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ORIGINAL ARTICLE

# Effect of Fungicide Application Timing on *Sclerotinia Sclerotiorum* Infection Rate and Yield in Winter Oilseed Rape

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**Abstract** In experiments on winter oilseed rape during 2013–2016, we examined the influence of timing of fungicides application at BBCH 61–63 and BBCH 65–67 for fungicides containing active ingredients based upon new triazoles, strobilurins and SDH inhibitors (cyproconazole 80 g/l a.i. + azoxystrobin 200 g/l a.i., picoxystrobin 200 g/l a.i. + cyproconazole 80 g/l a.i., boscalid 200 g/l a.i. + dimoxystrobin 200 g/l a.i., protioconazole 125 g/l a.i. + fluopyram 125 g/l a.i., and prochloraz 276 g/l a.i. + tebuconazole 133 g/l a.i.) on effectiveness against *Sclerotinia sclerotiorum* and yield. For cyproconazole 80 g/l a.i. + azoxystrobin 200 g/l a.i. and boscalid 200 g/l a.i. + dimoxystrobin 200 g/l a.i., two applications with divided and full doses were also performed. Applications at BBCH 65–67 resulted in a statistically insignificant 4% greater effectiveness against *Sclerotinia sclerotiorum*. All applications increased yields, but no significant difference was determined due to application timing by growth stages. Divided applications achieved the highest effectiveness, while yield was increased especially at full dosage. In practice, however, such split applications are difficult to perform.

**Keywords** Cyproconazole · Azoxystrobin · Protioconazole · Tebuconazole · Boscalid · Dimoxystrobin · Picoxystrobin · Fluopyram

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## Auswirkungen des Anwendungszeitpunkts von Fungizid auf die Infektionsrate mit *Sclerotinia sclerotiorum* und auf den Ertrag bei Winterraps

**Zusammenfassung** Bei Experimenten mit Winterraps von 2013 bis 2016 haben wir den Einfluss des Zeitpunkts der Fungizidanwendung zu BBCH 61–63 und BBCH 65–68 für Fungizide mit Wirkstoffen untersucht, die auf neuen Triazolen, Strobilurinen und SDH-Hemmern beruhen (Cyproconazol 80 g/l a.i. (active ingredient) + Azoxystrobin 200 g/l a.i., Picoxystrobin 200 g/l a.i. + Cyproconazol 80 g/l a.i., Boscalid 200 g/l a.i. + Dimoxystrobin 200 g/l a.i., Prothioconazol 125 g/l a.i. + Fluopyram 125 g/l a.i. und Prochloraz 276 g/l a.i. + Tebuconazol 133 g/l a.i.), und zwar bezüglich ihrer Wirksamkeit gegen *Sclerotinia sclerotiorum* sowie bezüglich des Ertrags. Für Cyproconazol 80 g/l a.i. + Azoxystrobin 200 g/l a.i. und Boscalid 200 g/l a.i. + Dimoxystrobin 200 g/l a.i. wurden außerdem zwei Anwendungen mit aufgeteilten und mit vollen Dosen durchgeführt. Die Anwendung zu BBCH 65–67 führte zu einer statistisch insignifikanten um 4 % stärkeren Wirksamkeit gegen *Sclerotinia sclerotiorum*. Alle Anwendungen erhöhten den Ertrag, aber es wurde kein signifikanter Unterschied nach Anwendungszeitpunkt im Wachstumsverlauf festgestellt. Die aufgeteilte Anwendung brachte die höchste Wirksamkeit, wohingegen der Ertrag insbesondere mit vollen Dosen erhöht wurde. In der Praxis ist die aufgeteilte Anwendung allerdings schwierig umzusetzen.

**Schlüsselwörter** Cyproconazole · Azoxystrobin · Protioconazole · Tebuconazole · Boscalid · Dimoxystrobin · Picoxystrobin · Fluopyram

## Introduction

*Sclerotinia sclerotiorum* (Lib.) 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's infection, and these are produced in spring months by fruiting bodies of the fungus growing from sclerotia in the soil. In addition to this method 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 and Boland 2010).

