

Effect of Fungicide Application Date against *Sclerotinia sclerotiorum* on Yield and Greening of Winter Rape

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Abstract

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In experiments with winter rape during 2004–2008, we examined the effectiveness of fungicides containing active ingredients based upon triazoles and SBI inhibitors (group A): flusilazole 250 g/l a.i., flusilazole 125 g/l a.i. + carbendazim 250 g/l a.i., and cyproconazole 120 g/l a.i. + carbendazim 300 g/l a.i. Fungicides based upon older and newer triazoles, strobilurins, and SDH inhibitors (group B) were also used: cyproconazole 80 g/l a.i. + azoxystrobin 200 g/l a.i., prothioconazole 250 g/l a.i., prothioconazole 125 g/l a.i. + tebuconazole 125 g/l a.i., and boscalid 200 g/l a.i. + dimoxystrobin 200 g/l a.i. The application of group A fungicides against *S. sclerotiorum* during BBCH 65–69 growth stage had demonstratively higher effectiveness, while for group B the application date had no effect. The greening (or “stay-green”) effect only occurred with group B. The correlation coefficient for greening effect and yield was $R = -0.3033$ for applications during BBCH 61–65 while it was $R = -0.3542$ for BBCH 65–69 stage, thus indicating a relatively weak relationship.

Keywords: flusilazole; carbendazim; cyproconazole; azoxystrobin; prothioconazole; tebuconazole; boscalid; dimoxystrobin; effectiveness

Sclerotinia sclerotiorum (Lib.) de Bary, (1884) is a fungal pathogen infecting more than 400 plant species worldwide, many of which are grown as agronomic field plants. These include potatoes, oilseed rape, poppy and sunflower (GARG *et al.* 2010). Ascospores comprise the primary source of oilseed rape infection, and these are produced in spring months by fruiting bodies of the fungus growing from sclerotia in the soil. In addition to this mode of spreading, carpogenic germination of sclerotia, and spread of infectious hyphae through the soil to plant roots also occur under certain conditions. This spreading long remains hidden and appears on the plants only later, in the ripening period (COWAN & BOLAND 2010).

Depending upon a number of weather and cultural factors, the damage to oilseed rape caused

by *S. sclerotiorum* can be up to 0.5% yield loss for each percentage point of the pathogen occurrence. DEL RÍO *et al.* (2007) defined 17% as the threshold infection rate at which it is cost-effective to apply a fungicide. In experiments with oilseed rape conducted by Agrotest fyto s.r.o. in Kroměříž, Czech Republic, this threshold was surpassed three times in total during 2004–2008 (SPITZER 2009) and every year during 2009–2011.

In the Czech Republic, the growing of winter rape (*Brassica napus* L.) has held steady at 300 000 ha in recent years and the crop is grown on more than 11% of the arable land. On many farms specialised in growing oilseed rape its proportion in the crop rotation is much higher and approaches 30% in the most extreme cases. An increasing proportion of winter rape in the crop rotation brings a height-

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ened risk for the fungal pathogen *S. sclerotiorum* to occur, and also therefore a risk of higher yield losses. A forecasting model for Sclerotinia stem rot in winter rape created in Germany calculates that with a 2-year rotation of oilseed rape in the crop sequence the infection threshold is decreased by a coefficient of 0.8 and, by contrast, in a 4-year rotation it is increased by a coefficient of 1.3 (KOCH *et al.* 2007).

Protection of winter rape stands against *S. sclerotiorum* is currently provided mostly by applications of fungicides during rape flowering. Fungicides containing active ingredients based upon older triazoles and inhibitors SBI (Sterol Biosynthesis Inhibitors) are used, as are newer fungicides with combinations of active ingredients from newly synthesised triazoles and strobilurins. Fungicides are applied in a period when no infection by the disease is visible in the stand. This is mainly due to the fact that the fields are inaccessible to machinery in later growth stages and during ripening of the oilseed rape, which would be significantly damaged by driving through the field. Therefore, the decision to use or not to use a fungicide and the application date during flowering are very important (BEČKA *et al.* 2011).

