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**Neal Evans and Bernd Ulber**

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## **Oilseed Crops in Poland – Past and perspectives**

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**Abstract:** Systematic study on oilseed crops after 2<sup>nd</sup> World War started in Poland in 1950, when the Oil Crop Department in Poznan was established in the Plant Breeding and Acclimatisation Institute. As broad a spectrum of species were examined as possible for oilseed crops for Poland. Possibilities of acclimatisation and expected economical values were investigated. Then the most promising species were chosen and research work and breeding were limited to the following crops:

- Winter and spring oilseed rape (*Brassica napus*), winter oilseed turnip rape (*Brassica rapa*) and sunflower (*Helianthus annuus*) – oils for edible purposes,
- Linseed (*Linum usitatissimum*) and false flax (*Camelina sativa*),
- Poppy (*Papaver somniferum*) – seed for nutritional purposes and raw material for pharmaceutical industry,
- Yellow mustard (*Sinapis alba*) - as a spice and phytosanitary aftercrop.

The work done up to the present time demonstrates that oilseed rape is the oil crop which performs best of all in the agroclimatic conditions of Poland. Research on the genetics and the biochemistry of *B. napus* formed the base for breeding of new, so called double low varieties characterised by a lack of erucic acid in oil and very low levels of glucosinolates in meal. Changeover to these new varieties was done in Poland in years 1985 – 1990. Now the changeover to hybrid varieties has subsequently taken place.

Direction of future research and breeding work:

- Adaptation of fatty acid composition to better fit the different oil uses;
- Increasing of fat and protein contents in seeds;
- Improvement of meal by further decreasing the content of antinutritive components like: alkenyl glucosinolates, dietary fiber, sinapine, polyphenols, phytin;
- Investigate sources of resistance or tolerance to diseases and to pests and to stress conditions (winterhardiness, drought resistance);
- Improvement of agronomic value e.g. yielding ability, resistance to lodging, resistance to shattering;
- Development and use of new method like tissue culture, doubled haploid production, protoplast fusion, vegetative propagation, interspecific crosses, embryo culture,
- Production of better hybrids using CMS or SI system
- Marker assisted selection, estimation of genetic distance;
- Improvement of oil stability – tocopherol content.

An increase of rapeseed production is expected in Poland in next years which highlights the importance of continued breeding work

## **Integrated pest management in oilseed crops in Pakistan**

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**Abstract:** In Pakistan the oilseed crops are seed cotton, rapeseed, sunflower, sesame seed, groundnut, soybean, linseed, castor bean, coconut and safflower. Over the last half century (from 1961 to 2004), the area under oilseed crops has increased by about 100% i.e. from 1,963,980 hectares in 1961 to 3,964,266 hectares in 2004. Although the yield of oilseed crops is quite low as compared to many other countries of the world, it has increased by about 270% (from 1054 kg/ha in 1961 to 2951 kg/ha in 2004), during the period under discussion. Abiotic and biotic factors responsible for lower yields in Pakistan are discussed, with particular emphasis on pests. Different approaches for integrated pest management in practice in Pakistan are discussed in this paper.

## Integrated oilseed rape protection in Belarus

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**Abstract:** The main oilseed crop in Belarus is winter and spring rape and is cultivated on 80-140, 000 ha area or 2.5-3.0% of cultivated area. In total there are 1.2 million ha in Belarus fit for cultivation of winter rape and 2.8 million ha for spring rape. Thus, oilseed rape can cover 400-450 million ha in Belarus annually within a crop rotation.

The crop capacity of winter rape oilseeds in average in 2005 was 18 dt/ha, spring rape – 16 dt/ha. A lot of farms receive 25-35 dt/ha and even 40-50 dt/ha.

Depending on the overwintering survival of winter rape, the area sown to spring rape covers 45-80%. For the last 10 years in our republic, winter rape losses over winter reached 80-90% in 1994, 1999 and 2003. Around 30% of these losses were due to the break in crop technology cultivation (soil preparation, sowing terms, sowing norms and so on).

Oilseed rape in Belarus is affected each year with a complex of diseases, the most significant of which are the following:

Alternaria blight (*Alternaria*) which annually affects 20-80% of the crop, seed infection – 37-100%, sclerotinosis (*Sclerotinia sclerotiorum*), fusarial wilting of *F. oxysporum*, gray mould (*Botrytis cinerea*). On spring rape in sprouts period – wire stem (*Rhizoctonia*, *Pythium*, *Olpidium*, *Botrytis*), on winter rape in a period of hibernation – snow mould (*Fusarium nivale*, *Typhula incarnata*, *Sclerotinia trifoliorum*), bacteriosis (*Xanthomonas*, *Pseudomonas*), root rots, phomosis (*Phoma lingam*). Potentially the dangerous diseases are disease caused by *Cylindrosporium* (*Cylindrosporium concentricum*), blue mould (*Peronospora brassicae*) and powdery mildew (*Erysiphe cruciferarum*).

In recent years, between 95-98% of winter and spring rape varieties cultivated in Belarus are from the institute's breeding programme. All of them are tolerant to the most wide-spread diseases.

Five fungicides are permitted in Belarus, however they are very seldom used in production. For rape seeds dipping preparations WITAVAX 200, VINCIT are used favorably. Of great importance in disease control is a crop rotation and a cultivation technology directed towards good establishment.

The most harmful pest in oilseed rape cultivation in Belarus is the pollen beetle (*Meligethes aeneus*). Yield losses in the absence of insecticides are 20-35%. Spring rape in particular is affected by *M. aeneus* with yield losses reaching 35-60%. The cruciferous flea-beetles (*Phyllotera* spp.) are serious pests of establishment, particularly in spring rape crops. Increasing numbers of stem and seed pod weevils (*Ceutorhynchus pallidactylus*, *Ceutorhynchus assimilis*, respectively) have been noted. In some years, in winter rape sowings, the rape sawfly (*Athalia rosae*) has been observed. In dry years, the cabbage aphid (*Brevicoryne brassicae*) can be a serious pest. In Belarus, oilseed rape crops are usually treated 2-3 times during the growing season with insecticides, especially KARATE, DECIS and FASTAK. Application of preparations are permitted in Belarus sowings. The most wide-spread of them are BUTISAN 400 (metazachlor), TROFI (acetochlor), TREFLAN (trifluralin), and TERIDOX (dimetachlor).

## **The occurrence of bees (Apoidea) on winter oilseed rape crops**

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**Abstract:** The aim of this study conducted in 2004-2005 on six oilseed rape crops through the entire spring - summer vegetation period, was to assess the phenology and the distribution of Apoidea guilds in different landscapes. The analysis was based on visual counting of bees coupled with the line transect method. A total number of 7648 individual bees were registered. The dominant species was *Apis mellifera* (63%). Among the wild bees, the most numerous group was Andrenidae (22%). The group Halictidae and *Bombus* spp. made up ca. 7% of the guild. The highest density of the honey bee was observed in transects running along neighbouring crops or along semi-natural plant assemblages, whereas the wild bees were recorded at their greatest numbers from transects close to field tracks, railway banks and drainage ditches. Wild species of bees appeared on oilseed rape crops in significant densities (32 % of the guild) before the flowering of the crop. At this early time, their recorded densities were the lowest in mid-field transects and at the edge-transects neighbouring semi-natural plant assemblages.

## **The EU project MASTER (Management STRategies for European Rape pests): An update**

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MASTER (MANagement STRategies for European Rape pests) is the acronym for the EU-funded project, entitled 'Integrated pest management strategies incorporating bio-control for European oilseed rape pests' (QLK5-CT-2001-01447). The project consortium has partners from six EU member states. The main aim of the project is to construct, develop, evaluate and promote an Integrated Pest Management (IPM) System for the European winter oilseed rape crop incorporating biological control of pests. The project is of four and a half years duration; it was initiated in December 2001 and will finish in May 2006. It is thus in its final year.

## **SECURE – Stem Canker of oilseed rape: Molecular methods and mathematical modeling to deploy durable resistance (QLK5-CT-2002-01813)**

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**Abstract:** SECURE aims to deliver a model for deployment of cultivars with resistance to *Leptosphaeria maculans* (phoma stem canker/blackleg) to improve durability of resistance and minimise risk that the resistance will be overcome. The project has four main parts. Firstly, a life-cycle model for *L. maculans* has been developed and validated. Secondly, the fitness of virulent/avirulent isolates of the pathogen is being investigated; the results indicate a fitness penalty resulting from loss of the avirulence function for at least two resistance genes. Genomic analysis of avirulence and virulence loci of the pathogen is also being done. In addition, two *L. maculans* avirulent alleles (*AvrLm1* and *AvrLm6*) have been cloned. Thirdly, the effects of plant genetic background and environmental factors on durability of resistance are being analysed in both field experiments at a number of sites across Europe and controlled environment experiments. Lastly, models have been developed to investigate the effects of different schemes for deployment of resistance on durability of resistance so that recommendations can be made for a sustainable strategy for managing the disease. Results are being disseminated using a website ([www.secure.rothamsted.ac.uk](http://www.secure.rothamsted.ac.uk)), scientific and popular publications and workshops.

## **Control of oilseed rape pests during flowering and pod development with combined application of insecticides and fungicides in 2003 – 2005**

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**Abstract:** In 2003 – 2004, at the Sośnicowice Branch of the Institute of Plant Protection, Poznań, investigations were made of tank-mix applications of modern production technologies of winter oilseed rape. This paper reports results from synchronous application of insecticides to control cabbage seed weevil (*Ceutorhynchus assimilis* Payk) and brassica pod midge (*Dasyneura brassicae* Winn.) and fungicides to control fungal pathogens at the flowering stages of the crop.

In 2003, one trial was conducted with two insecticides – Karate Zeon 050 CS (lambda-cyhalothrin) and Trebon 10 SC (ethofenprox). These insecticides were used individually or mixed with fungicides - Alert 375 SC (flusilazole + carbendazim), Horizon 250 EW (tebuconazole) and Amistar 250 SC (azoxystrobin). In 2004, three trials were conducted in which the same insecticides were used together with Proteus 110 OD (thiachlopid, deltamethrin), Calipso 480 SC (thiachlopid), and Trebon 10 SC (ethofenprox), and the same fungicides, together with Alert 375 SC and Amistar 250 SC. These three trials differed only in date and amount of protective treatments. In 2005, as in 2004, three trials were conducted with three insecticides - Calipso 480 SC, Patriot 100 EC (deltamethrin), Proteus 110 OD, and two fungicides - Alert 374 SC and Horizon 250 EW.

The most effective control of cabbage seed weevil and brassica pod midge was obtained in the trials which used two protective treatments in a one-week period. The highest increase in yield was obtained in the investigations with the treatments applied at BBCH growth stage 65 (trial 1) or at both growth stage 65 and 67 (trial 2).

## Differential effect of different nitrogen and sulphur fertiliser regimes on plant health and seed quality of winter oilseed rape grown in Poland

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**Abstract:** The effect of a number of doses of nitrogen and sulphur on the healthiness of two winter oilseed rape varieties (open-pollinated 'Gara' and composite hybrid 'Bufallo') was determined. Spring doses of nitrogen were applied at 80, 160 and 240 kg·ha<sup>-1</sup> and sulphur at 30, 60 and 90 kg·ha<sup>-1</sup>. Healthiness of plants was evaluated at flowering and during ripening using assessments of infection and then an index was calculated. After harvest the effect of fertilisation on the pathogenic fungal colonisation of seeds was assessed. *Alternaria* spp. were observed at higher levels on leaves and pods and *Phoma ligam* on leaves and stems.

There were observed differences of the healthiness of plants between varieties with the open-pollinated cultivar Gara being more infected. There were complex differential effects of fertilisation. Higher doses of nitrogen resulted in an increase in black spot (*Alternaria* spp.) severity. However, simultaneous fertilisation with nitrogen and sulphur resulted in lower intensity of symptoms of black spot and stem canker. The effect of sulphur fertilisation on plant health was not clear, but the use of this element together with higher doses of nitrogen may show some protective activity against plant infection. The main pathogenic species isolated from seeds was *Alternaria brassicae*. There was no relationship between fertilisation and occurrence of *A. brassicae* on seeds.

