Eesti Maaülikool
Estonian University of Life Sciences

INTEGRATED PLANT DISEASE MANAGEMENT IN SPRING BARLEY AND OAT PRODUCTION

INTEGREERITUD TAIMEKAITSE KASUTAMINE ODRAL JA KAERAL

PILLE SOOVÄLI

A Thesis
for applying for the degree of Doctor of Philosophy in Plant Protection

Väitekiri
Filosoofiadoktori kraadi taotlemiseks taimekaitse erialal

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals:

I Sooväli, P., Koppel, M. 2003. Genetic control of oat rust diseases. Agronomy Research 1: 245-251.

II Sooväli, P., Koppel, M. 2010. Efficacy of fungicide tebuconazole in barley varieties with different resistance level. Agriculture and Food Science 19: 34-42.

III Sooväli, P., Koppel, M. 2011. Timing of fungicide application for profitable disease management in oat (*Avena sativa* L.). Zemdirbyste=Agriculture 98(2): 167-174.

IV Sooväli, P., Koppel, M. 2008. Influence of fungicides and variety resistance on fungal flora of barley grain. Zemdirbyste=Agriculture 95(3): 158-165.

V Sooväli, P., Kangor, T., Tamm, I. 2010. The incidence of fungal diseases in oat leaves and yields as affected by fertilizer and chemical inputs in Estonia. Agronomy Research 8: 475-480.

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The contributions of the authors to the papers:

Paper	Idea and study design	Data collection	Data analysis	Manuscript preparation
I	PS, MK	PS	PS	All
II	MK, PS	PS	PS, MK	All
III	MK, PS	PS	PS, MK	All
IV	PS, MK	PS	PS	All
V	TK, IT	PS, TK	PS, TK	All

PS – Pille Sooväli; TK – Tiia Kangor; MK – Mati Koppel; IT – Ilmar Tamm;
All – all authors of the paper

ABBREVIATIONS

BBCH	A scale used to identify the phenological development stages of a plant
EMODN	The European and Mediterranean Oat Disease Nursery trial
IPM	Integrated Pest Management
GS	Growing stage
NORBARAG	Nordic-Baltic Resistance Action Group
PC-P	Personal Computer–Plant Protection based diseases forecasting system
T	treatment

1. INTRODUCTION

In 2010, the spring barley growing area in Estonia was 103.7 thousand ha and the oat growing area was 30.3 thousand ha, occupying 62% and 11%, respectively, of the total cereal growing area (www.stat.ee). Both crops are often grown as low input crops on productive land and in Estonian conditions both crops are inexpensive compared to wheat or oilseed rape. Therefore, in fungicide use, more attention should be paid to the concept of minimizing the input costs in barley and oat production.

Nowadays, fungicides are essential in agricultural production where they are used to control plant diseases during development of the crop and to increase its productivity; however, they constitute a potential risk to human health through fungicide residues in the environment (De Costa and Bezerra 2009). Fungicides, which are active against pathogens that have already infected the crop, tend to have a higher risk of pathogens developing resistance to the fungicide but a resistant pathogen is less sensitive to the action of the fungicide.

The serious problems in spring barley and oat production in Estonia are yield losses caused by infection by foliar diseases and the high cost of disease control in relation to the price of harvested grain. Therefore, it is important to find ways to lower production costs. Fungicides and varietal resistance are two main components, which influence disease management in spring barley and oat. Intensive protection is important for crop yields where prophylactic treatments at prearranged dates or stages of development have increasingly become part of the cereal growing system (Harris 1984). More economical is the integrated plant protection system, where fungicides are applied whenever the disease achieves a threshold incidence within a given crop. Considering the cost of fungicides, the tendency in future will probably be towards managed disease control or the development of more reliable disease-forecasting techniques (Cook 1984).

It is important to find efficient and economical ways to avoid foliar diseases in spring barley and oat by using optimal fungicide strategies according to the specific field situation. The current thesis covers studies on the possibilities of combining resistance of varieties and the use of fungicides to effectively control the main diseases on spring barley and oat. The influence of nutrition regimes on disease incidence and economic return of disease control are other aspects of the current thesis. Until now, there have been no studies in these aspects in Estonian conditions and only a relatively small number of studies have been made in similar areas in the whole Nordic-Baltic region. In this regard the current study provides novel scientific information on effective use of reduced fungicide dosages in disease control on spring barley and oat. The results of current research have high potential of applicability in farming practices in lowering environmental risks associated with pesticide use, and at the same time in increase of profitability of cereal production.

2. REVIEW OF THE LITERATURE

2.1. Major fungal pathogens in spring barley

Net blotch is caused by *Pyrenophora teres* (Drechsler, am *Drechlera teres* Sacc. Shoem) and spring barley (*Hordeum vulgare* L.) is the only known host of this pathogen. The fungus overwinters either as seed-borne mycelium or as perithecia in infected barley residue. During cool, humid weather, conidia are produced from mycelium of the infected seed and residue from where the spores are wind disseminated to healthy plants. The symptoms of the disease are net- and spot-type lesions whereas the development of different lesion types depends on barley genotype and pathogen strain. A typical characteristic of net blotch is a sudden increase in the production of conidia following a prolonged, steady increase in conidia proliferation as the weather remains cool and moist. Infection of barley leaves is greatest when humid conditions persist for 10–30 hours or longer and the optimal temperature range for infection is 15–25 °C (Motovilin 2000; Prigge *et al.* 2004).

Spot blotch is caused by *Cochliobolus sativus* ((Ito & Kurib.) Drechsler, am *Bipolaris sorokiana* (Sacc.) Shoem. Syn. *Helminthosporium sativum* P.K. et B). The disease agent is seed-borne and overwinters as mycelium in plant residue or as conidia in the soil. In the spring, conidia are produced on plant residue and are windborne to barley seedlings. Longer than a 16 hour-period of warm moist weather is conducive to epidemic development. Early and heavy infections of the flag leaf result in the greatest losses in yield because heavily infected leaves will dry up and heads may not emerge completely or kernels are poorly filled (Nyvall 1979; Zillinsky 1983; Mathre 1997).

Genetic variation and virulence of the net blotch pathogen population have been thoroughly studied in Finland, where *P. teres* population has changed very little in recent decades (Robinson and Jalli 1999; Serenius *et al.* 2005; Jalli 2011). The responses of barley varieties to the important diseases were studied in Latvia (Dreiseitl and Rashal 2004; Kokina *et al.* 2008; Bankina and Gaile 2009) and in Lithuania (Lazauskas *et al.* 2005; Leistrumaite and Razbadauskiene 2007; Liatukas and Leistrumaite 2007; Leistrumaite *et al.* 2008).

2.2. Major fungal pathogens in oat

Crown rust and leaf spot are major diseases in the oat (*Avena sativa* L.) growing areas in Estonia. Crown rust caused by *Puccinia coronata* (Cda f.sp. *avenae* Erikss.) requires the presence of the alternate host, buckthorn (*Rhamnus* sp.), to complete its life cycle (Nyvall 1979; Šebesta *et al.* 1997; Smith 2002). In early summer, a fungal spermacias develops from which the spores are carried by the wind to oat leaves. Crown rust develops best during mild to warm (20–25 °C), sunny days and mild nights (15–20 °C) with dew formation (Top Crop Manager 2008). Under optimal conditions, the spores germinate rapidly and in warm weather the following infection for new rust pustules may take only one week (Leonard 2002). The reproductive cycle is repeated several times while oat is growing which is the cause of the exponential increase in disease severity, particularly when susceptible varieties are grown during warm weather.

Oat leaf spot or seedling blotch caused by *Pyrenophora avenae* (Ito & Kurib., conidial stage: *Drechslera avenae*) is a seed-borne disease. The fungus survives on host plant debris but the main source of inoculum is a seed infested with long-lived resting mycelium (Zillinsky 1983; Clifford 1995; Smith 2002). The fungus may be carried over from one oat crop to the next on grain. Harvest residues are an ideal substrate for the fruiting bodies of the pathogen. In the soil, the spores can survive under ground for years. The disease starts with the coleoptiles becoming infected. When infected kernels germinate, the fungus infects seedling leaves. Disease infestation is favoured by rain and moist, humid periods as spores germinate at temperatures of 10–20 °C and at 100% humidity (Motovilin 2000). As the disease progresses, lesions gradually spread across most of the leaf blade. Because of the destruction of leaf tissue, photosynthesis is reduced in diseased plants, leading to yield decline (Motovilin and Strigekozin 2000).

To investigate oat crown rust, different isolates were collected in European countries (including Estonia) and virulence phenotypes were studied (Klenova *et al.* 2006; Klenova- Jirakova *et al.* 2010). The first report of virulence on Pg 13 (*Puccinia graminis*) in Europe was detected within the framework of the EMODN trials in Estonia in 1993 (Šebesta *et al.* 1998).

2.3. Effective disease control measures

In agriculture, integrated pest management (IPM) is a pest control strategy that aims to balance the benefits of pesticides with an ecological approach towards a main goal of significant reduction of application rates, with treatments applied only when the risk is real. Disease forecasting has become an important component of IPM and has helped to reduce the amounts of pesticides applied to crops without reducing yields (Agrios 1997). IPM has proven to be an important transitional strategy in the development of sustainable agriculture (Gips 1990).

Seed treatment is one aspect of crop management. It is an advanced and economic delivery system to protect the genetic potential of the seed against diseases from the moment of sowing, and also partial replacement of the conventional foliar application (Smiley *et al.* 2002; Clark 2008). Seed treatments with fungicides have antagonistic activity against pathogenic fungi on seed-borne and root rot diseases and are capable of suppressing root rots as well as other plant diseases (Bailey and Lazarovits 2003; Pauliz 2006). Common root rot pathogens live in the soil and are a serious threat to spring barley; most often they are caused by *C. sativus* and *Fusarium* spp. fungi and are responsible for yield losses (Piening 1997; Kumar *et al.* 2002).

One strategy is to improve the decision support system in order to manage with the minimum applied fungicide dose to achieve the desired effect. The computer based disease forecasting program PC–P (Jørgensen and Henriksen 2001; Jørgensen and Hagelskjær 2003; Jørgensen *et al.* 2003) (in Estonian 'I-Taimekaitse') enables reduction of direct costs, by adjusting fungicide dose according to varietal resistance, growth stage and weather conditions. The computer-based forecasting system has been developed to gain control of diseases using environmentally-sound and economically-viable fungicide strategies (Jørgensen *et al.* 2000). The aim is to use the dose, which gives the best margin over fungicide input (Dammer *et al.* 2007; Jørgensen *et al.* 2008). The economical benefit should be achieved from proper timing of reduced fungicide doses, which give substantial increases in net yield and cost-effectiveness.

Another strategy is the proper choice of fungicide; its dose and application time are important in achieving economic efficacy because of low returns caused by the low price of cereal grain (Leadbeater *et al.* 2000;

Wiik and Rosenqvist 2010). Efficacy of fungicides is highly dependent on the time of application. Fungicides can have a curative or preventative effect on diseases (Cook *et al.* 1999). It has been widely recognized (Dimmock and Gooding 2002; Jørgensen *et al.* 2003) that fungicide applied during the period from flag leaf emergence to ear emergence, just after the appearance of the first disease symptoms, often provides the best prospect for cost-effective control of foliar diseases. According to Yuen and Djurle (1998), an early spray time is preventive as chemicals remain outside the plant and hinder any new infections from taking place. Late treatment has an eradication effect, where fungicide enters the plant and kills or removes a portion of the pathogen within the plant tissue. Fungicide thus works by reducing the initial inoculum.

Chemical control is widely used to maintain green leaf area and increase grain yield. Fungicide treatment has been found to be effective when the infection level is visually more than 5% of the leaf area (Cook *et al.* 1999). However, fungicides remain the most popular method for controlling diseases; the other options for controlling include manipulation of agro-technology and the use of resistant varieties. The new regulation of the European Parliament an of the Council 1107/2009/EEC concerning the placing of plant protection products on the market (Official Journal of the European Union 24.11.2009) will potentially limit the choice of fungicides available to farmers within a 5-10 year time scale. Despite the reliance on fungicides as the primary method to manage disease, several disease surveys have indicated that fungicides are not sustainable long-term solutions to manage disease because resistance shifts have occurred to triazole fungicide (Hardwick *et al.* 2000; Oxley and Burnett 2008). A nutrient and disease interaction is essential in intensive cereal growing (Datnoff *et al.* 2007; Huber and Haneklaus 2007) because plant diseases are the major limitations to improved production efficiency and crop quality.

2.4. Effective plant resistance measures

Experiences from Denmark, Norway, and the Czech Republic have led to several findings which confirm that the severity of individual diseases is related to inoculum pressure, meteorological conditions and the popularity of susceptible varieties (Jørgensen *et al.* 1996; Henriksen *et al.* 2000; Jørgensen and Henriksen 2001; Jørgensen *et al.* 2003; Tvarůžek 2004). An essential way of reducing the rate of disease development is through the use of host plant resistance. Depending on the type of resistance and on the nature of the pathogen population, host plant resistance can affect the amount of inoculum as well as the rate of disease increase (Yuen and Djurle 1998; Das *et al.* 2007). The specifics of plant responses to pathogens are classified into horizontal resistance (non-host or basic resistance) and vertical resistance (race-specific resistance).

Horizontal resistance is controlled and affected by many genes and under different environmental conditions it may vary considerably more than vertical resistance. Vertical resistance is induced in response to a particular race of pathogen, but it occurs in all varieties of the host plant. This type of specific disease resistance is dependent upon genetic variation within the pathogen species and the production of proteins capable of altering the outcome of an otherwise compatible plant-pathogen interaction in only certain pathogen races (Agrios 1997; Jalali and Bhargava 2002; Wolpert *et al.* 2002).

Varieties with vertical resistance generally show complete resistance to a specific pathogen under most environmental conditions but a single or a few mutations in the pathogen may produce a new race that may infect the previously resistant variety.

3. AIMS OF THE STUDY

The main hypothesis of this thesis is that fungicide doses in cereal production can be reduced by using more resistant varieties; fungicide application can be beneficial in years favourable for disease; fungicide treatment has an impact on phytopathological conditions of the seed; disease infection and fungicide use are influenced by fertilization level.
The study is directed at finding opportunities for adjustment of fungicide dose according to field conditions, resulting in reduction of pesticide use and increase of economical benefit of disease control in cereals. For these reasons, there has been a need for thorough research of integrated control strategies, considering cultivation and weather conditions, host resistance and fungicide use.

The following objectives were posed:

- to study changes in rusts' resistance of oat genotypes (**I**).
- to investigate the efficacy of disease control in spring barley and oat varieties with different resistance levels using split and reduced fungicide doses, early and late treatment regimes and to provide advice for farmers on protective crop treatment according to the risk of imminent disease (**II, III**).
- to test the effects of reduced fungicide doses on the fungal contamination in harvested grain (**IV**).
- to investigate the effects of different fertilization and fungicide treatments on oat leaf diseases and grain yields and to recommend appropriate application for high yield and N use efficiency (**V**).
- to determine the efficacy of disease control in spring cereals for optimum yield and economical production (**II – V**).

4. MATERIALS AND METHODS

4.1. Experimental site and design

The current study was carried out at Jõgeva Plant Breeding Institute, Estonia (58°44'41"N, 26°23'41"E). The Calcaric (Eutric) Cambisol (K_0) (FAO classification) soil of the experimental area contained 190 mg P kg^{-1}, 180 mg K kg^{-1}, 1520 mg Ca kg^{-1}, 64 mg Mg kg^{-1}, 1.3 mg Cu kg^{-1}, 41 mg Mn kg^{-1}, 0.7 mg B kg^{-1}. Soil pH was 5.8. Trials on disease control of spring barley and oat were arranged in a randomized block design in 20 m^2 plots with three replicates; a sowing rate of 500 germinating seeds per 1 m^2 was used during 2003–2005 (**II, III, and IV**). All trials were sown at optimal time in the first week of May. Fertilizer rates of 80 kg N ha^{-1} Kemira Power 18 (18 N, 9 P_2O_5, 9 K_2O) were applied at 500 kg ha^{-1}. Additional nitrogen at AN 43 80 kg ha^{-1} was applied at shooting stage BBCH 21–23.

The trial to study the effects of fertilizers on fungal diseases infections and yield of oats was sown in 9 m^2 plots at the rate of 600 germinating seeds per 1m^2 in three replicates during 2006–2008 (**V**). Four levels of fertilization (N0 = untreated control $N_0P_0K_0$ kg ha^{-1}; N1 = $N_{60}P_{13}K_{23}$; N2 = $N_{100}P_{22}K_{39}$; N3 = $N_{140}P_{31}K_{54}$) were applied during soil preparation before sowing (basal N) using a complex fertilizer Kemira Power ($N_{18}P_4K_7$).

Weed control was chemical and all trials were harvested in late August in all years. The spring barley varieties Anni (moderately resistant to net and spot blotch), Barke (moderately susceptible to net and spot blotch), Extract (susceptible to net and spot blotch) and oat varieties Jaak, Villu, Flämingsprofi (moderately susceptible to crown rust and oat leaf spot), Hecht, and Belinda (susceptible to crown rust, moderately susceptible to oat leaf spot) were used. Untreated certified seed was used for all varieties.

The effectiveness of 32 oat genotypes as resistance sources to fungal diseases was studied during the field trial using late sowing time and hill plots consisting of 10–15 plants in 2 replications in 1996–2002 (**I**).

4.2. Experiments with foliar fungicides

The fungicide Folicur 250 EW consisted of 125 g l^{-1} tebuconazole. A split application of 0.5 l ha^{-1} at stages BBCH 32–51 (node 2 at least 2 cm above node 1 – beginning of heading) and BBCH 57–65 (70% of inflorescence emerged – full flowering) was compared with the effect of reduced dose at 0.3 l ha^{-1} (2003); 0.16 l ha^{-1} (2004); 0.15 l ha^{-1} (2005) based on the field specific infection level recommended by the decision support system PC–P for controlling disease infections on barley varieties (**II** Table 1), a single dose of 1.0 l ha^{-1} at BBCH 37–41 (flag leaf just visible, still rolled – flag leaf sheath extending) (Early T) or BBCH 59–63 (end of heading – beginning of flowering) (Late T) application was used in the oat trial (**III** Table 1). Fungicide applications were started when the first symptoms of infection appeared. The times when fungicides were applied varied between the trial years because of differences in meteorological conditions and disease development. All varieties were treated at the same time. The effect of different fungicides on the fungal flora of barley grains was tested in 2006, when ten fungicides were applied at ¾ rate of the registered full dose at growth stage BBCH 37–41 in the variety Barke (**IV** Table 1). Fungicides were applied with a bicycle sprayer equipped with 6 Hardy nozzles 4110–12 on a 2.5-m boom using 300 l of water per ha^{-1}.

4.3. Soil fertilizer and chemical inputs experiment

In the basic fertilization and chemical inputs trial, the plant growth regulator chlormequat chloride at 750 g l^{-1} 1.0 l ha^{-1} (BBCH 32) (node 2 at least 2 cm above node 1), the leaf fertilizer Folicare at 8 kg ha^{-1} ($N_{12}P_{20}K_7$ g kg^{-1}, BBCH 21–22), ($N_{18}P_8K_{15}$, BBCH 51–52) (beginning of heading), ($N_{10}P_2K_{33}$, BBCH 71–72) (watery ripe – early milk), and the fungicides propiconazole at 250 g l^{-1} 0.5 l ha^{-1} (BBCH 29–30) (end of tillering – beginning of stem elongation) were used in 2006; tebuconazole at 125 g l^{-1} 1.0 l ha^{-1} (BBCH 50–51) was used in 2007 and in 2008 (**V**). Fungicide treatments were applied with a bicycle sprayer equipped with 6 Hardy nozzles 4110–12 on a 2.5-m boom using 300 l of water per ha^{-1}.

4.4. Disease assessment

Assessments of the severity of the diseases in the field trials were visually scored as the percent leaf area infected by pathogens at BBCH 71–75 (watery ripe – medium milk) (Tekaus 1985; Fetch and Steffenson 1999). The three top leaves of the plant were assessed separately on three adjacent tillers at 10 randomly selected places on each plot. The infection level was expressed as the average of the infection score on second leaves (L2; the first leaf under the flag leaf) (**II, III, IV**). In the basic fertilization and the chemical inputs trial and the EMODN trial, ten randomly chosen tillers per plot in all replications on the 1–9 point scale (1–no infection, 9–highly infected) were visually assessed (EPPO 1981). The infection level was expressed as the average of the infection score at BBCH 75 on the top three leaves of the plant (**V, I**). Untreated plots were assessed as a control. Phenological growth stages were determined according to the BBCH scale for cereals (Meier 2001) when >50% of the plants had reached the target growth stage.

