samples during the field experiments.

Results and Conclusions

By means of the methods described it was observed over several years that in the first warmer days in the spring (if soil temperature 5 cm below ground is above 6° C and the average temperature of the day exceeds 10°C) peaks of flight activity of the Cabbage stem weevil were regularly recorded in the yellow traps (fig. 5; dashed lines with stars) which usually exceeded the damage threshold used in Germany (10 weevils/yellow trap and 3 days; Lauenstein 1992). Emergence traps (fig. 5; dotted lines with circles) however record increasing abundances of the weevils several weeks later on average (Büchs 1993).

Fig. 1a : not available

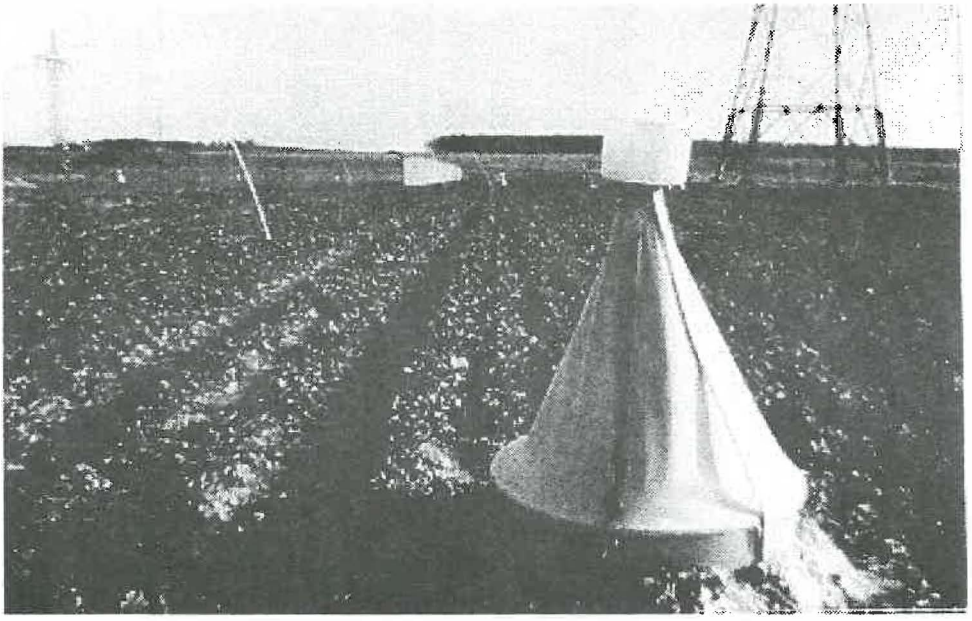

Figure 1a: Yellow trap (type Zeneca, Frankfurt/Main, Germany
Figure 1b: Emergence trap 1/4m² (type Ecotech, Bonn, Germany)

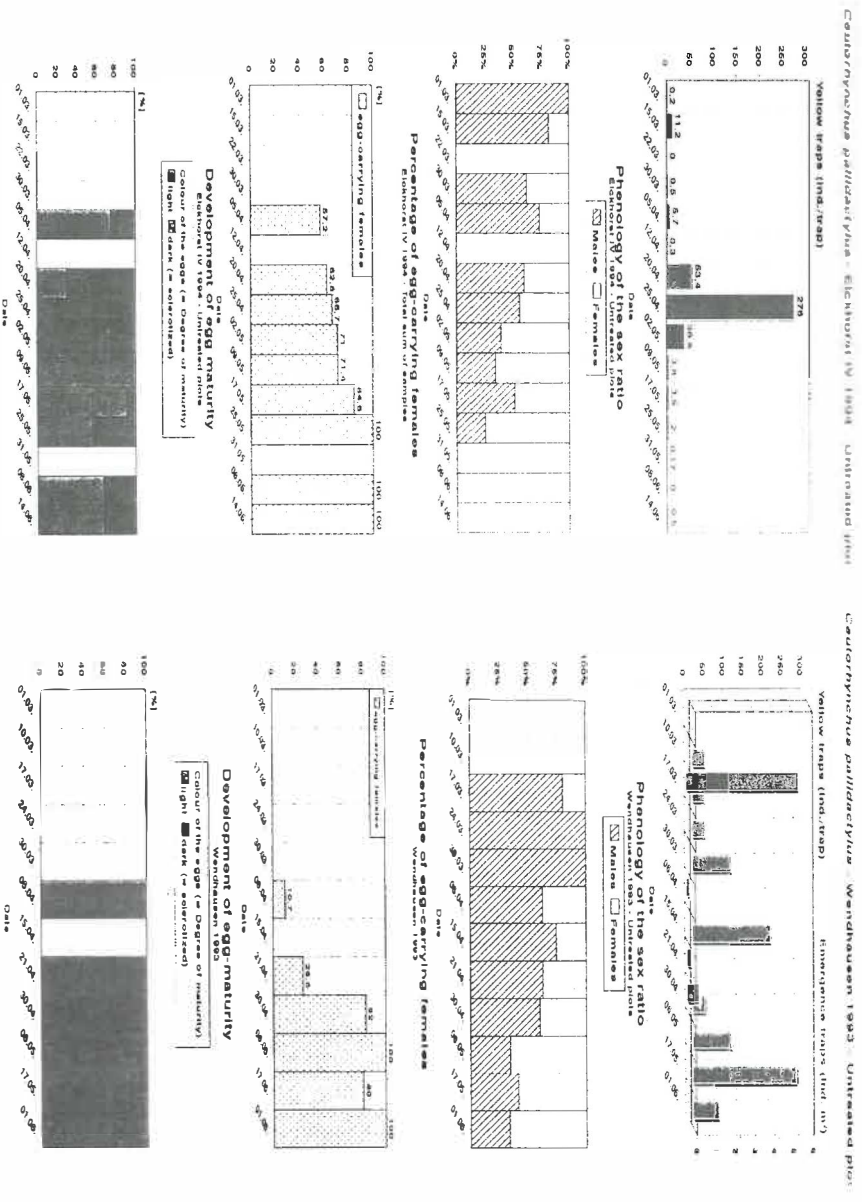

Figure 2a, b: Phenology of yellow trap samples (black columns), emergence trap samples (grey columns), sex ratio (striped bars = males; dotted bars = females), percentage of egg-carrying females (squared bars) and egg-maturity of *C. pallidactylus*. Sex ratio, Percentage of egg-carrying females and egg-maturity base on **yellow trap samples.**[1]

[1] The figures at the bottom are synchronized with the phenologies at the top. That means, in all graphs the data of one column belong to the same time period. The percentages shown in the lower figures refer to the yellow trap samples in the phenology at the top, except egg-maturity which refers to the percentage of egg-carrying females.

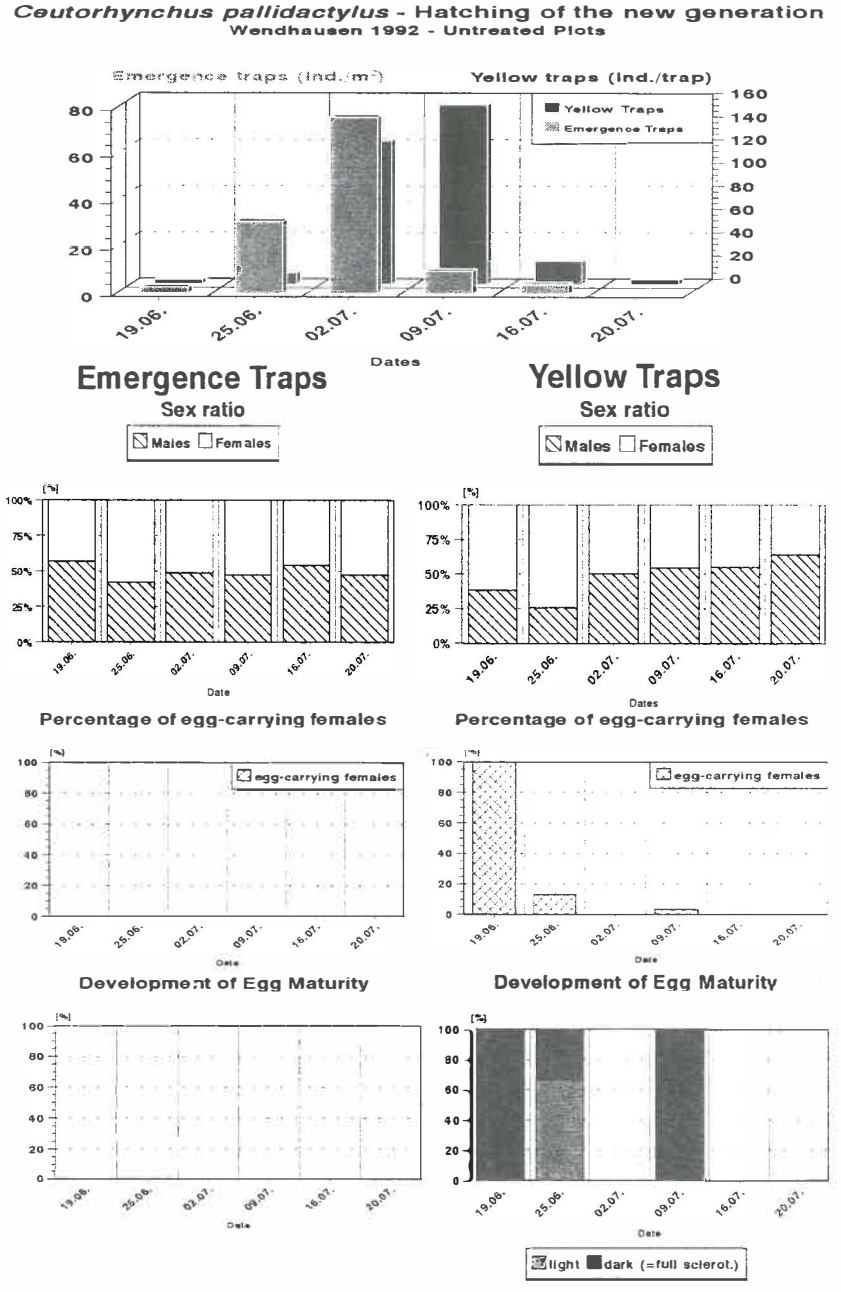

Figure 3: Phenology of yellow trap samples (black columns), hatching rate (emergence trap samples; grey columns), sex ratio (striped bars = males; dotted bars = females), percentage of egg-carrying females (sqared bars) and egg-maturity of the **new generation of *C. pallidactylus***. Further explanations see footnote at figure 2.