## Effects of mineral fertilisation and crop protection schemes on diseases of winter oilseed rape in Poland

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**Abstract:** Winter oilseed rape has a long growing season and during this time is attacked by various diseases. At present, the most common way to control diseases is through fungicide application but this is harmful to the environment and decreases crop profitability due to high cost of chemical products. According to the rules of integrated farming, plant cultivation should integrate biological and technological achievements with proper mineral fertilisation. The aim of this work described in this paper was to evaluate oilseed rape healthiness under different chemical protection and fertilisation regimes. Field experiments were done for two years at Baborowko and Grabow Experimental Stations. The first experimental factor was different levels of chemical protection of plants and the second factor was different levels of nitrogen fertilisation at rates that ranged from 0, 40, 80, 120, 160 to 200 kg N/ha. One treatment regime was characterised by applying mineral fertilisation and one fungicide treatment that contained sulphur. The experiments used two oilseed rape varieties: Kana (Grabow) and Lisek (Baborowko). The occurrence of fungal diseases of oilseed rape and their severities were different in both years of experimentation. An increase in chemical protection of oilseed rape caused a decrease in plant infection with most of diseases. However, greater doses of nitrogen fertilisation favoured the development of fungal diseases. Oilseed rape protected by fungicides and fertilized with sulphur showed, in general, lower infection by the main fungal diseases in comparison with treatments with fungicides without sulphur. The larger seed yield of rapeseed was found in the first experiment year, when lower infection of *Phoma lingam* was observed. Oilseed rape variety Kana cultivated at Grabow yielded higher than Lisek cultivated at Baborowko Station.

## Influence of fertilisation with sulphur, magnesium and boron on the content of glucosinolates and occurrence of *Alternaria* spp. on seeds of the spring oilseed rape 'Margo'

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### Abstract

Effects of soil or foliar fertilisation with sulphur as well as in combination of both together with foliar application with magnesium or boron on the content of glucosinolates and infection of seeds of the spring oilseed rape 'Margo' with *Alternaria* spp. were studied. Laboratory investigations were performed on seeds of oilseed rape of a three-year-long field experiment. The experiment was carried out in 2001-2003 using a design of randomised sub-blocks in 4 replications. It was shown that fertiliser treatments used did not significantly affect the total content of glucosinolates in the seeds. A high negative correlation between concentration of alkenyl glucosinolates, such as progloutrin and glucobrassicinapin, in the seeds and resistance toward the fungal pathogen *Alternaria brassicae* was shown. However, no significant correlation was observed between glucosinolates level and the infestation with the non-pathogenic *A. alternata* species.

## **Insect problems in European oilseed rape cultivation, and how to deal with them: The OSR farmers' perspective**

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**Abstract:** A survey was carried out among oilseed rape growers in six countries (Estonia, Finland, Germany, Poland, Sweden and the UK) concerning the growing season of 2002-2003, as part of the EU-project MASTER. In total, 1005 replies were obtained with 115-216 responses from each of the countries surveyed. Here the core results concerning the occurrence and importance of insect pests, the decision making criteria concerning the need for active control measures, and the impact of farming methods (conventional-integrated-organic) on these perspectives are presented. Europe-wide, only the pollen beetle was a problem requiring control in each country. The seed weevil, pod midge, and the cabbage stem flea beetle were the second most commonly mentioned as problems (important in 4 out of 6 countries), followed by the stem weevils (2-3 out of 6 countries). Six-seven different insect species were all very important in DE and PL, while in the UK 2-3 species and in SE, FI and EE only 1-2 species were problematic. This was reflected in the number of insecticide spray treatments: typically 2-4 per season in Poland, 1-4 in Germany, 0-3 in the UK, SE, FI, and EE. Paradoxically, the mean number of insecticide sprays was the same for conventional and IPM farmers (1.65 sprays/season), and in FI and PL farmers using IPM sprayed more often than conventional farmers. Also, the proportion of IPM farmers was highest in countries where the spraying rate also was highest (DE, PL, UK). Furthermore, overall, a higher proportion of IPM farmers used insecticide seed dressing than conventional farmers (77.3% vs. 69.7%). In all countries, the majority of farmers always sprayed all their OSR fields (81.0%), while overall, 14.7% sprayed some but not all fields, and a mere 4.3% reported having sprayed field edges only. Out of nine different decision-making criteria for determining whether active pest control was necessary, farmers were using most often simply seeing the pest on the crop (70.0%), followed by using economic thresholds (47.3%), recommendation by crop protection consultant (24.0%), plant growth stage (16.3%), advisory service message (16.3%), advisory service general recommendation (8.7%), advice from neighbours (8.2%), commercial decision support system (2.7%), or spraying by date (1.8%). Growers in PL used on the average 4.6 different criteria simultaneously to arrive at pest control decision, while in other countries clearly fewer criteria were sufficient: 2.7 in DE, 1.9 in SE and UK, and 1.6 in EE and FI.

## Damage of registered Polish winter oilseed rape cultivars caused by pests

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**Abstract:** The aim of our investigations was to assess the degree of damage caused by pests to 42 cultivars of winter oilseed rape registered in Poland. The cultivars tested comprised 5 restored hybrids, 4 composite hybrids and 33 populations and work was carried out at Experimental Stations: Krościna Mała and Rarwino, at the plant breeding station Małyszyn. Large differences were observed in the degree of damage caused to the different cultivars by *Ceutorhynchus pleurostigma* Marsh., *Phorbia brassicae* Bche., *Ceutorhynchus napi* Gyll., *Ceutorhynchus quadridens* Panz., *Meligethes aeneus* F., *Dasyneura brassicae* Winn. and *Ceutorhynchus assimilis* Payk.

## The endangerment of oilseed rape by pests in Poland

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**Abstract:** Winter and spring oilseed rape crops are damaged by different pests throughout development. Crop losses can be reduced in the absence of control by about 20%, and in extreme cases can lead to partial or even total crop destruction. The pests reduce the quality and quantity the seed yield as well as making harvesting difficult, due to unevenness in ripening caused by plant compensation. Pests of economic importance include *Meligethes aeneus* L., *Ceutorhynchus napi* Gyll., *Ceutorhynchus quadridens* Marsh., *Ceutorhynchus assimilis* Payk., *Dasyneura brassicae* Winn and occasional loss is attributed to *Phytomyza rufipes* Meig., Agrotinae, Thysanoptera, *Plutella cruciferarum* Zell. and also Gastropoda and birds. Endeavours to lower the cost and improve efficacy of crop protection methods depends mainly on: improvement monitoring to signal timing of interventions, qualification of thresholds of economic profitability of pest control, limitation of the number of executed interventions, using tank mixtures and correct choice of selective insecticides.

In this work the pests occurrence in winter and spring oilseed rape crops from 1999-2003 was studied across the different regions in Poland. Among the pests, 5 of them: *M. aeneus*, *C. napi*, *C. quadridens*, *C. assimilis* and *D. brassicae* caused most damage. These were summarized as a series of graphs and maps. The number of interventions registered to control pests in winter oilseed rape crops, the costs of spraying and insecticides as well as the proportional use of different groups of insecticides in Poland was also determined from historical records. Prior to the 1980s organophosphate preparations predominated. However from 1980 use of pyrethroids in prophylactic oilseed rape pest control increased. In the latter part of the 1990s the number of new biologically active substances registered for pest control in oilseed rape reduced. However, the number of generic insecticides is now increasing.

## **The influence of glucosinolate content variability in the seeds and green matter of winter oilseed rape on the attack by selected pests**

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**Abstract:** The glucosinolate content in the seeds of 30 cultivars of oilseed rape varied from 5.40 to 24.06  $\mu\text{mol/g}$  dry matter. The glucosinolate content of cotyledons of three selected varieties (with seed dry matters from 9.73 to 12.4  $\mu\text{mol/g}$ ) varied from 9.30 to 12.14  $\mu\text{mol/g}$  herbage dry matter. The changes in glucosinolate content were observed after mechanical injury to the leaves. Glucosinolate content varied on the third day after injury from 4.33 to 8.6  $\mu\text{mol/g}$  herbage dry matter of intact leaves and from 23.22 to 24.19  $\mu\text{mol/g}$  herbage dry matter of damaged leaves. The significant increase of indolyl glucosinolates was detected after leaf injury.

The relationship between seed glucosinolate content, emergence ability and size of plants to slug damage was observed using ten selected varieties oilseed rape. A scale with 6 degrees was used for the evaluation of slug injury: 1 = intact plant, 6 = whole plant or plant top is missing. The mean number of emerged plants varied from 22 to 31.5. Growth (size of plants) classification varied from degree 1 (small plant) to degree 3 (big plant). Slug damage varied from 2.14 to 3.55 degrees. Data from our 1-year study period showed that glucosinolate content, emergence ability and size of plants influence slug damage.

Our research on the influence of glucosinolate content in oilseed rape seeds on slug damage was completed in 2004. The results obtained will be used for further research on the influence of glucosinolate content of the green plant matter on the incidence of the rapeseed sawfly (*Athalia rosae*) and stem weewils (*Ceutorhynchus napi* and *C. pallidactylus*).

## **The damage of seeds and seedlings of winter oilseed rape cultivars caused by *Deroceras reticulatum* (Müller) (Gastropoda: Pulmonata: Agriolimacidae) and *Arion lusitanicus* Mabile (Gastropoda: Pulmonata: Arionidae)**

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**Abstract:** In laboratory conditions the susceptibility of 18 oilseed rape cultivars to damage caused by *Deroceras reticulatum* and *Arion lusitanicus* was estimated. For each slug species three experiments were carried out: one with seeds, one with seedlings at growth stage 08 – 10 BBCH and one with seedlings at growth stage 10 – 11 BBCH. Significant differences in damage to seeds and seedlings caused by *Deroceras reticulatum* and *Arion lusitanicus* were observed, but only on some days of the test. The most severely damaged cultivar, damaged by both slug species, was Lisek, and the least damaged cultivars were Bazyl, Extreme and Californium.

## Study of harmful Anthomyiidae in oilseed rape fields with different drilling dates

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**Abstract:** Over the past couple of years in Germany, the cabbage root fly (*Delia* spp.) has caused increasing damage in winter oilseed rape crops, particularly by the feeding of larvae on the roots of young plants in autumn. The intensity of infestation of the oilseed rape by root flies is a function of the development of the plant. In fields with different drilling dates, the Anthomyiidae spectrum was assessed by emergence traps. Overall, 7 Anthomyiidae species with the potential to feed on oilseed rape were recorded, four of them were abundant. It was shown that drilling date heavily influences the species assemblage of pest Anthomyiidae and their dominance. In early-drilled winter oilseed rape *Delia radicum* was dominant, while in later drilled winter oilseed rape fields *Delia platura* predominated. Other species like *Delia florilega* and *Botanophila fugax* were also more abundant in later-drilled oilseed rape fields. Thus, the change of Anthomyiidae assemblages and their dominance position according to drilling date can influence the success of control measures.

## Efficacy of *Trichogramma chilonis* (Ishii) and some new synthetic insecticides against *Helicoverpa armigera* (Hübner) in sunflower

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**Abstract:** The present studies were conducted to assess the effectiveness of the parasitoid *Trichogramma chilonis* against the pest *Helicoverpa armigera* and to examine the insecticidal effects of some new insecticides against *H. armigera*. Four new synthetic insecticides: Lannate 40 SP, Proclaim 019 EC, Steward 150 SC and Tracer 240 SC were used and the eggs of *T. chilonis* were released at 60,000, 80,000 and 1,00,000 acre<sup>-1</sup>. All the treatments controlled the pest but among the insecticides, Steward proved to be the most effective in reducing the larval population. *Trichogramma chilonis* suppressed the larval population of the pest and this effect was more pronounced with increased numbers of eggs released in the field. Maximum decrease in larval population was observed when the eggs of the parasitoid were released at 1,00,000 acre<sup>-1</sup>. It was concluded that the parasitoids might become an integral part of management package only by augmentation with new insecticides. But at the same time, to avoid resistance in the insect pests, conventional insecticides must be replaced with the newly emerging insecticides, which are safer.