This work aimed to:

- (1) compare the effectiveness of older versus newer fungicides;
- (2) compare application dates – during the first half of rape flowering versus applications in the second half of flowering;
- (3) evaluate the influence of the fungicide greening effect (green leaf area) upon oilseed rape yield.

MATERIAL AND METHODS

Experiments were conducted under field conditions using the winter rape (*B. napus* L.) cv. Asgard during 2006–2009. Seeding, fertilising, and protection from animal pathogens were carried out conventionally in accordance with good agricultural practices.

In experiments during all four years, fungicides with active ingredients based upon triazoles and SBI inhibitors (group A) were used: flusilazole 250 g/l a.i., flusilazole 125 g/l a.i. + carbendazim 250 g/l a.i., and cyproconazole 120 g/l a.i. + carbendazim 300 g/l a.i. Fungicides based upon older and new triazoles, strobilurins, and SDH inhibitors (the target enzyme of SDH inhibitors is succinate dehydrogenase in the

mitochondrial respiration chain) (group B) were also used: cyproconazole 80 g/l a.i. + azoxystrobin 200 g/l a.i., prothioconazole 250 g/l a.i., prothioconazole 125 g/l a.i. + tebuconazole 125 g/l a.i., and boscalid 200 g/l a.i. + dimoxystrobin 200 g/l a.i. Application rates of particular fungicides were in accordance with fungicide registrations and specific product labels.

Fungicides were applied on plots of 25 m², each variant in 4 repetitions in a random design. An R&D Sprayers-brand compressed-air precision sprayer (R&D Sprayers, Louisiana, USA) was used. The applications of the selected fungicidal materials were made on two dates: during the first half of rape flowering, BBCH 61-65 stage (MEIER 1997) and in the second half of rape flowering, BBCH 65-69 stage. The degree of *S. sclerotiorum* infection on stems and branches was evaluated in the BBCH 85 stage (50% of ripe pods, black and hard seeds) according to the EPPO PP 1/78(3) methodology (OEPP/EPPO 2003), green leaf area was evaluated before harvest, and yield was determined. Harvest was made using a Sampo 2010 plot combine harvester equipped with automatic weighing equipment and moisture detector. Yields were recalculated to standard 8% moisture and yield differences in the particular years versus the controls were calculated in percentage terms. These differences were then used in statistical processing in order to eliminate differences of year in the yield levels. From the values for degree of infection, effectiveness was calculated according to Abbot.

Statistica 7.0 software (StatSoft, Tulsa, USA) was used for statistical analysis by regression and analysis of variance (ANOVA).

Temperature and precipitation data for the Agrotest fyto, s.r.o. research institute are presented in Figure 1.

RESULTS

The yield level on the control in the trial with application period BBCH 61-65 was 4.39 t/ha and in the trial with application period BBCH 65-69 was 4.35 t/ha.

The effectiveness of group A formulations against *S. sclerotiorum* with application period BBCH 61-65 was in the 20–100% range, with the average around 60%. ANOVA found no statistically significant relationship between effectiveness and yield for this application period. The correlation