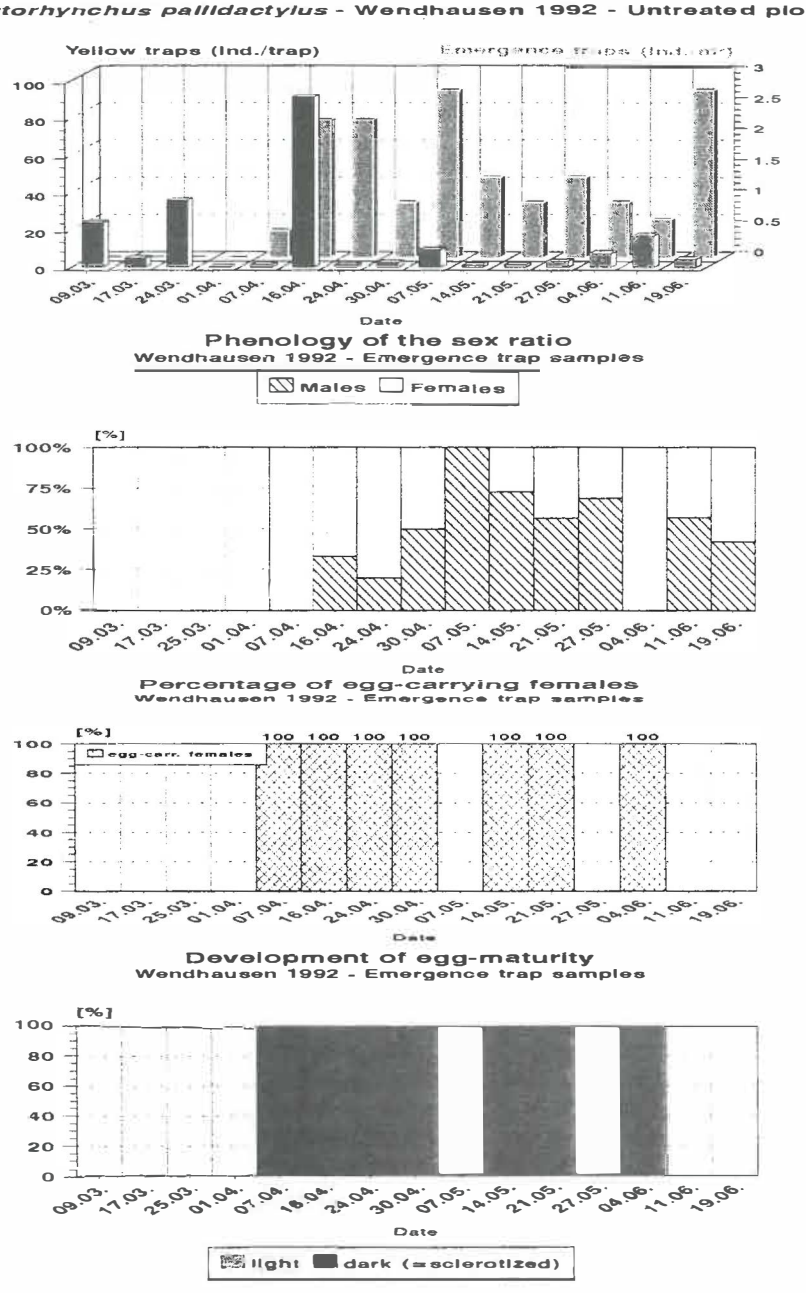

Figure 4: Phenology of yellow trap samples (black columns), emergence trap samples (grey columns), sex ratio (striped bars = males; dotted bars = females), percentage of egg-carrying females (sqaured bars) and egg-maturity of *C. pallidactylus*. Sex ratio, Percentage of egg-carrying females and egg-maturity base on **emergence trap samples**. Further explanations see footnote at figure 2.

Table 1: Description of the experimental design of those field trials which data are shown in the figures or which are mentioned in the text.

year	location	treatments	dates of insecticide application	plots (no.)	plot size	yellow traps (no.)	emerg. traps (no.)	cultivar
1990	Hötzum	untreated		3	ca. 0.7 ha	9	12	Ceres (8 kg/ha)
		1 x Decis 300 ml/ha	3.4.	3	ca. 0.7 ha	9	12	
1991	Wendhausen	untreated		2	48 m x 60 m	4	6	Ceres (5 kg/ha)
		1 x Decis 300 ml/ha	11.4.	4	48 m x 60 m	4	12	
		2 x Decis 300 ml/ha	17.3. / 11.4	4	48 m x 60 m	4	12	
1992	Wendhausen	untreated		3	48 m x 60 m	3	9	Ceres (5 kg/ha)
		1 x Decis 300 ml/ha	7.4.	6	48 m x 60 m	6	18	
		2 x Decis 300 ml/ha	10.3 / 7.4.	6	48 m x 60 m	6	18	
1993	Wendhausen	untreated		6	48 m x 60 m	6	6	Liberator
		1 x Decis 270 ml/ha	1.4.	6	48 m x 60 m	6	6	
		2 x Decis 270 ml/ha	16.3. / 1.4.	6	48 m x 60 m	6	6	
1994	Eickhorst	untreated		1	1.1 ha	6	8	Liberator (3,2 kg/ha)
		1 (2) x Decis 250 (300) ml/ha	21.4. (10.5.⇒D. brassicae)	1	1.6 ha	6	8	Liberator (3,2 kg/ha)
		2 (3) x Decis 250/150 (300) ml/ha	12.4. / 21.4. (10.5. ⇒ D. b.)	1	1.7 ha	6	8	Silvia (3,1 kg/ha)
1995	Abbenrode	untreated						
		1 x cypermethrin 300 ml/ha	15.5. (against Meligethes)	4	21 m x 40 m	in total	-	Synergie/Falcon (80:20)
		1 x lambda-cyhalothrin 100 ml/ha	30.5. (see above)	4	21 m x 40 m	4	-	
		4 x cyperm. (300 ml)/l-cyhal. 100 ml/ha	10.4. / 23.4. / 15.5. / 30.5. (s.a.)	4	21 m x 40 m		-	
1996	Wendhausen	untreated						Liberator
		1 x lambda-cyhalothrin 100 ml/ha	6.5.	4	ca. 0.65 ha	in total	-	
		2 x lambda-cyhalothrin 100 ml/ha	23.4. / 6.5.	4	ca. 0.65 ha	4	-	
		4 x lambda-cyhalothrin 100 ml/ha	23.4. / 6.5. / 20.5. / 30.5.	4	ca. 0.65 ha		-	

From this knowledge the experimental design and the concept for a control strategy with reduced insecticide input was developed: As a basic variant of the experiments an insecticide application was conducted immediately after the damage threshold decribed was exceeded as is usual in practical farming in Germany. This usually led in our experiments to two applications of pyrethroids in the spring (fig. 5; grey arrows). This variant was compared with a single insecticide application (fig. 5; black arrows), which was conducted only when the abundance values recorded by the emergence traps increased to a higher level, even if the damage threshold was already exceeded before in the yellow trap samples. There was also an untreated control.

In our experiments we observed that the infestation of the oil seed rape plants in the plots with only one insecticide application did always exceed the level of infestation of the plants in the plots with two insecticide applications and also both variants treated with insecticides showed a significant higher yield than the untreated plot, but there was no significant difference recognizable between the plot with one insecticidal treatment and those which were treated twice (see table in Büchs 1993).

So it could be assumed that the control strategy developed by us is feasible, but there was still no explanation of the delay of the „motion activity" of the weevils within the oil seed rape stands which, in contrast to the peaks of flight activity measured by the yellow traps, was not highly dependent on the weather conditions.

A separation of the yellow trap samples of *C. pallidactylus* into sexes showed that that male and female Cabbage Stem Weevils leave their hibernating sites at obviously different times: In the first yellow trap samples in the spring in all years and at all locations the male weevils predominate to a high extent. The percentage of female weevils increases only with time until they finally predominate (fig. 2a, b). The constancy of these relations for several years and locations indicate that the time difference of the immigration of both sexes from their hibernation sites into the rape fields is completely independent from the time of maximum flight activity, recorded by the yellow traps. This statement is illustrated by an example from two years in fig. 2a,b: It is obvious that in 1993 the maximum flight activity of *C. pallidactylus* in the yellow traps (fig.2a; black columns) was recorded very early (in the 1st half of March), in 1994 however (fig. 2b; black bars) it occurred relatively late at the end of April, that means six weeks after flight activity was recorded for the first time in the yellow traps in 1994. Independent of that in both years, mainly male weevils (fig. 2a,b; striped bars) were recorded first, later the percentage of female weevils (fig. 2a,b; dotted bars) increased.

The lack of females at the beginning of flight activity could explain that the „motion activity" in the oil seed rape stands, which is recorded by the emergence traps, starts considerably later (fig. 5). However, this low percentage of female weevils at the beginning of the immigration into the oil seed rape fields obviously limits the possibilities of copulation and egg laying of *C. pallidactylus* and so it finally limits also the possibilities of infestation of the oil seed rape plants at that early time.

The „normal" sex ratio of *C. pallidactylus* is shown by the hatching of the new generation at the end of June to the beginning of July, which can be recorded by emergence traps. In fig. 3 the results demonstrate for example in 1992 that the percentages of males and

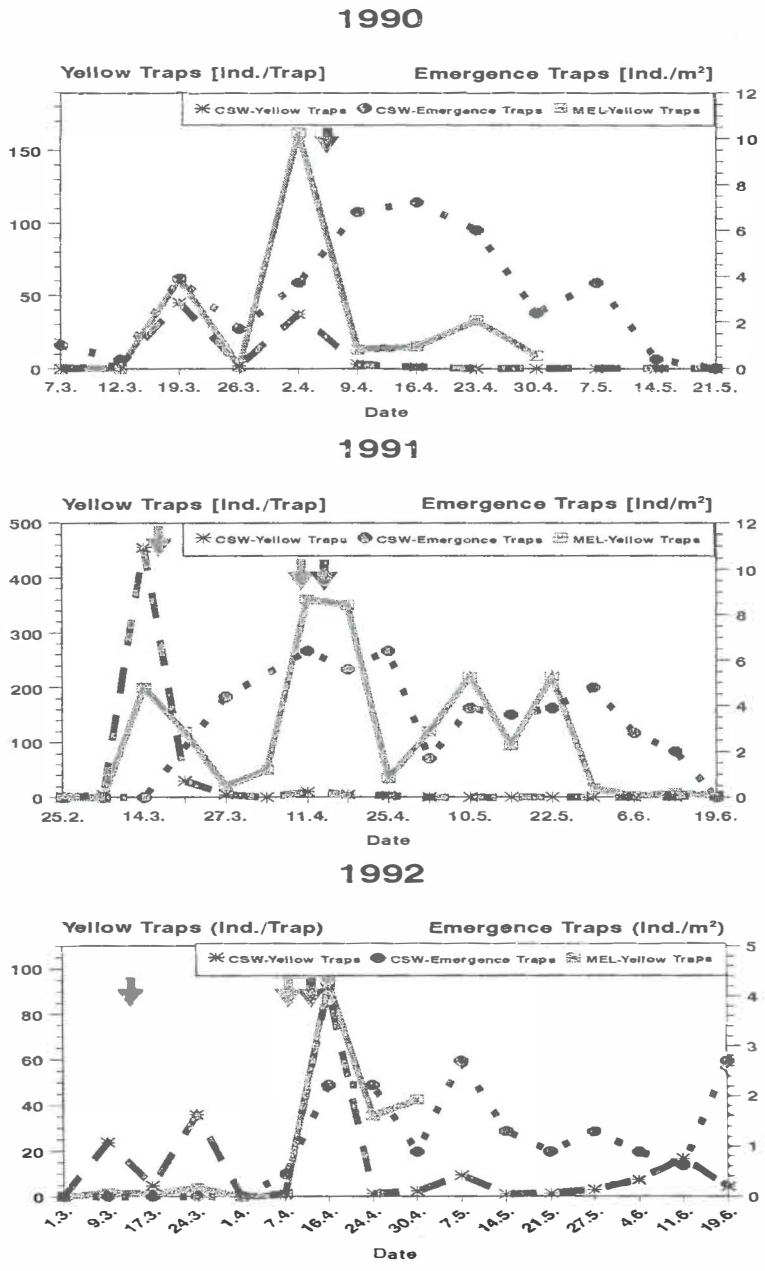

Figure 5: Comparison of yellow trap samples of the pollen beetle (*Meligethes aeneus*; grey line) and the phenology of the Cabbage Stem Weevil (*C. pallidactylus*) in yellow traps (dashed line with stars) and emergence traps (dotted line with spots) from 1990 to 1992. The arrows indicate the time of insecticide applications: grey arrows: intensive treatment (2 x deltamethin), black arrows: reduced treatment (1 x deltamethrin)

females of the new generation is nearly equal just after hatching, especially when the highest emergence rates are recorded (25th of June to 2nd of July). As it was expected the newly hatched female weevils carry no eggs. In principle similar relations are observed in yellow traps if one focuses on sampling periods with a high flight activity (25.06.-02.07. and 02.07.-09.07.). But concerning the whole sampling period shown in fig. 3 (12.06. 20.07.) a slight increase of the percentage of male weevils can be recognized. Besides that, in the yellow trap samples egg-carrying females occasionally occur, which obviously belong to the older generation.