## Subsequent effect of tau-fluvalinate (Maverik) and lambda-cyhalothrin (Karate) pyrethroids on the activity of carabid beetles (Coleoptera: Carabidae) in winter oilseed rape

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**Abstract:** Carabid beetles are important in agrocenosis as predaceous insects of some pests of winter oilseed rape, such as *Meligethes aeneus*, *Ceuthorhynchus pallidactylus*, *C. napi*, and *Dasyneura brassicae*. This study determined the effect of non-selective pyrethroids on reducing the number of phytophagous larvae (as a food) on the total amount and species diversity of carabid beetles. The experiment was carried out in 2002 and 2004 at Experimental Station Pawłowice (Agricultural University at Wrocław). Each year, the insecticides lambda-cyhalothrin (Karate 25 EC) and tau-fluvalinate (Mavrik 2S) were applied to control major pests larvae of the rapeseed crop. Data from two years of investigations prove indirectly the effect of pyrethroids application on dynamic seasonal appearance of beetles in winter rapeseed crop.

## First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany: An extended abstract

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**Abstract:** Due to EU regulations of plant protection products and increasing demands for human and environmental safety issues the number of active substances which can be used to control pest insects was reduced in the last year. In Germany only pyrethroids are available for the control of most pest insects in oilseed rape. Therefore resistance development of pest insects to pyrethroids is becoming more relevant because the resistance status of pest insects need to be known for IPM.

In the past years *Meligethes aeneus* has developed resistance to pesticides in different European regions. Actually resistant *M. aeneus* populations seem to spread in Europe. No information on possible development of resistance to the other pest insects of rape is available, though they often are exposed to more than one pyrethroid application per season similar to *M. aeneus*.

We conducted laboratory tests using active substances of pyrethroids on different pest insects of rape collected in different areas of Germany. Tests have been carried out on *Phyllotreta* spp., *Ceutorhynchus assimilis*, *C. quadridens*, *C. napi* until now.

Results obtained and methods used will be presented and discussed.

## **Resistance of pollen beetle (*Meligethes aeneus* F.) to pyrethroids, chloronicotinyls and organophosphorous insecticides in Poland**

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**Abstract:** Poland is a major producer of oilseed rape (*Brassica napus*). The pollen beetle (PB) (*Meligethes aeneus*) is the most serious oilseed rape pest in Poland and is considered to be the pest with the highest likelihood of developing insecticide resistance (Łakocy, 1967; Węgorzek, 2005). Pyrethroid, organophosphorous and chloronicotinyl insecticides have been widely used to control PB in Poland. However, during the last few years, a decrease in efficacy of chemical protection against PB was reported from many regions. Bioassays for resistance monitoring of PB to pyrethroid, chloronicotinyl and organophosphorous insecticides were performed in the Institute of Plant Protection, Poznań before the year 2004. Results demonstrated some level of resistance to alpha-cypermethrin, cypermethrin, deltamethrin, lambda-cyhalothrin, esfenvalerate, and also acetamiprid (Węgorzek, 2005). The results indicated that populations tolerant to pyrethroids and acetamiprid were not cross-resistant to chloropyrifos. The widespread use of pyrethroids in Poland could lead to control failure. It creates the need for constant monitoring of PB resistance and for further studies on resistance mechanisms.

## **Responses of pollen beetles (*Meligethes aeneus*) to conspecific odours**

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**Abstract:** As part of ongoing studies to investigate insect-derived semiochemicals for use in pest control strategies for oilseed rape (*Brassica napus*), the responses of male and female pollen beetles (*Meligethes aeneus*), to conspecific odours was assessed in laboratory and semi-field experiments. In laboratory bioassays using a linear track olfactometer, we found no evidence for the existence of a male-produced aggregation pheromone; male pollen beetles did not respond to the odours from groups of other males. However, female beetles were repelled by the odours from a large group of females (200 individuals). This response was not shown in tests involving male beetles. These results suggest that females show epideictic (spacing) behaviour in response to high densities of other females. This response was found to be at least partially due to the odour of the females themselves, rather than due to the volatiles from female-damaged plant material. The results from semi-field and field-scale experiments however, failed to support preliminary laboratory evidence for the existence of a female-produced epideictic pheromone in the pollen beetle.

## **Responses of pollen beetles (*Meligethes aeneus*) to petal colour**

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**Abstract:** It is well known that the pollen beetle (*Meligethes aeneus*), a major pest of oilseed rape (*Brassica napus*) inflorescences, is attracted to the yellow colour of the flowers. Little is known however, about how variation in flower colour might affect host plant location and subsequent infestation by this pest. We investigated the responses of new-generation pollen beetles to a range of differently-coloured traps and to flowers of a white-petalled oilseed rape cultivar that had been dyed different colours. In the field, beetles were significantly more attracted to sticky traps coloured yellow than other colours. Black and blue coloured traps caught fewest beetles. In wind tunnel and field experiments using racemes and potted plants with dyed flowers, flowers dyed blue were less attractive than yellow, untreated white, and red flowers. The potential for manipulation of petal colour in control strategies for this pest is discussed.

## **Assessment of staphylinid beetle larvae from oilseed rape flower stands and their influence on pollen beetle larvae**

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**Abstract:** In Germany, large numbers of staphylinid larvae (Coleoptera: Staphylinidae) are observed together with pollen beetle (*Meligethes aeneus*) larvae in oilseed rape flower stands. From 2002-2005 the quantitative and spatio-temporal relationship of staphylinid and *Meligethes* larvae was recorded in an Integrated Crop Management (ICM) system and a standardised oilseed rape management (STN) system. In all years there were more staphylinid larvae in the ICM-system than in the STN-system. In three years the number of *Meligethes larvae* was higher in the ICM- than in the STN-system. There was a higher temporal coincidence between the larval dropping of *Meligethes* and of Staphylinidae in the ICM-system than in the STN-system in all years. In 2003, the number of pollen beetle larvae was lower in the ICM- than in the STN-system, although the STN-system was treated with insecticides. In this year, the ratio and the temporal coincidence between pests and predators seems to have been favourable in the ICM-system, so that the staphylinid larvae were even more effective in reducing the pest population than the insecticides. The predators' influence on the *Meligethes* populations can also be seen in the emergence of the new pest generation. This was lower in the ICM-system than in the STN-system in three years and is evidence of the importance of the predators in regulation of the pest populations.

## Oilseed rape pests and their parasitoids in Estonia

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**Abstract:** The pests and their hymenopterous parasitoids present in spring and winter oilseed rape crop in Estonia were studied. *Meligethes aeneus* was abundant pest in the spring oilseed rape whereas *Ceutorhynchus assimilis* was more numerous in the winter oilseed rape. Other crucifer-specialist pests such as *Ceutorhynchus sulcicollis*, *C. pallidactylus*, *C. rapae*, *C. floralis* and *C. pleurostigma* were found but their abundance was very low. Also three parasitoids of *M. aeneus* larvae (*Diospilus capito*, *Phradis morionellus* and *P. interstitialis*) and three of *C. assimilis* larvae (*Mesopolobus morys*, *Stenomalina gracilis* and *Trichomalus perfectus*) were found. Larval parasitization rate of *M. aeneus* (0–7.4%) and *C. assimilis* (0–32%) was dependent on oilseed rape crop type and use of insecticides.

## Phenology of parasitoids (Hym., Ichneumonidae-Tersilochinae) of oilseed rape pests in northern Germany from 1995-1997

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**Abstract:** The phenology of the most abundant univoltine larval parasitoids of oilseed rape pests, *Tersilochus microgaster*, *Tersilochus fulvipes*, *Tersilochus obscurator*, *Phradis interstitialis* and *Tersilochus heterocerus* (Hym., Ichneumonidae-Tersilochinae), was studied at Goettingen, northern Germany, from 1995-1997. The emergence of adult parasitoids from overwintering sites was assessed by using ground photoelectors. Yellow water trap and Malaise trap catches were used to define the period of activity of adult parasitoids at emergence sites, as well as to monitor the time of arrival and duration of activity in current crops of oilseed rape.

The time of emergence and migration of female parasitoids to crops of oilseed rape varied between species. On many occasions, their arrival on the rape crop was found almost simultaneously with their emergence from overwintering from nearby fields. Females of *T. microgaster* were recorded from the early bud stage in March until mid-flowering. *Tersilochus fulvipes*, *T. obscurator* and *P. interstitialis* colonized the rape crops from mid-April onwards and adults of *T. heterocerus* occurred towards the end of April or the beginning of May. Few individuals were captured after the beginning of crop ripening. The main period of parasitoid activity coincided with the occurrence of susceptible larval instars of their hosts in the rape crop. Peak activity of all tersilochine parasitoids, except *T. microgaster*, was focussed during the flowering period of oilseed rape. Consequently, insecticide treatments during this period pose a high risk of damage to these parasitoid species. Adverse effects on parasitoid abundance may affect their impact on natural control of pest populations.

## **Upwind anemotaxis by the parasitoid *Tersilochus obscurator* (Hym., Ichneumonidae) on its migration flights to winter oilseed rape**

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**Abstract:** The direction of migration flights of parasitoids to a crop can affect their subsequent spatial distributions on the crop and hence their effectiveness in conservation biocontrol strategies. The effect of wind direction on the migration flights of natural populations of *Tersilochus obscurator* Aubert (Hym., Ichneumonidae), a parasitoid of the stem-mining pest of oilseed rape, *Ceutorhynchus pallidactylus* (Marsham) (Col., Curculionidae), to a crop of oilseed rape (*Brassica napus* L.) was investigated. Double-sided Malaise traps encircled a crop (20 m diameter) of winter rape, one at each of eight compass points and at 5 m from the crop periphery. Insects were trapped during eight alternate weeks from mid-March to late June 2003. Meteorological data were recorded close to the plot. *Tersilochus obscurator* were caught in the traps from mid April until mid June with a peak in late May. Most (88%) were caught in the external halves of the traps indicating a net movement towards the crop. On all five days analysed, there was a negative correlation between insect catch in the external halves of the traps and air flow through the traps, this was highly significant on three days. This study provides the first field evidence that *T. obscurator* utilizes upwind anemotaxis to locate its host habitat. Understanding the effect of wind direction on parasitoid migration flights to winter rape holds potential for forecasting their arrival and spatial distributions on the crop as well as for manipulating and enhancing their populations for IPM strategies incorporating biocontrol.

## ***Phradis morionellus* on *Meligethes aeneus*: Long-term patterns of parasitism and impact on pollen beetle populations in Finland**

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**Abstract:** Data on annual percent parasitism of *Meligethes aeneus* (Nitidulidae) larvae by *Phradis morionellus* (Ichneumoniidae) were collected in Finland for 11 years (1985-1995) from 35-70 different locations (13 regions) covering the total area of rapeseed cropping in Finland. Information on the level of pest attack at the national level (severity and frequency of attack) was obtained from the annual pest survey data, carried out by the Agricultural Research Centre of Finland until 1992. Total proportion of pollen beetles removed from the emerging new generation adult population by parasitism (%-parasitism at each region weighted by the area of rapeseed grown in that area) varied between 49.5% in 1987 to 7.5% in the following year (1988); usually the proportion was around 30%. Pollen beetle attack decreased from severe in the early 1980s to moderate in 1985-88 with rising levels of parasitism by *P. morionellus*. After the 1988 crash in *Phradis* populations, pollen beetle attack jumped again to severe until the early 1990s, after which some balance appears to have been reached. Insecticide sprays to control the all-time high populations of the cereal aphid *Rhopalosiphum padi* in 1988 are a likely explanation for the parasitoid crash in this year. A dynamic simulation model was constructed to describe the rapeseed plant, pollen beetle, and parasitoid interaction. This suggested that pollen beetle populations indeed can be held at a very low level by *Phradis*, if the interaction is not disrupted with pesticide applications, or if the parasitoid is enhanced by some other simple means. The parasitoid dynamics appear to follow a surprising pattern, being in synchrony over several large regions, but varying widely between these larger regions. Edaphic and climatic factors may explain such regional patterns. Also, several micro-level factors were identified as influencing the level of parasitism at an individual-field level. These include distance to forest edge, soil type, size of rapeseed field, and possibly the abundance of umbelliferous plants at the field edge.