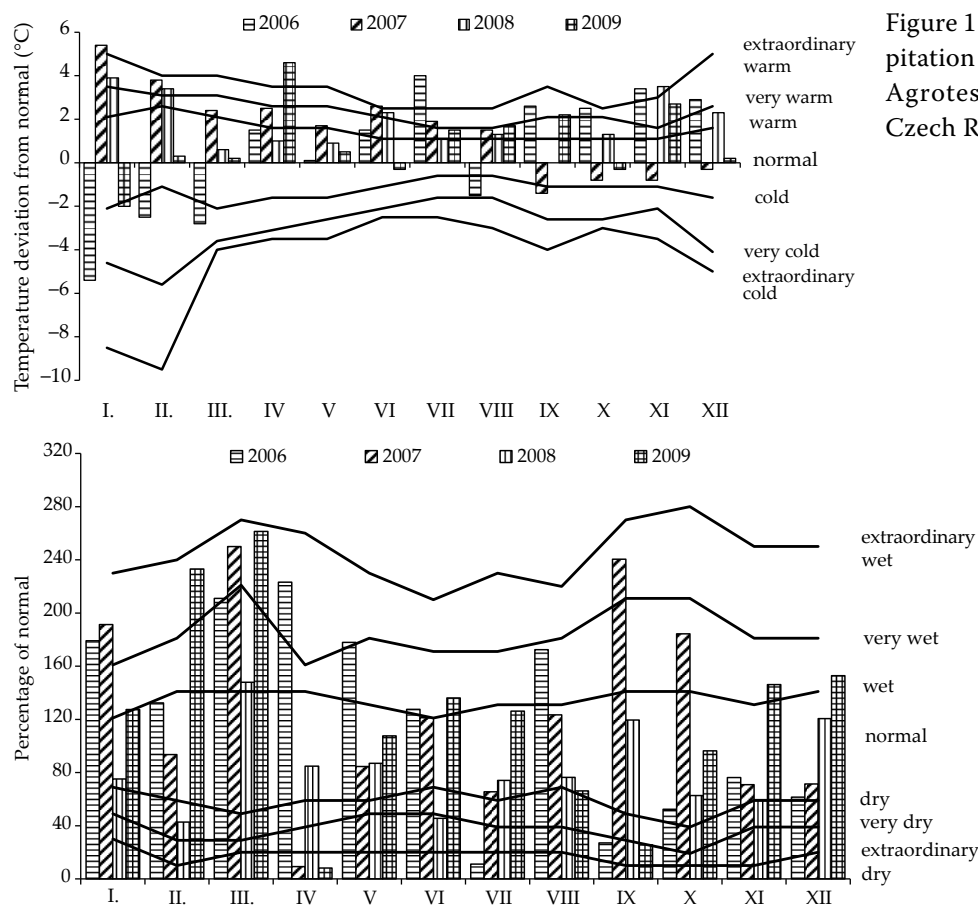


Figure 1. Temperature and precipitation at the Research Institute Agrotest fyto, Ltd., Kroměříž, Czech Republic

coefficient ($R = 0.05636$) indicates only a relatively weak relationship between the effectiveness of formulations and yield. For application period BBCH 65-69, effectiveness was in the range of 5–95%, with the average around 58%. ANOVA determined a statistically significant relationship between effectiveness and yield for this fungicide application date. The correlation coefficient ($R = 0.6284$) indicates a moderately strong relationship at this application period between the effectiveness of formulations and yield.

The effectiveness of group B formulations against *S. sclerotiorum* was in the 20–100% range for application period BBCH 61-65, with the average around 70%. ANOVA found no statistically significant relationship between the effectiveness and yield for this fungicide application date. The correlation coefficient ($R = 0.0604$) indicates a relatively weak relationship between the formulation effectiveness and yield. For application period BBCH 65-69, effectiveness was in the range of 35–100%, with the average around 75%. ANOVA determined no statistically significant relationship between effectiveness and yield for this period of

fungicide application. The correlation coefficient ($R = 0.1316$) indicates a relatively weak relationship between formulation effectiveness and yield. The results are presented in Table 1.

Greening (or “stay-green”) effect (green leaf area) was evaluated by visual comparison of the level of greenness between the treated versus control plants. The effect did not manifest itself for group A formulations. For group B formulations, it appeared in 2006 at a level below 5% at application period BBCH 65-69. In 2007, it was at a level of 0–10% and in 2008 at 10–25% for both application periods. In 2009, the greening effect did not occur.

ANOVA determined a statistically significant relationship between the greening effect and yield

Table 1. Correlation coefficients P for efficacy and dif. yield

Term of application	Group A	Group B
BBCH 61-65	0.0563	0.0604
BBCH 65-69	0.6284**	0.1316

** $P < 0.01$

Table 2. Correlation coefficients for green effect and dif. yield

Term of application	Group B
BBCH 61-65	–0.3033**
BBCH 65-69	–0.3542**

** $P < 0.01$

for both fungicide application dates. The results are shown in Table 2. The correlation coefficients (at $R = -0.3033$ for BBCH 61-65 and $R = -0.3542$ for BBCH 65-69) indicate, however, that the relationship between the greening effect and yield is relatively weak.