In their review of scientific literature pertaining to current Sclerotinia stem rot control practices, Derbyshire and Denton-Giles (2016) describe this as the most harmful disease of winter oilseed rape. Their findings indicate that, despite the development of a number of new technologies for suppressing *S. sclerotiorum* in winter oilseed rape, protection against this disease continues to be based on the use of fungicides. A problem remains in that fungicides must be used preventively due to an absence of reliable signalling as to the timing for precise and effective application. Although intensive work is being undertaken on signalization models, SklerPro remains the only model to have been introduced into agricultural practice (Koch et al. 2007) and it has not fared well in certain years. Other models are being developed, for example, on the basis of ascospore capture using the Burkard Multi-Vial Cyclone Sampler in connection with qPCR analysis (Parker et al. 2014) and on the basis of petal analysis as a source of *Sclerotinia sclerotiorum* infection using NIR hyperspectral imaging (Zhao et al. 2016).

Depending on a number of climatic and agricultural factors, the damage caused by *S. sclerotiorum* can reach levels as high as 0.5% loss in winter oilseed rape (*Brassica napus* L.) yield for every 1 percentage-point occurrence of the pathogen. According to del Rio et al. (2007), the threshold level of infection at which it is cost-effective to apply a fungicide is 17%. In experiments on winter oilseed rape conducted by Agrotest fyto s.r.o. in Kroměříž, Czech Republic, this threshold was exceeded three times in total during the period 2004–2008 (Spitzer et al. 2012) and every year during the period 2009–2016.

In recent years, winter oilseed rape cultivation in the Czech Republic has been stable at just above 300,000 ha and it already is grown on more than 11% of arable land. On many farms specialized in growing oilseed rape, its share in the crop rotation is much higher (in extreme cases, approaching 30%). An increasing proportion of winter oilseed rape in the crop rotation brings heightened risk for the fungal pathogen *S. sclerotiorum* to occur and thus also the risk of greater yield losses. A forecasting model for *Sclerotinia* stem rot in winter oilseed rape created in Germany calculates that with a two-year rotation of oilseed rape in the crop sequence the infection threshold is reduced by a coefficient of 0.8 and in a four-year rotation that rises by a coefficient of 1.3 (Koch et al. 2007).

Protection of winter oilseed rape stands against *S. sclerotiorum* is currently provided in the Czech Republic by applying fungicides during rape's flowering period. Fungicides containing older active ingredients based on triazoles and SBI inhibitors have been replaced with newer fungicides containing combinations of active ingredients from newly synthesized triazoles and strobilurins (Spitzer et al. 2012). Fungicides are applied in a period when no visible infection by the disease is apparent in the stand. This is mainly due to the fact that the fields are inaccessible to machinery in later growth stages and during rape maturation without causing substantial damage by driving through the field. The choice of fungicide and application timing during flowering is therefore a very important decision (Bečka et al. 2011).

The objective of this work was to compare the impact of fungicide application during the first half of flowering (BBCH 61–65), second half of flowering (BBCH 65–67), and divided versus full applications on the infection rate of *S. sclerotiorum* and yield in winter oilseed rape.

## Materials and Methods

The location where the field experiments were conducted (49°17′13.708″N, 17°22′13.296″E) lies among the most fertile lands in the Czech Republic. It is in a warm and slightly humid area with a mean annual temperature of 8.7 °C and total annual precipitation of 599 mm.

Experiments were conducted using the PX 114 winter oilseed rape variety during 2013–2016. Seeding, fertilizing, and protection against animal pests were carried out as conventional in accordance with good agricultural practices.

In all four years, fungicides commonly used in actual practice with active ingredients based on new triazoles, strobilurins, and SDH inhibitors were used in the experiments. The fungicides used are presented in Table 1. Individual fungicides were applied according to the fungicides' registrations and product labels.