The crucial point regarding the intensity of infestation of the oil seed rape plants is the occurrence of female weevils which carry eggs (fig. 2 a, b): Egg-carrying females of *C. pallidactylus* however occur (in respect to results from six field experiments) or average about 15 days after the first flight activity. On average it is only after 28 days that 50% of the female weevils carry eggs.

In addition, the maturity development of the weevil eggs has been investigated. From experiments with rove beetles (Zimmermann 1995, Zimmermann & Büchs 1993) it was known, that - referring to beetles which are conserved in alcohol, freshly reproduced eggs have a light colour, because they are not sclerotized, mature eggs however show a slightly more brownish colour.[2]

Only if a major part of the eggs is sclerotized, the main period of egg laying has to be expected. Analyzing the maturity development of eggs of the Cabbage Stem Weevil it could be observed for all years, that the eggs in the first occuring females which are carrying eggs had a light colour. Mainly sclerotized eggs occured in the *C. pallidactylus*-population in 1993 and 1994 only 5 to 6 weeks after the first major flight activity was recorded by the yellow traps (fig. 2a, b).

In addition, it was determined for all years that immediately after predominantly females with „dark" eggs were recorded again females with light eggs again appeared. This shows that after the first period of egg-laying the females of *C. pallidactylus* start again with the production of eggs. Actually it is not known whether these eggs are laid into oil seed rape plants (and in which part of them) or whether this „second wave" of egg-laying causes any serious damage to the oil seed rape plants.

Generally, it is conspicuous that the occurrence of egg-carrying female-weevils coincides always with an increase of their abundance and „motion activity" in the oil seed rape stand which is recorded by the emergence traps (fig. 2 and 4; grey columns).

In connection with these aspects, questions arise regarding the sex ratio, the percentage of egg-carrying females and the maturity development of eggs in the emergence trap-samples. Hitherto only results from yellow trap samples have been considered. In contrast to the results derived from yellow trap samples the emergence trap samples show (in fig. 4 for example illustrates results for 1992)

- that the female weevils predominate right at the beginning of their increased activity in the oil seed rape fields,
- all females in the samples are carrying eggs at that time,
- without exception the eggs are sclerotized and so obviously ready to be layed.

[2] In the case of *C. pallidactylus* and *C. napi* in the above mentioned field experiments more than two different stages of egg maturity could be determined, beginning with the forming of eggs in the ovarioles. To simplify the presentation of the results (fig. 2-4) these various stages have been (concentrated) summarized to two stages.

These results indicate, that emergence traps (fig. 4; grey columns) in contrast to yellow trap samples (fig. 4; black columns) record increasing numbers of weevils exactly at the time, when they start to lay eggs. So it can be assumed that an increasing abundance and „motion activity" recorded by the emergence traps marks the beginning of the main egg laying period and therefore it indicates the optimal time for an insecticide application (i.e. a considerable infestation of the pest insect has ocurred).

Furthermore our experiments showed that the increase of the abundance and „motion activity" of the Cabbage Stem Weevil within the oil seed rape stands (recorded by the emergence traps) always coincides with a peak of flight activity and immigration into the oil seed rape field of the pollen beetle (*Meligethes aeneus*) registered by the yellow traps (fig. 5). So there was no extra insecticide application necessary against the pollen beetle, because this pest insect as well as later immigrating specimens of *C. pallidactylus* were sufficiently controlled by the one and only, but delayed application of a pyrethroid. Therefore, also the time of occurrence of a pollen beetle peak in yellow trap samples is able to be brought in as an additional indicator for an optimal scheduling of a pyrethroid application against the Cabbage Stem Weevil.

Further results of Büchs (1993) showed that the density of later occurring oil seed rape pest insects such as Mealy Cabbage Aphid (*Brevicoryne brassicae* L.), the Brassica pod midge (*Dasineura brassicae* Winn.) and the Cabbage seed weevil (*C. assimilis*) are considerably reduced by pyrethroid applications even if those were conducted (against the Stem Weevils) at least four weeks before the occurrence of the above mentioned pest organisms. A repellent effect of pyrethroids against these later occurring pest insects is said to be responsable for this phenomenon.

Until this point, only results of the Cabbage Stem Weevil have been presented. The question then arises wether it is possible to transfer the results shown for the Cabbage Stem Weevil (*C. pallidactylus*) to the Oil Seed Rape Stem Weevil (*C. napi*), which occurs at nearly the same time as *C. pallidactylus*?

Fig. 6a-f demonstrate for the different years and locations that the results which had been observed for *C. pallidactylus* are obviously **not** transferable to *C. napi*: Whereas for *C. pallidactylus* the percentage of female weevils always increases continuously from the beginning of flight activity, the relations for *C. napi* are more or less variable. Commonly, right at the beginning of the immigration from the hibernating sites (rape fields of the previous year) into the rape fields, when a considerable flight activity is observed in the yellow trap samples, a comparatively high percentage of female weevils can be recorded for *C. napi* (fig. 6a-f).

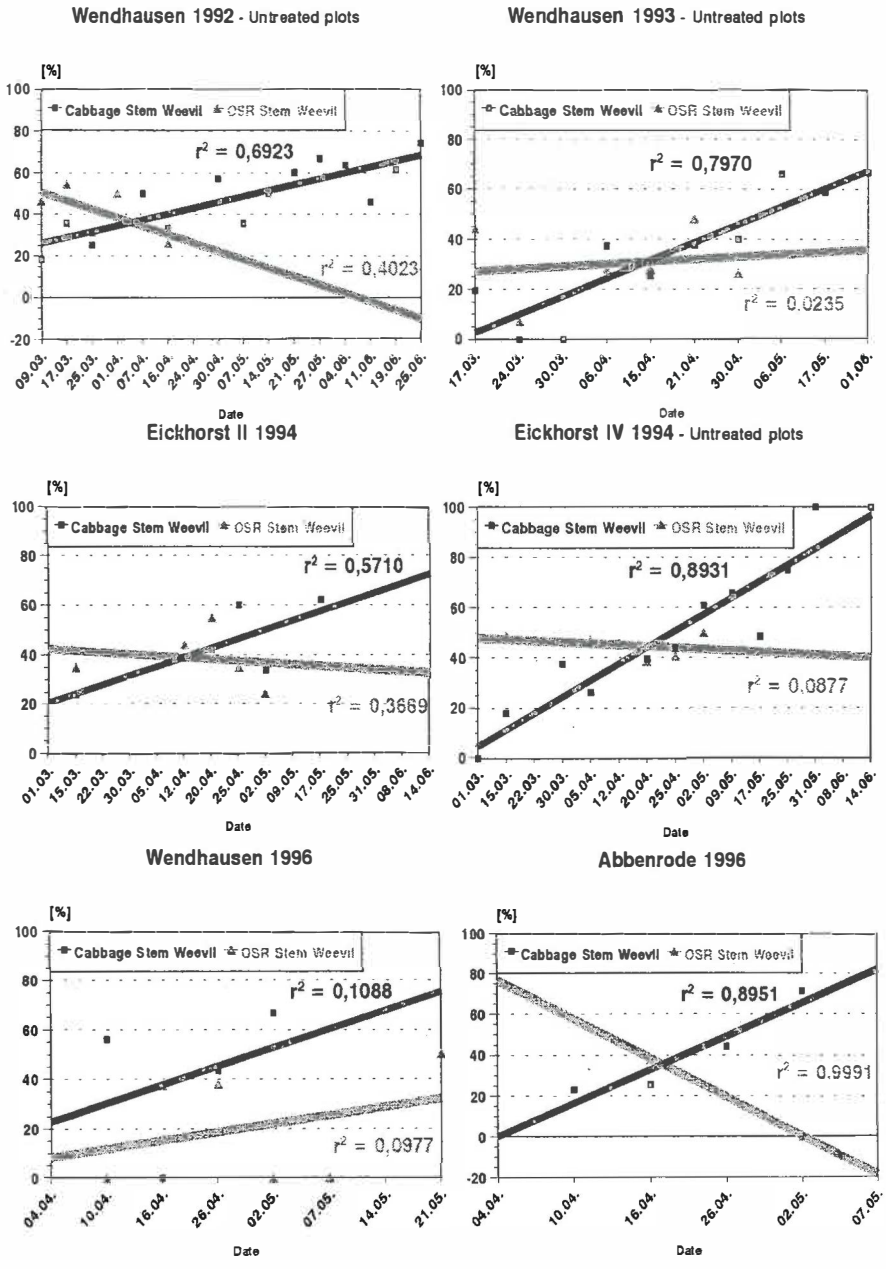

Figure 6a-f: Development of the percentage of females of the Cabbage Stem Weevil (*C. pallidactylus*; black line) and the Oil Seed Rape Stem Weevil (*C. napi*; grey line) in yellow trap samples during the immigration and infestation period. The percentage of females is represented as regression line for each species.