## Adult activity and larval abundance of stem weevils and their parasitoids at different crop densities of oilseed rape

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**Abstract:** The effect of oilseed rape plant density on adult activity, larval abundance and larval parasitism of stem weevils, *Ceutorhynchus napi* and *C. pallidactylus*, and their larval parasitoids, *Tersilochus fulvipes* and *T. obscurator* (Hym.: Ichneumonidae), respectively, was studied in a randomised field experiment of two contrasting plant densities (10 plants/m<sup>2</sup> and 70 plants/m<sup>2</sup>). Adult activity of weevils and parasitoids was monitored using water trays set up below the crop canopy. The abundance of stem-mining larvae was assessed from plant samples. The level of parasitism was determined by dissection of larvae.

In plots of low plant density, the numbers of adult *C. napi* and *T. fulvipes* trapped in water trays were significantly greater than at the higher plant density. The activity density of *C. pallidactylus* and *T. obscurator* was not affected by plant density. The number of *C. pallidactylus* larvae per plant in plots of low plant density was significantly higher than in plots of high density. The abundance of *C. pallidactylus* larvae per m<sup>2</sup> was similar at both plant densities, indicating that plant resources needed for oviposition and larval development were sufficiently available, even at low plant density. In contrast to our expectations, the number of *C. napi* larvae per plant did not increase with decreasing plant density. Corresponding to the activity density of adult *T. fulvipes*, percentage parasitism of *C. napi* at low plant density was significantly higher than at high plant density. The level of parasitism of *C. pallidactylus* was not affected by plant density.

## Larval parasitism of *Ceutorhynchus napi* Gyll. and *Ceutorhynchus pallidactylus* (Mrsh.) in plots of different crop density of oilseed rape

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**Abstract:** The rape stem weevil, *Ceutorhynchus napi* Gyll., and the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.), (Col., Curculionidae) are two of the most important stem-boring pests of oilseed rape in Germany. Natural enemies of *C. napi* and *C. pallidactylus* are the parasitic wasps *Tersilochus fulvipes* (Grav.) and *Tersilochus obscurator* Aub. (Hym., Ichneumonidae), respectively. The aim of this investigation was to examine the effect of crop density on the level of larval parasitism. Plant density may have an impact on plant architecture as well as on microclimate, thereby influencing the abundance and within-plant distribution of host larvae and hence the efficiency of parasitoids. In 2003/04 a field experiment was conducted including four sowing densities: 74, 49, 37 and 25 seeds/m<sup>2</sup>. Various densities were achieved by choosing two row spacings (22.5 cm and 45 cm) and two intra row spacings (6 cm and 9 cm). The experiment was laid out in a randomized block design with four replications. Plant parameters recorded were length of the main raceme, diameter of the main raceme at its base, and at 50 cm and 2/3 along the raceme length, the number of leaves per plant and the number of lateral racemes. Samples of 20 plants per plot were analyzed in May to assess the abundance and within-plant distribution of the target pests *C. napi* and *C. pallidactylus* in the upper, lower and middle part of the stems as well as in the lateral racemes and in the leaves. The level of parasitism was detected by dissecting the larvae. In plots of 25 seeds/m<sup>2</sup> the plant length, the diameter of the main raceme, the number of leaves and the number of lateral racemes was significantly increased compared to plots of 74 seeds/m<sup>2</sup>. The larval parasitism of *C. napi* was influenced by the sowing density as well. In the lower section of the main raceme, the level of parasitism was higher in plots of 74 seeds/m<sup>2</sup> than in the other treatments. Referring to the total number of larvae per plant, there was a tendency of decreasing levels of parasitism with lower sowing densities. The latter was also true for the level of parasitism in *C. pallidactylus*.

## Determining the sex of insect pests of oilseed rape for behavioural bioassays

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**Abstract:** Male and female insects of the same species often have different requirements for host plants, food resources and space. Therefore, insect responses to such stimuli in behavioural bioassays may vary according to the sex of the individuals tested. In order to compare differences in behavioural responses between female and male subjects, it is often necessary to determine the sex of an individual before its use in bioassays. Reliable techniques of determining the sex of live subjects which do not harm them are available for some species. We describe methods used to determine the sex of live, field-collected individuals of three major pest species of oilseed rape: pollen beetles (*Meligethes aeneus*), cabbage seed weevils (*Ceutorhynchus assimilis*) and cabbage stem flea beetles (*Psylliodes chrysocephala*).

## Rearing and identification of the larval parasitoids of *Psylliodes chrysocephala* and *Ceutorhynchus pallidactylus* from field-collected specimens

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**Abstract:** *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae) (cabbage stem flea beetle) and *Ceutorhynchus pallidactylus* (Marsh.) (Coleoptera: Curculionidae) (cabbage stem weevil) are two major stem-mining pests of oilseed rape (*Brassica napus*) in the UK. Little information is available on the occurrence, importance and efficiency of parasitoids in the biocontrol of these pests, due to sampling difficulties and inadequate knowledge on rearing and identification methods for the adults.

In this study, we developed an efficient method of rearing the larval parasitoids of *P. chrysocephala* and to a lesser extent, *C. pallidactylus* from field-collected specimens in order to identify the active larval parasitoids of these pests. Plant samples were collected in 2002 and 2003 from unsprayed winter oilseed rape crops at ~10-day intervals from mid-April until July. The samples were kept in cages in an outdoor insectary and the pest larvae were collected as they left naturally from the plant stems when ready to pupate. The larvae were identified to species and put into either 'pot emergence trap' or 'corked tube' containers containing sterilised soil, then kept under natural environmental conditions in the insectary. The adult pests were collected and counted as they emerged. The soil of the containers was then examined and parasitoid cocoons sorted from it, collected and counted. The cocoons were dissected and the pre-emergence adult parasitoids were removed and identified.

Adding 20% sharp sand to the soil increased the proportion of parasitoid cocoons found and reduced mortality in both containers. More parasitoid cocoons were found and lower mortality occurred in corked tubes than pot emergence traps in both years. These results suggest that the addition of sand to the soil and the use of corked tube containers contributed to the rearing success of the parasitoids by providing better conditions for their development. *Tersilochus microgaster* Szép. and *T. obscurator* Aub. (Hymenoptera: Ichneumonidae) were reared from *P. chrysocephala* and *C. pallidactylus* larvae, respectively. These parasitoids were identified for the first time in the UK as active parasitoids of these pests.

## **OREGIN collection of oilseed rape fungal pathogen isolates managed by a relational database accessible to stakeholders via the Internet**

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**Abstract:** One aim of the Defra-funded Oilseed Rape Genetic Improvement Network (OREGIN) is to establish, characterise and catalogue a collection of *Leptosphaeria maculans* (phoma stem canker) and *Pyrenopeziza brassicae* (light leaf spot) isolates from around the world and to determine genetic diversity in the world-wide populations of these pathogens. Five hundred and twenty isolates of *L. maculans* and 253 isolates of *P. brassicae* have been assembled at Rothamsted Research from sources representative of the world-wide distribution of phoma stem canker and light leaf spot. Information on these isolates (including geographical origin, host species and cultivar of origin, pathogenicity group, mating type and other properties such as culture medium, stock type [slope culture/ glycerol spore suspension stocks]) is now being collected and saved to a Rothamsted Research MySQL open source relational database server. This is being made accessible to the research community and stakeholders via the OREGIN website ([www.oregin.info](http://www.oregin.info)). Once characterised and described, the isolates will be available and the website will generate the appropriate Material Transfer Agreement (MTA) forms. The OREGIN website is being expanded to include access to information relating to other public domain plant resources being made available via OREGIN. This includes information about the *Brassica napus* Diversity Fixed Foundation Set (BnDFFS) and reference genetic mapping populations that are held in secure storage at Warwick HRI.

## **Detection of *Pyrenopeziza brassicae* (light leaf spot) infection of winter oilseed rape**

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**Abstract:** After initial infection of winter oilseed rape leaves in autumn by air-borne ascospores, *Pyrenopeziza brassicae* has a long symptomless phase before the first visible necrotic lesions appear, in January/February in the UK or March/April in Poland. Assessment of symptomless *P. brassicae* infection visually is not reliable before necrotic lesions appear, unless plants are first incubated for several days at high humidity, in polyethylene bags, to encourage *P. brassicae* sporulation. Visual methods for assessment of light leaf spot in winter oilseed rape were compared with PCR for detection of *P. brassicae* infection. PCR diagnosis on leaves sampled from field experiments was more sensitive than visual assessments, even after incubation, with symptomless infection of leaves detected 2 months earlier by PCR than by incubation in polyethylene bags.

## **Development of light leaf spot and phoma stem canker on a range of current and historical oilseed rape cultivars at Rothamsted in 2003/04 and 2004/05 growing seasons.**

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**Abstract:** Field trials in successive growing seasons (2003/04 and 2004/05) were monitored for light leaf spot (*Pyrenopeziza brassicae*), phoma leaf spot and phoma stem canker (*Leptosphaeria maculans*) infection. Each experiment included 42 current, historical or exotic cultivars/breeding lines. Weekly assessments examined leaf, stem and pod disease as % plants affected and % area affected. Significant differences were found between resistant and susceptible cultivars, suggesting resistance to be under discrete genetic control. Many of the cultivars that were susceptible or resistant in the 2003/04 season gave similar responses in 2004/05. However, material gave a range of responses to the two pathogens; for example, cv. Aviso was very resistant to *L. maculans* in both seasons whilst Bronowski was the most susceptible cultivar in both seasons. For *Pyrenopeziza brassicae*, cv. Tapidor was consistently susceptible whilst the line PR45W05 was consistently resistant, when % leaf area affected was assessed. The results are discussed in relation to current knowledge of the reported oilseed rape resistance gene mediated response to *L. maculans* and the suggested resistance mechanism of oilseed rape to *P. brassicae*.

## **Phomadidacte: A computer-aided training program for the severity assessment of phoma stem canker of oilseed rape**

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**Abstract:** Phoma stem canker (caused by *Leptosphaeria maculans*) is one of the main diseases that affect oilseed rape world-wide. The disease is usually characterised by the visual assessment of the severity of cankers created by the pathogen at the crown level of infected plants. In order to avoid arbitrary categories and to maintain standardisation of assessment keys, a rating scale based on the percentage severity estimates of cross-section cankered crowns has been recently proposed in France. This scale consists in 6 severity classes, defined as a function of the percentage of the discoloured cross-section: 1, healthy plant, no visible lesions; 2, 0-25%; 3, 25-50%; 4, 50-75%; 5, 75-100% of discoloured section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling. However, like many other rating systems for the severity of diseases, assessor bias effects have been reported. The aim of this paper is first to give a description of Phomadidacte, a computer-aided training program to guide assessors on how to use this rating scale; and second, to report an evaluation of its efficacy in training assessors. The basic principle of the program consists of displaying pictures of cankered cross-sections of oilseed rape that are to be rated by the user. These pictures had been previously rated by a panel of eight experts from four different research or extension units (2 from INRA, 1 from CETIOM, and 1 from GEVES), and the mean of the severity classes assigned to each picture were taken as the "true" or correct severity class for each picture. At the end of a training session, the program will either suggest the assessor continues with training or will declare that there was good agreement between the experts and the user, based on two evaluation thresholds: the percentage of pictures correctly rated (75%) and the percentage of pictures for which the severity class given by the user and the expert differ by more than one severity class (5%). A graph summarising the differences between the user and the experts' grades is displayed and an ASCII file containing the data of the training session is created. The user can then browse all the pictures to compare the grades that the user gave with the experts' grades. Phomadidacte runs under Windows® and an on-line version is available. An experiment was conducted to test the efficacy of Phomadidacte in improving assessor accuracy. This experiment compared the grades given by two groups of ten assessors who have been evaluated either with or without using Phomadidacte with the grades assigned by a panel of three experts (INRA, CETIOM, GEVES) on actual diseased field samples. The group evaluated without Phomadidacte used one picture per severity grade and diagrams illustrating the diversity of symptoms that can be encountered. Plants were first graded by experts who chose twenty plants for each severity grade. The plants were ordered randomly from the field and then independently rated by all assessors. The distribution of errors (experts minus the assessors' grade) was significantly different between the two groups of assessors (Kolmogorov-Smirnov test,  $P < 10^{-4}$ ). The assessments for the group that had not received Phomadidacte training was in agreement with the experts in 62% of the cases, whereas the group trained with Phomadidacte achieved 69% agreement. Three assessors trained using Phomadidacte succeeded in having a percentage of agreement with experts greater than 75%, whereas none of the non-Phomadidacte group assessors succeeded in achieving this threshold. No assessor, within the two groups, had more than 4.2% of the cases with a difference with the experts that was greater than one severity class, which indicates that the scale is quite easy to use. The generic method described in this paper could be successfully applied to other pathosystems that are difficult to visually characterise.