To assess mycological contamination of harvested barley grain, 25 seeds in three replications per treatment, were analyzed for the presence of fungal spores under a microscope (Olympus CX 31, 40x enlargement) after incubation using the moist chamber method (10 days in Petri dishes at 20 °C under a 12 h dark/light regime). The percentage of occurrence of fungal species was evaluated for each trial variant: (number of grains with occurrence of species / total number of grains) x 100 (**IV**).

4.5. Meteorological conditions

Weather factors are generally considered to have a vital influence on efficacy. Meteorological data were obtained from the field meteorological weather station Metos Compact. 2003 was characterised by a rainy May, moderate temperature in June, warmer and dryer weather in July and very rainy August. 2004 was characterised by drought in May, a colder than usual but very rainy June, a dry July and a rainy August. 2005 was dry and hot, characterised by below average precipitation in June and July and above average temperatures in July and August (**III** Table 2). The average temperature was higher during the trial period in 2006 and 2007 but the long-term average of the same time period was lower. There was little precipitation in 2006 and 2007. Precipitation exceeded the long-term average in 2008. A month of drought was observed during June in 2006 and 2007 and July 2007. The grain harvest period was wet and rainy in 2008 (**V** Table 1).

4.6. Yield and yield revenue

Field trials were harvested with a plot combine harvester and grain yield (kg ha^{-1}) was measured on dried and cleaned seeds and expressed on the basis of 14% moisture content (**II**, **III**, **V**).

The net yield kg ha^{-1} was calculated to analyze the economical benefit of fungicide use by subtracting from the harvest yield the amount of grain equal to the cost of fungicide and its application. Fungicide prices, the cost of a fungicide application, and the average purchase prices of spring barley and oats in Estonia for the period 2003–2005 were used in calculations of yield revenue. All prices were presented without VAT (**II**, **III**).

4.7. Data analysis

The data were subjected to factorial analysis of variance (ANOVA) using statistical software Agrobase™ 20 (1999). Standard analysis of variance was performed to determine the main factors and interactions. Nearest Neighbour Analysis (NNA) was used to adjust yield data for soil fertility trends. Data obtained from each treatment were analysed for mean separations by least significant difference (LSD) at the 0.05 level of probability (**II – V**).

5. RESULTS

5.1. Temporal changes in virulence of oat crown rust

Results of infection of 32 oat genotypes provided information about changes in the virulence spectrum of oat crown rust in Estonia (**I** Table 1, 2). The greatest breakdown of race specific resistance to *P. coronata* was recorded in the differential variety Pirol, lines Pc 58 and Pc 61 and varieties Alo, Jaak and Edit, which had lost resistance to crown rust in 1998. A widely spread variety Alo was resistant to crown rust until 1997; it became highly infected for the first time in 1998 after the appearance of new rust races, and has been infected, since then, on the level of the most susceptible varieties. A similar infection pattern can also be seen in the varieties Jaak, Edit and Pirol. The lines Pc 58 and Pc 61 were infected for the first time in 2001.

5.2. The importance of the timing, optimal fungicide dose and fertilizers

Net blotch (*P. teres*) infection in spring barley trial was more severe in 2003 and 2004 (**II**). The best control over the disease was achieved by split application of half fungicide doses (Figure 1). A split-treatment of tebuconazole resulted in significantly better disease control than the use of reduced dose according to PC–P also in conditions of low disease pressure in 2005. The best control over *P. teres* was achieved by application of half-dose of tebuconazole in all varieties, the infection level varying between 1.2–4.3% (2003), 6.7–12.7% (2004) and 0.6–1.21% (2005), respectively. The level of spot blotch (*C. sativus*) infection was low in 2003 and 2005, the infection reached a significant 19.3–42.8% leaf area infection of untreated varieties in 2004 (**II** Figure 2). The rate of *C. sativus* infection after the first treatment with the half fungicide dose varied in different varieties between 0.2–4.7% (2003), 8.7–10.4% (2004) and 0.5–1.1% (2005). Two fungicide applications were needed in 2004 to control severe disease pressure. Results of ANOVA indicated that in all the test years the year had the biggest influence on intensity of infection of both diseases (p=0.000) (*P. teres*) and (p=0.000) (*C. sativus*) (**II** Table 2).

Application of reduced fungicide dose according to PC–P was sufficient for disease control in 2003 when the right time for the application and the optimal dose according to the disease pressure were chosen.

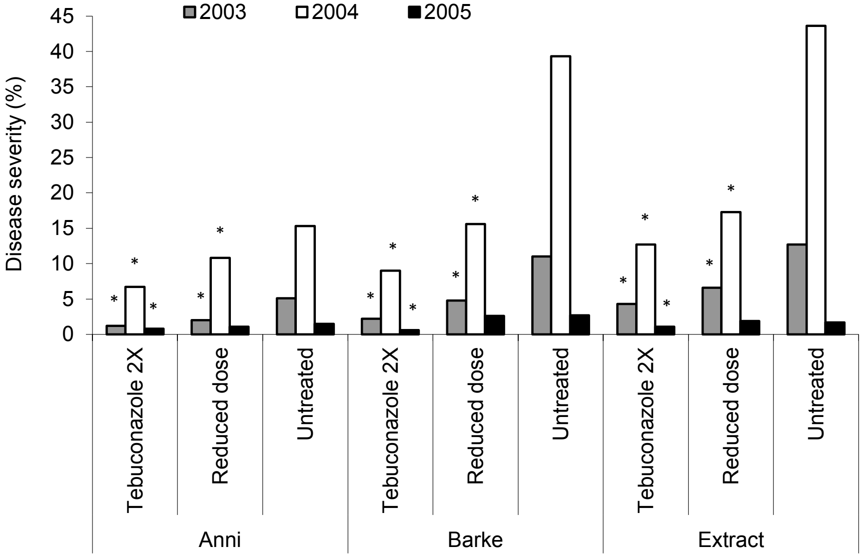

Figure 1. Efficacy of tebuconazole application against *P. teres* and untreated control on L-2 leaves in spring barley varieties in 2003– 2005, significant difference at the 0.05 level of probability as compared with the untreated control.

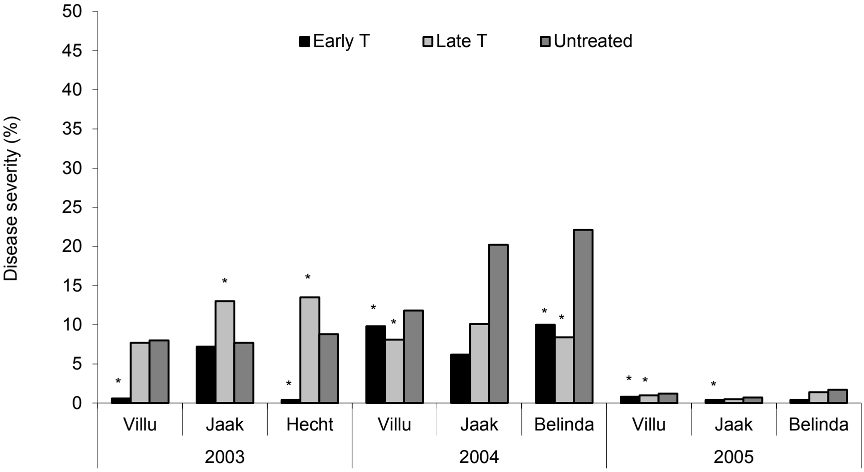

Figure 2. Oat leaf spot severity (%) caused by *P. avenae* in field trials (2003–2005) assessed at BBCH 71–75, significant difference at the 0.05 level of probability as compared with the untreated control. Treatment (T).

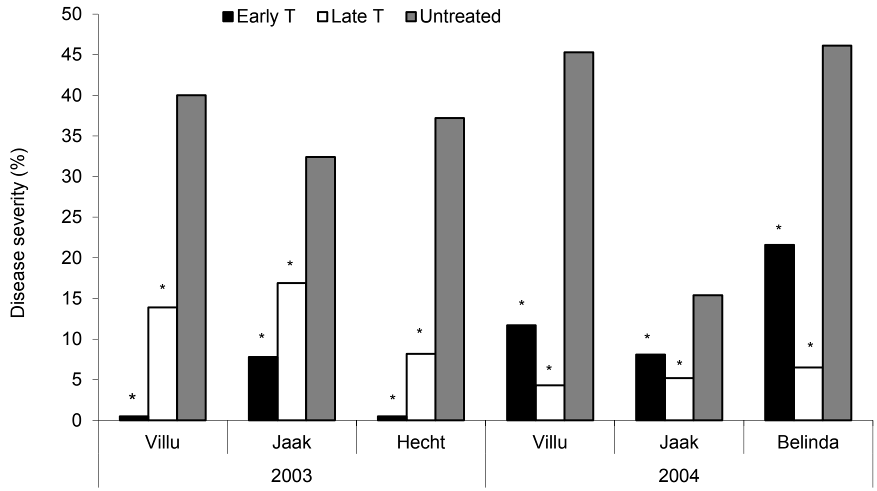

Figure 3. Oat crown rust severity (%) caused by *P. coronata* in field trials (2003–2004) assessed at BBCH 71–75, significant difference at the 0.05 level of probability as compared with the untreated control. Treatment (T).

In oat trial series (**III**), tebuconazole application at BBCH 37–41 achieved good disease protection against oat leaf spot in 2003, significantly reducing infection from 8.0% to 0.6% in variety Villu and from 8.8% to 0.4% in variety Hecht (p<0.001), whereas late treatment application at BBCH 59–63 gave lower disease control on all varieties in 2003 and 2005 (Figure 2). Treatment at BBCH 59–63 had none or very limited effect on *P. avenae* control because infection occurred in early stages of plant development and fungicide was unable to control the already established infection. Only in conditions of the fast epidemic in 2004, late treatment resulted in slightly better oat leaf spot control.

Early treatment was more effective than late treatment in controlling the oat crown rust in the warm and moderately rainy summer of 2003 with infection reduction to 7.8% in variety Jaak and to 0.5% in varieties Villu and Hecht (p<0.001) (Figure 3). Late treatment was more effective in the conditions of 2004 when air temperature remained lower and host reactions to crown rust fungi were weak until July. Disease infection decreased to 5.2% in variety Jaak, to 4.2% in variety Villu and to 6.5% in variety Belinda (p<0.001). The lowest effect on disease control was observed in the most resistant variety Jaak in both treatment times and in both years.

The results of the effects of fertilizers on the infections of fungal diseases on oat revealed that, in the basic fertilization conditions, severity of *P. coronata* correlated highly with yearly climatic conditions, a variety, a fertilizer input and their interactions ($R^2=0.933$, $p<0.001$) and severity of *P. avenae* correlated highly with a year, a fertilizer input, the interactions between a variety and a year and between a year and a fertilizer input ($R^2=0.838$, $p<0.001$) (**V** Table 2). In soil, fertilizer and chemical treatment variant, fungicide treatment, had a significant protective effect against diseases, whereas N fertilization levels apparently had a non-significant effect in disease severities (**V** Table 3). Leaf spot infection mostly depended on genotype, on year and on the interaction between year and fertilizer input, whereas crown rust infection was mostly associated with weather conditions.

5.3. The impact of fungicides on yield

The intensity of plant protection caused significant differences in yield of fungicide treated plots (**II, III, V**). The split treatment had a positive effect on spring barley yield. The split treatment strategy gave a yield increase in the more susceptible genotypes Barke and Extract in all years. The more resistant variety Anni gave relatively low returns, because of much higher level of biological disease resistance (**II**) (Figure 4).

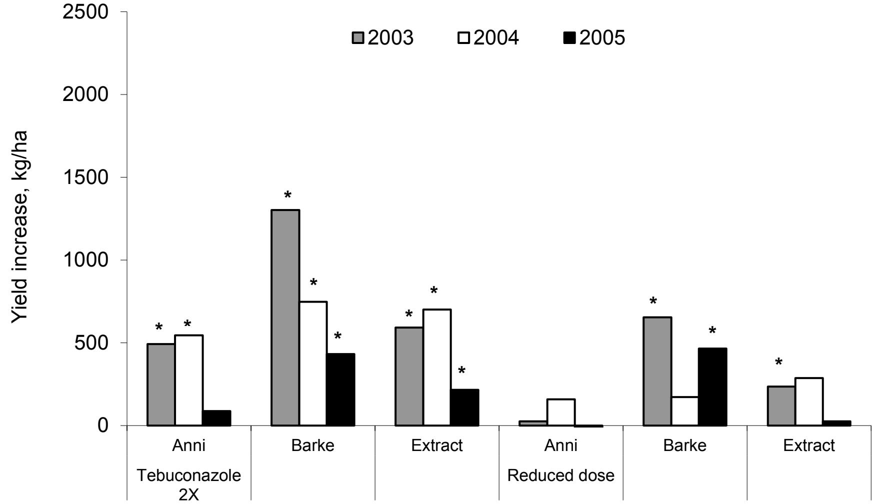

Figure 4. Yield increase of spring barley kg ha^{-1} in fungicide treatments in comparison with untreated control crop in 2003–2005, significant difference at the 0.05 level of probability as compared with the untreated control.

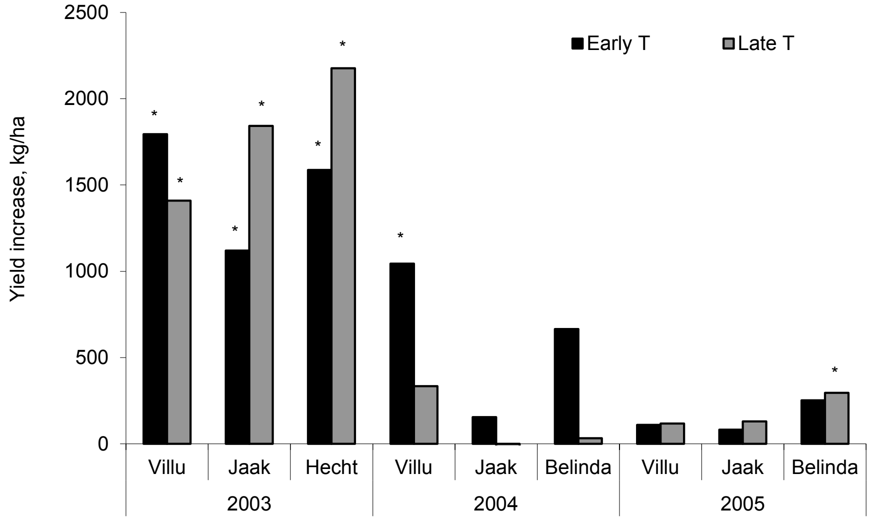

Figure 5. Yield increase of oat kg ha^{-1} in fungicide treated plots in comparison with the untreated control (2003–2005), significant difference at the 0.05 level of probability as compared with the untreated control. Treatment (T).

The three-year average yield of spring barley control was 4334 kg ha^{-1} and the average yield increase due to fungicide treatment above the control was 569 kg ha^{-1} (split treatment) and 219 kg ha^{-1} (reduced application); on average, increases were 11.6 and 2.7%, respectively.

Fungicide application in the oat trial provided significant yield increase only in 2003 (**III** Figure 3) (Figure 5). The highest yield increase from fungicide use in 2003 was obtained at BBCH 59-63 in varieties Hecht (2176 kg ha^{-1}, $p<0.001$) and Jaak (3092 kg ha^{-1}, $p<0.05$).

The opposite was observed in variety Villu, where a higher yield increase was obtained at BBCH 37–41. In conditions of very low disease pressure, in 2005, higher yield increases were obtained from late treatment. A significant yield increase was obtained only in variety Belinda (296 kg ha^{-1}, $p<0.01$). Over the trial period, the average yield of the control was 4512 kg ha^{-1} and the average yield increase due to fungicide treatment above the control was 756 kg ha^{-1} (14.2%) in early treatment and 697 kg ha^{-1} (14.8%) in late treatment.

The results of the fertilizer and chemical input trial indicated that average grain yields at N1–N3 fertilizer levels were quite similar either with or without chemical treatment but considerably higher compared to yields at N0, the intensity of fertilizer input caused a significant differentiation in yields of tested varieties (V Table 4).

5.4. The influence of the fungicide application on fungal contamination of harvested barley grain

The choice of fungicide had a significant impact on contamination and on the proportion of fungal species occurring on the harvested grain of spring barley. Climatic conditions influenced the extent of fungal contamination more than varietal resistance (**IV**). The prevailing fungi in untreated plots in 2004 on the harvested grain were necrotrophic *C. sativus* 76%, saprotrophic members of genus *Alternaria* spp. and *Cladosporium* spp. 79% occurrence on seeds, facultative saprotrophic *Fusarium* spp. covered the range from 8 to 31% on seeds. The highest observed occurrence of *C. sativus* was 44%, *Fusarium* spp. 37%, *Alternaria* spp. and *Cladosporium* spp. 81% on seeds in 2005 (**IV** Figure 1). The split application of tebuconazole was effective in reducing kernel contamination with *C. sativus* in all treatments except in variety Extract in 2005. This is an indication of the activity of tebuconazole in control of phytopathogenic fungi in later stages of barley development. The single application of reduced dose decreased kernel contamination with *C. sativus* only in the more susceptible varieties. The application of five fungicides had 100% effect in control of *C. sativus* and *Fusarium* spp. in 2006 (**IV** Figure 2). Yearly climatic conditions had a highly significant effect on the occurrence of the great majority of phytopathogenic fungi. The effect of year was not significant only for genus *Alternaria* (**IV** Table 5).

5.5. Economic profitability

The net yield of the moderately resistant variety Anni did not increase, but was reduced as a result of being treated by two strategies (**II** Table 3). The yields of the fungicide treated plots of the more susceptible varieties were either equal to or exceeded the yield of the untreated control. Fungicide use in oat gave a highly significant financial benefit in 2003. Half the treatments increased income in 2004, in the other half the costs of fungicide application were higher than the value of the additional yield (**III** Table 3). None of the treatments were profitable in 2005 when the cost of fungicide application exceeded the value of the extra yield.

6. DISCUSSION

Our studies showed that the optimal input of fungicide in spring barley and oats depends on the disease pressure in the field.

6.1. Changes in rust resistance over time (Paper I)

Our results of testing the resistance sources to rust diseases in 1996–2002 showed that the variety Jaak lost its resistance to *P. coronata* in 1998. The first report of virulence on the resistance gene Pg 13 in Europe was detected within the international framework of the EMODN trials in Estonia in 1993 (Šebesta *et al.* 1999). Integrated selection for agronomic performance and quantitative resistance to crown rust requires an understanding of their genetic relationships (Holland and Munkvold 2001; Klenova *et al.* 2006). Growing rust resistant genotypes create the evolutionary pressure for the selection of new more virulent pathogen strains, sometimes very soon after the new variety is released. A large number of resistance genes have been isolated from wild *Avena* sp. since the mid-1960s but virulence by rust towards all these genes has been detected (Harder and Haber 1992). In breeding for resistance, it is not necessary to seek for complete resistance. According to Simmonds (1988), resistance must be reliable over years and it matters only so far as it protects yield. However, for mobile pathogens like cereal rusts, the use of horizontal resistance offers only short-term solutions. Yield damaging epidemics are a clear sign that more resistance is needed (Stuthman *et al.* 2007). When new virulence's emerge, fungicides have to be used to supplement the protection of plants.

6.2. The effect of variety resistance and treatment time on disease severity (Papers II, III)

The studies indicated that fungicide dose may be lowered to achieve better disease control but it can be effectively improved by proper timing of fungicide. The results also revealed that the occurrence and severity of the disease are influenced more by the biology of pathogens, the resistance level of varieties and weather conditions, than by the use of fungicide. Similar results have been found in trials made in the United Kingdom and Denmark (Hardwick *et al.* 2000; Hossy *et al.* 2000; Newton *et al.* 2004). The fungicide dose for effective disease control may be lower than the registered standard dose. According to Jørgensen *et al.* (1996), Hardwick *et al.* (2000), Henriksen *et al.* (2000) and Jørgensen *et al.* (2003), the use of reduced fungicide doses was effective depending on weather conditions of the year and on the resistance level of the variety. Under high disease pressure, the variety with a low susceptibility is not able to delay infection. Better protection was ascribed to the prolonged protective effect of split doses where the second application is used to control any infection that survived the first application. On the other hand, using preventative fungicide applications too early may lessen their effect on late disease establishment. Therefore, application of fungicides at the heading stage could result in better disease control at late disease development. There are no single solutions for timing fungicide application. Weather conditions and varietal resistance should be considered in the timing of fungicides in order to achieve the best economic results (Jørgensen *et al.* 2003).