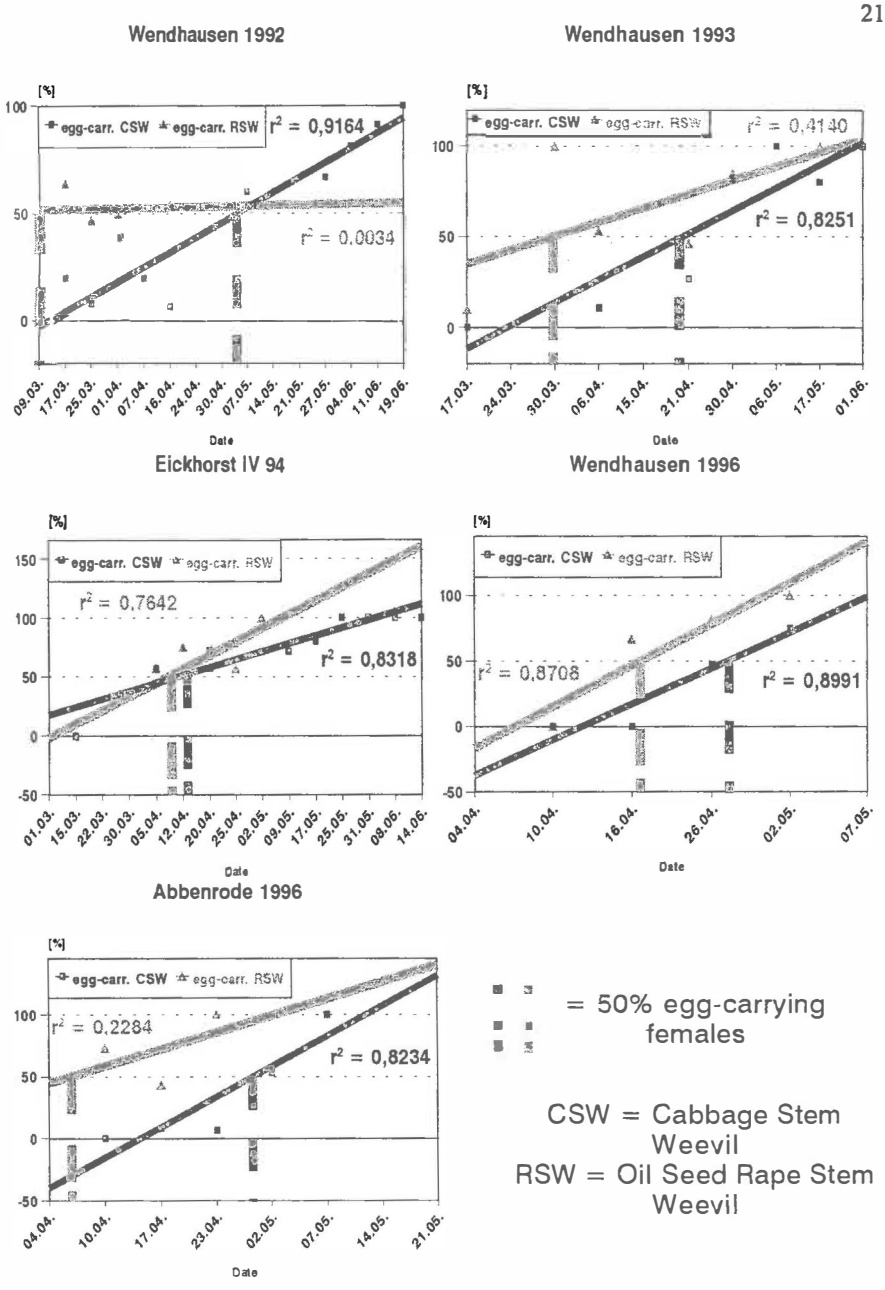

Figure 7a-e: Development of the percentage of egg-carrying females of the Cabbage Stem Weevil (CSW = *C. pallidactylus*; black line) and the Oil Seed Rape Stem Weevil (RSW = *C. napi*; grey line) in yellow trap samples during the infestation period. The percentage of egg-carrying females is represented as regression line for each species. The time when 50% of females are carrying eggs is pointed out by a dashed line.

Similar relations can be observed for the percentage of egg-carrying females (fig. 7a-e): The time when 50% of the female weevils are carrying eggs is always reached recognizably earlier by *C. napi* in comparison to *C. pallidactylus*: For *C. napi* this is recorded on 9 to 11 days after the first peak in the yellow traps, but for *C. pallidactylus* only after 28,5 days on average. For *C. napi* however this period (reaching a percentage of 50% egg-carrying females) varies greatly from year to year, as it can be seen in fig. 7a-e.

Because of these different aspects regarding the biology of both species, it can be assumed that the infestation of the plants, which is relevant for damage of economical importance, starts considerably earlier for *C. napi* than *C. pallidactylus*. In addition the damage of *C. napi* is estimated as much more worse than that of *C. pallidactylus* (Günthart 1949, Oppermann 1990).

The following conclusions relevant for practical oil seed rape growing in Germany can be drawn:

1. The usual practice to combine the two weevil species *C. napi* and *C. pallidactylus* in one damage threshold (10 weevils/trap in 3 days) is common in Germany, but with respect to their different biology (see above) it is not a way to a more sustainable oil seed rape production with a reduced input of insecticides. Therefore, the opportunities for farmers in exercising the determination of the relevant *Ceutorhynchus*-species should be enhanced by the plant protection services and offered frequently during the winter.

2. According to the present status of knowledge, there is a greater variability in several biological and ecological aspects for *C. napi* in comparison to *C. pallidactylus.* In regions, where *C. napi* occurs more abundantly (that is in Germany mainly the southern part), an insecticide application immediately after exceeding the damage threshold is still required. In regions however with predominance of *C. pallidactylus* (that is in Germany, more or less the northern parts and especially the lowland areas) an application of a pyrethroid is necessary only at the time, when an increase of the abundance and of the „motion activity" within the oil seed rape field is recorded by the emergence traps. That means that in these regions the farmers are able to wait before applying pyrethroid for at least two or three weeks after the first major flight activity of *C. pallidactylus* recorded by yellow traps, even if the action threshold is exceeded several times.

As was demonstrated, the safest and most exact way to determine the best time for a most effective insecticide application against the Stem Weevils is to record their abundance and „motion activity" within the oil seed rape stands by emergence traps. However, this is a very laborious way because a sufficient number of traps has to be installed in the fields and has to be controlled and shifted to another location each week. Because of such reasons emergence traps as a method for monitoring of stem weevils might be only suitable in some reference fields for the plant protection services or in research activities, but not for the commercial grower.

A comparatively easy alternative method which nearly everybody is able to learn is the control of a random sample of (about) 10 weevils per yellow trap for the occurrence of egg-carrying females by crushing their abdomen and looking for the presence of eggs. According to preliminary experiences at the region of Braunschweig, we would recommend an insecticide application if more than 20% of the random sample consists of egg-carrying female weevils. However, only if egg-carrying females occur in considerable percentages is an application of an insecticide appropriate. Because this is valid for the Cabbage Stem Weevil

(*C. pallidactylus*) as well as for the Oil Seed Rape Stem Weevil (*C. napi*) farmers could be able to monitor these pest insects without the determination of the *Ceutorhynchus*-species which is technically difficult for anyone who is not familiar with the identification of insects. Nevertheless the validity of this preliminary threshold has to be improved by further experiments and seperately determined for each oil seed rape growing region in Europe.

In total, the aspects demonstrated showed that we are able by exploitation of our knowledge of the population dynamics and the biology and the ecology of pest insects of the species level, to save up to two insecticide applications against Stem Weevils. Including such phenomena as the above mentioned „repellent effect", in a lot of cases the complete spectrum of oil seed rape insect pest which occur in the spring could be controlled sufficiently by only one optimally scheduled pyrethroid application.

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References

Broschewitz, B. 1985: Untersuchungen zur Biologie und Schadwirkung des Gefleckten Kohltriebrüsslers (*Ceutorhynchus quadridens* PANZER) an Winterraps (*Brassica napus* L. var. *oleifera* METZG.). PhD-Thesis, Wilhelm-Pieck-University Rostock, 149 pp.

Broschewitz, B.; Steinbach, P.; Goltermann, St. 1993: Einfluß stengelbewohnender tierischer Schaderreger auf den Befall von Winterraps mit *Phoma lingam* und *Botrytis cinerea*. Gesunde Pflanzen **45 (3),** 106-110.

Büchi, R. 1988: Neue Bekämpfungsschwelle für den Rapsstengelrüssler *Ceutorhynchus napi* Gyll. Mitt. Schweiz. Landwirtsch. **36 (3),** 110-117.

Büchs, W. 1992: Versuche zur Erarbeitung von Grundlagen für die Entwicklung von Schadensschwellen für die Bekämpfung von Rapsschädlingen. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem **283,** 70.

Büchs, W. 1993: Investigations on the occurence of pest insects in oil seed rape as a basis for the development of action thresholds, concepts for prognosis and strategies for the reduction of the input of insecticides. IOBC/WPRS Bulletin **16 (9)** IPM in Oilseed Crops, 216-234.

Büchs, W. 1994: Reduzierter Insektizideinsatz im Winterraps gegen den Gefleckten Kohltriebrüssler (*Ceutorhynchus pallidactylus* [MRSH.]) und andere im Frühjahr auftretende Rapsschädlinge. Rapssymposium zu Fragen der Phytopathologie und des Pflanzenschutzes anläßlich des 65. Geburtstages von Herrn Prof. Dr. habil. Franz Daebeler, 13./14. Okober 1994, Rostock, 115-119.

Büchs, W. 1996: Möglichkeiten des reduzierten Insektizideinsatzes gegen den Gefleckten Kohltriebrüssler (*Ceutorhynchus pallidactylus* [MRSH.]) und den Großen Rapsstengelrüssler (*Ceutorhynchus napi* GYLL.) im Winterraps. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem **321,** 175.

Büchs, W. 1997a: Gefleckten Kohltriebrüßler und Großen Rapsstengelrüßler gezielt bekämpfen. Raps **15 (1)**, 47-49.

Büchs, W. 1997b: Tierische Schädlinge und ihre Antagonisten in Rapskulturen - Arbeiten zu Biologie, Epidemiologie, natürlicher Regulation und chemischer Bekämpfung in der 100-jährigen Geschichte der Biologischen Bundeanstalt für Land- und Forstwirtschaft. Mitteilungen aus der Biologischen Bundeanstalt für Land- und Forstwirtschaft Berlin-Dahlem (in press).

Günthart, E. 1949: Beiträge zur Lebensweise und Bekämpfung von *Ceuthorrhynchus quadridens* Panz. und *Ceuthorryhnchus napi* Gyll. Mitt. Schweiz. Ent. Ges. **22**, 441-591.

Keim, M.; Waiczak, B. 1997: The relationship between the stem weevil (*Ceutorhynchus quadridens* Panz.) injury and losses of flower buds. IOBC/WPRS Bulletin (this issue).

Lauenstein, G. (1992): Schwellenwerte für die Bekämpfung von Rapsschädlingen. Raps **10 (1)**, 28-31.

Lerin, J. 1995: Assessment of yield losses caused by insects in winter oilseed rape, a critical review. in: Paul, V. H.; Gladders, P.; I. H. Williams eds.: Integrated control in oil seed crops. IOBC/WPRS Bulletin **18 (4)**, 95-101.

Oppermann, Ch. 1990: Untersuchungen zum Auftreten des Rapsstengelrüßlers (*Ceutorhynchus napi* Gyll.) an Winterraps (*Brassica napus* var. *oleifera* Metzg.) und Möglichkeiten der Überwachung und Bekämpfung. PhD-Thesis, University of Rostock, 100 pp.

Ulber, B. 1994: Wirkung des kombinierten Befalls von Winterraps mit dem Großen Rapsstengelrüßler (*Ceutorhynchus napi* Gyll.) und dem Gefleckten Kohltriebrüßler (*Ceutorhynchus pallidactylus* (Mrsh.)) auf den *Phoma lingam*-Befall und Ertrag. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft **301**, 96.

Zimmermann, J. 1995: Biologisch-Ökologische Untersuchungen an Kurzflügelkäfern (Coleoptera: Staphylinidae) einer abgestuft intensiv bewirtschafteten Agrarfläche unter Berücksichtigung methodischer und ökotoxikologischer Aspekte. PhD-Thesis TU Berlin, 199 pp.