## **Molecular detection of *L. maculans* and *L. biglobosa* spores from Burkard tapes**

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**Abstract:** Oilseed rape in Poland is exposed to two fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*, which cause stem canker of cruciferes and are responsible for considerable yield losses. Infected stems that stay unploughed, remain on the soil surface and are the source of pseudothecia-fruiting bodies of the perfect stage that contain ascospores. Released ascospores are the main source of infection for young plants of winter oilseed rape in the following season. Information about timing of an ascospore release is a basis of decision support systems used to control the disease.

One of the standard tools used to detect timings of first and mass ascospore release, is a seven day volumetric spore trap by Burkard Manufacturing Ltd. (Rickmansworth, UK). Analysis of presence and concentration of airborne fungal spores may be performed either by means of conventional microscopy methods or PCR-based molecular techniques. In contrast to time consuming microscopy, PCR assays are faster and more accurate. This experiment was designed to optimise methods used for molecular detection of *L. maculans* and *L. biglobosa* spores from Burkard tapes. We have performed multiplex PCR using previously described primers, LmacA, LmacB and LmacRev as well as our own primers, LmF, LmR, LbF and LbR. Both Lmac, Lm and Lb primers were species specific. The primers worked very well in the presence of one of the two species. However, it was not possible to detect any spore DNA in case of imbalanced ratio between *L. maculans* and *L. biglobosa*. Freezing of a spore tape, which is done to store tapes for a long time, has increased the resolution of the method by 10 times. For *L. maculans*, the sensitivity of the method was ca. 8 spores on a frozen tape or fresh pycnidiospore suspension or 100 pycnidiospores, when the fresh tape was used for processing. The resolution for *L. biglobosa* was lower: 100 pycnidiospores from fresh and frozen tape. The detection of 4 and 10 pycnidiospores of *L. biglobosa* has been achieved, but the method was not reproducible.

## Distribution and change in *L. maculans* / *L. biglobosa* populations in Poland (2000-2004)

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**Abstract:** For four seasons (from 2000/01 to 2003/2004) we have collected fragments of oilseed rape plants with symptoms of infection with *Phoma lingam*. In 2000/01 samples were collected from 157 fields, in 2001/02 from 222 fields, 2002/2003 from 46 fields and in 2003/04 from 158 fields of winter oilseed rape. Sampling was done in most of the main areas of intensive cultivation of oilseed rape, including Lower Silesia, Opole region, West Pomerania and Pomerania with Zulawy (delta of Vistula river), Kujavia and Great Poland. Less intensive sampling was done in Lublin region, Varmia and Mazuria, Upper Silesia and the Lodz region located in central Poland. Sampling was done three times a year: in the autumn at rosette stage (symptoms on leaves), in the spring after start of vegetation extension (symptoms on leaves and stems) and before harvest (symptoms on stems). Pathogens were isolated after surface sterilisation with ethanol and calcium hypochlorite. In total, isolations resulted in 2841 fungal strains with 962, 256, 666 and 957 isolates for respective subsequent growing seasons. Isolates were characterised with colony morphology and pigment production on Czapek-Dox or Fries liquid media and identified as *Leptosphaeria maculans* and *L. biglobosa*. In general, half of the isolates belonged to one species and the other half to the other one. However, there were great differences between isolates from different seasons and years. The majority of isolates obtained in the autumn belonged to *L. maculans* with the average of 71.9% and a variation from 67.3 % in autumn 2003 to 8.2.6 % in autumn 2001. Spring samplings resulted in comparable numbers of isolates from either of the two species, with 55.7 % isolates of *L. maculans*. However, isolations from stems before harvest resulted in more *L. biglobosa* than *L. maculans* in a proportion reversed to this obtained in the autumn. Before harvest it was only 21.4 % of *L. maculans*, with variation from 16.9 % in summer 2001 to 25.9 % in summer 2003. Results obtained in this experiment support the hypothesis of possible reduction of *L. maculans* infections due to slow growth of the fungus in leaf tissues and petioles combined with leaf shearing due to frost damage. It is noteworthy, that in contrast to previous reports, *L. maculans* was detected in all regions of intensive cultivation of oilseed rape, including the east of Poland.

## **Patterns of *Leptosphaeria maculans*/ *L. biglobosa* ascospore release in the season 2004/2005 in Poland**

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**Abstract:** Ascospores of *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa* Nov. are the main cause of stem canker of crucifers, one of the most damaging diseases of oilseed rape in Poland and worldwide. In the season 2004/2005 monitoring of airborne ascospores occurrence of these fungi was done in six regions of Poland (West Pomerania, Pomerania, Great Poland, Lower Silesia, Upper Silesia, Carpathian Foothills) within the framework of the System for Forecasting Disease Epidemics (SPEC). Spores were detected using 7-day volumetric spore traps or volumetric pollen and particle sampler surrounded by a circle of oilseed rape debris infected with *Leptosphaeria* spp. In West Pomerania, Pomerania and Lower Silesia monitoring was done from 1 September to 30 November 2004 and from 21 March to 7 June 2005. In Great Poland and Upper Silesia spore trapping has continued since 1 September 2004. In Carpathian Foothills, monitoring began in the spring 2005 and has continued to the present time. In autumn 2004, the difference between the date of the first spore detection in various regions was 12 days. Spores were first observed in Lower Silesia on 11 September 2004 and the latest date of detection was in Great Poland on 23 September 2004. The lowest numbers of spores were detected in Upper Silesia and the highest numbers (up to 320 spores/m<sup>3</sup> of air) in Lower Silesia. Lower Silesia had the longest duration of ascospore release (73 days from the detection of first spores in September to the detection of last spores in November 2004, including 54 days with spore release). In winter 2004/2005, no spores were captured either in Poznan (Great Poland) or in Sosnicowice (Upper Silesia). In spring 2005 ascospores were trapped at very low quantities only in Rarwino (West Pomerania), Tarnow (Lower Silesia) and Rzeszow (Carpathian Foothills).

## **Polymorphisms within *Leptosphaeria maculans* and *Leptosphaeria biglobosa* revealed with rep-PCR fingerprints**

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**Abstract:** The method of rep-PCR was originally designed for species or strain differentiation of prokaryotes, but later it was successfully used to generate DNA fingerprints for numerous fungi, including '*Leptosphaeria maculans* species complex'. The method allows amplification of sequences between randomly dispersed repetitive sequences in a genome and it proved its usefulness in discriminating different components of the species complex and in evaluating a level of polymorphism within members of this complex. In a current study, 200 isolates, including 111 isolates of *L. biglobosa* and 89 isolates of *L. maculans*, were studied using primers derived from the 'repetitive extragenic palindromic' (REP) sequence, the 'enterobacterial repetitive intergenic consensus' (ERIC) sequence and the conserved repeated DNA element 'BOX'. In *L. biglobosa*, two polymorphic bands were found for REP and three polymorphic bands were found for both ERIC and BOX. Surprisingly, more variation was found for *L. maculans*, with five polymorphic bands for REP, twenty nine for ERIC and three for BOX. This polymorphism was not connected with any known character of two species in study. Isolates differing from by a substitution in ITS1-5.8S-ITS2 region did not form any specific group with a unique rep-PCR fingerprint. Different fingerprints were not specific to geographical location.

## **Development of a decision support system for control of stem canker of oilseed rape in Poland**

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**Abstract:** System for Forecasting Disease Epidemics (SPEC; System Prognozowania Epidemii Chorób) is a decision support system for improved control of stem canker of oilseed rape in Poland. The system is a joint initiative between the Institute of Plant Genetics PAS and DuPont Poland. Activities of the system are supported by several research organisations, such as the Institute of Plant Protection, Central Cultivar Testing Station, University of Rzeszow, Agricultural University of Poznan, Institute of Soil Science and Plant Cultivation and two commercial enterprises: Arenda in Charbielin and Agro-Fundusz Mazury in Drogosze.

Stem canker of crucifers is a serious disease of oilseed rape in Poland and worldwide. The disease is caused by the ascomycete fungi *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. SPEC aims to optimise fungicide sprays against stem canker. To achieve this, the system uses a series of seven day volumetric spore traps (Burkard Manufacturing, UK and Lanzoni, Italy) to monitor the concentration of fungal ascospores in the air. The system has been operating since 1 September 2004 with five spore traps operating during the 2004/05 season and ten traps in the 2005/06 season. The traps are located across the main oilseed rape growing areas of Poland, namely Lower Silesia, West Pomerania, Pomerania and west Varmia, Great Poland, Upper Silesia, the north part of the Opole region, the south part of the Opole region, the Carpathian Foothills, Mazuria with east Varmia and the area encompassing the Mazovia Plain and Lublin region. Data on ascospore release are published on the websites of the SPEC system ([www.spec.edu.pl](http://www.spec.edu.pl)) and DuPont Poland ([www.dupont.pl](http://www.dupont.pl)). The results are updated weekly from September to November and from March to May. Information is also distributed free of charge *via* e-mail, SMS text messages or fax to registered users.

Since the beginning of the 2005/2006 season, a network of sampling points to examine maturity of fungal fruiting bodies on infected straw has been established. Monitoring of pseudothecia maturity is performed in 44 locations and covers all provinces of Poland.

To investigate the efficiency of fungicide treatments an experiment has been done with the timing of application being based on spore sampling results. In parallel, monitoring is accompanied by two field experiments located in regions with different weather conditions located *ca.* 330 kilometres apart.

The target end users of SPEC are oilseed rape farmers, breeders, extension services and the commercial companies who distribute agrochemicals.

## SimMat, a new dynamic module of Blackleg Sporacle for the prediction of pseudothecial maturation of *L. maculans*/*L. biglobosa* species complex. Parameterisation and evaluation under Polish conditions

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**Abstract:** The dynamics of pseudothecial maturation is a key process of phoma stem canker epidemics. The prediction of ascospore showers, which strongly depends on the state of pseudothecial maturity, could be useful to help decision making for fungicide application. This paper presents the structure, the parameterisation, and the evaluation of SimMat, a model that represents the dynamic of *Leptosphaeria maculans*/*L. biglobosa* complex species for pseudothecial maturity, in Poland. The data used in this study were collected in Poznan over four years (1998, 1999, 2000, 2001). The model uses three input variables: average harvest date, mean daily temperature, and mean daily rainfall. SimMat uses the same concept of favourable day to pseudothecial maturation as in the Blackleg Sporacle, a model developed under Australian conditions. However, the criteria used to define a favourable day for pseudothecial maturation are different and the biological variability in the requirement of a number of favourable days to maturation has been introduced differently. A day is considered to be favourable for pseudothecial maturation if the mean temperature is below a given threshold ( $\theta_{\max}$ ) and if the accumulated rainfall over the last  $n_r$  days is greater than a threshold ( $r_{\min}$ ). SimMat assumes that the number of favourable days required for pseudothecial maturation follows a Gaussian distribution. SimMat has five parameters: the expected number of favourable days required for pseudothecial maturation,  $N_{FD}$ ; the standard deviation of the number of favourable days required for pseudothecial maturation,  $\sigma_{FD}$ ;  $\theta_{\max}$ ;  $n_r$ ; and  $r_{\min}$ . Firstly, the model was tested using parameter values established in Australian conditions (in the Blackleg Sporacle model), with addition of  $\sigma_{FD}$ , which was chosen to ensure that the simulated proportion of mature pseudothecia at harvest is null. Without a calibration, as expected, the quality of prediction was poor (Root Mean Squared Error of Prediction, RMSEP = 0.44). In order to parameterise SimMat for Polish conditions, each possible combination of parameters to be adjusted was tested using cross-validation (in all 32 combinations). The best quality of prediction was obtained by adjusting just two parameters:  $N_{FD}$  and  $\sigma_{FD}$  (RMSEP = 0.17; bias =  $-4.3 \cdot 10^{-2}$  for cross-validation over the four years). Fitting these two parameters to the whole dataset led to a Root Mean Squared Error of 0.15, and a bias of  $-4.4 \cdot 10^{-4}$ . Although this parameter estimation appears to be acceptable, the robustness of the model still has to be enhanced by increasing the size of the dataset used for parameterisation. Using this approach, the possibility of prediction of ascospore showers is discussed.