6.3. The effect of fertilization and chemicals on oat diseases (Paper V)

In our study, we found that it is important to use optimal fertilization to provide high soil fertility and high yields of oats. Our results revealed that the basic fertilization applied to the soil in moderate and higher NPK-doses determines the degree of disease attack when compared to the non-fertilized treatments and the fungicide applications, which demonstrated a reduction of disease attack in all fertilizer levels. Balanced and adequate fertilization of a crop reduces plant stress, improves physiological resistance and decreases disease risk (Krupinsky *et al.* 2002). Suitable conditions for the growth and development of the plant, including necessary supplies of nutrients, improve the plant's resistance to infectious diseases and enable realization of the grain yield (Datnoff *et al.* 2007; Huber and Haneklaus 2007; Mohr *et al.* 2007). Disease severity may differ considerably at different N rates. Better supply of available nutrients contributes to the plants' resistance to attack by the pathogen (Krupinsky and Tanaka 2001). If fungicides are not used against fungal diseases, it is not worth fertilizing cereals with large nitrogen rates because cereal productivity does not increase significantly (Lisova *et al.* 1996).

6.4. The influence of fungicide on fungal contamination of harvested grain (Paper IV)

Our results affirmed that the choice of fungicide had a significant impact on contamination and on the proportion of fungal species occurring on the harvested grain of spring barley. In our trials, the applied fungicides had a highly significant effect in controlling the occurrence of most saprophytes during the whole trial period. The yearly conditions gave a highly significant effect on occurrence of the great majority of phytopathogenic fungi.

Harvested grain commonly carries an abundant microbial fungal population, which requires organic nutrients for their energy source, like carbon nutrients for cellular synthesis (Deacon 2006). Similarly to our results, significant increase of *Fusarium* infection was detected in fungicide treated plots compared to untreated plots (Henriksen and Elen 2005). *Fusarium* contamination might be influenced by a long lasting fungicidal effect; the plants remain greener for a longer period and serve as a good growing medium for *Fusarium* species (Mesterhazy and Batok 2001). Therefore, fungicide use is not recommended in cases of low or medium disease pressure, as it may lead towards increased *Fusarium* contamination of the grain.

6.5. Achievement of economical benefit in disease control (Papers II, III)

We found that the application of fungicides increased grain yields significantly compared with untreated controls. Fungicide treatment had a favourable effect on weight and chemical composition of grain compared with untreated controls also tested by several authors (Conry and Dunne 2001; Kelly 2001; Hrivna 2003). Intensive protection is important for barley yielding but an integrated plant protection system is more economical since the use of the decision support system enables lowering of direct costs (Jørgensen *et al.* 2000). In less favourable years for disease development, disease control in spring barley can result in negative net revenue (Tischner and Schenkel 2006). The high price of fungicide reduces the profit in more resistant varieties during years when disease severity is low throughout the whole growing season. The investigations have indicated that fungicide doses can be reduced by 50–70% without essential loss in disease control (Jørgensen *et al.* 2003). The use of reduced fungicide doses giving the same level of disease control will increase the economical profitability of the crop.

6.6. Effective protection recommendations

The results of this thesis have great value for farmers to protect spring barley and oat crops against diseases. Since the aim was to find optimal fungicide strategies and lessen direct costs, the following general recommendations can be made to farmers:

– Taking into account the resistance level of the spring barley variety, the prevailing weather conditions and infection situation in the field, the fungicide dose may be lowered to achieve disease control but it has to be applied at the proper time to lower direct costs and achieve economic profitability.

- Weather conditions and varietal resistance should be considered when timing fungicide use for achievement of best economic result on oats. Fungicide use is most reasonable in susceptible varieties in weather conditions favourable for disease development. Usually more resistant oat varieties do not need a foliar application of fungicides.

6.7. Future prospects

Future management approaches include the use of molecular diagnostics, risk management tools and delivery of advice to growers for management of diseases in an integrated way. Better knowledge and implementation of the principles of practical plant pathology increases the cost-efficiency of farmers.

The knowledge about the varietal resistance, fungicide activity and phytopathological and agrometeorological situations in the field have to be combined to make correct decisions about fungicide dose and application time. The studies covered in the present thesis are currently continued within the framework of the project Development of an Internet Based Decision Support System for Crop Protection with the aim of improving effective disease management and to provide recommendations for practical solutions in farmers' fields. Considering fungicide use, the tendency in future will probably be towards more widespread use of integrated plant protection measures. Fungicides are recommended to be used only when the disease achieves a threshold of incidence. The continuation of the studies in these areas is needed to provide scientific support for implementation of integrated plant protection.

Improved knowledge about racial composition of pathogen populations in cereals as well as timely detection of new emerging plant diseases is needed to enable better management of biological resistance.

The current studies have a direct connection with efficacy evaluation of fungicides, organised in direct contact with chemical companies. Studies on fungicide resistance are continued in the NORBARAG framework to guarantee the effective and sustainable use of fungicides.

Grain yield was reduced as a result of fungicide use in several trials in the current study. To provide safe and sustainable use of fungicides we are planning to start new studies on the influence of fungicides on plant physiology.

CONCLUSIONS

The current Thesis provides results of studies on several aspects of environmentally friendly crop protection measures in spring barley and oat, what have not been studied in Estonian conditions previously.

Effective management of foliar diseases in cereals depends on an integrated approach, which combines pathogen identification and infection (population) assessments in the field with cultural and chemical methods and choice of either resistant or tolerant varieties.

The results of our study indicate that considering the cost of chemical control, fungicides are recommended to be used only when the disease achieves a threshold of incidence to gain economical benefit.

The study emphasized the importance of matching the variety to local field conditions. The wrong choice of variety may be reflected in lower economical benefit.

From the results of the present Thesis we can present the following general conclusions:

- Pathogenic situation of *Puccinia coronata* Cda. F. sp. *Avenae* Erikss. has changed in Estonia during the last decade with the appearance of new races. This has caused the need for using fungicides in formerly resistant varieties (**I**).

- Chemical control is the most profitable for moderately susceptible spring barley varieties with the use of reduced fungicide doses. In susceptible varieties diseases cause bigger yield reduction than could be compensated by fungicide use, in resistant varieties cost of fungicide use usually exceeds returns from yield increase (**II**).

- There are no universal recommendations for timing of fungicide application in oats. Weather conditions and varietal resistance should be considered in order to achieve the best economic results. Fungicide use is mostly reasonable in susceptible varieties in weather conditions favourable for disease development. Smaller economical returns are achieved in more resistant varieties. It was found that more resistant oat varieties do not usually need foliar application of fungicides (**III**).

- There will be little benefit from fungicide application at low yield potential, under unfavourable conditions for spread of diseases, or when grain price is low (**II**, **III**).

- Weather conditions of the growing period, resistance of the variety, choice of fungicide and its application regime all have influence on the contamination of barley grains with phytopathogenic fungi. Fungicides do well in controlling the foliar infection of barley diseases but have minor influence in reducing kernel contamination with saprotrophic fungi. We found that certain fungicides or fungicide application regimes can increase grain contamination with saprotrophic fungi (**IV**).

- The basic fertilization applied to the soil at moderate and higher fertilizer levels determines the degree of increase of the oat crown rust attack compared to that of non-fertilized controls (**V**).

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SUMMARY IN ESTONIAN

Integreeritud taimekaitse kasutamine odral ja kaeral

Integreeritud taimekaitse eesmärgiks on pestitsiidide kasutamise piiramine, vältides seejuures võimalikke negatiivseid kõrvalmõjusid. Euroopa Nõukogu ja Komisjoni direktiiv 3607/09, millega kehtestatakse ühenduse tegevusraamistik pestitsiidide säästval kasutamisel, seab eesmärgiks vähendada taimekaitsevahendite kasutamisega seotud ohtusid inimese tervisele ja keskkonnale ning sõltuvust pestitsiidide kasutamisest. Ühe olulisema komponendina direktiivi eesmärkide saavutamiseks nähakse integreeritud taimekaitse laiaulatuslikku rakendamist, rõhutades seejuures seonduvate teadusuuringute olulisust. Eesti tingimustes ei ole fungitsiidide vähendatud kulunormide kasutamise efektiivsust keskkonnasõbraliku taimekaitse meetodina seni teaduslikult uuritud. Selles osas pakub käesolev töö uudset teaduslikku informatsiooni.

Taimehaiguste arenemiseks on vajalik nii peremeestaime kui patogeeni populatsioonide olemasolu. Taimehaiguste esinemise sageduse ja arengu kiiruse piiramisel omab olulist rolli haiguskindlate sortide kasvatamine. Olenevalt resistentsuse tüübist ja haigustekitaja populatsioonist võib haiguskindlam sort mõjutada nii patogeeni kogust kui nakkuse arengu kiirust.

Haiguskindlate sortide aretuses on kasutada kahte tüüpi resistentsust. Rassispetsiifiline resistentsus tagab täieliku haiguskindluse patogeeni teatud rasside suhtes. Taime nakatavad ainult komplementaarset virulentsusgeeni (uue terminoloogia kohaselt effektorit) kandvad patogeenid. Praeguseks on rassispetsiifilise resistentsuse kasutamisest loobutud kiiresti ja suurte vahemaade taha levivate, taime kasvuperioodi jooksul mitut põlvkonda tootvate ja suure geneetilise muutlikkusega haigustekitajate suhtes, mille populatsioonides arenevad kiiresti uued, resistentseid sorte nakatavad genotüübid. Polügeenne e. horisontaalne resistentsus tagab osalise, kuid mitte täieliku haiguskindluse haigustekitaja kõigi genotüüpide suhtes ning kontrollib infektsiooni ulatust ja nakatumise kiirust peremeestaimes. Polügeenne resistentsus vähendab patogeeni nakatamisefektiivsust, nakkuse arengu kiirust taimes ja haigustekitaja sporulatsiooni hulka. Parima ja pikemaajalise haiguskindluse tagab mõlema resistentsustüübi kooskasutamine.

Taimehaiguste tõrjeks kasutatakse kaitsva ja tõrjuva toimega fungitsiide. Kaitsva toimega fungitsiidid tagavad tõrje uute infektsioonide eest, vähendades sellega patogeeni kogust. Fungitsiidi mõjuperioodi möödudes on patogeenid võimelised jällegi taime nakatama, kuid taim on vahepeal edasi kasvanud haigustekitaja poolt vähem kahjustatavasse kasvufaasi. Tõrjuva toimega fungitsiidid hävitavad haigustekitajad nakatunud taime kudedes. See grupp fungitsiide töötab haiguse koguse vähendajana, kuid mõju on lühemaajaline. Kiiresti progresseeruvate taimehaiguste suhtes omavad patogeeni kogust vähendava tõrjuva toimega fungitsiidid väikest efekti.

Seega omavad haiguskindlad sordid ja fungitsiidid sarnaseid mõjusid haigustekitajate populatsioonide piiramisel ja taimehaiguste tõrjel. Taimede haiguskindluse ja fungitsiidide oskuslik kooskasutamine võimaldab tagada taimehaiguste efektiivse ja keskkonnasäästliku tõrje.

Eestis on odral enam levinumateks ja saagikadu põhjustavateks lehestiku haigusteks võrklaiksus (haigustekitaja *Pyrenophora teres*) ja kõrreliste pruunlaiksus (haigustekitaja *Cochliobolus sativus*). Kaera kahjustavad ja saagikust mõjutavad kasvuaegselt kõige enam kaera kroonrooste (haigustekitaja *Puccinia coronata*) ja pruunlaiksus (haigustekitaja *Pyrenophora avenae*). Kuna haigustekitajad võivad geneetiliselt muutuda ja sellega seoses väheneb taimede haiguskindlus või fungitsiidi toime efektiivsus, on vaja anda teaduslikult põhjendatud praktilisi täpsustusi taimekaitse soovituste kohta. Käesoleva uurimuse eesmärgiks oli selgitada esmakordselt Eesti tingimustes fungitsiidide vähendatud koguste efektiivse kasutamise võimalusi.

Töö eesmärgid olid järgmised:

- haigustekitaja virulentsuse monitooring ja uute virulentsuste tuvastamine kaera kroonrooste näitel ;
- sordi resistentsuse ja fungitsiidi kooskasutamise võimaluste väljaselgitamine efektiivse haigustõrje tagamisel, sordi resistentsuse täiendamine fungitsiidide vähendatud normide kasutamisega epidemioloogiliselt olulisel ajal;
- kasvuaegse haigustõrje mõju selgitamine seemnega edasikanduvate haigustekitajate nakkuse vähendamisele;
- väetamise ja taimekaitse koosmõju selgitamine kaera haiguste esinemisele ja saagikusele;
- kultuuripõhine majandusliku tasuvuse analüüs.

Esmakordselt Eestis selgitati kaera genotüüpide resistentsust aastatel 1996–2002. Eestis ei olnud varem tehtud efektiivsuskatseid majanduslikult optimaalsete fungitsiidi koguste ja kasutamisaegade selgitamiseks odral ja kaeral, need viidi läbi aastatel 2003–2005, kasvuaegse haigustõrje mõju odraterade saastumisele mikroorganismidega selgitati Eesti oludes esmakordselt aastatel 2004–2006, uurimata oli ka väetisfooni ja fungitsiidide kasutamise mõju kaerahaiguste esinemisele, seda katsetati aastatel 2006–2008. Kõik katsed viidi läbi Jõgeva Sordiaretuse Instituudis.

Odra ja kaera haigustõrje katsetes (**II, III**) kasutati triasoolide rühma fungitsiidi Folicur 250 EW (toimeaineks 250 g tebukonasooli), odral jagatud (2 x 0.5 l ha^{-1}) ja programmi I-Taimekaitse soovitatud kulunorme 0.3 (2003), 0.16 (2004), 0.15 l ha^{-1} (2005) ning kaeral täisnormi (1.0 l ha^{-1}) erinevatel pritsimisaegadel, lisaks kontrollvariandid. Mõlema kultuuri puhul hinnati taimehaigustesse nakatumist visuaalselt sajaprotsendilise skaala alusel kolmelt ülemiselt lehelt vahetult enne igat pritsimist ja kaks nädalat pärast tõrjet. Viimane hindamine tehti vahaküpsuse kasvufaasis. Väetise ja fungitsiidide katses (**V**) ja resistentsuse taseme selgitamise katses (**I**) hinnati kaera haigustesse nakatumist visuaalselt üheksapallilisel skaala alusel. Fungitsiidi kasutamise majandusliku tasuvuse arvutamisel arvestati odra ja kaera 2003–2005. a. keskmist kokkuostuhinda 0.1 € kg^{-1}, pritsimiskuludeks 7.7 € ha^{-1} ja Folicur 250 EW täisdoosi maksumuseks 33.55 € ha^{-1} (kõik hinnad ilma käibemaksuta). Terasaagid koristati kombainiga Hege 125 C, kaaluti ja sorteeriti. Saagid arvutati ümber 14% niiskusesisaldusele. Koristatud terade pinnalt määrati haigustekitajate liigiline koosseis niiskuskambri meetodi ja mikroskopeerimisega (**IV**). Ilmastikuandmed saadi katsepõllule paigaldatud agrometeoroloogilise automaatilmajaama Metos Compact abil.

Andmete statistiliseks analüüsiks kasutati tarkvaraprogrammi Agrobase^{TM} 20. Tulemuste statistilisel analüüsil kasutati dispersioonanalüüsi, mille abil leiti determinatsioonikoefitsiendid. Sortide keskmise saagikuse ja piirdiferentside leidmiseks kasutati NNA (Nearest Neighbours Analysis) meetodit. Olulisusnivooks võeti 0.05.

32 kaera genotüübi resistentsuse selgitamise katse näitas, et kroonrooste rassispetsiifiline resistentsus oli 1998. a. vähenenud kõige enam diferentsiaatorsordil Pirol, liinidel Pc 58, Pc 61 ja sortidel Alo, Jaak ja Edith (**I** tabelid 1, 2). Diferentsiaatorliinid Pc 58 ja Pc 61, mis olid haiguskind-

lad katsetsükli alguses 1996. a., olid kaotanud suures osas resistentsuse 2001. aastaks.

Odra haigustõrje katses (**II**) saavutati võrklaiksuse (haigustekitaja *Pyrenophora teres*) levikuks soodsamatel 2003. ja 2004. aastal kõige efektiivsem tõrje Folicur 250 EW täisdoosi jagatud pritsimisega, sama tendents esines ka võrklaiksuse levikuks vähemsoodsal 2005. aastal (joonis 1). Kuid ka vastavalt I-Taimekaitse programmi poolt soovitatud kulunorm oli haiguse tõrjeks piisav 2003. a. õigeaegse tõrje korral. Kõrreliste pruunlaiksuse (haigustekitaja *Cochliobolus sativus*) infektsiooni intensiivsus jäi madalaks 2003. ja 2005. a., aga 2004. a. kahjustas taimi olulisel tasemel, vajades kõige efektiivsemaks tõrjeks kahekordset jagatud kulunormiga pritsimist (joonis 2). Ka kõige väiksemad preparaatide kogused vähendasid haiguse levikut, eriti haiguste levikule soodsal 2004. a. I–Taimekaitse soovituste järgimine oli igati põhjendatud, kuna programm arvestas sordi resistentsuse ja ilmastiku näitajatega. Esmakordselt näidati, et taimekaitse ja tootmine on efektiivsed, kui nad on kohandatud konkreetse sordi vajadustele konkreetsetes tingimustes. I–Taimekaitse soovitas kasutada väiksemat vastuvõetavat soovituslikku doosi võrreldes tavapritsimisega. Haiguskindlamal sordil Anni jäid fungitsiidi erinevate koguste kasutamisel tõrjeefektiivsused küllaltki sarnasteks, seega täisnormi kasutamine oli enim õigustatud ainult vastuvõtlike sortide Extract ja Barke puhul haiguste levikule soodsal aastal. Katsetulemuste dispersioonanalüüsi andmed näitasid, et mõlema taimehaiguse intensiivsusele oli kõige suurem aasta mõju (**II** tabel 2). Katsetulemused näitasid, et haiguskindlamad odrasordid vajavad vähem intensiivset kaitset.

Kaera haigustõrje katses (**III**) saavutati 2003. a. oluline pruunlaiksuse infektsiooni vähenemine Folicur 250 EW täisdoosi kasutamisel lipulehe kuni viljatupe avanemise kasvufaasides (BBCH 37–41) haigusele vastuvõtlikumatel sortidel Villu ja Hecht (joonis 2). Pruunlaiksuse tõrje hilisemates kasvufaasides loomise lõpust õitsemise alguseni (BBCH 59–63) ei olnud efektiivne ühelgi katseaastal. Kaera kroonrooste tõrjel oli varane pritsimine (BBCH 37–41) efektiivsem 2003. a. soojal ja keskmiste sademetega suvel kõikidele erineva resistentsuse tasemega sortidele (joonis 3). 2004. a. jahedamal suvel, kui haiguse lööbimine hilines ja peremeestaimede nakatumise tase jäi madalamaks, osutus efektiivsemaks hiline pritsimine (BBCH 59–63). Kroonrooste virulentsuse spekter ja sellele soodsad levimise tingimused mõjutasid pritsimisaja efektiivsust. Üldiselt jäi keemilise tõrje mõju nõrgaks olukordades, kus haigustekitaja juba kahjustas taime, kuid vältis efektiivselt uut eoste põlvkonda. Kõige madalam efekt haigustõrjele saadi resistentsemal sordil Jaak. Esmakordselt tõestati Eesti oludes, et oluline on pritsimise ajastamine, mis põhineb taimehaiguste survele. Kui haigus lööbib hilja, ei ole selle bioloogiline mõju nii suur. Sageli arenevad taimehaigused juulis sademete tõttu hüppeliselt, õigeaegne tõrje võimaldab selle ära hoida. Katsetulemused andsid selge tõenduse suurtest hooajalistest erinevustest haigustõrje ajastamises.