Zimmermann, J.; Büchs, W. 1993: Verzögerte Einflüsse von Pflanzenschutzmitteln auf die Kurzflügelkäfer (Coleoptera: Staphylinidae) eines Zuckerrübenfeldes unter besonderer Berücksichtigung eines Bodeninsektizides. Verhandlungen der Gesellschaft für Ökologie **22**, 183-190.

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Effect of different nitrogen fertilization and absence of pest control on health status of rapeseed

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Abstract In the period 1993-1995 rape health status was studied in trials on which intensive chemical treatments were performed in comparison with those without pest control. Different nitrogen fertilisation: 40, 80, 120, 160, and 200 kg N/ha and two varieties: 'Mar' and 'Ceres" were additional factors. the most severe pathogens were evaluated. These were: *Phoma lingam, Botrytis cinerea, Sclerotinia sclerotiorum, Alternaria* spp., and *Erysiphe* sp. Per cent of *prematurely ripened* plants was also assessed. The absence of insecticide use significantly affected the rate of rape infestation with *Botrytis cinerea, Phoma lingam, Alternaria* spp., and *Sclerotinia sclerotiorum.* Moreover, the occurrence of *premature ripening* was found to be higher. However, there was no effect on the infestation of *Erysiphe* sp. Different nitrogen doses did not affect the presence of *Sclerotinia sclerotiorum, Phoma lingam or Alternaria* spp. Infection caused by *Erysiphe* sp. increased with higher amounts of nitrogen, while a reverse tendency was found in relation to *premature ripening.* No significant infection differences werenotedbetween the varieties studied.

Keywords: control, pesticide removal, fertoilisation, fungus, nitrogen, oilseed rape

Introduction

During the period 1993-1995 the Department of Plant and Soil Cultivation of the Poznañ Agricultural University carried out investigations to determine possibilities for cultivation of winter oilseed rape without chemical pest control. It have been suggested that higher infestation of the pests and plant injuries caused by it can result in higher number of fungal pathogens (Newman 1984, Schultz and Daebeler 1984). Therefore, health status of plants cultivated under such conditions was the objective of this study. Health status of rape on which the pest control treatments were carried out was compared with that of rape from objects where chemical pest treatments were completely removed. Moreover, assessment of the effect of differentiated nitrogen fertilisation on pathogen occurrence was also investigated.

Methods

Evaluation of health status was carried out on the experimental field of the Przybroda AES. The experiment was established in four replications in a "split plot" design and it included three factors: 'Mar' and 'Ceres' varieties, differentiated nitrogen fertilisation (40, 80, 120, 160

and 200 kg N/ha), and alternatively: intensive pest control or absence of control.

Pathogens occurring at the highest levels were taken into consideration. Infection of stems and siliques with fungal pathogens was evaluated about two - three weeks before seed harvest. Each time health status of fifty randomly chosen plants from every plot was analysed. Infection caused by *Botrytis cinerea* Pers., *Phoma lingam* (Tode) Desm., *Sclerotinia sclerotiorum* (Lib.) de Bary was estimated as per cent of infected stems, that caused by *Alternaria* spp. in percent of infected siliques, while the *Erysiphe* sp. infection in degrees of infection (0 - 5°) and *premature ripening* as per cent of infected plants. Symptome caused by *Erysiphe* sp. were observed only in 1993 and 1994, whereas those of *Alternaria* spp. in 1994 and 1995. The part of the results presented here deal only with the 'Mar' variety.

Results and Discussion

Lack of insecticide application caused an increase of the occurrence of the majority of the pathogens observed, both pests and diseases. The detailed results are given in Fig. 1 - 6. In every year of the experiment removal of pest control significantly increased the infestation of *Botrytis cinerea*, *Phoma lingam*, *Alternaria* spp., *Sclerotinia sclerotiorum* and *premature ripening*, regardless of nitrogen fertilisation level. However, no differences in the occurrence of *Erysiphe* sp. were noted despite it being observed in two years at a high level (Fig. 4). The presence of *Alternaria* spp. varied in different years. In 1993 only traces of the pathogen were found, while a severe infection of siliques was noted in 1994. On the trials with an intensive pest control on average 12.3% siliques demonstrated disease symptoms, whereas on those without the level was 30.4%. In 1995 the figures were 2.9% and 7.4%, respectively (Fig. 5). The results have clearly indicated the existence of a positive correlation between infestation by pests and health status of rape in the case of the majority of diseases, regardless of nitrogen fertilisation applied. Similar results were reported by Garbe (1995), Tobola et al. (1994), Giamoustaris and Mithen (1995), Sadowski and Budzyñski (1995).

Different nitrogen fertilisation did not significantly affect the occurrence of *Sclerotinia sclerotiorum*, *Phoma lingam* and *Alternaria* spp. in all years of observations. However, an effect of the fertilisation rate on infestation of powdery mildew (*Erysiphe* sp.) was noted. Its lowest infection was found at fertilisation with 40 kg N/ha and it was increasing with higher nitrogen amounts. Significant differences were noted in 1994 and 1995 in the case of *premature ripening* of plants where a reverse tendency was observed - less severe symptoms were found at the level 200 kg N/ha (Fig. 6). A considerably higher occurrence of *Botrytis cinerea* was noted only at the level 200 kg N/ha, while the infection observed at other levels of nitrogen fertilisation was similar.

Relations similar to these reported here for *Phoma lingam* and *Alternaria* spp. were also noted by Przylêcka et al. (1995). Different conclusions about effects of nitrogen fertilisation on infestation of diseases are present in literature. Total rainfall is a crucial factor (Barszczak et al. 1994). The same observations and conclusions relating to removal of pest control and nitrogen fertilisation were made for the 'Ceres' variety.

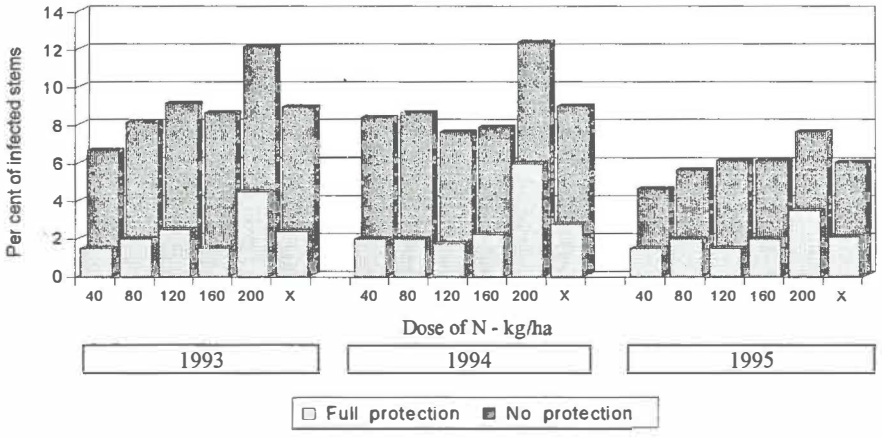

Figure 1. Effect of removal of pest control on the occurrence of *Botrytis cinerea* (Przybroda AES, 1993 - 1995).

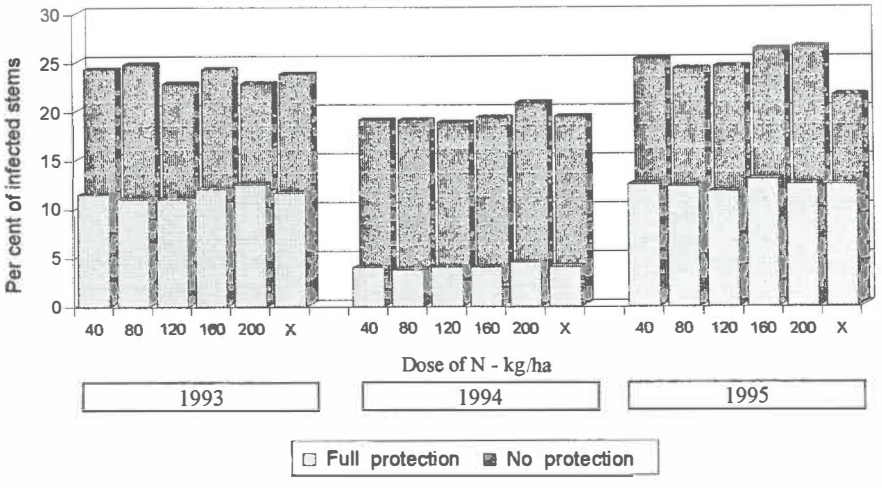

Figure 2. Effect of removal of pest control on the occurrence of *Phoma lingam* (Przybroda AES, 1993 - 1995).

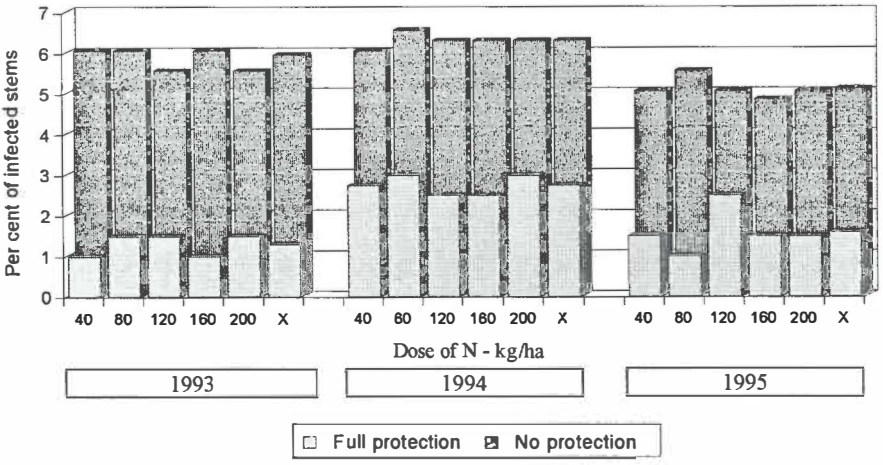

Figure 3. Effect of removal of pest control on the occurrence of *Sclerotinia sclerotiorum* (Przybroda AES, 1993 - 1995).

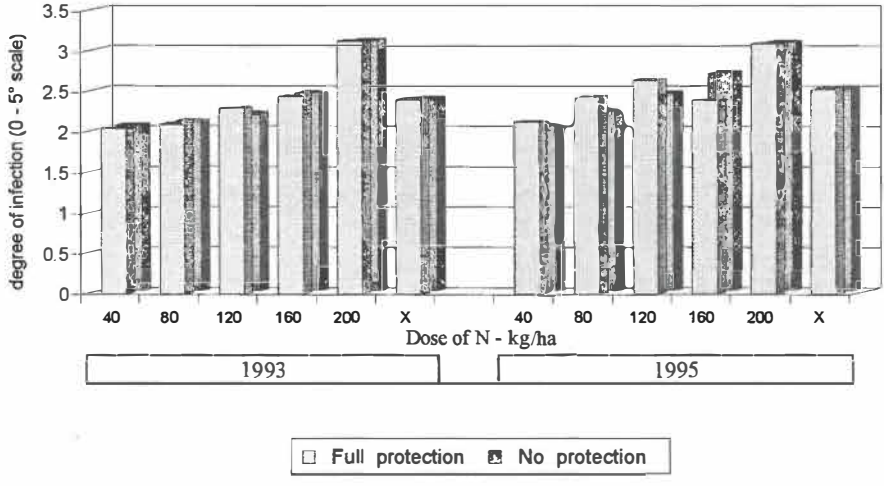

Figure 4. Effect of removal of pest control on the occurence of *Erysiphe* sp. (Przybroda AES, 1993, 1995).