## Prediction of the date of onset of phoma leaf spot epidemics on oilseed rape in the UK

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**Abstract:** To reduce reliance on fungicides for control of oilseed rape diseases, Decision Support Systems (DSS) are being developed to target applications as part of an integrated disease management strategy. This paper reports on the development of an empirical model for predicting risk of severe phoma stem canker epidemics. Meteorological data are used to predict the date when incidence (% plants affected) of phoma leaf spot can be expected to reach 10%. This incidence has been suggested as an appropriate threshold to guide timing of fungicide applications against this disease to prevent pathogen spread from leaf to stem and the subsequent development of damaging stem cankers. The 10% phoma leaf spot forecast can be extended, using further meteorological and cultivar resistance rating data, to predict the date of canker onset, canker severity at harvest and subsequent yield loss.

## Studies on the contribution of cultivar resistance to the management of stem canker (*Leptosphaeria maculans*) in Europe

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**Abstract:** The development of phoma leaf spot and stem canker was investigated on a range of 7-10 cultivars at 9 sites in France, Germany, Poland, Sweden and UK in 2002/2003 and 2003/2004. The cultivars selected differed in their major gene and quantitative resistance to stem canker and included near isogenic lines of two cultivars without (Darmor, and Eurol) or with (Darmor MX, Eurol MX) major resistance gene *Rlm6* and their parent cultivars. The lines with *Rlm6* gave very effective control of phoma leaf spot and stem canker caused by *Leptosphaeria maculans*. At some sites, there was a significant increase in the incidence of *Leptosphaeria biglobosa* on leaves and stems on the *Rlm6* lines. There were differences in the severity of stem canker between the other cultivars, which reflected the differences in their quantitative resistance. Populations of *L. maculans* in Europe are known to have a high frequency of virulence to overcome resistance genes *Rlm1-4* and *Rlm9* and therefore quantitative resistance makes an important contribution to stem canker control. Strategies are required to ensure that the durability of resistance to stem canker is improved.

## Controlling the deployment of a new specific resistance Rlm7 to *Leptosphaeria maculans* in a small production area, in the Centre of France

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**Abstract:** Recently new oilseed rape cultivars resistant to stem canker have been made commercially available. Their efficiency against the pathogen *Leptosphaeria maculans* is mainly due to a new specific resistance gene Rlm7. However, such varieties could rapidly be exposed to a risk of resistance break down. To prevent such an event, an extension approach of deployment of resistance cultivars has been carried out in a small area (15 km<sup>2</sup>) in the Central region of France where stem canker pressure used to be high. This approach of durable management of genetics resistance has involved about twenty oilseed rape fields with a cropping ratio of Rlm7 resistant cultivars of about 5%. Observations are focused on technical practices, agronomic diagnosis, foliar and stem symptoms and population structure of *L. maculans*. A particular attention is paid to the emergence of leaf spot symptoms on the Rlm7 cultivars. These observations will be associated with extension recommendations for controlling stem canker to the farmers and local advisors. Evolution in time will be followed over four years. This paper presents results of the initial year.

## Effects of temperature on Rlm6-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*

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**Abstract:** Near-isogenic lines of *Brassica napus* lines with/without the resistance gene Rlm6 were used to investigate the effects of temperature on Rlm6-mediated resistance to *Leptosphaeria maculans*. Leaves were inoculated with ascospores of *L. maculans* carrying the corresponding avirulence gene AvrLm6. Inoculated plants were incubated at 15°C or 25°C. DarmorMX (with Rlm6) was resistant to *L. maculans* at 15°C but susceptible at 25°C, and Darmor (without Rlm6) was susceptible at both temperatures. On Darmor, large grey leaf lesions developed at both 15 and 25°C. On DarmorMX, small dark necrotic spots were produced at 15°C but large grey lesions were produced at 25°C. The incubation period of *L. maculans* (from inoculation to the appearance of first lesion) was longer on DarmorMX than on Darmor. The infection efficiency (number of lesions resulting from inoculation with 100 ascospores) was greater on Darmor than on DarmorMX at 15 and 25°C. Further characterisation of the Rlm6-mediated resistance showed that the resistance was effective at 25°C if inoculated plants were pre-incubated at 15°C for 5 days before moving them to 25°C, but not if inoculated plants were pre-incubated at 15°C for only 2 days before moving them to 25°C. We conclude that temperature affects Rlm6-mediated resistance to *L. maculans* in *B. napus* leaves.

## ***In vitro* comparison of fitness of *AvrLm1* vs. *avrLm1* isolates of *Leptosphaeria maculans***

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**Abstract:** One of the main diseases of rapeseed (*Brassica napus*) in Europe and across the world is stem canker of crucifers, caused by *Leptosphaeria maculans* (*Phoma lingam*). Stem canker contributes to great losses of winter oilseed rape yield. The newly introduced resistance of cultivars can be overcome by the pathogen within a few years.

Experiments in controlled temperature environment were performed to compare growth rate of near isogenic isolates of *L. maculans*, differing with avirulence gene towards resistance gene *Rlm 1*. The studies were done using six avirulent isolates (*AvrLm1*) and six virulent isolates (*avrLm1*). The isolates were cultivated on six media: Malt Extract Agar (MEA), Luria Broth Agar (LBA), Water Agar (WA), Potato Dextrose Agar (PDA), Campbell's V8 Juice Agar (V8) and Synthetic low Nutrient Agar (SNA). Twenty ml of medium was poured on a 9 mm diameter Petri dish. Experiments were performed in controlled environment at 20 °C and 12 hour photoperiod. Three replicates for each variant (isolate x medium) were used. Growth rate was measured every two days from 3 days to 3 weeks after depositing the fungus on the medium.

Statistically significant differences between growth rate of virulent and avirulent isolates were observed at the early phase of the experiment – three days after subculturing fungi on media V8, PDA and LBA and 5 days after subculturing on V8, with avirulent isolates growing faster than virulent ones. The fastest growth rate was observed on V8 medium and the slowest growth was on MEA medium. One week after depositing an agar disc on a medium, differences between avirulent and virulent isolates were no longer significant, but avirulent isolates tended to grow faster on most of the media.

The experiment confirms the hypothesis of loss of fitness connected with the gain of new virulence of an isolate. Similar results were previously reported for *AvrLm4* vs. *avrLm4* isolates of *L. maculans*, although the differences between two groups of isolates were mostly observed *in vivo*.

## Detection and quantification of *Leptosphaeria maculans* in the leaf petiole of *Brassica napus*

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**Abstract:** In controlled environment experiments winter oilseed rape (*Brassica napus*) cultivars Canberra and Courage did not differ significantly in leaf lesion size and systemic growth in petioles, measured after inoculation of leaves with conidia of *Leptosphaeria maculans* isolate ME24 (transformed to express green fluorescent protein). In field experiments (2003/2004) quantification of *L. maculans* within petioles of six doubled haploid (DH) lines of oilseed rape derived from Canberra x Courage showed differences in the number of colony forming units obtained from maceration of petiole parts. Differences between lines did not relate to differences in stem canker severity at harvest.

## Impact of rape stem weevil, *Ceutorhynchus napi*, on the early stem infection of oilseed rape by *Phoma lingam*

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**Abstract:** The rape stem weevil, *Ceutorhynchus napi* Gyll., is one of the most destructive insect pests of winter oilseed rape (*Brassica napus*) in Central Europe. Females deposit their eggs into the top of elongating stems mainly in March and April, thereby inducing substantial changes of stem growth like swelling, deformation and splitting. Moreover, wounding of plant tissue by egg-laying females and stem-mining larvae is thought to predispose the stems to early secondary infections by fungal pathogens, particularly *Phoma lingam*.

To test the hypothesis that *C. napi* is capable of increasing early stem infections by *P. lingam* at the time of oviposition, the relation between the number of oviposition punctures on stems and the incidence of *P. lingam* within the stem pith was determined. In addition, controlled experiments were done under standardised conditions in the laboratory to determine whether the fungal inoculum is being transmitted by *C. napi* females. Results showed that the severity of *P. lingam* stem infection increased significantly with increasing number of oviposition punctures of *C. napi*. Experiments in the laboratory provided evidence for the transmission of *P. lingam* by *C. napi*.

## The complete-genome sequencing project of *Leptosphaeria maculans*

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**Abstract:** Until now *Leptosphaeria maculans*, which is responsible for major losses on oilseed rape crops, has been poorly characterized at the genomic level. This fungus belongs to the dothideomycete class, which encompasses numerous important phytopathogenic fungi, such as the wheat pathogen *Stagonospora nodorum*, the apple scab agent *Venturia inaequalis*, and the oilseed rape pathogen *Alternaria brassicicola*. The species belonging to this group share some common life traits, such as their mode of infection and colonization, the frequent production of secondary toxic metabolites, or the frequent occurrence of a sexual stage in their life cycle. They also differ by their host range, and include pathogens of both monocots and dicots. The whole genome sequence of *S. nodorum* was very recently released, while genome initiatives for the whole sequencing of *L. maculans* at Genoscope, and *A. brassicicola* in the USA were recently successful. Therefore the complete sequences of three closely related phytopathogenic fungi will soon be available. In this communication, the *L. maculans* genome initiative will be presented, along with the perspectives in terms of new fields of research offered by these sequence data. In particular, comparative genomic studies between *L. maculans*, *S. nodorum* and *A. brassicicola* are expected to allow identification of pathogenicity genes either common or specific to each of these plant/fungus interactions. Also, the complete sequence of *L. maculans* will allow the development of a large set of new micro- and minisatellite markers. These will be used both to develop population genetic studies and therefore help answer unresolved epidemiological questions, and to speed up map-based cloning of avirulence genes, and therefore help develop molecular markers of specific races of the pathogen, which will be useful for a better management of specific resistance genes. The complete sequence data will also speed up the molecular analyses of pathogenicity genes identified either via random insertional mutagenesis, or via a systematic genome-wide search of candidate genes, including avirulence genes, and genes involved in toxin production.

## Significance of *Leptosphaeria maculans* and *Sclerotinia sclerotiorum* incidence of winter rapeseed in the Czech Republic

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**Abstract:** Phoma root rot (causal agent *Leptosphaeria maculans*) and white mold (causal agent *Sclerotinia sclerotiorum*) are serious pathogens of oilseed rape in the Czech Republic. In test years we confirmed, the strong dependence of disease occurrence on the position of rape in crop rotation system, weather conditions, characteristics of locality and growing technology (fungicidal treatment). In 2000 and sporadically also in 2001, the occurrence of visual symptoms of white mold attack was observed before fungicidal treatment in early spring and at the beginning of fast prolongation growth especially on plants having the overgrown symptoms at autumn

In growing season 2001/2002 heavy infestation of oilseed rape by phoma root rot was observed. In field trials, the mean disease infestation was 57 % on treated plots in comparison with 67 % on untreated controls. This result corresponds with the low effect of fungicidal treatments in autumn and early spring on increase of seed yield (+3.6 %). Infestation of oilseed rape by white mold in 2002 was about 30 %. Targeted fungicidal treatment against this disease at the full flowering stage increased seed yield by 7.65 %.