Odra genotüüp ja kasvuaegne fungitsiidi kasutamine mõjutavad koristatud terade saastumist mikroseentega (**IV** joonised 1, 2). Esmakordselt näidati, et sortide ja fungitsiidide valikuga saab lisaks kasvuaegsete taimehaiguste tõrjeefektiivsuse tõstmisele mõjutada ka järgmistesse aastatesse edasikanduvate fütopatogeensete ja saprotroofsete seente esinemist. Samuti on kasvuperioodi ilmastikutingimused tugevas seoses fütopatogeensete seente esinemisega odra teradel. Ainult *Alternaria* perekonna liikide puhul ei omanud aasta usutavat mõju (**IV** tabel 5).

Kaerahaiguste esinemist mõjutavate faktorite analüüs (**V** tabel 2) kinnitab, et ainult külvieelse väetamise korral korreleerub kroonrooste intensiivsus tugevasti aasta ilmastiku, sordi resistentsuse ja mulda viidud väetise kogusega ($R^2=0.933$, $p<0.001$). Pruunlaiksuse esinemist mõjutas enam aasta, väetamine, sordi haiguskindluse ja aasta koosmõju ning aasta ja väetamise koosmõju ($R^2=0.838$, $p<0.001$). Intensiivsel väetise- ja taimekaitsefoonil oli suurim seos aasta ja kroonrooste esinemise vahel. Samal foonil oli ka sordi mõju märkimisväärsem võrreldes ilma taimekaitseta väetamise variantidega. Kaera terasaaki mõjutas ilma taimekaitseta variandis kõige rohkem väetamine ($R^2=0.552$, $p<0.001$) ja väetamise ning taimekaitse kooskasutamisel omas suurimat mõju aasta ($R^2=0.712$, $p<0.001$). Võrreldes kontrolliga intensiivistus väetiskoguste suurenemisel nii kroonroostesse kui pruunlaiksusesse nakatumine (**V** tabel 3). Oodatult vähendas keemilise taimekaitse kasutamine mõlemasse haigusesse nakatumise taset kõikides väetamise variantides.

Suurema enamsaagi saamise tõenäosus oli suurem kõrgema saagitaseme korral. Odra ja kaera enamsaagi (väljendatud hektari saagina kg ha^{-1}) väärtuse ja majandusliku tasuvuse (€ ha^{-1}) analüüs näitas, et väga olulised olid fungitsiidi hind ja kultuuri oodatav realiseerimishind. Võrreldes enamsaagi hinda fungitsiidile ja pritsimisele tehtud kulutustega selgus, et fungitsiidi kasutamine oli majanduslikult kasulik väiksema haiguskindlusega odrasordil Barke kõigi katseaastate I-Taimekaitse ja 2003.,

2004. a. jagatud pritsimistega variantides ning sordi Extract 2003., 2004. a. mõlemas töödeldud variandis (**II** tabel 3). Arvestades odra realiseerimishindu oleks haigustõrjes mõttekas suurendada I-Taimekaitse soovituslikku kulunormi ja loobuda suure kuluga kahekordsest pritsimisest. Kuigi täisnormiga pritsimisel saadakse suure tõenäosusega suurem saak, olid keskmise haiguse levikuga aastatel kulutused fungitsiidile suuremad võrreldes saagilisast saadud kasumiga (joonis 4). Täisnormiga haigustõrje oli õigustatud ainult vastuvõtliku sordi puhul haiguste levikule soodsal aastal. Kaera katses oli saagitõus positiivne enamikes töödeldud variantides (joonis 5). 2003. a. kattis kõigi variantide puhul enamsaagist saadud tulu fungitsiidi ja töö maksumuse (**III** tabel 3). 2004. a. oli majanduslikult tulus haigustele vastuvõtlikumate sortide varane e. ennetav tõrje. Fungitsiidi tõrjeaja valik ennetava või kaitsva toimena, vastavalt sordi haiguskindlusele, aitas vähendada taimehaiguse intensiivsust ning mõjutas haiguse levikut. Nendes odra ja kaera haigustõrje katsetes ei ole välja toodud terade kvaliteedi ja fungitsiidi kasutamise vahelisi seoseid, kuid fungitsiidi kasutamisel võib paraneda terade kvaliteet sellisel määral, et sellest tõuseb teravilja hind ja muutub ka tasuvus, kuna kõrgem kvaliteet võimaldab realiseerida saaki kõrgema hinnaga.

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I

PUBLICATIONS

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GENETIC CONTROL OF OAT RUST DISEASES

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Genetic control of oat rust diseases

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Abstract. Oat grain is indicated to be of great value, especially for its favourable effects on the health of humans and animals. Food and feed industries can utilise only fully developed and faultless oat grain that can be harvested from healthy, unattacked plants. Cultivating disease-resistant varieties seems to be an optimum alternative to chemical control. Growing of the resistant varieties is the most effective biological control of diseases. It is highly economic and ideal from the ecological point of view. Disease resistant varieties are the basic precondition for successful sustainable (organic) agriculture. Stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Erikss. et Henn) and crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Erikss.) are the potentially destructive diseases of oat crop in Estonian conditions. The effectiveness of resistance sources to *Puccinia coronata* and *Puccinia graminis* was tested in the framework of the European and Mediterranean Oat Disease Nursery (EMODN) at Jõgeva Plant Breeding Institute in 1996–2002. Highly resistant to crown rust were Pc-gene lines Pc 39, Pc 54-2, Pc 59, Pc 60, Pc 68 and Pen2xCAV1376. The greatest change in crown rust incidence was recorded for Pc 58 and Pc 61. These lines were completely free from disease infection at the beginning of the trial cycle, but, in 2001, were attacked at a moderate level (5–6 points in 9-point scale). The differential 'Pirol' and the varieties 'Alo', 'Jaak' and 'Edit' of the Estonian Variety List lost resistance to crown rust in 1998. Effective stem rust resistance against *Puccinia graminis* f. sp. *avenae* were conferring Pg-gene lines Pg 15, Pg a and Rodney ABDH. The first report of virulence on Pg 13 in Europe was detected in the framework of EMODN trials in Estonia in 1993.

Key words: Disease resistance, disease incidence, oat crown rust, *Puccinia coronata* f. sp. *avenae*, oat stem rust, *Puccinia graminis* f. sp. *avenae*

INTRODUCTION

Rust diseases are mostly the greatest harmful diseases of cereals. All above-ground parts of a plant can be infected from seedling to mature stadium. Fungi of *Puccinia* species are obligatory parasites with a high level of life cycle complex (alternate host). Symptoms of disease occur on stem and petiole, blade and spike may also be infected. Host plants give three spore forms. On oats two spore stages of rust can occur: the uredial and the telial. Urediospores are mainly responsible for epidemics of rust diseases. Urediospore formation is maintained as long as green plants are available. At cereal ripening rusts cease to produce urediospores and begin to produce teliospores (Sebesta et al., 1997). Uredial pustules develop tear epidermis and bare red-brown spores. Pustules are oval or oblong, open or chark epidermic tissues on the edges. Development of dark brown teliospores begins when plant is almost ripe. Teliospores remain on straw debrises. *Berberis vulgaris* and *Mahonia* species are alternate hosts to *Puccinia graminis*. Urediospores of stem rust are supposed to be realised by wind from a long distance. During plant growing period, new generation of uredia can be released in every 14 to 21 days (Zillinsky, 1983).

Crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Erikss.) belongs to the most widespread and damaging diseases of oat. It affects leaves, cheaths and panicles. The uredial pustules (uredinia) are oblong and yellow-orange. The telia of *Puccinia coronata* are dark and form rings around uredinia. The inoculum, responsible for epidemics of crown rust in Europe, comes either from wild oat species, other host grasses and volunteer oat plants, or from an alternate host, *Rhamnus catharticus*. The transport of urediospores like in other cereal rusts is supposed to be realised by wind from a long distance, from southeastern Europe (Sebesta et al., 1999).

Stem rust (*Puccinia graminis* Pers. sp. *avenae* Erikss. et Henn) is a potentially damaging oat disease. Stem rust is a more destructive pathogen than crown rust. The reduction of grain yield on a susceptible cultivar, resulting from the effect of stem rust, can vary between 27–29%. The 1000-grain weight can be decreased by 25–29%. Percentage of hulls can be increased by 30–39% as a result of stem rust infection (Sebesta et al., 1999). Stem rust affects all above-ground parts of an oat plant: leaves, sheaths, stems and panicles. The uredial pustules of stem rust are dark red-brown in colour and elongated. The urediospores of stem rust are elliptical. Epidermics of stem rust are incited by *Puccinia graminis* f. sp. *avenae* inoculum that comes either from wild oat species, other host grasses and volunteers, especially from *Berberis vulgaris* bushes. Like in other cereal rusts, urediospores are supposed to be transported from a long distance, from southeastern areas of the European Continent (Sebesta, 1998).

MATERIALS AND METHODS

The European and Mediterranean Oat Disease Nursery (EMODN) trials for testing the incidence of fungal diseases on oat differential cultivars were carried out at Jõgeva Plant Breeding Institute, where 32 oat lines with resistance or tolerance to either crown rust or stem rust were grown in provocative conditions during 1996–2002. Oat lines were sown on hill plots consisting of 10–15 plants in two replications. Late sowing time (beginning of June) was used to maintain green foliage at the time of a spread of urediospores from naturally infected plants. During the same time, oat varieties included in the Estonian Variety List were assessed for the incidence of rust infection under the conditions of natural infection and compared with the results of the EMODN trial. Field trials were carried out on 10-m^2 plots in 4 replications with 500 germinating seeds/m^2. The disease assessment time was selected according to the appearance of infection in most susceptible lines. Disease scoring was made *ca* two weeks after the establishment of infection in susceptible lines. The incidence of disease infection was estimated according to the 1–9 scale (1- no disease, 9- severe infection) on the whole plot.

RESULTS AND DISCUSSION

The evaluation of new germplasms to identify potential donors of disease resistance is of prime importance in crop improvement. Obtained information on host reactions to pathogenic fungi is especially valuable.

The incidence of *Puccinia coronata* f. sp. *avena* in EMODN varieties in 1996–2002 has been presented in Table 1. There was no virulence to any of the gene lines used in the EMODN set in earlier trials conducted at Jõgeva in 1992 and 1993. Moderate to high incidence of *Puccinia coronata* was recorded in 1996 and 1997 when conditions for disease incidence were very favourable. Pc-gene lines Pc 50, Pc 55, Pc 63, Pc 67, Kr 3813/73 and Garland were very susceptible. Pathogenic situation was different in the dry conditions of 1998, when infection pressure was weak until the end of August. The trial in 1999 failed because of the hot and dry June and July. Disease incidence was moderate in 2000, when dry and warm weather during sowing was followed by a cool and rainy summer. The warm and rainy summer of 2001 favoured spread of the infection. Warm and droughty weather with foggy nights favoured development and spread of *Puccinia coronata* in 2002.

Lines Pc 39, Pc 54-2, Pc 59, Pc 60 and Pen2xCAV1376 were resistant throughout the whole trial period 1996–2002. Susceptibility of the oat lines Pc 48, Pc 54-1, Pc 58, Pc 61, Kr 288/73L/569 and variety 'Pirol' against crown rust was increased during the trial period. The effective resistance of these genotypes was lost in the last years. Lines Pc 50, Pc 55, Pc 56, Pc 63, Pc 64, Pc 67, KR 3813/73 and 'Garland' have been highly susceptible and were severely attacked.

Moderate to high incidence of *Puccinia coronata* was also recorded in natural conditions (Table 2). Very high incidence of crown rust was recorded in 1998, when the rainy and warm June was a reason for the strong attack of the pathogene. Natural infection was at a moderate level in 1999 and 2000, when both, main and side shoots became infected. Favourable conditions for spread of crown rust were in the warm and rainy summer of 2001. All the varieties were infected up to a moderate level in the droughty and warm vegetation period of 2002.

A breakdown of the race specific resistance of the variety 'Alo' is considerable. 'Alo' was resistant to crown rust until 1997, but it became highly infected the first time in 1998 after the appearance of new rust races, and, during the last years, has been infected at the level of the most susceptible varieties. Similar infection pattern is also shown by the varieties 'Jaak', 'Edit' and 'Pirol'. Considerable similarity is found in the infection pattern of these varieties and the line Pc 58. Supposedly, the varieties 'Alo', 'Jaak', 'Edit' and 'Pirol' have the same crown rust resistance gene as the differential Pc 58.

The differential Pc-gene lines Pc 39, Pc 55, Pc 58 and Pc 68 have been resistant to local populations of *Puccinia coronata* f. sp. *avenae* in field trials carried out in different regions of Europa. Genotypes with these genes were completely resistant also in laboratory infection tests with pathotypes of *Puccinia coronata* f. sp. *avenae* isolated from different European countries.

Table 1. Incidence of *Puccinia coronata* on EMODN oat differential Pc-gene lines at Jõgeva in 1996–2002. Data from 1999 are not presented because of the absence of the disease infection.

Variety/line	Disease incidence (1–9 points)					
	1996	1997	1998	2000	2001	2002
Pc 38	7	8	2	3	5	4
Pc 39	2	2	2	2	2	3
Pc 48	3	2	2	2	5	3
Pc 50	3	5	1	4	7	3
Pc 50–2	5	3	2	6	X	3
Pc 50–4	5	5	2	1	5	5
Pc 54–1	2	2	2	1	5	4
Pc 54–2	2	2	2	3	2	2
Pc 55	3	7	2	3	2	7
Pc 56	7	7	1	4	8	7
Pc 58	1	2	2	2	5	6
Pc 59	2	2	2	1	1	2
Pc 60	2	2	2	1	X	2
Pc 61	2	2	2	2	5	6
Pc 62	5	3	1	5	5	4
Pc 63	3	8	1	4	7	7
Pc 64	7	7	1	7	5	7
Pc 67	3	6	1	5	X	6
Pc 68	5	2	2	2	1	X
Pen2xCAV1376	3	2	2	1	2	2
KR 3813/73	3	7	1	7	7	6
Pirol	2	2	2	5	7	6
KR288/73L/569	3	2	1	2	3	5
Garland	2	8	1	5	7	7

The lines Pc 48, Pc 50-2, Pc 50-4, Pc 54-1 and Pc 59 are of importance for the European crown rust resistance breeding of oats. In most countries they have been resistant or have shown only moderate infection in certain regions (Sebesta et al., 1998). Pc 48 has shown somewhat higher effectiveness against crown rust compared to Pc 50-2 in Estonian trials. Pc 54-1 was only slightly infected until 2000, after that the susceptibility increased. The most resistant lines in Estonia have been Pc 39, Pc 54-2, Pc 59, Pc 60 and Pen2xCAV1376. They have importance in crown rust resistance breeding. The Pc-gene lines Pc 50-4 and Pc 67, effective in Europe, are susceptible in Estonia (Table 1).

Table 2. Incidence of *Puccinia coronata* on oat varieties of the Estonian Variety List at Jõgeva in 1996–2002.

Variety	Origin	Disease incidence (1–9 points)						
		1996	1997	1998	1999	2000	2001	2002
Miku	Estonia	7	6	7	3	6	7	4
Alo	Estonia	1	2	6	3	2	7	4
Jaak	Estonia	3	3	7	3	2	7	3
Villu	Estonia	7	7	9	3	4	7	5
Salo	Sweden	7	6	9	2	4	7	7
Freja	Sweden	7	5	8	4	3	7	5
Edit	Sweden	2	3	5	2	5	7	5
Lena	Norway	5	6	8	4	3	6	4
Leila	Norway	6	3	8	4	2	7	5
Revisor	Germany	7	6	9	3	3	7	4

Table 3. Incidence of *Puccinia graminis* on EMODN oat differential Pg-gene lines at Jõgeva in 1996–2002. Data from 1999 are not presented because of the absence of the disease infection.

Variety/line	Disease incidence (1–9 points)					
	1996	1997	1998	2000	2001	2002
Rodney A (Pg 2)	2	1	1	1	1	2
Rodney B (Pg 4)	5	1	1	1	1	1
Rodney H (Pg 9)	3	2	1	1	1	1
Rodney M (Pg 13)	2	2	1	1	1	1
Pg 15	2	1	1	1	1	1
Pg 16	3	2	1	1	1	1
Pg a	1	1	1	1	1	1
Rodney ABDH	2	1	1	1	1	1

Compared with the situation in the foregoing periods, the virulence spectrum of oat stem rust populations and the effectiveness of resistance genes has changed considerably during the last decade in Central Europe (Sebesta et al., 1998). The same has occurred in Estonia.

Stem rust (*Puccinia graminis* f. sp. *avenae)* incidence on EMODN differential Pg-gene lines was low throughout the trial period 1996–2002 (Table 3). Favourable conditions for stem rust infection were in 1996, when moderate infection of lines Rodney B, Rodney H and Pg-16 (3–5 points) were recorded. Almost no stem rust infection was observed during the last five years. The trial failed totally in the droughty year 1999.

Table 4. Incidence of *Puccinia graminis* on oat varieties of the Estonian Variety List at Jõgeva in 1996–2002.

Variety	Origin	Disease incidence (1–9 points)						
		1996	1997	1998	1999	2000	2001	2002
Miku	Estonia	3	4	2	1	3	1	1
Alo	Estonia	1	2	1	1	1	1	1
Jaak	Estonia	1	2	2	1	1	1	1
Villu	Estonia	3	2	3	1	1	1	1
Salo	Sweden	3	4	4	1	1	2	1
Freja	Sweden	1	5	3	1	1	1	1
Edit	Sweden	1	1	1	1	2	1	1
Lena	Norway	2	3	1	1	1	2	1
Leila	Norway	3	2	2	1	1	1	1
Revisor	Germany	3	3	4	1	1	2	1

More severe infection was recorded in trials with oat varieties of the Estonian Variety List (Table 4). All the varieties were infected at a moderate level in 1996–1998. The weather of the following years did not favour development and spread of stem rust. Almost the absence of or very low stem rust infection has not allowed monitoring of changes in race compositon of this pathogen.

The Pg-gene lines Rodney A, Rodney M, Pg 15, Pg a and Rodney ABDH were highly effective in Estonia. The line Rodney M carries the major resistance gene Pg 13 and the line Rodney A gene Pg 2. Line Pg a has 3 recessive resistance genes (Table 3).

The recessive gene Pg 13 has been considered one of the most effective stem rust resistance genes available to breeders. The first stem rust isolate able to overcome resistance of gene Pg 13 was detected in the framework of the EMODN trial network in Estonia (Sebesta et al., 1999).

CONCLUSIONS

Early teliospore formation could be included in the genetic control of those cereal rust populations in which it functions. Selection for this trait might be carried out in the field or even in seedling tests. However, more research on both pathogens – host relationships and the effect of external factors on the early development of teliospores – process is needed (Sebesta et al., 1999).

The pathogenic situation of *Puccinia coronata* Cda. f. sp. *avenae* Eriks. has changed in Estonia during 1996–2002. The Pc-gene lines Pc 39, Pc 59, Pc 60, Pc 68, Pen2 x CAV1376 were resistant throughout the trial period. Pc 58 and Pc 61 were relatively resistant up to 2000 and attacked moderately (5–6 points) in 2001. The variety ‘Pirol’ was resistant up to 1998, but highly (5–7 points) infected in 2000. The lines Pc 38, Pc 50, Pc 56, Pc 62, Pc 63, Pc 64, Pc 67 and the varieties Kr 3813/73 and

‘Garland’ were highly susceptible to crown rust all the years. The varieties ‘Alo’, ‘Jaak’ and ‘Edit’of the Estonian Variety List have lost resistance to *Puccinia coronata* f. sp. *avenae*. They were first time infected in 1997.

Oat stem rust (*Puccinia graminis* Pers f. sp. *avenae* Erikss. et Henn) is potentially a more destructive disease than crown rust (*Puccinia coronata* Cda f. sp. *avenae* Erikss.), however, fortunately, it occurs at a high incidence only in some areas in Europe (Sebesta et al., 1998). High or moderate levels of stem rust were recorded at Jõgeva in 1995 and 1996. Uredial samples of *Puccinia graminis* f. sp. *avenae* from Estonia were analysed for their virulence in relation to Pg lines.

Testing of the effectiveness of resistance sources to *Puccinia coronata* and *Puccinia graminis* creates a good basis for a selection of initial material for resistance breeding of oat.