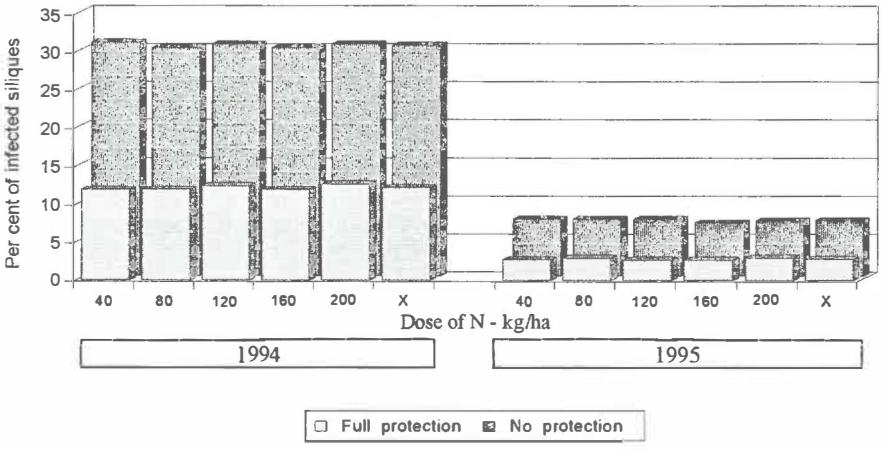

Figure 5. Effect of removal of pest control on the occurence of *Alternaria* spp. (Przybroda AES, 1994, 1995).

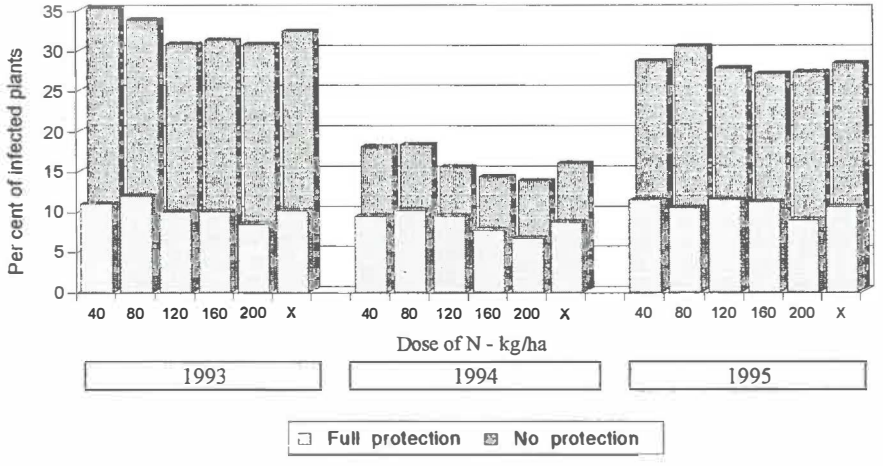

Figure 6. Effect of removal of pest control on the occurrence of *premature ripening* of plants (Przybroda AES, 1993 - 1995).

References

Barszczak, Z.; Barszczak, T.; Kasjanowicz, W. 1994: Effect of periodical drought and nitrogen rates on seed yields and chemical composition of winter oilseed rape cultivars. Rosliny Oleiste-Oilseed Crops XV, 2: 9-16.

Garbe, V. 1995: Influence of insect damage on the infestation degree of blackleg. Proc. of 9th Intern. Rapeseed Congress, Cambridge, UK, Vol. 2: 667-669.

Giamoustaris, A.; Mithen, R. 1995: Modifying leaf glucosinolates in oilseed rape and its effects upon pest and pathogen interactions. Proc. of 9th Intern. Rapeseed Congress, Cambridge, UK, Vol.4: 1220-1222.

Newman, P.L. 1984: The effects of insect larval damage upon the incidence of cancer in winter rape. Proc. Brit. Crop Prot. 2: 815-822.

Przylêcka, E.; Walkowski, T.; Wnêkowski, S.; Grala, B. 1995: The incidence of fungal diseases on Polish oilseed rape cultivars as related to the level of nitrate fertilization. Workshop on *Brassica napus*. Helsinger, Denmark. Book of Abstracts 1: 6.

Sadowski, Cz.; Budzyñski, W. 1995: Health status of winter oilseed rape at desistance from pest control in Olsztyn region. Rosliny Oleiste-Oilseed Crops XVI 2: 229-234.

Schultz, R.; Daebeler, F. 1984: Zum Schaden durch den Rapserdfloh (*Psylliodes chrysocephala* L.) insbesondere seiner Imagines. Nachrichtenbl. Pflanzenschutz DDR 3: 113-115.

Tobola, P.; Musnicki, Cz.; Budzyñski, W.; Malarz, W. 1994: Conseqences of desistance from pest control in oilseed rape as depends on nitrogen fertilization intensity. Rosliny Oleiste-Oilseed Crops XV 2: 41-48.

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Health status of winter oilseed rape as affected by nitrogen fertilization and the intensity of protection against pests

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Abstract Infestation of pathogens on winter oilseed rape was studied at different levels of nitrogen fertilisation and pest control. Nitrogen rates were: 0, 60, 120, and 180 kg N/ha. Four variants of pest control were used: A - intensive control, B - treatment for *Meligethes aeneus* and silique pests, C - stem and silique pests control, D - silique pests control. During two years of observations the following pests and deseases were most common: *Phoma lingam*, *Botrytis cinerea, Alternaria* sp., *Sclerotinia sclerotiorum*. It was found that intensity of pest control affected the occurrence of fungal diseases. Plants from the "A" combination plots were less infected then those from the "C" combination.

Keywords: control, diseases, fertilization, fungus, oilseed rape, partial control, pests

Introduction

Earlier studies on the possibilities of rape cultivation without pest control gave negative results. In Poland winter rape is attacked by numerous pests, especially in spring. Therefore, a total reduction of pest control makes rape cultivation unprofitable (Budzyński et al. 1994, Musnicki et al. 1995). Moreover, the plants from objects without chemical treatments are infested with fungal pathogens to a higher degree (Sadowski et Budzyński 1995). On the other hand application of numerous sprayings is cost-consuming and has a negative effect on the environment. A system to determine possibilities for eliminating some treatments against pests with a simultaneous application of different nitrogen fertilisation levels was developed at the Department of Plant Production of the Olsztyn University of Agriculture and Technology. The occurrence of pests causes plant injuries, therefore an attempt was made to determine plant health status in such an experiment.

Methods

Health status of plants on which 4 variants of pest control were used was analysed.

Variant	Pest controlled		
	stem pests	*Meligethes aeneus*	silique pests
A	x*	x	x
B	-	x	x
C	x	-	x
D	-	-	x

*one or several treatments

A full protection, a -control of stem pests (*Ceutorhynchus napi, C. Quaridens*), b - control of *Meligethes aeneus*, c - control of silique pests (*C. Assimilis, Dasineura brassicae, Brevicoryne brassicae*),

B control of *Meligethes aeneus* (b) and silique pests (c),

C control of stem pests (a) and silique pests (c),

D only control of silique pests (c).

There were four nitrogen fertilisation levels in every variant: 0, 60, 120, and 180 kg N/ha and four replications. Health status was evaluated on fifty randomly chosen plants from each plot. Pathogens of highest infestation rate were evaluated. The occurrence of *Phoma lingam, Botrytis cinerea* and *Sclerotinia sclerotiorum* was evaluated as per cent of infected plants, while that of *Alternaria* spp. was estinated in degrees of infection (0-5) evaluating separately infection of leaves, shoots and siliques. The results were analysed statistically using analysis of variance and Tukey's test.

Results and Discussion

Despite the variable occurrence of fungal pathogens in the experimental years sampled, an effect of the factors studied can be noticed, especially that of intensity of pest treatments. Control of silique pests alone (D) and removal of treatments for stem pests and *Meligethes aeneus* (B and C) in both years and on both varieties increased the infestation of *Phoma lingam*. In 1996 lack of the treatment against *Meligethes arneus* (C) on the 'Leo' variety did not increase this pathogen in comparison with the plants with full control (A), but it was still higher on the plots without treatments against stem pests (B) (Tab.1). Similar relations were found in case of infestation of *Sclerotinia sclerotiorum* in 1995 (Tab. 2).

Table 1. Effect on the occurrence of *Phoma lingam*

Variety	Dose of N (kg/ha)	Per cent of infected stems									
		1995					1996				
		A	B	C	D	mean	A	B	C	D	mean
Lirajet	0	7.0	6.3	6.5 ab	17.3 c	9.3 b	10.0	11.0	10.8	13.0	11.2
	60	7.5 a*	6.3 a	7.0 ab a	12.0 b b	8.4 b	9.8	10.5	10.3	12.0	10.7
	120	6.0 a	7.0 ab	8.8 b bc	10.8 ab c	8.2 b	10.5	9.5	10.5	12.0	10.6
	180	6.8 ab	5.3 a	5.3 a a	8.5 a b	6.5 a	8.3	11.8	9.8	12.0	10.5
	mean	6.8 a	6.2 a	6.9 a	12.2 b	-	9.7 a	10.7 bc	10.4 ab	12.3 c	-
Leo	0	9.0	10.0	11.0	11.8	10.5 b	9.5	12.3	10.0	14.8	11.7
	60	8.5	8.8	10.5	12.8	10.2 ab	9.5	10.3	9.8	12.0	10.4
	120	7.8	9.0	10.0	11.8	9.7 ab	9.0	10.3	9.0	12.8	10.3
	180	6.0	6.8	11.0	9.5	8.3 a	9.3	11.3	9.3	11.5	10.4
	mean	7.8 a	8.7 ab	10.6 bc	11.5 c	-	9.4 a	11.1 b	9.5 a	12.7 c	-

* - values in the same line and column followed by different letters are significantly different at $p<0.05$

The method of rape protection against pests resulted in clear differences in the occurrence of *Botrytis cinerea* (Table 3). Limiting pest control in every case caused higher infestation of grey mould. The smallest number of symptoms was noted at full control (A), while the highest one in combination without treatments against stem pests and *Meligethes aeneus* (D). However, it should be pointed out that in combinations B and C the occurrence of pathogen was higher than in combination A with full control. Only in case of 'Leo" in 1995 infection of the plants in combinations A and C was similar.

Higher occurrence of *Alternaria* spp. was noted only in 1996. Infection of shoots of both varieties in combinations A, B, and C was similar and statistically more significant than for combination D. Infection of siliques was almost the same for all combinations studied (Table 4). Different methods of pest control had also a significant effect on *premature ripening* (Tab. 5). Those plants were more numerous in a combination with control of stem pests (D).