In growing season 2002/2003, heavy damage of roots occurred as a result of temperature variation in early spring. The surface of cracked roots had deep furrows. This caused the difficult evaluation of phoma root rot infestation. It was not possible by visual evaluation to differentiate if the furrows on roots were caused by the weather conditions or by fungal disease. Roots of plants from plots with lower crop density and plots untreated in autumn were most damaged. Occurrence of phoma root rot on rapeseed root crown in autumn 2002 was not observed. Infestation of phoma root rot before harvest on untreated controls was in Opava 85 %, in Šumperk 73 %. Average infestation on plots treated in autumn was in Opava 78 %, in Šumperk 50%. Infestation by white mold was not observed in Opava, in Šumperk was 7 % only at untreated crops.

Sporadic phoma infection was observed at test localities Opava and Šumperk at autumn 2003; the infection was observed on the leaves only. In 2004 (BBCH 85 - the seeds becoming brown) significant infection, appearing as dark brown or black spots at the stem base was observed. The infection of roots was minimal. The infection of controls was about 50% at Opava and 15% at Šumperk. The mean infection of crops treated by combined autumn and spring treatments: in Opava about 42% and at Šumperk 4 - 8 %. The infection of *Sclerotinia* on untreated control crops was Opava 21%, Šumperk 39%, the mean infection of treated variants was: Opava 5 %, Šumperk 18.5 %. First symptoms of infection were observed in the second half of June.

In autumn 2004, phoma root rot symptoms were observed only on leaves, with sporadic and medium occurrence. The dependence of disease on position of oilseed rape in the crop rotation and on locality was monitored. A similar situation was also observed in spring 2005. Phoma root rot incidence on stem bases and roots of plants has not been realized so far. Infestation by white mold in this year was very high. The early start of plant infestation was recorded, occurrence of necroses, mycelia and sclerotia was observed (24th May 2005 - Slapy u Tabora). Types of infestation were different: infestation of roots and stem base was similar to 2000 and 2001. High occurrence of stem lesions to the middle or in the second third of the plant was also observed. Mostly the main stem is infected. Infestation of lateral branches and siliques is sporadic. Evaluation of infestation by both pathogens in 2005 has not been completed and the exact data are not yet available.

## **Development of a new disease and yield loss related forecasting model for Sclerotinia stem rot in winter oilseed rape in Germany**

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**Abstract:** *Sclerotinia sclerotiorum* the causal pathogen of Sclerotinia stem rot is a common problem in winter oilseed rape production in Germany. Fungicide treatments against the disease are routinely carried out during flowering. Prediction systems can help to reduce the costs of oilseed rape production by predicting the necessity and exact timing of a fungicide application. The major aim of prediction systems is to reduce fungicide use and to avoid crop loss. A new prediction model for Sclerotinia stem rot has been developed, involving weather data, field specific factors and damage thresholds. Data from different field trials of the state extension services from 1994-2004 in Germany were analysed to investigate the impact of different field factors as cultivar, soil type, sowing time, crop rotation, fertilization and tillage. In laboratory experiments, the effect of the microclimate in plant cover was determined. The examination of the field specific factors showed that the disease incidence is dependent on various factors which are crop rotation, sowing time, cultivar and soil type. The temperature range for an infection with ascospores is between 7 - 22°C. The new simulation model SkleroPro was validated by a retrospective calculation using data of different field trials of the state extension services with meteorological data of the last ten years with regard to the economic efficiency of fungicide applications against *S. sclerotiorum*. SkleroPro is the first disease forecasting model for a Sclerotinia disease providing a crop-loss related, field-site and time-point specific decision support. SkleroPro will be made available to growers via the internet portal 'ISIP' in 2006.

## Petal test – Success and disappointments in sclerotinia stem rot forecasting in Poland and China

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**Abstract:** Sclerotinia stem rot or white mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most damaging fungal diseases of oilseed rape worldwide. In China, the worlds largest producer of rapeseed, this disease causes considerable yield loss. In Poland, sclerotinia stem rot and stem canker of crucifers are the two most damaging diseases of winter oilseed rape (*Brassica napus* L. forma *biennis*), whereas spring oilseed rape (*B. napus* L. forma *annua*) is mostly infected by black spot and sclerotinia stem rot. Primary sources of plant infection are ascospores produced in apothecia. These fruiting bodies of the perfect stage germinate in the spring on sclerotia, the dormant mycelium formed in black lumps or nodules, inside stems infected with the pathogen. At first, ascospores infect petals, which then fall down onto leaf surfaces and cause disease within the infected plant tissue.

The petal test serves as the basis of all forecasting systems for sclerotinia stem rot on oilseed rape. The test uses selective media with pH indicators, that change the colour of the medium when it is acidified with oxalic acid, secreted by *S. sclerotiorum* during its growth.

We studied discolouration of Steadman's medium supplemented with different pH indicators which change pH in the range 4.5 – 5.5. Based on experiments using mycelial discs obtained from pure cultures of *S. sclerotiorum*, we have chosen bromophenol blue and bromocresol green as two supplements showing clear and easily assessable results. These two reagents were subsequently used for tests with petals of different cultivars and field situations in China and Poland.

## **Comparison of results of a petal test and natural infection of oilseed rape with sclerotinia stem rot in Poland**

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**Abstract:** In spring 2005, petals of oilseed rape (*Brassica napus* L.) were sampled from flowering plants from commercial fields and cultured on BB and BCG media, supplemented with bromophenol blue or bromocresol green pH indicators. Samples were collected 24 times from 19 different fields located in different places throughout Poland. Some fields were treated and some were untreated with fungicides. Each time, a field sample consisted of 30 Petri dishes containing 20 ml of a given medium with 7 petals uniformly placed on each dish. Petals from the same inflorescence were placed on the same Petri dish, with one petal from a randomly chosen flower on BB medium and a petal from the same flower on BCG medium. Petals were placed on a Petri dish in the same order on all plates, which allowed us to study the infection of the same flowers and inflorescences on two media in parallel. Sampling was performed over 20 days in May, with one field sampled 3 times, every nine days. Sampling was also done from four separate replicates of control fields of two experiments using different cultivars in one location. There were also studies to compare the infection of petals of inflorescences located in upper and lower parts of the oilseed rape canopy, as well as studies comparing the infection of different parts of flowers and also studies of infection of leaves and siliques. For comparison, petals were placed on media directly in the field and a few hours later, in laboratory conditions.

In general, upper flowers were more infected than flowers which were collected from lower parts of the canopy. Petals were the most infected parts of flowers. The use of fungicides decreased petal infection by half. Considerable differences were found between the two media on intermediate days of observation (day 3 to day 5), but the final results (obtained on day 4 to day 6) were comparable, with results slightly higher for BCG than for BB medium. The highest infection level of petals was observed at the beginning of the flowering period. There were no considerable differences concerning the final result, irrespective of the place used to put petals on the media. Two different cultivars at one location were infected with the same percentage. At final assessment, the percent infection of petals varied from 19.9% to 90.5%.

## Studies on the germination of sclerotia and formation of apothecia of *Sclerotinia sclerotiorum*

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**Abstract:** A method has been developed and optimized to study germination of sclerotia and apothecia formation under continuous temperature conditions. This method allowed differentiation of the origin of 33 different sclerotia isolates and also the behaviour of germination of the origin of *S. sclerotiorum* isolates. Wheat kernels are highly suitable for production of sclerotia. Development of apothecia was optimal under conditions of 10°C and 500 lux and sclerotia germinated after 2-3 months of incubation. Germination of sclerotia and formation of apothecia could be differentiated according to the origin of sclerotia.

In general, sclerotia of isolates originally coming from warmer areas germinated more readily. Sclerotia germinated in different substrates, such as 'Einheitserde', sand and vermiculite. Germination of sclerotia which had been developed on especially nutrient rich or nutrient poor media was impaired. One apothecium produced more than 1 million ascospores and was viable more than 15 days at 10°C and 500 lux.

## Importance of application mode for the efficacy of CONTANS (*Coniothyrium minitans*) in biocontrol of *Sclerotinia sclerotiorum*

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**Abstract:** CONTANS WG®, a biofungicide containing the living conidia of *Coniothyrium minitans* (CM), is registered in Germany for biocontrol of *Sclerotinia sclerotiorum* since 1998. Its main application is in oilseed rape for control of stem rot. Here, we report laboratory and field experiments conducted in order to evaluate the biocontrol efficacy of CM under practice conditions. CM was capable of degrading living sclerotia of *S. sclerotiorum* at soil conditions ranging from 10 to 90% water holding capacity (WHC) and from 1 to 29 °C. Significant reduction in antagonistic activity only occurred at dry conditions below 20% WHC and at soil temperatures below 5 °C. At conditions from 13 to 25 °C and 20 to 90% WHC, sclerotia were degraded within 8 weeks at rates of 90% or more. However, preservation of CM conidia increased at lower soil temperatures. In mini-plots in the field, the antagonist initially applied at a rate of 3.3 x 10<sup>5</sup> cfu g<sup>-1</sup> soil, was still detectable after 72 months at rates sufficient to effectively reduce the survival of sclerotia to levels between 6.6 and 0.2%. The minimum rate of CM in soil for a more than 90% degradation of sclerotia within six months was 1.9 x 10<sup>3</sup> cfu g<sup>-1</sup> soil. In order to optimise the biocontrol efficacy of CM, the timing of application within a rapeseed-cereal crop rotation was varied by either applying CONTANS WG® on the oilseed rape stubble (SR), on the stubble of the previous barley crop (SB) or on the soil prior to sowing of oilseed rape (PS). Appearance of apothecia was recorded in the three following seasons. Three years after application, no apothecia were found in SR, while in SB and PS apothecia still occurred at elevated rates. Further laboratory studies revealed complete inhibition of CM conidia germination in unsterile natural soil, clearly implying a lack of potential of the antagonist to reach sclerotia at any distance apart. Therefore the direct targeting of this antagonist appears essential for realizing its biocontrol potential.

## Verticillium wilt on Brassica oilseed crops

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**Abstract:** This presentation on Verticillium wilt in Brassica oilseed crops in Sweden aims to give a historic background to the disease problem as well as to present recent work on both the disease causing organism, its biology and plant breeding actions taken. The severe disease incidence present in Skåne and Östergötland is most likely due to the very intensive cultivation, in some places monoculture, of Brassica oilseed crops between 1945 and 1955. Molecular studies of Verticillium isolates from diseased plants have revealed that *Verticillium longisporum* is the disease causing organism. *V. longisporum* is closely related to *V. dahliae* and *V. albo-atrum* and can easily be confused with *V. dahliae*, especially when considering morphological characters. The overall colonization pattern of *V. longisporum* in oilseed rape seems to be in concordance with earlier reports concerning *V. dahliae* in a range of host species. However, *V. longisporum* preferentially infect members of Brassicaceae. The dispersal, propagation, and long-term survival of this pathogen is mediated through the microsclerotia. Analyses of soil samples from Skåne and Östergötland have revealed high levels of microsclerotia and a presence of *V. longisporum*, *V. dahliae* and *V. tricorpus* in the soil. All *Brassica napus* germplasm is susceptible to Verticillium wilt. Thus, gene bank accessions of both *B. oleracea* and *B. rapa* origin have been evaluated to identify new resistant material. Enhanced levels of resistance were found within both species and these novel genotypes are now being incorporated in *B. napus* breeding programmes. This widening of the gene-pool of *B. napus* will also be of great value to future hybrid breeding programs.

## Verticillium wilt in Sweden – Incidence, field scoring and importance

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**Abstract:** The aim of this paper on Verticillium wilt is to show the difficulties with the assessment of the disease. Often plants are infected not only by Verticillium wilt but also with *Phoma lingam*. Therefore, determination of disease severity by visual assessment is uncertain.

Verticillium wilt, caused by *Verticillium longisporum*, is a very serious disease in some regions of Sweden. The problem is mainly due to intensive oilseed cultivation in the early 1950s, sometimes even as a second crop. The high inoculum density of microsclerotia has been maintained by weeds and crop rotation. A crop rotation with four years between every oilseed crops has been practiced since the late 1950s. The yield of winter oilseed rape in Sweden has increased very slow since the 1970s and has varied between 22-36 dt/ha. There are many reasons for this big variation in yield, but Verticillium wilt is definitely one explanation. The impact on yield from Verticillium wilt varies very much between different years. When infected plants show visible microsclerotia and senescence, the yield can be reduced by 30-50 %. But in other years, when only bronze-coloured symptoms appear, the yield is little affected.