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EFFICACY OF FUNGICIDE TEBUCONAZOLE IN BARLEY VARIETIES WITH DIFFERENT RESISTANCE LEVEL

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Efficacy of fungicide tebuconazole in barley varieties with different resistance level

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Efficacy of the fungicide tebuconazole was tested in 2 treatment regimes in 3 spring barley varieties over three years (2003–2005). The impact of the fungicide on the control of major barley pathogens *Pyrenophora teres* and *Cochliobolus sativus*, as well as kernel yield was studied in the course of field trials. The fungicide treatments had a strong impact on the control of infection of *P. teres* and increased kernel yield in variable disease infection conditions. For the more resistant genotype, fungicide application had relatively low returns because of the much higher level of biological resistance and small disease-related yield reductions. For the susceptible genotype, severe disease infection caused bigger yield reduction, not compensated by the use of fungicides. Use of fungicide demonstrated the highest economic return in the case of the moderately susceptible barley variety.

Key-words: spring barley, net and spot blotch, tebuconazole, application time, yield, quality

Introduction

Spring barley is the prevailing spring crop in Estonia, with growing area of 128.2 thousand ha, occupying 48.2% of the total area under cereals in 2004 (www.stat.ee). In Estonian conditions, spot blotch, caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsler, am *Bipolaris sorokiana* (Sacc.) Shoem. Syn. *Helminthosporium sativum* P.K. et B) and net blotch, caused by *Pyrenophora teres* Drechsl. am. *Drechlera teres* (Sacc.) Shoem. (Palmer 1989, Mathre 1997), are serious foliar diseases of barley (*Hordeum vulgare*), causing serious yield and quality reduction. Both pathogens are mainly controlled by fungicide treatments. Estonian disease monitoring of the last decade has shown that the occurrence of net blotch is increasing, which is directly related

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to the grown cultivars (Tamm 2003). Other common barley diseases - powdery mildew (*Erysiphe graminis* f. sp. *hordei* E.M. Syn. *Blumeria graminis* (DC) E.O. Speer f.sp. *hordei* E.M.) (Mathre 1997) and scald (*Rhynchosporium secalis* (Oudem.) J.J. Davis) (Mathre 1997) – have been rather infrequent during the last years and have rarely crossed the threshold of economic importance.

Net blotch has been widely spread in particular years and is a serious problem in untreated fields. The first symptoms of *P. teres* can be seen in barley leaves starting from GS 12, the symptoms of *C. sativus* normally develop at later growth stages, in Estonian conditions after GS 37–39. The time and level of disease infection in the field depends on the susceptibility of the used variety, therefore the resistance of varieties has a great importance in the control of plant diseases. Use of fungicides reduces the occurrence of fungal diseases and thereby reduces yield losses, increasing the economic profit. Economic profitability of fungicide use in spring barley is questionable during the years less suitable for disease development. The best disease control and yield increase are achieved by fungicide application at the early and late development stages of the crop plant (split application) but because of the high costs of fungicide application, the economic result could often be negative. Trial results from several countries have shown that despite of achieved yield increase, the high cost of fungicide application does often result in negative net revenue (Jørgensen 2006, Tischner et al. 2006, Laine et al. 2007). Higher net revenue is achieved by use of reduced fungicide doses at a later stage of plant development (after GS 37).

The aim of the study was to find out the efficacy of fungicides at different disease control intensities on spring barley varieties differing in the resistance level.

Fungicide trials usually deal with the effect of different fungicides and their doses on reduction of disease incidence and increase of yield or net revenue. Less attention is paid to the influence of the variety on the size of harvested yield and formation of net revenue. The objective of this study was to find out whether fungicide treatments would be justified in relation to the low grain prices in Estonia.

In our study we used split application of tebuconazole (250 g. a.i.; trade name in Estonia: Folicur 250EW) and reduced doses of the fungicide recommended by decision support system PC–Plant Protection (PC–P) (Jørgensen et al. 2003). PC–P adjusts the fungicide dose according to the variety resistance, growth stage, disease pressure and efficacy of fungicide. Split fungicide application provides long-lasting protection and has the best effect in control of diseases but compared to other treatment regimes, the cost of application is higher. PC–P is designed to recommend the minimum fungicide dose during the critical stage of disease development to restrict the development of diseases and to achieve the highest economic returns.

Implementation of integrated control strategies needs comprehensive studies on the efficacy of plant protection on varieties with different resistance levels and at different application intensities.

Material and methods

Field trials on disease control of spring barley were arranged with three replicates in a randomized design 20m² plots at the rate of 500 germinating seeds per 1m² at Jõgeva Plant Breeding Institute during the three seasons of 2003–2005. Three two-row spring barley varieties with different resistance levels were used: Anni (moderately resistant to net and spot blotch), Barke (moderately susceptible to net and spot blotch), Extract (susceptible to net and spot blotch). Untreated certified seed was used for all varieties. The varieties were selected based on data from previous disease scoring trials in the same region (Tamm 2003). Fungicide application was started upon the first symptoms of infection (Table1). The effect of split application of tebuconazole 0.5 l ha^{-1} at stages BBCH 32–51 (T1) and BBCH 57–65 (T2) was compared with the effect of reduced fungicide dose recommended by the decision support system PC–P for controlling disease infections on moderately susceptible barley varieties. For the PC–P treatments, the same fungicide dose and application time was used for

Table 1. Time of fungicide application and assessment of disease infection (dates, growth stages BBCH) for trials of 2003–2005.

Number of Assessment	Date	Growth stages (BBCH)	Fungicide dose, l ha^{-1}	Term of disease scoring
2003				
I	27/6	GS 32–33	T1 – tebuconazole 0.5 l ha^{-1}	
II	16/7	GS 57	T2 – tebuconazole 0.5 l ha^{-1} Reduced dose – tebuconazole 0.3 l ha^{-1}	
III	25/7	GS 71–73	-	30 days after T1, 10 days after T2 and Reduced dose
2004				
I	9/7	GS 51	T1 – tebuconazole 0.5 l ha^{-1}	
II	21/7	GS 65	T2 – tebuconazole 0.5 l ha^{-1}	
III	16/7	GS 59	Reduced dose – tebuconazole 0.16 l ha^{-1}	
IV	17/8	GS 75	-	40 days after T1, 30 days after T2 and Reduced dose
2005				
I	26/6	GS 35–37	T1 – tebuconazole 0.5 l ha^{-1}	
II	18/7	GS 61–65	T2 – tebuconazole 0.5 l ha^{-1} Reduced dose– tebuconazole 0.15 l ha^{-1}	
III	1/8	GS 73–75	-	40 days after T1, 10 days after T2 and Reduced dose

T1 – first, T2 – second treatment.

all three varieties. Phenological growth stages were determined according to BBCH-identification keys for cereals (when > 50% of the plants had reached the target growth stage). Fungicides were applied with a bicycle sprayer equipped with 6 Hardy nozzles 4110-12 on a 2.5-m boom using 300 l of water per ha^{-1}.

Disease infection was scored as the percent of leaf area infected by *P. teres* and *C. sativus* at GS 71–75. The three top leaves of the plant were assessed separately on three adjacent tillers at 10 randomly selected places on each plot. The infection level was expressed as an average of the infection score on second leaves (L-2; the first leaf under the flag leaf). The lesions of net blotch were determined according description of Tekauz (1986). The symptoms caused by *C. sativus* were distinguished from net blotch spot type according to relative size of lesion and presence of necrosis and chlorosis. Lesions with marginal chlorosis bearing character of moderately resistant or more susceptible infection response according to scales of Fetch and Steffenson (1999) had classified to be caused by *C. sativus*. The identification was confirmed by examination of lesions under the microscope for spore production after incubation of leaves with disease symptoms in moisture chamber.

Trials were harvested with a plot combine harvester and the grain yield was adjusted to kgha^{-1}. Qualitative and quantitative analysis of the yield parameters was conducted on dried and cleaned seeds and expressed on the basis of 14% moisture content. The net yield (harvested yield minus the cost of fungicide and application) was calculated in kg ha^{-1}. The average price of barley (0.1 EUR/kg^{-1}) in Estonia for the period 2003–2005 was used for calculating the yield revenue. The costs of the fungicide and work (7.7 EUR/ha) were subtracted from the value of the yield increase achieved with the fungicides. All prices were used without VAT.

The data were analyzed with ANOVA, using Agrobase 20 software package.

Results

Disease development

Net blotch (*P. teres*) infection dominated during all the years. The infection was more severe in 2003 and 2004 when it was promoted by high relative humidity and high air temperature (Fig.1–a). As expected, in both years, the highest infection levels were observed in the variety Extract (untreated 12.7 and 43.6% respectively). The biggest difference between varieties in terms of infection was observed in 2004 (Fig.1–b) when more susceptible varieties Barke and Extract were strongly infected. Hot and dry July limited development of *P. teres* in 2005 when only slight damage by net blotch infection was observed. Only minimal infection occurred on Anni and Extract (untreated 1.5 and 1.7% respectively), infection level in Barke was 2.7% (Fig.1–c).

Spot blotch infection caused by *C. sativus* was observed during all the years (Fig. 2). The infection reached a significant level only in 2004 when *C. sativus* occupied 19.3–42.8% of leaf area for untreated varieties. Spot blotch infection level was low in 2003 and 2005, being the highest in the susceptible variety Extract. Based on infection levels of both diseases, the trial years can be classified in the following terms: 2003 – medium infection, 2004 – severe infection and 2005 – slight infection.

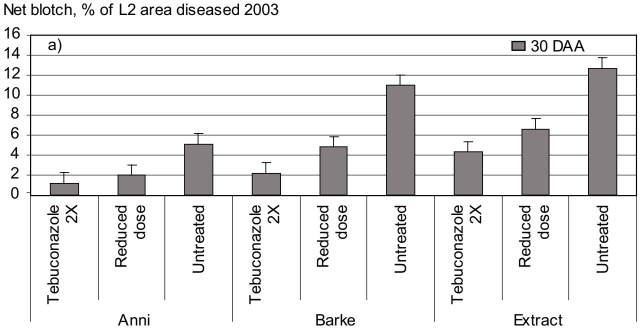

Fig. 1 a. Efficacy of tebuconazole application against *P. teres* and untreated control on L-2 leaves in spring barley varieties in 2003. L-2= first leaf under flag leaf. DAA – days after application. Anni -moderately resistant, Barke - moderately susceptible, Extract - susceptible to net blotch. I – LSD0.05 =1.04.

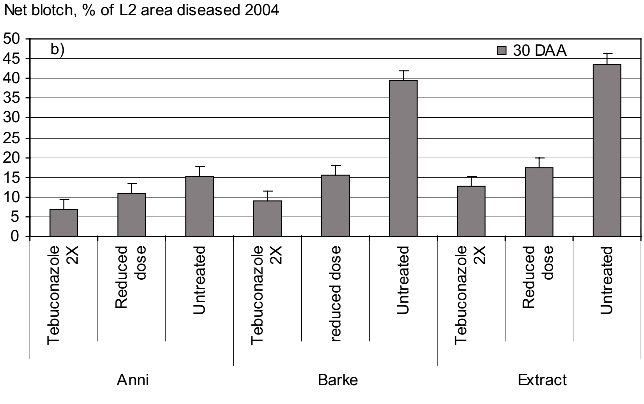

Fig. 1 b. Efficacy of tebuconazole application against *P. teres* and untreated control on L-2 leaves in spring barley varieties in 2004.
L-2= first leaf under flag leaf. DAA - days after application. Anni -moderately resistant, Barke - moderately susceptible, Extract - susceptible to net blotch. I – LSD0.05 =2.54

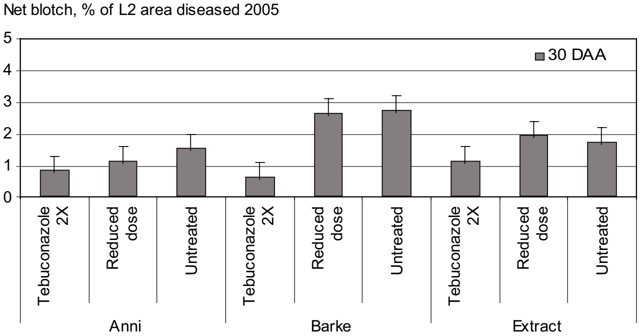

Fig. 1 c. Efficacy of tebuconazole application against *P. teres* and untreated control on L-2 leaves in spring barley varieties. L-2= first leaf under flag leaf. DAA - days after application. Anni -moderately resistant, Barke - moderately susceptible, Extract - susceptible to net blotch. I – LSD0.05 = 0.51.

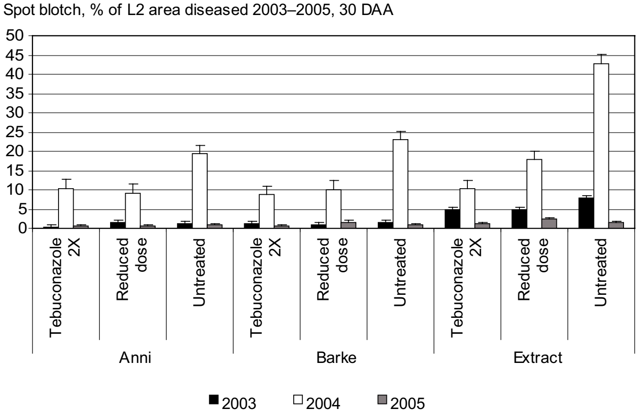

Fig. 2. Efficacy of tebuconazole application against C. sativus and untreated control on L-2 leaves in spring barley varieties during 2003-2005.
L-2= first leaf under flag leaf. DAA -days after first treatment. Anni - moderately resistant, Barke - moderately susceptible, Extract - susceptible to spot blotch. I – LSD0.05 = 0.71 (2003); 2.25 (2004); 0.42 (2005).

Effect of fungicide application on disease control

As expected, the best disease control effect was achieved with two treatments per season. Two fungicide applications were needed in 2004 to control severe disease pressure. Also in conditions of low disease infection in 2005, split application of Folicur 250EW resulted in significantly better disease control effect than the use of reduced dose according to PC–P. Application of reduced fungicide dose according to PC–P was sufficient to control the spread of *P. teres* and *C. sativus* in spring barley in 2003 when the right timing for the application and the optimal dose for the disease pressure were chosen.

Effect of different factors on disease incidence. Results of ANOVA verified that the impact of the year had the biggest influence on the infection intensity of *P. teres* and *C. sativus* (Table 2). The year and treatment were major factors determining the infection level with *P. teres*. The infection level with *C. sativus* was mostly determined by year, variety and year by variety interactions. Other factors' influence on the infection level was smaller. The coefficients of determination indicate that environmental and genetic factors' contribution to the occurrence of *P. teres* was 72% (R^2=0.72). The occurrence of *C .sativus* was less dependent on environmental and genetic factors (R^2=0.46). The rest is related to some other factors.

Table 2. Mean squares of ANOVA of infection data of *P. teres* and *C. sativus.*

	Net blotch	*p*>F	Spot blotch	*p*>F
Treatment	20.8	0.000	4.0	0.000
Year	58.0	0.000	38.8	0.000
Variety	7.7	0.000	37.0	0.000
Year by treatment	8.7	0.000	2.0	0.000
Year by variety	2.8	0.000	16.4	0.000
Year by variety by treatment	2.1	0.000	1.8	0.000
R^2	0.7236		0.4608	

Grain yield

The intensity of plant protection caused significant differentiation in yields of tested varieties by treatment variants (Fig. 3). Two applications with the half-dose of tebuconazole improved disease control and resulted in the best yield in all varieties in all years. In all years, the split-treatment strategy brought about higher yield increase for more susceptible varieties Barke and Extract. Yield increase resulting from PC–P –based fungicide application was significantly lower than that from split application. The moderately resistant variety Anni had relatively low returns on both treatment regimes because of the much higher level of biological resistance.

Fungicide impact on net revenue. Comparing the economic benefit of the extra yield produced by the barley varieties treated by the two strategies, the net yield of moderately resistant variety Anni did not increase, but was even reduced in result of PC–P treatment (Table 3). The yields of the other varieties were equal to or exceeded the control crop. In accordance with these results, we have to examine the possibility that use of fungicides at later growth stages may reduce yield formation in more resistant varieties. Because of low disease pressure, the use of fungicides was not profitable on any of the varieties in 2005.

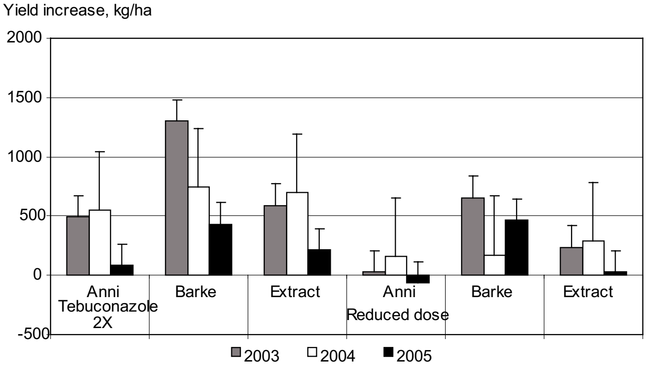

Fig. 3. Yield increase kg ha^{-1} in fungicide treated variants in comparison with untreated control crop in 2003–2005.
I - LSD0.05 = 182 (2003); 494 (2004); 178 (2005). Yields of untreated control crop (kg ha^{-1}) in the trials were 1) in 2003: Anni–4451, Barke–3356, Extract–3693, 2) in 2004: Anni–5924, Barke–3452, Extract–4869, 3) in 2005: Anni–4980, Barke–4012, Extract–4271.

Table 3. Yield profit in terms of money (EUR ha^{-1}) of spring barley varieties in 2003-2005.

Variety		Net yield kg/ha		Net revenue EUR		Benefit in monetary terms EUR/ha	
		Tebuconazole 2X	Reduced dose	Tebuconazole 2X	Reduced dose	Tebuconazole 2X	Reduced dose
Anni	2003	4419	4320	442	432	-8	-16
	2004	5945	5120	595	512	-4	-73
	2005	4543	4838	454	484	-42	-15
Barke	2003	4133	3852	413	385	59	37
	2004	3675	3541	368	354	13	1
	2005	3920	4399	392	440	-13	29
Extract	2003	3760	3772	376	377	0.3	2
	2004	5046	5072	505	507	9	11
	2005	3963	4218	396	422	-31	-8

Anni - moderately resistant, Barke - moderately susceptible, Extract - susceptible to net and spot blotch; Net yield = harvest yield minus the cost of fungicide and application. Price of chemical control 49 (Tebuconazole 2x); 18 (Reduced dose 2003); 13 (Reduced dose 2004); 10 (Reduced dose 2005) EUR/ha.

Discussion

In recent years, intensive cereal cultivation with limited crop rotation and suitable seasonal weather factors have increased the occurrence of net blotch and, to a lesser extent, spot blotch infection in spring barley. Chemical control measures are needed to avoid yield reduction by disease infections. To achieve economic profitability, it is important to deploy integrated pest management practices among production methods. In the future, the tendency will probably be towards management of disease control under integrated protection methods whereby the fungicide dose and time of application are calculated based on the resistance level of the variety, the prevailing weather conditions and infection situation in the field, taking into account economic profitability. It has been found in the UK that fungicide treatment is effective when the infection level is visually more than 5% of leaf area (Cook et al. 1999), thus indicating that in the case of low-intensity infection, yield loss is smaller than the cost of fungicide application. Infection level of a specific variety may exceed the infection threshold because of weather conditions and/or susceptibility. For effective control and maximal net yield, the minimal dose of fungicide may be smaller than the standard dose, if adjusted at the point of time when the disease normally emerges.

According to Jørgensen et al. (1996), Hardwick et al. (2000), Henriksen et al. (2000), and Jørgensen et al. (2003) it was found that the use of reduced fungicide doses was effective depending on the weather conditions of the year and on the resistance level of the variety. The PC–P system has previously been described to combine information on thresholds with recommendations for treatments using adjusted fungicide dose (Jørgensen et al. 1996, Henriksen et al. 2000, Jørgensen et al. 2003).

The results of this trial showed that the yield had tendency to display high returns upon application of high fungicide rates, but the high costs of fungicide application reduced the net revenue. Intensive protection was important for barley yielding but the integrated plant protection system was more economical, as the use of PC–P method enables to lower direct costs. Danish trials (Jørgensen et al. 2000) have produced similar results. In our trials, the PC–P variants had significantly higher net yields in 2005, in conditions of low disease infection. This indicates that the fungicide dose may be lowered to achieve disease control but has to be applied at the proper time to be highly effective.