DISCUSSION

The results from comparing the two groups of fungicides against *S. sclerotiorum* and the two application periods indicate that application date is more important for older fungicides (group A) than it is for the second group of newer fungicides (group B). The older fungicides had a greater influence on yield if they were applied at BBCH 65-69. For the newer fungicides, the application date did not play an important role. This allows broadening the optimal application date for the new fungicides on oilseed rape to include the entire flowering period. The average rate of effectiveness also indicates that the new fungicides achieve higher effectiveness than do the old ones. DEL RÍO *et al.* (2007) monitored the use of fungicides against *S. sclerotiorum* at various concentrations and various application intervals during the oilseed rape flowering period and determined that the disease developed with various levels of intensity depending on the time of application.

The statistical evaluation of the relationship between effectiveness and yield shows that the two are not always directly correlated. For group B fungicides, there is a weak correlation for both application periods. There may be a problem with using BBCH 85 as a point in time for evaluating fungicides in relation to the development of *S. sclerotiorum* in the particular years. Depending upon weather, the disease can occur at any time from the end of flowering until harvest, and late occurrences have a low effect on yield but can significantly affect the assessment of infection rate.

Based upon results from experiments examining the effects of fungicides on *S. sclerotiorum*

and on yield, BRADLEY *et al.* (2006) stated that the pathogen rate of occurrence differed by year and location. A generally marked decrease in infection was recorded for the active ingredients azoxystrobin, benzyl, boscalid, iprodione, prothioconazole, tebuconazole, thiophanate-methyl, trifloxystrobin, and vinclozolin. Nevertheless, a significant decrease in infection by *S. sclerotiorum* did not always affect the yield. Different results were observed at various application timings according to the stage of flowering.

Postponing of senescence and intensified pigmentation of green leaf area – so-called greening effect – are known side-effects of applying certain fungicides, e.g. in cereals. A marked greening effect occurs especially after the application of strobilurins-based fungicides. In the case of kresoxim-methyl, inhibition of ethylene production occurs in wheat leaves and production of cytokinins increases, thus resulting in retardation of senescence and intensified pigmentation (GROSSMANN & RETZLAFF 1997). JAMIESON *et al.* (1999) reported a positive influence of azoxystrobin upon yield via the longer preservation of green leaf area (greening effect). Postponing of senescence resulted in larger grain. In two experiments, BERTELSEN *et al.* (2011) compared the effects of azoxystrobin and epoxiconazole on a spectrum of saprophytes and phytopathogenic fungi on wheat leaves, the influence on leaf senescence, and yield. Both active ingredients prolonged the green leaf area, but azoxystrobin did so more markedly and for a longer time; yield was higher only in one experiment after the application of azoxystrobin as opposed to epoxiconazole.

There are no data in the available literature to show whether greening effect also occurs in winter rape and whether it affects yield. The analysis of results from experiments during 2006–2009 showed that greening effect has a demonstrable influence on yield for newer fungicides based upon azoles and strobilurins that is independent of application date, but surprisingly this influence is negative. A negative correlation was determined for both application periods, even though the correlation coefficients are low.

The available literature concerning the topic of the influence of strobilurins on green leaf area and yield only includes works dealing with cereals. Most authors observed a positive effect on green leaf area, postponing of senescence, and increase in yields. Postponing of senescence in

wheat chiefly means extending the vegetation of the ear and flag leaf, which crucially influence kernel formation in the last stage of ripening. PETR *et al.* (1980) reported the ratio of ear, upper internode and flag leaf to the resulting amount of assimilates in the kernel to be 90%. In winter rape, the highest number of leaves occurs before flowering. Vegetative growth thereafter sees the leaf growth quickly replaced by the growth of stems, branches and siliques, which take over the assimilation of nutrients (VAŠÁK *et al.* 2000).

Strobilurin fungicides applied to cereals during the flowering period move to the ear and flag leaf and by increasing their vegetation period they also increase the time period during which the caryopses can accept assimilates. This results in higher yield, or higher 1000-grain weight and quality.

In the case of winter rape, the application is made especially onto the leaves, stems and part of the branches and siliques, which are formed during the time of application. Shortly after application, however, the source of assimilates changes and branches and siliques become the main suppliers of nutrients to the seeds, most of which had not yet been created at the time of application.

This may be one of the possible factors responsible for different influence of greening effect from strobilurin fungicides in cereals and in oilseed rape.

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