## **Determination of risk-factors for the occurrence of *Verticillium longisporum***

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**Abstract:** In a two-year monitoring experiment the occurrence of *Verticillium*-wilt in oilseed rape could be confirmed for all surveyed regions in Germany. However, there are some regional differences in the infestation frequency. In north- and northeast-Germany, traditionally areas where oilseed rape production is highly-intensive, the disease was more frequently diagnosed. The data obtained from the determination of risk-factors showed a clear effect by the percentage of infected oilseed rape during crop rotation with *V. longisporum*. By extending the oilseed rape crop rotation to longer intervals (more than three years) and an additional growing of non-host plants in close cereal/oilseed rape crop rotations, the risk of an infestation by this pathogen could be lowered. In addition, a longer rotation period between oilseed rape crops had a positive effect on subsequent yield. However, the importance of conservation tillage systems as a risk-factor could only be basically confirmed and further investigations are still required. The same conclusions were reached with respect to differences in varietal susceptibility which recently became apparent. There was no effect associated with the application of organic nitrogenous fertilizer, soil type, pH-value and the application of fungicides on the occurrence of *Verticillium*-wilt disease.

## ***Verticillium longisporum* in winter oilseed rape – Impact on plant development and yield**

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**Abstract:** In the last ten years, an increasing incidence of *Verticillium longisporum* has been observed in the German winter oilseed rape production. The impact of this pathogen on yield and plant morphological parameters, the disease development as well as the reaction of different cultivars is still not known. In field trials done over two-years, the yield effect of *V. longisporum* in relation to disease severity was investigated in plots artificially inoculated with different amounts of infested rapeseed straw. Disease development was investigated on plant samples during the whole growing season using an ELISA test. In agreement with the visual disease assessment in the field, fungal spread in the plants could not be detected before the beginning of maturity. In glasshouse experiments, plants of a susceptible and a moderately susceptible cultivar were inoculated using two different methods (root dip inoculation or microsclerotia inoculation). In contrast to the lack of symptom development in the field, an intense stunting effect was observed in infested plants two weeks after inoculation. Disease severity was higher in the susceptible cultivar and fungal spread was faster and more extensive than in the moderately susceptible cultivar. For plants inoculated with microsclerotia, fungal spread was delayed compared to the root dip inoculation.

## **Comparative histological studies on the interaction of *Verticillium longisporum* and *V. dahliae* with roots of *Brassica napus***

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**Abstract:** *Verticillium longisporum*, the causal agent of Verticillium wilt on oilseed rape (*Brassica napus*) differs from *V. dahliae* in being host-specific to Brassica crops. In order to compare the colonisation and infection processes of these two fungi on roots of *B. napus*, the green fluorescent protein (GFP) was used to label them. Additionally, a conventional fluorescence dye was applied in order to compare the suitability of these techniques for plant-pathogen interaction studies. Using confocal laser scanning microscopy, the colonisation and infection processes were analysed in detail and the following aspects could be observed: (i) the first steps of contact between root and fungi take place at the root hairs, (ii) the preferred colonisation sites on the root surface for *V. longisporum* are the grooves along the junctions of the epidermal cells, (iii) *V. longisporum* does not show a preferation for any specific infection sites, such as wounded tissue or sites of emergence of secondary roots and does not form any specific infection structures, like appressoria. (iv) In contrast to this, *V. dahliae* shows an undirected growth on the root surface and very quickly forms resting structures. Until now, it is not proven whether *V. dahliae* is able to penetrate into the roots or not. Further studies on this non-pathogenic interaction are in progress.

## **Analysis of systemic signals in the xylem of *Brassica napus* infected with *Verticillium longisporum***

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**Abstract:** *Verticillium longisporum* (VL) is a soilborne, pathogen with host specificity on the Brassicas. Typical symptoms on oilseed rape are premature ripening and stunting under greenhouse conditions. Compared to other *Verticillium* diseases no wilting occurs on crucifers. There are signalling molecules assumed to cause disease symptoms by altering the developmental physiology of the host plant. A likely candidate is nitric oxide (NO). NO is reported to induce various physiological alterations, such as de-etiolation, inhibition of hypocotyl growth and ethylene production. Therefore, we examined the induction of stunting by application of the NO donor sodium nitroprusside (SNP). SNP, applied over a period of 28 days (three times per week) at different concentrations (10, 50, 100 µM), induced stunting on 'rapid cycling rape' in the climate chamber. Plant growth was reduced in each SNP treated variant and plants were smaller than the ones infected with VL, with the greatest growth reduction at 10 µM SNP. However, after 30 days of growth a difference in shoot length was no longer detectable. At present, it is unclear whether the nitric oxide level in the plant is (i) up- or down-regulated during infection with the vascular pathogen, (ii) whether the pathogen produces NO scavenging proteins, or (iii) whether VL directly alters the NO metabolism of the plant so that NO is no longer produced. First results indicate that the NO level in VL infected plants is lower than in uninfected plants. As NO is known to delay senescence, a lower NO level may result in premature flowering, an effect observed on field-grown oilseed rape upon infection with VL. Hence the physiological effects of VL infection and NO application were analysed with relation to NO levels in plant tissue. Therefore, we developed an indirect NO analytical method by using an NO specific scavenger, carboxy-PTIO, which reacts with NO in a stoichiometric manner. NO can be detected by measuring the specific reaction product (cPTI) with HPLC mass spectrometry. We extracted xylem sap from infected plants with a pressure bomb and used the samples for the quantitative analysis of NO. Alternatively, measurements were conducted with electron-resonance-spectroscopy (ESR). Here, NO is scavenged and stabilised by 'spin trap' molecules and measured due to its properties as a free electron radical, by recording the changes in a magnetic field, which creates specific signal peaks. Since NO can derive from different sources e.g. nitrate/nitrite reductase or a presumed NO synthase, the level of nitrite in infected and uninfected plants in relation to NO levels was recorded. In a further analytical approach NO is indirectly determined by using a nitrite specific fluorescence dye diaminonaphthalene (DAN), whose derivate NAT can be detected by fluorescence- HPLC.

## Consequences of oilseed rape infection with phytoplasma like organisms

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**Abstract:** On winter oilseed rape plantations in Poland and in other countries, some “shaggy” looking plants can sporadically be observed. These deformations are usually caused by phytoplasmas, that settle the sieve tubes (phloem) of affected plants. Morphological changes of inflorescences and single flowers was described for the first time by Schmidt (1955). In the following years other authors: Lehmann (1969), Horvath (1969), Gundersen et al. (1994) described the etiology and symptomatology of the pathogen which was responsible for growth aberration and irregular organogenesis. Initially, it was thought that yellow type viruses were responsible for such a situation (Valenta & Musil 1963). But further investigations excluded this hypothesis and pointed at mycoplasma-like organisms as the actual perpetrators (Sears & Kirkpatrick 1994, Gundersen et al., 1994). To distinguish them from bacterial animal pathogens, so called mycoplasmas, bacteria that settle on plants was termed “phytoplasmas”. The vectors of phytoplasmas are insects of *Jasside* family. In the insect the pathogen occurs as inclusions. At present the identification of possible phytoplasmas and their pathotypes is possible with the use of molecular techniques.

Isolation of total DNA was done by the Doyle and Doyle method (1990). For PCR analysis two pairs of universal primers for identification of *Phytoplasma* were used: rU3/fU5 that amplifies about 880 bp (Lorenz et al., 1995) and rA16/fA16 (Ahrens & Seemüller 1992, Schneider et al. 1993) that amplifies 558 bp. As a standard of phytoplasma from group AAY (Kamińska & Korbin, 1999) DNA of infected plant *Catharanthus roseus* L. was used (Kamińska et al., 1996).

Additionally from some of less infected plants seeds were received, which were sown again. After the vernalisation period, only “shaggy” plants were isolated in the flowering phase and put to haploidisation (Cegielska-Taras & Szała 1997). Observations of haploids development were made from seedling stage (*in vitro*) to grown-up stage (*in vivo*). The same treatment was applied to diploid plants received from seeds that were descendent from self-pollinated “shaggy” plants.

## Effect of temperature on development of lesions caused by *Alternaria* spp. on leaves of oilseed rape

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**Abstract:** Leaves, stems, pods and seeds of oilseed rape (*Brassica napus* L.) can be infected by species belonging to the genus *Alternaria*. Pathogenic species are responsible for black spot disease which can cause considerable problems, especially in cases of pod infection before harvest. Heavy infection of pods may result in poor quality of seeds used for processing, poor storage and low quality of sowing material. In Poland, black spot is regarded as an important problem in cultivation of both spring and winter oilseed rape.

In our experiment we have used 12 isolates belonging to six following species: *A. brassicae* (3 isolates), *A. brassicicola* (3), *A. alternata* (3), *A. radicina* (1), *A. porri* (1) and *A. dauci* (1). The aim of this experiment was to compare development of disease symptoms on leaves of oilseed rape at different temperature regimes. Experiments were performed in a controlled environment at 16°C, 18°C and 22°C. Fungal isolates were cultured on SNA medium, that allowed to form numerous spores. Symptom development was studied on the third and fourth leaves of cv. Bosman. Leaves were cut and placed in plastic containers, with petioles wrapped up in filter paper soaked with distilled water. Special supports prevented contact of leaf surfaces with the wet paper. Leaves were inoculated with 6 mm diameter agar discs placed in the middle of each half of a leaf. Each isolate was placed on intact leaves and on leaves wounded with a needle. Each variant (isolate x temperature x leaf treatment) had twelve replicates. Experiments were run for two weeks. Symptoms resulting from inoculation were scored every three days. Discolouration of a leaf was measured in two perpendicular directions: along the side vein and across the leaf. Measurement of symptoms on leaf was done separately for black, yellow and light green zone.

The largest symptoms on leaves were caused by *A. brassicicola* with the mean size of the whole leaf symptom reaching 29.5 mm for wounded leaves and 22.4 mm for intact leaves (observation 11 days after treatment). Symptoms caused by *A. brassicae* were about half the size of symptoms caused by *A. brassicicola*. Symptoms produced by other species were negligible or non existent when the leaf surface was not wounded prior to depositing of an inoculum. Symptom development greatly depended on temperature, with the smallest spot sizes at 16°C and the largest symptoms at 22°C. Wounding speeded up the infection process and resulted in large symptoms on leaves.

## **The incidence of *Alternaria* spp. on seeds of chosen population and hybrid oilseed rape cultivars**

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**Abstract:** Often in Poland, on leaves, stems and siliques of oilseed rape plants, symptoms of black spot are observed. The casual agents of black spot are fungi of the genus *Alternaria*, mainly *Alternaria brassicae*, *Alternaria brassicicola* and *Alternaria alternata*. Often from diseased siliques diseased seeds are obtained. Spores and mycelium of *Alternaria* spp. are present on the seed surface or the mycelium cover completely the seed surface.

In our investigation, the main aim was to estimate the occurrence of *Alternaria* fungi on 16 cultivars of oilseed rape (8 population and 8 hybrid cultivars). Surface disinfected and nondisinfected seeds were incubated on potato dextrose agar (PDA) and on malt extract agar (MA). After 8-9 days of incubation (at 18 °C and 12 hours photoperiod) the incidence of fungi were observed and identification was performed. The disinfected seeds incubated on both medium were little infected (mean 2.3 % of seeds). Of the obtained isolates, 1.4% were fungi from the genus *Alternaria*. The most frequently observed was *A. alternata* and *A. brassicicola* and to a lesser extent *A. brassicae* (mainly on PDA medium). From nondisinfected seeds 16 % on MA and 10% on PDA isolates of fungi were obtained. The incidence of fungi from genus *Alternaria* on MA medium reached 50% of seeds (6.7% – *A. alternata*, 1.2% – *A. brassicicola*), and on PDA over 90% ( 7% – *A. brassicicola*, 2% – *A. alternata*). On these seeds *A. brassicae* was absent. The occurrence of *Alternaria* spp. on investigated oilseed rape cultivars were similar in the case of seed disinfection and slightly different in case of nondisinfected seeds.