It is important to note that fungicide application is, in many cases (low disease pressure, resistant cultivar), not profitable for the farmer and the correct decision would be to refrain from it. In order to predict the need of fungicide use, it is important to use a DSS, e.g. PC–P. Comparison of fungicides on the market for spring barley in Finland in 2006 has shown that fungicide applications did not improve net revenue in any of the spring barley trials during the dry season of 2006, how ever the negative effect compared to the untreated plots was not significant either (Laine et al. 2007). Trial results from Bavaria also indicate that in years less favorable for disease development, disease control in spring barley can result in negative net revenue (Tischner and Schenkel 2006).

Our trials demonstrated a significant impact of variety resistance on net revenue. Fungicide use in the moderately resistant variety Anni resulted in negative net revenue for all years and doses, the moderately susceptible variety Barke produced the highest and significant net returns and the susceptible variety Extract produced medium net revenue. The variety's tolerance towards disease infection could be a reason for differences in net yield between the studied varieties. According to definition, tolerance is an ability of plants to endure severe disease without severe losses in yield and quality (Schafer 1971). Yield reduction in the variety Anni seems to be lower than could be expected based on the disease infection level and therefore chemical control is too costly for this variety. On the other hand, disease infection seems to cause more severe yield reduction for the susceptible variety Extract than could be compensated by fungicide application. Trials performed in Finland have also shown that the relationship between net blotch symptom expression and yield maintenance in spring barley genotypes was stronger in the case of higher yields and less severe net blotch infection. In conditions of lower yields and/or severe disease infection, the relation between the level of disease infection, yield losses and net revenue was less clear (Robinson 2000).

Multiyear trials in Northern Ireland with a range of fungicides, applied at a range of doses, have demonstrated that the overall profitability was higher for resistant cultivars than for susceptible cultivars and that treatment of resistant cultivars with fungicides did not significantly increase profitability of winter wheat and spring barley any further (Mercer and Ruddock 2002, 2005). Results indicate that the potential of disease resistance of cultivars should be fully exploited and prophylactic spraying is unlikely to be profitable (Mercer and Ruddock 2002).

Our previous trials on spring wheat have shown that chemical disease control is most complicated in relation to moderately susceptible varieties grown in conditions of medium disease pressure (Koppel et al. 2003). Results of the current study indicate that chemical control is most profitable for moderately susceptible spring barley varieties with the use of different fungicide doses, thus differing from the chemical disease control of spring wheat. DSS programs should take more account of relations between severity of disease symptoms and yield reduction.

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TIMING OF FUNGICIDE APPLICATION FOR PROFITABLE DISEASE MANAGEMENT IN OAT (*AVENA SATIVA* L.)

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Timing of fungicide application for profitable disease management in oat (*Avena sativa* L.)

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Abstract

The efficacy of tebuconazole fungicide for the control of oat leaf diseases caused by *Pyrenophora avenae* and *Puccinia coronata* was studied during 2003–2005. Two application times and four varieties belonging to three resistance categories were used in the trial series. The aim of the study was to test the effect of fungicide timing and variety resistance on the disease control efficacy and yield increase. The trial series provided diverse results in terms of disease control and economic profitability. There were no single solutions for timing of the fungicide application. Depending on the weather conditions, better efficacy in disease control or higher economic return was achieved from fungicide use at flag leaf stage or at heading stage.

Key words: *Avena sativa*, chemical control, crown rust, oat leaf spot, tebuconazole, application time, yield.

Introduction

Crown rust and leaf spot are major diseases in oat (*Avena sativa* L.). Oat leaf spot, caused by *Pyrenophora avenae* (Ito & Kurib., conidial stage: *Drechslera avenae* syn. *Helminthosporium avenae*) is a seed-borne pathogen, the fungus also survives on host debris but the main source of inoculum is seed infested with the long-lived resting mycelium (Zillinsky, 1983; Clifford, 1995). When infected kernels germinate, the fungus infects the seedling leaves. Produced spores are dispersed to other leaves and they produce new spots. The disease infects oat plants at early stages of development under conducive weather conditions. Disease infestation is favoured by rains and moist, humid periods as spores germinate at temperatures of 10–20°C and 100% humidity (Motovilin, 2000). Because of the destruction of leaf tissue, photosynthesis is reduced in diseased plants leading to yield decline (Motovilin, Strihekozin, 2000).

Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae* is a long-cycled rust fungus having buckthorn (*Rhamnus* sp.) as alternate host (Nyvall, 1979; Šebesta et al., 1997). Crown rust develops best during mild to warm (20–25°C) sunny days and mild nights (15–20°C) with adequate moisture for dew formation (Top crop manager, 2008). In Estonian conditions, the infection usually occurs at heading stage. Weather conditions being favourable for oat growth also favour crown rust, therefore greatest yield losses commonly occur in years when oat yields should be highest. Moderate to severe epidemics can reduce grain yield by 10% to 40% (Top crop manager, 2008).

Both diseases are increasing problem in oat production worldwide, therefore chemical control is widely used to maintain green leaf area and increase kernel yield. A number of fungicides are used to control crown rust and leaf spot, but their use is often limited by economic constraints (Clifford, 1995).

Proper choice of fungicide, its dose and application time are important in achieving the economic efficacy because of low returns caused by low price of oat grain. Efficacy of fungicides is highly dependent on the time of application. Fungicides can have a curative or preventive effect on diseases (Cook et al., 1999). It has been widely recognized (Cook et al., 1999; Dimmock, Gooding, 2002; Jorgensen et al., 2003) that fungicides applied during the period from flag leaf emergence to ear emergence, just after the appearance of first disease symptoms often provide the best prospect for cost-effective control of foliar diseases.

The use of preventive fungicide applications to prolong the duration of green leaf area and to increase the yield has been an increasing trend in farmer's practice over the last decade. The use of fungicides has been effective in disease control but represents an added input cost for producers. At the same time much research has been put into optimization of pesticide use in main cereals. The adjustment of application to complement the general resistance of a variety is important to improve the economics of disease control. Several studies have covered aspects of reduction of environmental concerns and increased economic profitability (Wale, 1994; Hedge, Verrelt, 1999; Mercer, Ruddock, 2003). However, very limited information is available about chemical control of oat leaf diseases and fungicide impact on oat yield and economic profitability.

Oat leaf spot and crown rust differ in their epidemiology – differing in weather conditions favouring the development of epidemics and having different infection times. Therefore these diseases trigger the need of fungicide treatment at different stages of plant development and at different weather conditions.

The aim of the current study was to identify the proper timing of fungicide treatment in control of leaf diseases in oats and to study the effect of variety resistance in control of oat diseases. Other targets were to find the best time for a single fungicide application to control both diseases and to study the effect of fungicide application on yield and economy. We compared two fungicide application times, widely accepted time at flag leaf emergence (BBCH 37) and late application after the heading (BBCH 59). Because of low oat grain price, the avoidance of unnecessary expenses on disease control is of great importance in order to achieve economic profitability.

Materials and methods

Field trials on disease control of oat were arranged in a randomized block design in 20 m² plots with three replicates at Jõgeva Plant Breeding Institute, Estonia during 2003–2005, on *Calcaric cambisol* (*CMc*) soil (K_0, pH_{KCl} 5.8; available P 190, K 180, Ca 1520, Mg 64, Cu 1.3, Mn 41 and B 0.7 mg kg^{-1}). A sowing rate of 500 viable seeds per 1 m² was used in all trials seeded at optimal time in the first week of May. Fertilizer application was 80 N ha^{-1}, 40.5 P_2O_5 ha^{-1} and 40.5 K_2O ha^{-1} using complex fertilizer Kemira Power 18 (18 N, 9 P_2O_5, 9 K_2O) at planting. Additional nitrogen AN 43 80 kg ha^{-1} was applied at shooting stage BBCH 21–23. Previous crop was potato in all years. Four oat varieties with different resistance level were used: 'Jaak', 'Villu' (moderately susceptible to crown rust and oat leaf spot) and 'Hecht', 'Belinda' (susceptible to crown rust, moderately susceptible to oat leaf spot). Varieties 'Jaak' and 'Villu' were in trial in all three years, variety 'Hecht' was used in 2003 and variety 'Belinda' in 2004 and 2005. The varieties were selected based on the data from previous disease scoring trials in the same region (Tamm, 2003). Untreated certified seed was used for all varieties.

Fungicide tebuconazole (250 g a.i. tebuconazole; trade name in Estonia: Folicur EW 250) was used in a single dose of 1.0 l ha^{-1} in an early (BBCH 37–41) or late (BBCH 59–63) application in all trials. Tebuconazole is a broad spectrum fungicide with special strength against several phytopathogenic fungi including *Puccinia* and *Helminthosporium* (Bayer Crop Science). Phenological growth stages were determined according to BBCH-identification keys for cereals (Meier (ed.), 2001). Fungicide applications were started upon the first symptoms of infection and application times are presented in Table 1; all varieties were treated at the same time. The early application (Early T) of tebuconazole was targeted on control of oat leaf spot disease and late application (Late T) was targeted on control of crown rust. First symptoms of oat leaf spot were observed at time Early T in 2003 and 2005, and first symptoms of oat crown rust were observed at Late T in 2003. Fungicides were applied with a bicycle sprayer equipped with 6 Hardy nozzles 4110-12 on a 2.5-m boom using 300 l of water per ha^{-1}.

Disease infection was scored on 30 randomly selected plants in every plot as the percent of leaf area infected. Disease incidence was scored separately for *P. avenae* and *P. coronata* at the time of fungicide applications and 10–15 days after the late treatment at BBCH 71–75. The infection level was expressed as an average of the infection score on second leaves (L-2; the first leaf under the flag leaf). For proper identification of pathogens, leaves with symptoms of *P. avenae* were incubated in a moisture chamber for 6–7 days at 22°C, and formed spores were examined with the microscope. Crown rust was identified by direct examination of rust pustules by light-microscopy for identification of characteristic spores of *P. coronata*.

Table 1. Dates and growing stages of fungicide applications and disease assessment times in 2003–2005

Year	Fungicide treatment (T)				Disease assessment	
	Early T	BBCH	Late T	BBCH	Third	BBCH
2003	5th July	37–41	15th July	59	26th July	71–73
2004	1st July	41	15th July	63	30th July	75–77
2005	1st July	37–41	15th July	59	1st August	71–73

Trial years differed in weather conditions and their suitability for the development of oat diseases. Year 2003 was characterised by rainy May, moderate temperature in June, warmer and dryer weather in July and very rainy August (Table 2). Such conditions favoured early infection of oat leaf spot and moderate development of oat crown rust. Year 2004 was characterised by droughty May, colder than usual but very rainy June, dry July and rainy August. Especially rainy June favoured infection and development of oat diseases. Year 2005 was dry and hot, it was characterised by lower than average precipitation in June and July and higher than average temperatures in July and August. Weather conditions were suitable for lower level infection of oat leaf spot. Oat crown rust did not develop in droughty conditions.

Table 2. Mean temperature and precipitation for growing seasons 2003–2005 and long term average (1922–2005) at Jõgeva

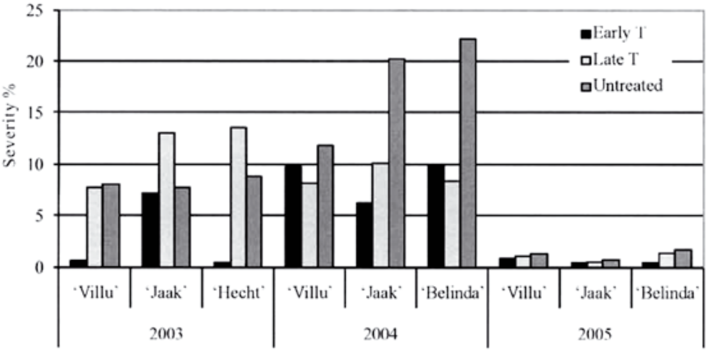
Month	Air temperature °C			Long term average	Precipitation mm			Long term average
	2003	2004	2005		2003	2004	2005	
May	11.3	10.1	10.5	10.2	101	16	84	50
June	13.0	13.1	14.0	14.4	59	184	50	66
July	19.4	16.2	17.9	16.7	54	68	60	81
August	15.0	16.5	16.0	15.3	187	113	116	87

Development of diseases depends on the presence of indigenous primary inoculum and on existing weather conditions. Oat leaf spot development is favoured by cool and wet weather. The overall sparse oat leaf spot in the present study probably resulted from low inoculum densities and not from weather factors, which were moderately and highly conductive to *P. avenae* in 2003 and 2004, respectively. Germination of urediospores of *P. coronata* needs presence of free water on the leaf surface (Simons, 1985). Oat crown rust is most problematic when the disease develops early and the weather conditions are mild to warm during the day and mild at night with rains or frequent dews. Drier and warmer first half of the vegetation period hindered development of oat crown rust in 2005. As *P. coronata* exists in the form of different races, the appearance of new races affects performance of varieties over time. Trials were harvested with a plot combine harvester "Hege 125" at the full maturity in a single day. The grain yield was adjusted to the 14% moisture content and expressed in kg ha^{-1}. The net yield kg ha^{-1}, harvest yield subtracted amount of grain equal to the cost of fungicide and application, was calculated to analyze the economic benefit of fungicide use. The price of the fungicide Folicur EW 250 (33.55 EUR l^{-1}), cost of fungicide application (7.70 EUR ha^{-1}) and the average purchase price of oat (0.10 EUR kg^{-1}) in Estonia for the period 2003–2005 were used in calculations of yield revenue. All prices were used without VAT.

Statistical analyses were performed with software package *Agrobase* (Agrobase™, 1999) using the factorial analysis of variance. Standard analysis of variance (ANOVA) was performed to determine the main factors and the interactions. Mean separations were made for significant effects with LSD at probability $P \leq 0.05$.

Results and discussion

Oat leaf spot control. Severe infection of *P. avenae* developed in 2003 and 2004 (Fig. 1). All varieties were infected in equal level in 2003 and 2005, disease incidence was significantly lower in variety 'Villu' in 2004 compared to other varieties. Early application of tebuconazole had high biological effect in 2003, significantly reducing infection from 8.0% to 0.6% in variety 'Villu' and from 8.8% to 0.4% in variety 'Hecht' ($R^2 = 0.21$, d.f. = 4, $p = 0.0000$). Late T exhibited very limited effect in variety 'Villu' reducing infection from 8.0% to 7.7%. No effect on disease control compared with untreated was achieved in varieties 'Jaak' (13.0%) and 'Hecht' (13.5%) where infection level exceeded that in the untreated control. Late T exerted no or very limited effect on disease control because infection occurred in early stages of plant development and fungicide was unable to control already established infection. Early T significantly reduced oat leaf spot infection in all varieties at more severe infection in 2004, infection was reduced from 20.2% to 6.2% in variety 'Jaak' and from 22.1% to 10.0% in variety 'Belinda' ($R^2 = 0.11$, d.f. = 4, $p = 0.0709$). Under conditions of fast epidemic, Late T resulted in slightly better oat leaf spot control than Early T in 2004. Late T decreased infection from 22.1% to 8.4% in variety 'Belinda' and from 11.8% to 8.1% in variety 'Villu' ($R^2 = 0.33$, d.f. = 4, $p = 0.0000$). Significant disease control effect was achieved with Early T in conditions of weak infection pressure in 2005, disease infection was reduced from 1.7% to 0.4% in variety 'Belinda', from 1.2% to 0.8% in variety 'Villu' and from 0.7% to 0.4% in variety 'Jaak' ($R^2 = 0.04$, d.f. = 4, $p = 0.0312$). Late T resulted in significant reduction of disease infection only in 'Belinda' this year. In summary of trial years and test varieties, the Late T had lower disease control effect than Early T. Higher disease reduction was achieved in more susceptible varieties 'Hecht' (was grown in 2003) and 'Belinda' (grown in 2004 and 2005). Significantly lower disease control efficacy was observed in variety 'Villu'.

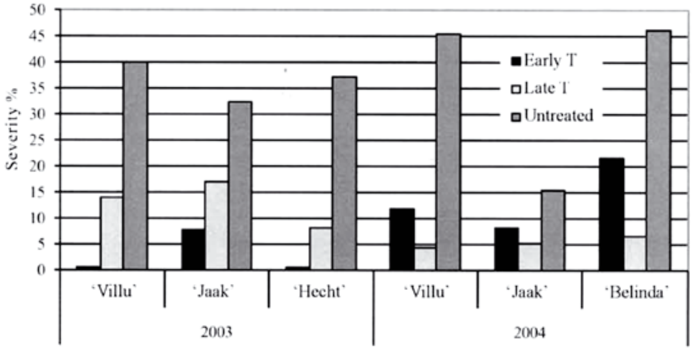


$LSD_{0.05}$ = 0.94 (2003), 1.99 (2004), 0.40 (2005); T – treatment

Figure 1. Oat leaf spot severity caused by *P. avenae* in field trials (2003–2005) assessed at BBCH 71–75

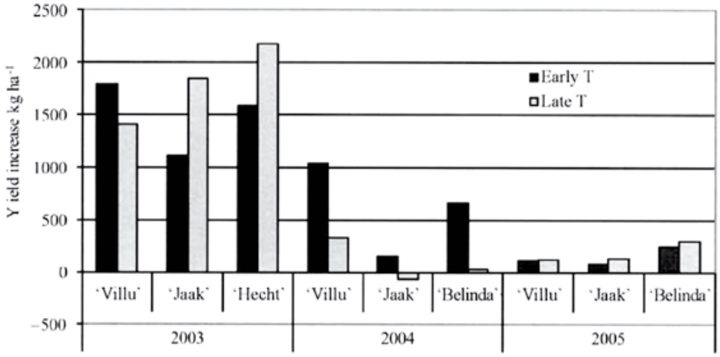
Crown rust control. Oat crown rust caused by *P. coronata* was observed in 2003 and 2004, the disease was absent in 2005. Variety 'Jaak' had lower disease infection than other varieties in both years of crown rust incidence (Fig. 2). All fungicide treatments resulted in significant reduction of oat crown rust infection and there were significant differences between efficacies of different treatment times. Early T was more effective than Late T in 2003 with infection reduction to 7.8% in variety 'Jaak' and to 0.5% in varieties 'Villu' and 'Hecht' ($R^2 = 0.55$, d.f. = 4, $p = 0.0000$). Late T was more effective than Early T in 2004, with infection reduction to 5.2% in variety 'Jaak', to 4.2% in variety 'Villu' and to 6.5% in variety 'Belinda' ($R^2 = 0.70$, d.f. = 4, $p = 0.0000$). Lowest disease control effect was observed in most resistant variety 'Jaak' for both treatment times and both years.

Grain yield. The yields of untreated plots were 2654 kg ha^{-1} (2003), 5673 kg ha^{-1} (2004), 4633 kg ha^{-1} (2005) in variety 'Jaak', 2557 kg ha^{-1} (2003), 5783 kg ha^{-1} (2004), 4718 kg ha^{-1} (2005) in variety 'Villu', and 2957 kg ha^{-1} (2003) in variety 'Hecht', and 6601 kg ha^{-1} (2004), 5034 kg ha^{-1} (2005) in variety 'Belinda'. Fungicide application provided significant yield increase only in 2003. Highest yield increase from fungicide use in 2003 was obtained from Late T in varieties 'Hecht' (2176 kg ha^{-1}) ($R^2 = 0.90$, d.f. = 8, $p = 0.004$) and 'Jaak' (3092 kg ha^{-1}) ($R^2 = 0.85$, d.f. = 8, $p = 0.0136$) (Fig. 3). An opposite was observed in variety 'Villu', where higher yield increase was obtained from Early T. The yields were greatly affected by lodging in 2004, especially in variety 'Jaak'. Early T resulted in higher yield increases in 2004 in all varieties. Higher yield increase was observed in varieties 'Villu' and 'Belinda'.



LSD$_{0.05}$ = 2.22 (2003), 2.50 (2004); T – treatment

Figure 2. Oat crown rust severity caused by *P. coronata* in field trials (2003–2004) assessed at BBCH 71–75



LSD$_{0.05}$ = 849 (2003), 756 (2004), 292 (2005); T – treatment

Figure 3. Yield increase in fungicide treated plots in comparison with untreated control (2003–2005)

In conditions of very low disease pressure in 2005 higher yield increases were obtained from Late T. Significant yield increase was obtained only for variety 'Belinda' (296 kg ha^{-1}) ($R^2 = 0.88$, d.f. = 8, $p = 0.0051$).