Fungicides were applied on plots of 25 $m^2$, each variant in 4 randomly arranged repetitions. An R&D Sprayers compressed-air precision sprayer was used. The applications of the selected fungicidal substances were made at two growth stages: during the first half of rape flowering BBCH 61–65 (Meier 1997) and in the second half of flowering BBCH

**Table 1** Description of fungicides used

| Trade name | Active ingredient and rate | Permitted dose per ha |
|---|---|---|
| Amistar Xtra | Azoxystrobin 200 g/l a.i + cyproconazole 80 g/l a.i. | 1 l |
| Pictor | Boscalid 200 g/l a.i. + dimoxystrobin 200 g/l a.i. | 0.5 l |
| Propulse | Fluopyram 125 g/l a.i. + prothioconazole 125 g/l a.i. | 1 l |
| Acanto Plus | Picoxystrobin 200 g/l a.i. + cyproconazole 80 g/l a.i. | 1 l |
| Zamir 40 EW | Tebuconazole 125 g/l a.i. + prochloraz 276 g/l a.i. | 1 l |

65–67. The two most commonly used fungicides were also applied in divided application of full and half doses at the two stages stated.

The degree of *S. sclerotiorum* infection on stems and branches was evaluated at the BBCH 85 phase (50% of pods ripe, seeds black and hard) according to the EPPO PP 1/78(3) methodology (OEPP/EPPO 2003) before harvest and yield was determined. Harvesting was done using a Sampo 2010 plot combine harvester equipped with automatic weighing equipment and moisture sensor. Yields were recalculated to standard 8% moisture and yield differences in the individual years versus the controls were calculated as percentages. These differences were then used in statistical processing of the results. From the values for degree of infection, effectiveness was calculated according to Abbot (1925). Statistica 7.0 software was used for statistical analysis of variance (ANOVA).

Temperature and precipitation data for the Agrotest fyto, s.r.o. research institute are presented in Figs. 1 and 2.