Table 2. Effect on the occurrence of *Sclerotinia sclerotiorum*

Variety	Dose of N (kg/ha)	Per cent of infected stems									
		1995					1996				
		A	B	C	D	mean	A	B	C	D	mean
Lirajet	0	1.8	4.0	2.5	4.3	3.2	5.0	5.5	5.8	6.0	5.6
	60	1.8	3.3	3.0	5.8	3.5	4.8	5.3	4.8	4.5	4.9
	120	1.8	4.5	3.8	5.8	4.0	3.5	4.5	4.5	3.5	4.0
	180	2.3	6.0	3.3	4.0	3.9	5.3	4.8	4.3	4.3	4.7
	mean	1.9 **a***	4.5 **b**	3.2 **b**	5.0 **b**	-	4.7	5.0	4.9	4.6	-
Leo	0	1.8	3.0	1.8	2.5	2.0	5.5	4.5	5.0	5.3	5.1
	60	2.0	3.5	1.5	3.8	2.7	5.0	5.8	5.8	5.0	5.4
	120	1.3	4.0	2.0	3.8	2.8	3.8	5.8	5.5	4.3	4.9
	180	1.3	4.3	1.8	2.5	2.5	5.0	4.8	4.8	6.0	5.2
	mean	1.6 **a**	3.7 **b**	1.8 **a**	3.2 **b**	-	4.8	5.2	5.3	5.2	-

* - values in the same line followed by different letters are significantly different at $p<0.05$

Different N amounts had an effect on the occurrence of diseases. Infestation of *Phoma lingam* in one year of the experiments was the lowest at the highest nitrogen rate (Tab. 1), while reaction of *Botrytis cinerea* was reverse. The occurrence of this pathogen in 1996 increased with higher nitrogen amounts, and the differences were statistically significant for the rate 180 kg N/ha for the 'Leo' variety and for the rates 120 and 180 kgN/ha for 'Lirajet' (Tab. 3). No effect of nitrogen fertilisation was found on the infestation of *Sclerotinia sclerotiorum*.

In an integrated plant protection system there is a need for limiting chemical treatments. Control of pests is an important element of modern agriculture. There is a question to what extent we can limit application of insecticides and the effects this produces on yield of rape. According to Musnicki et al. (1995) a total removal of insecticide use causes a substantial drop of the yield, and also weakens rape reaction to nitrogen fertilisation. Sadowski et Budzyński (1995) have suggested that such a measure increases infestation of fungal pathogens. Therefore, if total reduction causes such unequivosal negative consequences in the yield and plant health status there arises an interest in what results may appear after a partial reduction of some treatments. Our results varied. It was found that the occurrence of *Phoma*

lingam is strongly linked with the control of stem pests and the effects of rape injuries caused by pests on the infestation of this pathogen were reported by Weber et al. (1992), Steinbach (1995) and Garbe (1995). Also infestation of other fungal pathogens was related to pest control. Because of their less intense infestation it is difficult to draw definite conclusions concerning what treatments are necessary, and what are not. Preliminary, two-year-long observations proved that under some conditions reduction of insecticide use did not increase the occurrence of diseases at some dates. It has indicated a possibility of limiting number of sprays against pests without risking higher infestation of fungal pathogens on plants. However, more detailed studies would be necessary to get more information.

Table 3. Effect on the occurrence of *Botrytis cinerea*

Variety	Dose of N (kg/ha)	Per cent of infected stems									
		1995					1996				
		A	B	C	D	mean	A	B	C	D	mean
Lirajet	0	2.3 a*	4.5 ab	3.3 a	5.8 b	4.0	4.5	6.0	5.0	7.5	5.8 a
	60	1.5 a	6.0 b	3.0 a	4.8 ab	3.8	3.8	5.8	5.5	7.8	5.7 a
	120	2.0 a	6.8 b	2.8 a	5.3 b	4.2	5.5	7.8	6.5	9.8	7.4 b
	180	2.0 a	7.3 b	2.0 a	6.0 b	4.3	6.0	9.0	7.3	12.5	8.7 b
	mean	2.0 a	6.2 c	2.8 b	5.8 c	-	5.0 a	7.2 b	6.1 b	9.4 c	-
Leo	0	2.0	4.5	1.5	6.3	3.6	4.0 a	7.0 bc	5.0 ab	8.0 a c	6.0 a
	60	2.5	4.3	2.5	7.5	4.2	4.3 a	7.0 b	5.0 a a	8.3 a b	6.2 a
	120	2.0	4.0	2.0	5.5	3.4	5.5 a	7.0 a	5.5 a a	9.3 ab b	6.8 a
	180	2.0	4.8	1.8	5.5	3.5	5.5 a	6.5 a	9.0 b	11.8 b c	8.2 b
	mean	2.1 a	4.4 b	2.0 a	6.2 c	-	4.8 a	6.9 b	6.1 b	9.3 c	-

* - values in the same line and column followed by different letters are significantly different at $p<0.05$

Table 4. Effect on the occurrence of *Alternaria* spp. (Balcyny, 1996)

Variety	Dose of N (kg/ha)	Degree of infection (0 - 5°)									
		Stems					Siliques				
		A	B	C	D	mean	A	B	C	D	mean
Lirajet	0	0.6 a*	1.1 a	1.0 a	1.6 b	1.1	0.7	0.8	0.8	1.0	0.8
	60	0.7 a	1.0 a	0.9 a	1.7 b	1.0	0.7	0.7	0.8	0.9	0.8
	120	0.6 a	1.0 a	0.9 a	1.6 b	1.0	0.8	0.9	0.9	1.1	0.9
	180	0.6 a	1.1 a	1.0 a	1.7 b	1.1	0.7	0.8	0.8	1.0	0.8
	mean	0.6 a	1.1 a	1.0 a	1.7 b	-	0.7	0.8	0.8	1.0	-
Leo	0	0.6 a	1.0 a	1.0 a	1.7 b	1.1	0.6	0.8	0.9	0.9	0.8
	60	0.6 a	1.0 a	1.1 a	1.8 b	1.1	0.7	0.9	0.8	1.1	0.9
	120	0.7 a	1.1 a	1.0 a	1.7 b	1.1	0.8	0.9	0.9	1.0	0.9
	180	0.7 a	1.1 a	1.1 a	1.7 b	1.2	0.6	0.8	0.9	1.1	0.9
	mean	0.7 a	1.1 a	1.1 a	1.7 b	-	0.7	0.9	0.9	1.0	-

* - values in the same line followed by different letters are significantly different at $p<0.05$

Table 5. Effect on the occurrence of *premature ripening*

Variety	Dose of N (kg/ha)	Per cent of infected plants									
		1995					1996				
		A	B	C	D	mean	A	B	C	D	mean
Lirajet	0	6.3	12.0	7.0	19.8	11.3 b*	7.8	9.0	11.0	14.8	10.7 b
	60	6.0	10.8	8.0	19.0	11.0 ab	5.4	6.5	9.3	13.5	8.7 a
	120	6.5	10.5	9.8	17.8	11.2 b	5.8	6.8	9.0	12.5	8.5 a

	180	4.8	8.8	6.5	14.3	8.6 a	3.8	7.3	8.3	10.3	7.4 a
	mean	5.9 a	10.5 b	7.8 a	17.7 c	-	5.7 a	7.4 b	9.4 c	12.8 d	-
Leo	0	8.0	12.5	8.5	19.5	12.1	7.5	10.0	11.0	14.3	10.7 c
	60	8.5	12.5	7.5	19.5	12.0	5.0	6.3	9.0	12.8	8.3 b
	120	7.3	11.8	6.8	19.5	11.4	6.8	7.3	9.5	11.8	8.8 b
	180	7.5	14.5	10.3	19.5	13.0	3.5	4.3	7.5	9.0	6.1 a
	mean	7.8 a	12.8 b	1.8 a	19.5 c	-	5.7 a	7.0 b	9.3 c	12.0 d	-

* - values in the same line and column followed by different letters are significantly different at $p<0.05$

References

Budzyñski ,W.; Musnicki, Cz.; Kotecki, A.; Jankowski, K. 1994: Nitrogen productivity in rape protected or not protected against insects. Rosliny Oleiste-Oilseed Crops XV, 2:35-40.

Garbe, V. 1995: Influence of insect damage on the infestation degree of blackleg. Proc. of 9th.Intern. Rapeseed Congress, Cambridge, UK, Vol. 2: 667-669.

Musnicki, Cz., Tobola P., Musnicka B., 1995: Quality of harvesed oilseed rape seeds depending on protection intensity against pests. Rosliny Oleiste-Oilseed Crops XVI,2:209-216.

Sadowski, Cz.; Budzyñski, W. 1995: Health status of winter oilseed rape at desistance from pest control in Olsztyn region.. Rosliny Oleiste-Oilseed CropsXVI, 2: 229-234.

Steinbach, P. 1995: "Early wilting" of winter oilseed rape caused by abiotic factors and secondary pathogens in the spring of 1994 in Northern Germany, 9th. Inter. Rapeseed Congress Cambridge, UK, 4-7 July, Vol. 2: 592-594.

Tobola, P.; Musnicki, Cz.; Budzyñski, W.; Malarz, W.£. 1994: Conseqences of desistance from pest control in oilseed rape as depends on nitrogen fertilization intensity. Rosliny Oleiste-Oilseed Crops XV,2: 41-48.

Weber, Z.; Karolewski, Z.; Zygmunt, B. 1992: Wystêpowanie chorób na rzepaku ozimym w rejonie Poznania. Ochrona Roslin, 5: 11-12.

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Molecular diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape.

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Abstract Degenerate polymerase chain reaction (PCR) primers, designed to amplify a region of the SFI1 (Sex Factor-Induced 1) gene from the oilseed rape fungal pathogen, *Pyrenopeziza brassicae*, were assessed to determine their suitability for use in specific detection of the pathogen. Use of the SFI1 primers in PCR facilitated the amplification of 700 and 550 base pair (bp) products from *P. brassicae* template DNA. Amplification of *Botrytis cinerea* DNA also generated a faint 700 bp product but no amplification products were generated from DNA extracted from *Brassica napus* or from the phytopathogenic fungi *Leptosphaeria maculans*, *Alternaria brassicae* and *Sclerotinia sclerotiorum*. These preliminary results suggest that the SFI1 primers can be used as a molecular diagnostic tool to identify *P. brassicae* in pure culture.

Keywords: light leaf spot, molecular diagnosis, polymerase chain reaction, *Pyrenopeziza brassicae*.

Introduction

The discomycete fungus *Pyrenopeziza brassicae* Sutton and Rawlinson (anamorph *Cylindrosporium concentricum*) is the causal agent of light leaf spot disease of brassicas. In particular, light leaf spot is considered to be one of the most damaging diseases of winter oilseed rape *Brassica napus* L. subsp. *oleifera* in the UK (Hardwick *et al.*, 1991), causing estimated losses of >£30M per annum (Sansford *et al.*, 1996). Evidence has shown that to control light leaf spot efficiently, fungicide treatment should be first applied to crops during winter (November/ December) when infection first occurs (Fitt *et al.*, 1997). However, due to the latent period between initial infection by *P. brassicae* during autumn and subsequent appearance of disease symptoms in spring, it is often difficult to assess the extent of initial infection. As a result, the amounts of fungicide applied to crops do not always relate well to the actual disease present (Fitt *et al.*, 1996). To enable early detection of light leaf spot infections in the field, work has begun on the development of a molecular diagnostic technique, based upon the polymerase chain reaction (PCR). This paper describes the preliminary stages in the development of this technique.

Methods

Origin and culture of fungal isolates and plant material

The isolates of *P. brassicae* and other fungi used in this study are given in Table 1.