Fungicide impact on net revenue. Fungicide applications resulted in highly significant financial benefit in 2003, when highest benefit (176.25 EUR ha^{-1}) was obtained from Late T in variety 'Hecht'. Lowest benefit (70.75 EUR ha^{-1}) was in Early T in variety 'Jaak' (Table 3). This was the only non-significant difference.

Very diverse financial results were obtained in 2004 when one half of treatments increased the income in the case of the other half of treatments the cost for fungicide application was higher than the price of obtained extra yield. The highest benefit (63.15 EUR ha^{-1}) was obtained from Early T in variety 'Villu', in opposite side was Late T in variety 'Jaak' and 'Belinda', where the cost of fungicide application exceeded the benefit by 37.95 to 47.65 EUR ha^{-1}. None of the differences was significant.

None of the fungicide treatments was profitable in 2005 when cost of fungicide application exceeded the price of extra yield by 11.65 ('Belinda' Late T) to 33.05 ('Jaak' Early T) EUR ha^{-1}. Both applications in variety 'Villu' and Early T in variety 'Jaak' caused significant reduction of yield and income.

Several factors have to be considered in order to achieve the profitability of pesticide treatments. Application of fungicides is particularly critical for small crop where costs of protection must be kept low in order to remain profitable even if it controls the pathogen. Oat is relatively low-input crop and fungicide application is therefore commonly not desirable (Newton et al., 2003). In the current trial series none of the fungicide applications provided yield increase conditions of low disease spread in 2005. Results of some oat experiments have shown that disease control can have economically significant effect on kernel yield (White et al., 2003). The chemical protection against *P. avenae* and *P. coronata* is commonly considered to be economically most reasonable with single fungicide application at growth stages 37–41. In the current trial series, a good disease protection and high profitability with fungicide application at growth stages 37–41 was achieved in variety 'Villu' in 2003. Control measures reducing the amount of initial inoculum will reduce the amount of disease at any particular time (Cook et al., 1999). Seed treatment will reduce the initial inoculum of *Pyrenophora avenae* and the need for foliar fungicide application. This allows postponing the fungicide application or decreasing the amount of fungicide dose. Optimum application timing is at flag leaf emergence, when all leaves are formed. Earlier application could result in insufficient fungicide transport to the forming leaves. Most fungicides provide protection against fungal pathogens for two-three weeks. In practice, fungicide does not cover whole plant area with total efficacy; especially the most sensitive varieties cannot be protected fully under heavy epidemic conditions. Too early preventative fungicide applications can lose the effect for time of late disease establishment. Therefore application of fungicides at heading stage could result in better disease control and better economic profit in conditions of late disease development. In the current trial series, a good disease protection with fungicide application at growth stage 63 was achieved in varieties 'Villu' and 'Belinda' in 2004.

Table 3. Net yield and financial benefit of fungicide treated (T) variants (2003–2005)

Variety	Year	Yield kg ha^{-1}	Net yield kg ha^{-1}		Financial benefit EUR ha^{-1}	
		untreated	Early T	Late T	Early T	Late T
'Villu'	2003	2557	3939.5	3554.5	138.25	99.75
'Jaak'	2003	2654	3361.5	4084.5	70.75	143.3
'Hecht'	2003	2957	4131.5	4720.5	117.45	176.35
'Villu'	2004	5783	6414.5	5705.5	63.15	−7.75
'Jaak'	2004	5673	5415.5	5196.5	−25.75	−47.65
'Belinda'	2004	6601	6853.5	6221.5	25.25	−37.95
'Villu'	2005	4718	4416.5	4423.5	−30.15	−29.45
'Jaak'	2005	4633	4302.5	4350.5	−33.05	−28.25
'Belinda'	2005	5034	4873.5	4917.5	−16.05	−11.65

Notes. Net yield – harvested yield subtracted the amount of grain equal to the price of fungicide and cost of fungicide application (41.25 EUR ha^{-1}). Benefit – net revenue subtracted the cost of yield in untreated control.

Our results indicate that demand for fungicide control is clearly minimal for more resistant genotype (variety 'Jaak'). However, the previous study conducted in Jõgeva PBI has shown that variety 'Jaak' is losing the resistance to *P. coronata* and increased fungicide use could be needed for this variety in the future (Sooväli, Koppel, 2003). The increase of susceptibility of variety 'Jaak' is reflecting a rapid change in frequency of matching pathotypes of *P. coronata*. The same has also been observed between yellow rust and wheat (Hovmoller, 2001).

It is likely that the use of other fungicides can give different results. Full dose of tebuconazole was used in the current trial series. Several investigations have indicated that fungicide doses can be reduced by 50–70% without essential loss in control of diseases (Jorgensen et al., 2003). Providing the same level disease control, the use of reduced fungicide doses will increase the economic profitability.

Conclusions

The results of the current trial series indicated that fungicide use in oat could provide diverse results in terms of disease control and economic profitability.

1. There is no single solution to timing the fungicide application.

2. Weather conditions and variety resistance should be considered when timing fungicide use for achievement of best economic results.

3. Fungicide use is most reasonable in susceptible varieties in weather conditions favourable for disease development.

4. Smaller economic returns are achieved in more resistant varieties. Usually more resistant oat varieties do not need foliar application of fungicides.

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Fungicidų purškimo laiko svarba, siekiant efektyvios sėjamosios avižos (*Avena sativa* L.) ligų kontrolės

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Santrauka

Fungicido tebukonazolo efektyvumas nuo avižų lapų ligų *Pyrenophora avenae* ir *Puccinia coronata* tirtas 2003–2005 m. Bandymų metu tirta du purškimo laikai, naudotos keturios veislės, priskiriamos trims atsparumo kategorijoms. Tyrimų tikslas – nustatyti fungicido purškimo laiko ir veislės atsparumo poveikį ligų kontrolės efektyvumui bei derlingumui. Tyrimų metu gauti skirtingi ligų kontrolės ir ekonominio efektyvumo rezultatai. Nenustatyta esminių skirtumų tarp skirtingų fungicido purškimo laikų. Priklausomai nuo oro sąlygų, geresnis ligų kontrolės ir ekonominis efektyvumas gautas fungicidą purškiant paskutinio lapo arba plaukėjimo tarpsniais.

Reikšminiai žodžiai: *Avena sativa*, cheminė kontrolė, vainikuotosios rūdys, avižų lapų dėmėtligė, tebukonazolas, purškimo laikas, derlius.

IV

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INFLUENCE OF FUNGICIDES AND VARIETY RESISTANCE ON FUNGAL FLORA OF BARLEY GRAIN

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Abstract

The effects on disease incidence, grain yield and quality are commonly studied in fungicide trials. Less attention is paid to the effect of fungicides on fungal contamination of harvested grain. The aim of the current studies was to identify the effects of used fungicide treatment and the resistance of variety on seed contamination by phytopathogenic and saprophytic fungi. The harvested grain of three spring barley varieties was examined for the fungal contamination at Jõgeva Plant Breeding Institute in 2004 to 2006. Eight fungal genera were identified; the most common pathogens were *Cochliobolus sativus* and *Fusarium* spp. and saprophytes *Alternaria* spp*.,* *Cladosporium* spp. and *Phoma* spp. It was found that the fungicide and genotype factors contributed to the variance seen in fungal contamination. The time of fungicide application had clear effect on the incidence of phytopathogenic fungal species. Fungal contamination was also highly influenced by environmental conditions of the experimental year. The results illustrate the possibility of use of fungicide and variety resistance based disease control strategy for reduction of seed contamination by fungal spores.

Key words: spring barley, fungicides, phytopathogenic fungi, saprophytes.

Introduction

Chemical control strategies are general tools to keep plants free of diseases and to avoid yield losses. Fungicide application can be beneficial by decrease fungi by a direct preventive and curative effect /Bertelsen et al., 2001/. Barley grain carries commonly a numerous microbial fungal populations. The extent and the activity of this mycoflora are determined by the state of the grain and the environmental conditions /Noots et al., 1999; Lõiveke et al., 2004/. All fungi require organic nutrients for their energy source and as carbon nutrients for cellular synthesis /Deacon, 2006/. Increased water sensitivity may be the result of microorganisms living on the surface of the grain, which compete with the embryo for available oxygen /Kelly, Briggs, 1992/. Early application of fungicides against foliar diseases on spring barley is common practice among farmers in Estonia. Whether this has any effect on infection of the mature grain has been little investigated. The overall aim of the study was to test the effect of fungicide application on the presence and amount of pathogenic and non-pathogenic fungi on harvested barley grain. The side objectives were to evaluate the effect of reduced fungicide dose, timing of fungicide application and variety resistance on the fungal contamination in harvested grain.

Materials and Methods

Field trials on disease control on spring barley were arranged with three replicates in a randomized design 20m² plots at the rate of 500 viable seeds per 1m² at Jõgeva Plant Breeding Institute. The trials were organized in two series.

The effect of fungicide dose, timing of application and resistance of varieties were tested in trials of growing seasons 2004–2005. Spring barley varieties 'Anni', 'Barke' and 'Extract' and two – treatment regimes of tebuconazole fungicide were tested in the trial. The split application of dose 0.5 l ha^{-1} at growth stage BBCH 32–51 and of 0.5 l ha^{-1} at stage BBCH 57–65 was compared with single application of reduced dose 0.16 l ha^{-1} at stage BBCH 59 (2004) and 0.15 l ha^{-1} at stage BBCH 61–65 (2005). The effect of different fungicides was tested in 2006, when ten fungicides were applied in 3/4 rate of registered full dose at growing stage BBCH 37–41 in variety 'Barke'. Untreated plots were used as control. The dosages and content of active ingredients of used fungicides are given in Table 1.

Table 1. Product names, doses and active ingredients of fungicides used in the field trials

Product	Active ingredients per litre	Dose applied l ha^{-1}
2004		
Folicur EW 250	250 g tebuconazole	2 x 0.5; 0.16
2005		
Folicur EW 250	250 g tebuconazole	2 x 0.5; 0.15
2006		
Amistar Opti 480 SC	80 g azoxystrobin + 400 g chlorothalonil	2.5
Amistar Xtra 280 SC	200 g azoxystrobin + 80 g cyproconazole	0.75
Fandango 200 EC	100 g protioconazole + 100 g fluoxastrobin	0.8
Duett Ultra	310 g metyltiophanat + 187 g epoxiconazole	0.6
Tango Super	84 g epoxiconazole + 250 g fenpropimorph	1.5
Sphere 267,5 EC	80 g cyproconazole + 187.5 g trifloxystrobin	0.6
Delaro 325 SC	150 g trifloxystrobin + 175 prothioconasole	0.8
Prosaro 250 EC	125 g protioconazole + 125 g tebuconazole	0.8
Falcon 460 EC	167 g tebuconazole + 43 g triadimenol + 250 g spiroxamin	0.6
Dukes 475 EC	60 g prothioconazole + 250 g spiroxamin 165 g tebuconazole	0.8

Disease occurrence in the field was scored as per cent of infected leaf area by *P. teres* and *C. sativus* and expressed as an average of the infection score on second leaf (L-2; first leaf under the flag leaf). Samples of harvested grain were analyzed for presence of fungal spores separately for each single fungal species. Seed samples were examined under a microscope Olympus CX 31 (40x enlargement) after the incubation by the method of moist chamber (10 days in Petry dishes at 20° C under 12 h dark/light regime) according to the identifying manual /Barnett, 1956/. Analyses were done for 25 seeds in three replications per treatment. The percentage of occurrence of fungal species was evaluated for each trial variant: (number of grains with occurrence of species / total number of grains) x 100. The achieved results were analyzed by the analysis of variance, Agrobase (Agrobase^{TM} 20, 1999), p = 0.05.

Field meteorological weather station Metos Compact was used for recording the meteorological data from the beginning of the vegetation period until the harvest. The weather conditions varied quite significantly during the trial period (Table 2). The summer was cooler than average in 2004; the sum of effective temperatures exceeded the long-term average by 26 degrees in 2005 and the sum of effective temperatures remained 12 degrees below the long-term average in 2006. June 2004 was very rainy (207 mm respectively – long-term average 65 mm) as well May 2005, when the sum of precipitation was 84 mm respectively (long-term average 47 mm). In general, the vegetation periods of 2005 and 2006 were considerably drier and warmer and were not favourable for the development of high occurrence of foliar diseases.

Table 2. Decade average air temperature (^{o}C) and sum of precipitation (mm) of summers 2004–2006 and a long – term (1964–2005) average at the Jõgeva PBI

Average t° C	05. I	05. II	05. III	06. I	06. II	06. III	07. I	07. II	07. III	08. I	t° Σ
2004	15	6.5	8.7	12.7	12.2	14.5	14.8	15.9	18	19.6	1406
2005	7,4	9.4	14.6	12	15.3	14.7	17.5	19.4	16.7	17.2	1472
2006	12,5	8.9	10.2	11.7	18.1	18.3	20.4	17.8	16.2	16.7	1434
1964–2005	8,8	10.7	11.7	14.1	14.5	15.6	16.1	16.3	16.9	16.4	1446
Precipitation mm	05. I	05. II	05. III	06. I	06. II	06. III	07. I	07. II	07. III	08. I	mm Σ
2004	2	9	6	38	54	115	18	12	48	6	307
2005	17	34	33	9	20	13	3	10	40	75	253
2006	0	13	33	9	0	26	0	0	8	20	109
1922–2006	11	17	19	12	26	27	23	28	32	31	255

Results and Discussion

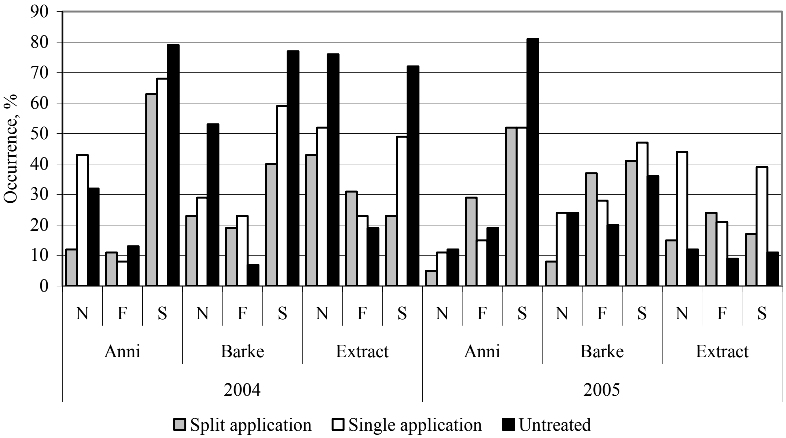
Fungicide applications and resistance of varieties caused significant differentiation in foliar infection level in trials of 2004–2005. The dominating fungi were major barley pathogens *Pyrenophora teres* causing net blotch and *Cochliobolus sativus* causing spot blotch (Table 3). Tebuconazole fungicide was effective in control of net blotch in both years in single and split applications. The significant effect in control of *C. sativus* was achieved only in 2004. Tebuconazole applications had no effect in control of low level infection of *C. sativus* in 2005. Significant effect of variety resistance on infection level of foliar diseases was detected in both diseases in both years.

Table 3. The impact of tebuconazole fungicide on foliar disease infection in different barley varieties at Jõgeva PBI 2004–2005. Disease incidence on the second leaf (%, assessed at BBCH 71–73)

Variety	Treatment	*P. teres* %		*C. sativus* %	
		2004	2005	2004	2005
Anni	Tebuconazole 2 x 0.5	6.7	0.8	10.4	0.5
	Tebuconazole 0.15	10.8	1.1	9.2	0.6
	Untreated	15.3	1.5	19.3	0.9
Barke	Tebuconazole 2 x 0.5	9.0	0.6	8.7	0.6
	Tebuconazole 0.15	15.6	2.6	10.1	1.6
	Untreated	39.3	2.7	23.0	0.8
Extract	Tebuconazole 2 x 0.5	12.7	1.1	10.3	1.1
	Tebuconazole 0.15	17.3	1.9	17.9	2.4
	Untreated	43.6	1.7	42.8	1.4
PD 0.05		2.5	0.5	2.3	0.4

Occurrence of necrotrophic fungus *C. sativus,* facultative saprophytes *Fusarium* spp. and saprophytic fungi *Alternaria* spp.*,* *Cladosporium* spp. and *Phoma* spp. were identified on the harvested grain. Weather and growing conditions favoured occurrence of foliar diseases and contamination of harvested grain with fungal spores in 2004. The prevailing fungi on the harvested grain were *C. sativus* – 76% occurrence on seeds of variety Extract, members of genus *Alternaria* spp. and *Cladosporium* spp. – 79% occurrence in seeds of variety 'Anni'. *Fusarium* spp. covered the range from 8% in seeds of variety 'Anni' to 31% in variety 'Extract' (Figure 1). The disease occurrence was medium in 2005 when weather conditions were warm and droughty at the grain formation stage. The highest occurrence of *C. sativus* was observed in variety 'Extract' by 44%, *Fusarium* spp. in 'Barke' by 37% and *Alternaria* spp. and *Cladosporium* spp. in 'Anni' by 81% respectively (Figure 1).

Despite the low incidence of foliar infection of *C. sativus* and small differences between infections in different years, the grain contamination of untreated variants varies significantly between the years and varieties. Split application of tebuconazole was effective in reduction of kernel contamination with *C. sativus* in all variants except in variety 'Extract' in 2005. This is an indication of activity of tebuconazole in control of phytopathogenic fungi in later stages of plant development Single application of reduced dose decreased kernel contamination with *C. sativus* only in more susceptible varieties 'Barke' and 'Extract' in more humid 2005.



N = necrotrophes, F = facultative saprophytes, S = saprophytes
LSD0.05 = 0.10 (N); 0.11 (F, S)

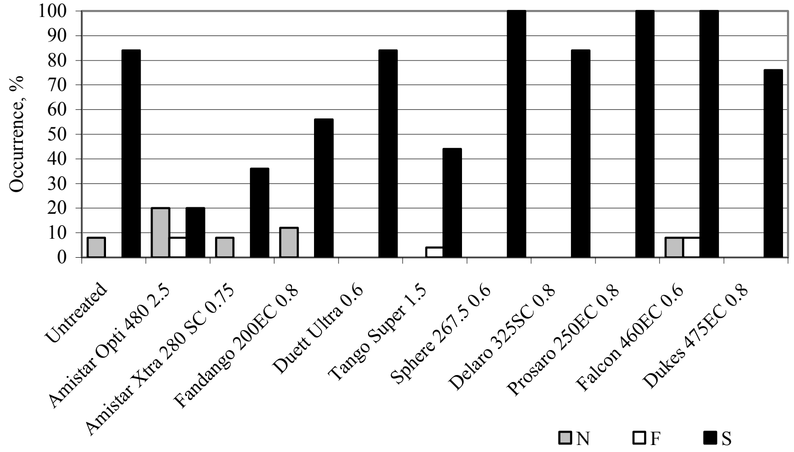
Figure 1. Occurrence (%) of phytopathogenic and saprophytic fungi on grain of spring barley varieties with different tebuconazole applications at growth stages 32–51 + 57–65 (split) and 59 (2004) or 61–65 (2005) (single)

The disease infection level of spring barley variety 'Barke' was low in 2006 (Table 4). The majority of used fungicides had very limited effect in reduction of infection of foliar diseases. Only applications of fungicides containing metyltiophanat + epoxiconazole and epoxiconazole + fenpropimorph resulted in significant decrease of net blotch. No significant differences were detected in foliar infection of *C. sativus*.

Table 4. The impact of fungicides on foliar disease infection of spring barley variety 'Barke' at Jõgeva PBI 2006. Disease incidence on the second leaf (%, assessed in BBCH 71–73)

Treatment	*P. teres %*	*C. sativus %*
Untreated	1.9	1.6
Amistar Opti 480 2.5	1.7	1.4
Amistar Xtra 280 SC 0.75	1.9	1.3
Fandango 200 EC 0.8	1.7	1.5
Duett Ultra 0.6	1.3	1.9
Tango Super 1.5	1.3	1.3
Sphere 267.5 0.6	1.8	1.0
Delaro 325SC 0.8	1.4	1.3
Prosaro 250EC 0.8	1.5	1.2
Falcon 460EC 0.6	1.8	1.3
Dukes 475EC 0.8	1.5	1.3
PD 0.05	0.6	0.8

The evaluation of the extent of mycological contamination of harvested barley grain showed, that five fungicides had 100% effect in control of *C. sativus* and *Fusarium* spp. in 2006 (Figure 2). The application of other five fungicides resulted in low occurrence of *C. sativus* (up to 20%) and *Fusarium* spp. (up to 8%). Fungicides containing azoxystrobin + chlorothalonil, azoxystrobin + cyproconazole, epoxiconazole + fenpropimorph and prothioconazole + fluoxastrobin worked effectively in reduction of contamination with saprophytes. Application of fungicides containing cyproconazole + trifloxystrobin, protioconazole + tebuconazole and tebuconazole + triadimenol + spiroxamin resulted in increase of grain contamination with *Alternaria* spp. and *Cladosporium* spp. In these treatments all kernels were contaminated with saprophytic fungi.



N = necrotrophes, F = facultative saprophytes, S = saprophytes
LSD0.05 = 0.23 (N); 0.27 (F); 0.54 (S)

Figure 2. Occurrence (%) of phytopathogenic and saprophytic fungi in grain of spring barley variety 'Barke' with different fungicide applications at growth stage 37–41

Influence of environmental conditions, fungicide treatment and variety on contamination of grain with pathogens and saprophytes is presented in Table 5. Focusing on data of mycoflora, it was confirmed that yearly conditions gave high significant effect on the occurrence of great majority of phytopathogenic fungi. The effect of year was not significant only for genus *Alternaria.*

Applied fungicides had high significant effect in control of occurrence of most saprophytes during the whole trial period. Only the occurrence of *Phoma* spp. was not influenced with fungicide applications. Significant increase of occurrence of *Fusarium* spp. was observed in varieties 'Barke' and 'Extract' in result of tebuconazole applications in 2004–2005 trials. *Fusarium* contamination was absent in untreated control, but was detected in three fungicide treated variants in 2006.

Table 5. Sums of squares of analysis of variance of contamination of harvested barley grain with pathogens and saprophytes at Jõgeva PBI in 2004–2006

Source of variation		C. sativus	*Fusarium* spp.	*Alternaria* spp.	Cladosporium spp.	Phoma
2004–2005	d.f.	SS	SS	SS	SS	SS
Year	1	2.15***	1.28***	0.04 ns	0.80***	2.46***
Treatment	2	1.76***	1.93***	0.89**	0.58**	0.13 ns
Variety	2	1.96***	0.22 ns	3.54***	0.59**	0.43 ns
Residual	1344	94.13	96.57	95.53	98.03	96.98
2006						
Treatment	10	8.27**	20.06***	14.65***	13.23***	2.86 ns
Residual	264	91.73	79.93	85.35	86.77	97.14

*** – significant at 0.01; ** – significant at 0.05; ns – non-significant

The results from the trials using eleven fungicides and three varieties showed that choice of fungicide had significant impact on the contamination and proportion of fungal species present on the harvested grain of spring barley.

Climatic conditions influenced the extent of mycological contamination more than varietal resistance. This indicates that variety in general has a lower impact on the saprophytes and conditions of vegetation period and fungicide treatment have greater impact on pathogens than saprophytes. According to Deacon (2006) more humid growing periods are related to spread of fungal species. The majority of economically damaging fungal species undergoes multiple cycles of infection in a single season and can cause serious damage to a range of cereal crops. Infection by C. sativus may occur at any stage of plant development. The disease develops faster at temperatures above 20° C. The fruiting structures develop readily on moistened diseased plant tissue /Zillinsky, 1983/.

Fusarium species can survive in the soil in the form of thick-walled resting spores for years. The plants are infected at the tillering stage or into crown node. In the case of prolonged rainfall at the flowering stage rain splashes carry the conidia up to the flowering heads /Zillinsky, 1983/. The more common saprophytic genera such as Alternaria and Cladosporium invade leaves and heads of ripening plants. These organisms are aggressive spore producers. Under conditions favourable to the fungi they may invade living plant tissues or developing grain, usually during the maturation stage /Zillinsky, 1983/. Analysis of the weather conditions of our study period illustrates again that the high precipitation in vegetation period increases the need of fungicide use in spring barley. The proportion of Fusarium spp. infection was lower than that with C. sativus in all trials. Notable is presence of Fusarium contamination only in three fungicide treated variants in trial of 2006. Fusarium contamination might be influenced by long lasting fungicidal effect; the plants remain greener for longer period and serve as a good growing medium for Fusarium species /Mesterhazy, Batok, 2001/. The differences in grain infection level between years leads to the conclusion that there was strong influence of weather conditions at grain maturing time as suggest Legzdina and Belicka, 2001. Our results agree with the experience from Norway where significant increase of Fusarium infection was detected in fungicide treated plots compared with untreated plots /Henriksen, Elen, 2005/.

Conclusions

Weather conditions of the growing period, resistance of the cultivated variety as well as choice of fungicide and its application regime influence the contamination of barley grain with necrotrophic and saprotrophic fungi. Fungicides do well in controlling the foliar infection of barley diseases but have minor influence in reduction of kernel contamination with saprotrophic fungi. Certain fungicides or fungicide application regimes can increase the grain contamination with saprophytic fungi

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The incidence of fungal diseases in oat leaves and yields as affected by fertilizer and chemical inputs in Estonia

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Abstract. Field experiments were conducted for three years and were designed to study the effects of fertilizers on fungal disease infections and yield of two oat genotypes. The impact of the different levels of fertilization has been noticed at the level of crown rust (induced by *Puccinia coronata*) and oat leaf spot (induced by *Pyrenophora avenae*). Four fertilizer doses (N0 = untreated control $N_0P_0K_0$ kg ha^{-1}; N1 = $N_{60}P_{13}K_{23}$; N2 = $N_{100}P_{22}K_{39}$; N3 = $N_{140}P_{31}K_{54}$) and two variants of chemical treatments (variant 1 – without chemicals; variant 2 – with chemicals as growth regulator, fungicide and thrice with foliar fertilizer) were used. The significant differences in levels of disease infection and grain yields between inputs and varieties were observed. The infection level of both oat diseases was mostly influenced by the yearly weather conditions. By using variant 2, including fungicide, the infection of *Puccinia coronata* decreased considerably. The fertilizer input increased the grain yield of the oat varieties. Oat grain yields were higher in treated plots in variant 1 than in variant 2, due to weather conditions.

Keywords: oat, fertilization, fungicides, diseases, yield

INTRODUCTION

Nutrients from the rhizosphere may aid foliar pathogen survival in soil and influence disease epidemiology. Balanced and adequate fertility for any crop reduces plant stress, improves physiological resistance and decreases disease risk (Krupinsky et al., 2002). The effect of disease control on yield is of economic significance (White et al., 2003). Oat leaf spot and crown rust are diseases that have often been found in Estonia. Oat leaf spot or seedling blotch, caused by *Pyrenophora avenae* (Ito & Kurib., conidial stage: *Drechslera avenae* syn. *Helminthosporium avenae*) is a seed-borne pathogen; the fungus also survives on host debris (Clifford, 1995). Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae,* is a long-cycled rust fungus that has a species of buckthorn (*Rhamnus* sp.) as an alternate host (Šebesta et al., 1997).

There is comparatively little published on the influence of the basic and foliar fertilization on the diseases' intensity and the degree of attack on oat plants; traditionally, oat has been viewed as a minor crop and has received little research attention. If chemicals are not used against crop lodge and fungal diseases, it is not worth fertilizing cereals with large nitrogen rates because cereal productivity does not increase significantly (Lisova et al., 1996). In general it is suggested that oat requires less nitrogen fertilizer and chemicals to optimize yield than other spring cereals.

The present experiments were designed to study foliar disease management options in basic fertilization and chemical inputs in oat. The objective was to determine the yield responses of oat to basic fertilization applied to the soil and to determine if the oat crown rust and leaf spot incidence and severity would be reduced with chemical inputs.

MATERIALS AND METHODS

The trials were made on Calcaric (Eutric) Cambisol (FAO classification) soil (clay loam; pH_{KCl} 5.8; P 170–190 mg kg^{-1}; K 153–206 mg kg^{-1}; Ca 1440–1600 mg kg^{-1}; Mg 57–71 mg kg^{-1}; Cu 1.2–1.3 mg kg^{-1}; Mn 36–46 mg kg^{-1}; B 0.48–0.92 mg kg^{-1}). Two oat varieties Villu (Estonia) and Flämingsprofi (Germany) were included in the trial. Non-treated seeds were sown in 9 m² plots at the rate of 600 germinating seeds per 1m² in three replicates using a randomized block design, in early May. Four levels of fertilization (N0 = untreated control $N_0P_0K_0$ kg ha^{-1}; N1 = $N_{60}P_{13}K_{23}$; N2 = $N_{100}P_{22}K_{39}$; N3 = $N_{140}P_{31}K_{54}$) were applied to the soil before sowing using a complex fertilizer Kemira Power ($N_{18}P_4K_7$). Additionally, for fertilization, two different variants were utilized (1 – only soil fertilizer doses and non treatment; 2 – soil fertilizer doses and chemical treatment). In variant 2 the full dose of 1.0 l ha^{-1} (GS 32) foliar spraying with plant growth regulator CCC (a.i. 750 g l^{-1} chlormequat chloride) and leaf fertilizer Folicare 8 kg ha^{-1} ($N_{12}P_{20}K_7$ g kg^{-1} at GS 21–22; $N_{18}P_8K_{15}$ at GS 51–52; $N_{10}P_2K_{33}$ at GS 71–72) were applied. Fungicides Tilt 250 EC (a.i. g l^{-1} 250 propiconazole) 0.5 l ha^{-1} (GS 29–30) in 2006 and, in 2007 and 2008, Folicur EW 250 (a.i. g l^{-1} 125 tebuconazole) 1.0 l ha^{-1} (GS 50–51) were used against foliar diseases in variant 2.

Disease observations were made using a modified septoria and rust disease assessment keys (James, 1971). The assessments of the diseases' severity were carried out on ten randomly chosen tillers per plot in four replications. Visual scoring was made on the 1–9 point scale (1 – no infection, 9 – highly infected). The infection level was expressed as an average of the infection score at milk ripening stage (GS 75) on the top three leaves of the plant. Phenological growth stages were determined according to Zadoks scale of cereals (Zadoks et al., 1974) when > 50% of the plants reached the target growth stage. Grain yield (kg ha^{-1}) was measured on dried and cleaned seeds and expressed on the basis of 14% moisture content. Data were analyzed by factorial analysis of variance using the Agrobase statistics software (Agrobase™, 1999).

Field meteorological station Metos Compact recorded the weather data during the trial period. The average temperature of 15.4°C during the trial period in 2006 and 2007 was higher than the 14.2°C long-term average; 13.9°C for the same period in 2008 was lower, respectively (Table 1). There was little precipitation in 2006 and 2007. A monthly period of drought was observed during the tillers in 2006 and 2007 and during stem elongation in 2007. The grain harvest period was wet and rainy in 2008; precipitation exceeded the long-term average period by 96 mm.

RESULTS AND DISCUSSION

The relations between the plant and the pathogen are of a nutritional nature. The influence of the diseases on the plants depended on a particular genotype, fertilizer input and climatic conditions, which varied yearly (Krupinsky et al., 2007). The influence of the year, variety, fertilizer input and their joint effect on the infection severity are shown in Table 2. By focusing on pathogen data, it was confirmed that in the basic fertilization conditions crown rust correlated highly with yearly climatic conditions, variety, fertilizer input and their interactions (R^2 = 0.933***). In both variants, the year had the most impact on the infection of crown rust: 0.704 and 0.449 respectively. By using the chemical treatment the effect of the fertilizer input on crown rust distribution was non-significant. In variant 2, the influence of variety on trial results was more considerable than in variant 1.

Table 1. Mean temperature (ºC) and precipitation (mm) for growing seasons 2006–2008 and long-term average (1922–2007) at Jõgeva PBI.

Month	Air temperature ºC			85-years average	Precipitation mm			85-years average
	2006	2007	2008		2006	2007	2008	
May	10.5	11.5	10.4	10.2	36	62	22	50
June	16.0	15.7	14.2	14.4	40	42	107	66
July	18.1	16.8	15.6	16.7	11	86	56	81
August	16.8	17.7	15.4	15.3	74	64	195	87
May-August	15.4	15.4	13.9	14.2	161	254	380	284

Table 2. Infection of oat varieties by *Puccinia coronata*, *Pyrenophora avenae* and grain yield at different variation sources (ANOVA).

Source of variation	*P. coronata*	SED(df)	*P. avenae*	SED(df)	Yield	SED(df)
Variant 1						
Year	0.704***	0.1866 (2)	0.610***	0.1741 (2)	0.139***	124.2 (2)
Variety	0.054***	0.1523 (1)	0.007 ns	0.1421 (1)	0.037***	101.4 (1)
Fertilizer input	0.025***	0.2154 (3)	0.085***	0.2010 (3)	0.552***	143.4 (3)
Variety by year	0.090***	0.2638 (2)	0.063***	0.2462 (2)	0.046***	175.6 (2)
Year by fertilizer input	0.058***	0.3731 (6)	0.067**	0.3482 (6)	0.134***	248.3 (6)
R^2	0.933***		0.838***		0.914***	
Variant 2						
Year	0.449***	0.1445 (2)	0.196***	0.1132 (2)	0.712***	107.0 (2)
Variety	0.142***	0.1180 (1)	0.157***	0.0924 (1)	0.004*	87.4 (1)
Fertilizer input	0.012 ns	0.1668 (3)	0.155***	0.1307 (3)	0.158***	123.6 (3)
Variety by year	0.087***	0.2043 (2)	0.064**	0.1601 (2)	0.007*	151.4 (2)
Year by fertilizer input	0.069*	0.2890 (6)	0.142***	0.2264 (6)	0.074***	214.1 (6)
R^2	0.779***		0.739***		0.958***	

* significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; ns – non-significant

Results revealed that the oat leaf spot correlated highly with year, fertilizer input, the interactions of variety x year and year x fertilizer input (R^2 = 0.838***). This conclusion was also reported by Krupinsky et al. (2002). The impact of the variety on oat leaf spot was insignificant in the untreated variant and this adverted to the fact that in natural conditions the leaf spot resistance of varieties was quite similar. The leaf spot infection depended on several factors, such as genotype, year and the interaction of year x fertilizer input in variant 2, whereas crown rust infection was primarily associated with weather conditions. The grain yield of oat was influenced mostly by fertilizer input (0.552***) in variant 1 and by year (0.712***) in variant 2. The variation of average oat yields of the two variants was determined by both factors, 0.369*** (year) and 0.291*** (fertiliser input) respectively. The influence of other factors turned out to be much less.

The results demonstrated that the increased level of fundamental fertilization in variant 1 increased the intensity of *P. coronata* and *P. avenae* (Table 3). A trend of crown rust infection decreased in variant 2; this was apparent at higher N-doses. This indicates that, to some degree, fungicides help to prevent the increase in the disease infection level normally associated with fertilizer use.

Table 3. Average infection of foliar diseases of oat varieties at different input levels during the years 2006–2008.

Variety	Variant	*P. coronata* (1–9 scale)				*P. avenae* (1–9 scale)			
		2006	2007	2008	average	2006	2007	2008	average
Villu	1+N 0	1	4	6	3.7	3	3	4	3.3
	1+N 1	1	5	6	4.0	4	4	5	4.3
	1+N 2	1	6	5	4.0	3	4	6	4.3
	1+N 3	1	6	7	4.7	4	5	5	4.7
	2+N 0	1	2	3	2.0	3	3	3	3.0
	2+N 1	1	2	3	2.0	3	2	3	2.7
	2+N 2	1	3	3	2.3	3	3	3	3.0
	2+N 3	1	4	2	2.3	3	4	4	3.7
Flämingsprofi	1+N 0	1	3	4	2.7	3	3	5	3.7
	1+N 1	1	5	3	3.0	3	4	5	4.0
	1+N 2	1	6	3	3.3	2	4	6	4.0
	1+N 3	1	6	3	3.3	2	4	7	4.3
	2+N 0	1	2	2	1.7	2	2	3	2.3
	2+N 1	1	2	2	1.7	2	2	3	2.3
	2+N 2	1	2	1	1.3	2	3	3	2.7
	2+N 3	1	2	1	1.3	2	3	4	3.0
LSD 0.5		0.0	0.8	0.6	0.4	0.5	0.4	0.6	0.3
Average of Villu		1.0	4.0	4.4	3.1	3.3	3.5	4.1	3.6
Average of Flämingsprofi		1.0	3.5	2.4	2.3	2.3	3.1	4.5	3.3
LSD 0.5		0.0	0.4	0.3	0.1	0.2	0.2	0.3	0.1

As to the adjusted average for the experimental years, more severe crown rust infection was recorded in trials with the Estonian variety Villu. The infection level of oat leaf spot was more severe and exceeded the average of both varieties in variant 1 in 2008. Oat crown rust occurs worldwide, infecting cultivated oat (Šebesta et al., 1997) and has often been found in Estonia (Sooväli & Koppel, 2003). In our trials, oat crown rust occurred in two years (2007, 2008) out of three. The incidence and severity of the degree of crown rust attack in the untreated variants demonstrated that weather conditions for disease development in natural conditions were favourable in 2008. In variant 2, fungicide treatment had a significant protective effect against diseases in both varieties. The decrease in disease severity accomplished with chemical treatments was consistent in the study. In the case of fungicide treatment, N fertilization levels apparently had no significant effect on disease severities.

The grain yields of oat varieties based on different inputs are shown in Table 4. Some earlier studies had suggested that the amount of N fertilizer needed to maximize yield has varied between years (May et al., 2004; Mohr et al., 2007). The trial yield level average varied significantly by years from 3988 kg ha^{-1} in 2006 to 6064 kg ha^{-1} in 2008. The average yield level of 2007 was 4540 kg ha^{-1}. The weather conditions during the experimental years were diverse, causing differentiation of yields. In 2006 and 2007 the grain yields in variant 2 were lower compared to the yields in variant 1, which may have resulted from warm and dry growing conditions causing crop losses due to environmental stresses. In the 2008 trial the opposite effect was observed. As the results obtained indicate, the response of grain yield to soil fertilization depends greatly on moisture (Peltonen-Sainio, 1997). The results of our study support previous research (Mohr et al., 2007) indicating that the average grain yields at the N1–N3 fertilizer levels were quite similar in both variants but were considerably higher compared to yields at the N0. The intensity of fertilizer input caused significant differentiation in yields of the tested varieties, where the variety Flämingsprofi had higher yield over the average of the tested years.

Table 4. Dependency of grain yield of oat on different inputs at the Jõgeva PBI in 2006–2008.

Variety	Variant	Grain yield, kg ha^{-1}			
		2006	2007	2008	average
Villu	1+N 0	2726	3077	4214	3339
	1+N 1	4556	5200	6411	5389
	1+N 2	4274	6251	5458	5328
	1+N 3	5102	6594	4447	5381
	2+N 0	2397	3198	4587	3574
	2+N 1	3153	4028	6897	4693
	2+N 2	3299	4041	7215	4852
	2+N 3	3675	4745	6476	4965
Flämingsprofi	1+N 0	3354	2907	4550	3603
	1+N 1	4985	5063	6949	5666
	1+N 2	5195	5969	6948	6037
	1+N 3	5792	6185	6278	6085
	2+N 0	3252	2914	4615	3594
	2+N 1	3302	3669	7300	4757
	2+N 2	3963	4063	7636	5219
	2+N 3	4241	4733	7041	5338
LSD 0.5		430	294	418	275
Average of Villu		3715	4,642	5713	4690
Average of Flämingsprofi		4260	4438	6414	5037
LSD 0.5		215	147	209	138

CONCLUSIONS

We conclude that the basic fertilization applied to the soil in moderate and higher NPK-doses determines the increase of the disease attack degree as compared to the non-fertilized variants. As expected, the fungicide application demonstrated a reduction of crown rust and oat leaf spot attack in all fertilizer levels. In this study there was absolutely no infection of crown rust in the case of the variety Flämingsprofi in N100 and N140. The oat leaf spot attack in variety Villu decreased to very low when fungicides were used in years of moderate infection severity, 2006 and 2007, when the basic fertilization applied to the soil brought about a higher yield increase for both varieties. The yield increase resulting from intensive fertilizers with fungicide and application of growth regulator was significantly higher only in 2008 than that from soil fertilization.

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Inventions

Küüts, H., Küüts, I., Zakrisson, M., **Sooväli, P.**, Tamm, Ü. Spring barley variety 'Roosi' (application for variety protection 13.12.2000)

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