Isolate	Fungus	Origin	Source
JH26	Pyrenopeziza brassicae	Derbyshire, UK	A. M. Ashby
PB23	Pyrenopeziza brassicae	Suffolk, UK	A. M. Ashby
NH10	Pyrenopeziza brassicae	Northern UK	A. M. Ashby
Lm A4	Leptosphaeria maculans	Hertfordshire, UK	B. D. L. Fitt
Lm A7	Leptosphaeria maculans	Hertfordshire, UK	B. D. L. Fitt
Bc B7	*Botrytis cinerea*	Unknown	M. Jarman
Bc B13	*Botrytis cinerea*	Cambridgeshire, UK	M. Jarman
Ab A53	*Alternaria brassicae*	Unknown	Unknown
Ab A55	*Alternaria brassicae*	Canada	J. P. Tewari
Ss S3	*Sclerotinia sclerotiorum*	Hertfordshire, UK	S. E. Mitchell
Ss M24	*Sclerotinia sclerotiorum*	Hertfordshire, UK	S. E. Mitchell

Table 1. Codes and origins of fungal isolates used in this study.

Isolates of *P. brassicae* were routinely maintained on 3% MA [3% malt extract (Oxoid), 1.2% Difco Bacto agar] at 16 ^{o}C in the dark. Isolates of *Leptosphaeria maculans*, *Alternaria brassicae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* were maintained on potato dextrose agar (PDA) under the same incubation conditions. *Pyrenopeziza brassicae* mycelium for DNA extraction was obtained by inoculation of macerated mycelium into 20 ml of potato dextrose broth (PDB), followed by static incubation at 16 ^{o}C for 14 days. DNA from the other fungi was obtained from mycelium peeled from cellophane discs placed on PDA.

Apical meristems of glasshouse grown *B. napus* cv. Shogun were surface sterilised for 15 minutes in 10% hypochlorite, rinsed three times in sterile distilled water and allowed to grow on Murashige and Skoog basal media (Sigma Chemical Co., Dorset, UK) supplemented with 30 g/L sucrose (Murashige & Skoog, 1961). Plant material infected by *P. brassicae* was obtained from a field near Bedford, UK.

DNA extraction

DNA was extracted from plant and fungal material according to the protocol of Raeder and Broda (1985), diluted to 100 ng/ml and stored at –20 ^{o}C prior to use in PCR reactions.

Polymerase chain reaction (PCR)

The degenerate PCR primers used in this study, SFI1:1 [5'-AACGTTCA(A/G)GCICCIGA(T/C)AA(T/C)TC–3'] and SFI1:6 [5'-AACGTTATGGAGTAIAGICGIATGTT–3'], were designed from internal amino-acid sequences obtained from the sex factor-induced (SFI1) protein (Ashby, 1997). This protein is found to be present in extracts taken from single mating-type isolates of *P. brassicae* which have been exposed to a lipoidal sex factor (SF) (Ashby, 1997). PCRs were performed in 50 ml volumes, each reaction volume containing 5 ml 10x PCR buffer [Bioline, London, UK; 160

mM $(NH_4)_2SO_4$, 670 mM Tris-HCl pH 8.8, 0.1% Tween 20], 4.0 mM $MgCl_2$, 200 mM each of dATP, dCTP, dGTP and dTTP, 50 pmol each of primers SFI1:1 and SFI1:6, 1.25 units of *Taq* polymerase (BioTaq, Bioline, London, UK) and 125 ng template DNA. Each reaction was overlaid with 30 ml mineral oil. Thermal cycling was performed using a Techne PHC-3 thermal cycler (Techne Ltd., Cambridge, UK) using cycling parameters of 95 ^{O}C – 5 minutes, 55 ^{O}C – 1 minute, 72 ^{O}C - 2 minutes (1 cycle); 95 ^{O}C – 1 minute, 55 ^{O}C – 1 minute, 72 ^{O}C – 2 minutes (30 cycles) followed by an extended period of 3 minutes at 72 ^{O}C. Amplification products were visualised on 1.2% agarose gels containing 0.1mg/ml ethidium bromide.

Results

Amplification of *P. brassicae* DNA using the SFI1 primers resulted in the production of 700 base-pair (bp) and 550 bp PCR products from isolates JH26 and PB23 (Figure 1, lanes 1 & 2). The 700 bp product has also been obtained using template DNA from several other isolates taken from locations within the UK, although it has not been observed with every isolate tested (e.g. isolate NH10 – Figure 1, lane 3). The 550 bp product, however, has been produced with every *P. brassicae* isolate tested thus far. Further experiments involving reamplification of the 700 bp product have shown that the 550 bp band is produced as a result of primer SFI1:1 annealing to an additional site within the 700 bp product (data not shown).

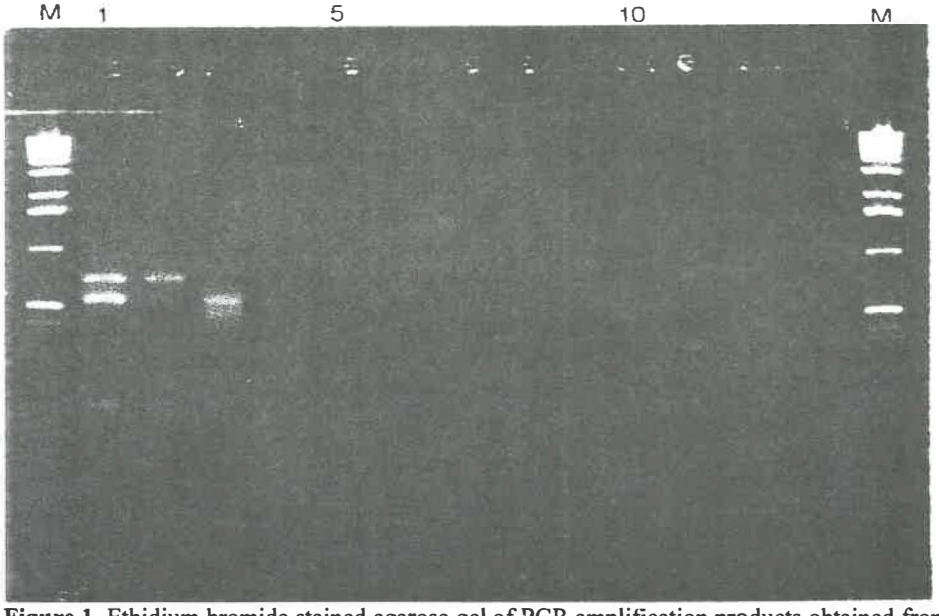

Figure 1. Ethidium bromide stained agarose gel of PCR amplification products obtained from fungal and plant DNA using SFI1 primers. M: size marker (1Kb ladder, Boehringer Mannheim Ltd, East Sussex, UK): lanes 1-3: *Pyrenopeziza brassicae* isolates JH26, PB23, NH10; lanes 4-5: *Leptosphaeria maculans* isolates Lm A4, Lm A7; lanes 6-7: *Alternaria brassicae* isolates Ab A53, Ab A55; lanes 8-9: *Sclerotinia sclerotiorum* isolates Ss S3, Ss M24; lanes 10-11: *Botrytis cinerea* isolates Bc B7, Bc B13; lane 12: *Brassica napus* cv. Shogun; lane 13: *P. brassicae* infected *B. napus*. Arrows indicate positions of 700 and 550 bp amplification products.

Neither of the amplification products obtained from *P. brassicae* DNA were produced upon amplification of DNA from *B. napus* (Figure 1, lane 12) or from isolates of the oilseed rape pathogens *L. maculans*, *A. brassicae* and *S. sclerotiorum* (Figure 1, lanes 4-9). A faint band of approximately 700 bp was produced upon amplification of DNA from *B. cinerea* isolate Bc B13 (Figure 1, lane 11); however, the 550 bp band DNA was not evident. Amplification of DNA extracted from an oilseed rape plant exhibiting light leaf spot symptoms did not result in the production of either the 700 or 550 bp bands observed with *P. brassicae* DNA (Figure 1, lane 13).

Discussion

The polymerase chain reaction has been used successfully as a tool for identification and diagnosis of a number of economically important phytopathogenic fungi such as *Gaeumannomyces graminis* var. *tritici* (Ward & Akrofi, 1994), *Microdochium nivale* (Nicholson *et al.*, 1996) and *Stagonospora nodorum* and *Septoria tritici* (Beck & Ligon, 1995). The preliminary results presented here suggest that the SFI1 primers can be used in PCR to specifically detect *P. brassicae* in pure culture. Use of the primers with DNA from *P. brassicae* result in the generation of two amplification products, which are not produced using DNA from three other fungal pathogens of oilseed rape or from oilseed rape itself. Although one of the bands (700 bp) appears to be produced using template DNA from *B. cinerea*, this band is not as clearly defined as that produced by *P. brassicae* and hence may be produced as a result of the degeneracy of the SFI1 primers. The fact that the 550 bp band specific to *P. brassicae*, which is a truncated version of the 700 bp product, is not produced from *B. cinerea* also suggests that the *B. cinerea* product may not be a true product of the SFI1 primers.

Further work is required to ascertain whether the bands produced using the SFI1 primers are truly specific to *P. brassicae*. The 700 bp band has recently been sequenced and this sequence is currently being used to design primers with complete homology to this product. These primers will then be tested to determine their specificity with respect to *P. brassicae*. It is hoped that these primers will form the basis of a molecular diagnostic technique, which will be able to detect *P. brassicae* in newly infected oilseed rape material. Such a technique will prove invaluable for early diagnosis of light leaf spot infections and, in conjunction with the forecasting model currently under development (Fitt *et al.*, 1996), may enhance the ability to predict and thus control the severity of light leaf spot epidemics.

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References

Ashby, A. M. 1997: A molecular view through the looking glass: the *Pyrenopeziza brassicae-Brassica* interaction. Advances in Botanical Research 24: 31-70.

Beck, J. J.; Ligon, J. M. 1995: Polymerase chain reaction assays for the detection of *Stagonospora nodorum* and *Septoria tritici* in wheat. Phytopathology 85: 319-324.

Fitt, B. D. L.; Gladders, P.; Turner, J. A.; Sutherland, K. G.; Welham, S. J. 1996: Predicting risk of severe light leaf spot (Pyrenopeziza brassicae) on winter oilseed rape in the UK. 1996 Brighton Crop Protection Conference – Pests and Diseases, British Crop Protection Council: 239-244.

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

Hardwick, N. V.; Fitt, B. D. L.; Wale, S. J.; Sweet, J. B. 1991: Oilseed rape diseases. Home Grown Cereals Authority Research Review OS4, London, Home Grown Cereals Authority.

Murashige, T.; Skoog, F. 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Nicholson, P.; Lees, A. K.; Maurin, N.; Parry, D. W.; Rezanoor, H. N. 1996: Development of a PCR assay to identify and quantify *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus* in wheat. Physiological and Molecular Plant Pathology 48: 257-271.

Raeder, U.; Broda, P. 1985: Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17-20.

Sansford, C. E.; Fitt, B. D. L.; Gladders, P.; Lockley, K. D.; Sutherland, K. G. 1996: Oilseed rape: disease development, forecasting and yield loss relationships. Home Grown Cereals Authority Project Report OS17, London, Home Grown Cereals Authority.

Ward, E.; Akrofi, A. Y. 1994: Identification of fungi in the *Gaeumannomyces-Phialophora* complex by RFLPs of PCR-amplified ribosomal DNAs. Mycological Research 98: 219-224.