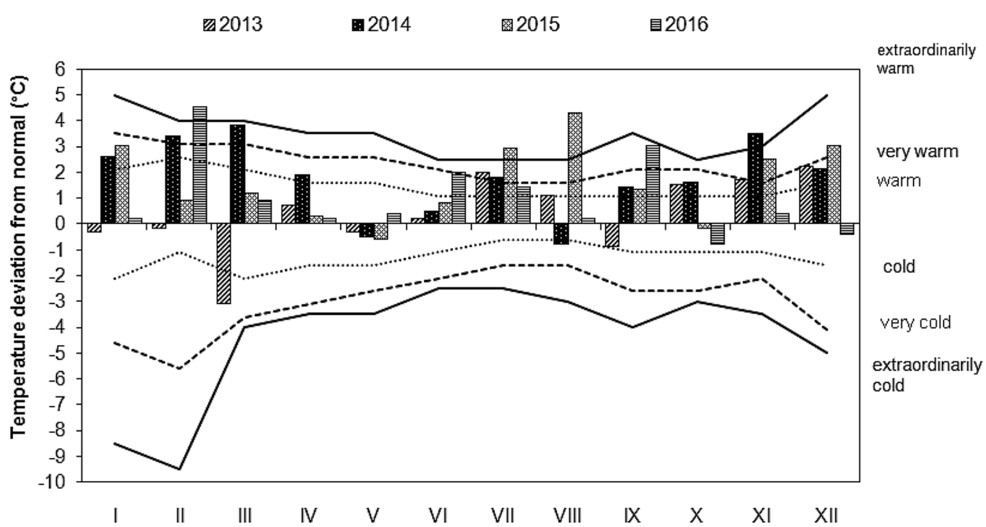


**Fig. 1** Temperature at the Agrotest fyto, s.r.o. research institute

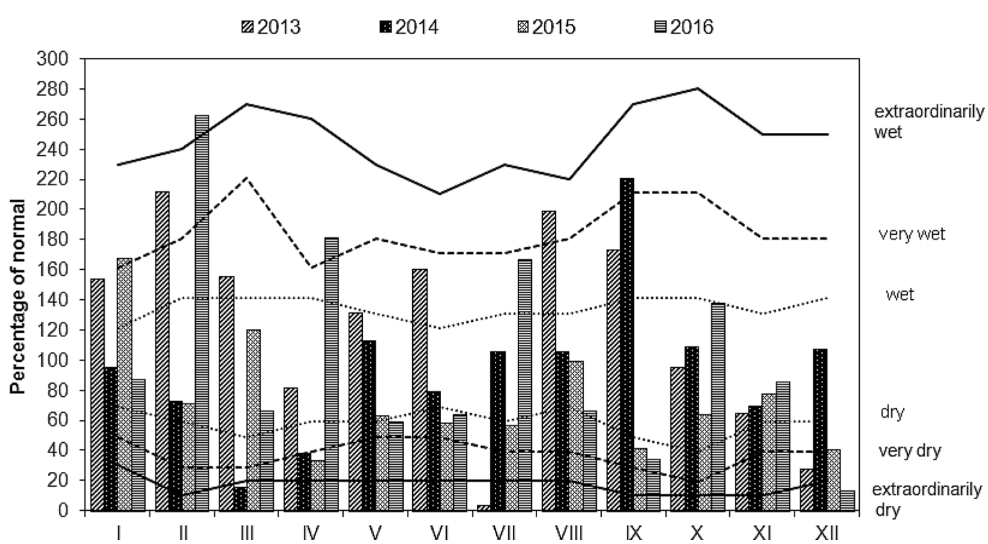


**Fig. 2** Precipitation at the Agrotest fyto, s.r.o. research institute

## Results

### Summary

results of fungicide effectiveness in the individual application periods against *S. sclerotiorum* and yields are presented in Table 2 and 3. The infection rates during the study years ranged from 19.4% in 2013 to 50% in 2016. The effectiveness rates for individual fungicides differed in individual years. To compare individual application terms, the effectiveness rates and yields for individual applications were averaged and statistically evaluated.

The effectiveness of the preparations against *S. sclerotiorum* in the BBCH 61–63 application term ranged between 14 and 100% with average of 52% for all applications. For the BBCH 65–67 application term, the effectiveness ranged from 0 to 100% with average of 48% for all applications. No significant difference was demonstrated in the effectiveness of fungicides against *S. sclerotiorum* in application periods BBCH 61–65 and BBCH 65–67, nor was any significant difference recorded in comparing the effectiveness of individual fungicides in application periods BBCH 61–65 and BBCH 65–67.

Among fungicides applied during both application periods (BBCH 61–65 and BBCH 65–67), effectiveness against *S. sclerotiorum* was higher for both full and reduced doses as compared to the one-time application of the same fungicide at either BBCH 61–65 or BBCH 65–67. Those differences were, however, statistically insignificant.

Yields during the study years fluctuated between 3.05 t/ha in 2015 and 4.38 t/ha in 2014. Yields for the BBCH 61–65 application period ranged 10–13% higher than the control and on average 12% higher for all applications. For the BBCH 65–67 application period, yields ranged 10–14% higher than the control, again averaging 12% higher for all applications. Most applications increased yield significantly, but no significant difference between application stages BBCH 61–65 and BBCH 65–67 was recorded.

For fungicides applied in both application periods (BBCH 61–65 and BBCH 65–67), the yields were higher for both full and reduced doses as compared to the control and the application of two full doses exhibited the highest yield in the study (four-year average of 17% and 19% above the control) as compared to the single application of the same fungicide at either BBCH 61–65 or BBCH 65–67. However, the differences were not statistically significant.

**Table 2** Yield results

| Treatment | | | | Yield | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of | Dosage | Application at | v t/ha | | | | | in % of control | | | | | Mean |
| | applications | Per ha | BBCH | 2013 | 2014 | 2015 | 2016 | Mean | 2013 | 2014 | 2015 | 2016 | Mean | BBCH 61–63 |
| Control | – | – | – | 4.16 | 4.38 | 3.05 | 4.19 | 3.94 | 100 | 100 | 100 | 100 | – | – |
| AX | 1 | 1 l | 61–63 | 4.36 | 5.12* | 3.73* | 4.55* | 4.44 | 105 | 117 | 122 | 109 | 113 | – |
| Pic | 1 | 0.5 l | 61–63 | 4.04 | 5.00* | 3.96* | 4.55* | 4.39 | 97 | 114 | 130 | 108 | 113 | – |
| Pro | 1 | 1 l | 61–63 | 4.12 | 5.00* | 3.50* | 4.69* | 4.33 | 99 | 114 | 115 | 112 | 110 | 112 |
| AcP | 1 | 1 l | 61–63 | 4.08 | 5.10* | 3.58* | 4.75* | 4.38 | 98 | 116 | 118 | 113 | 111 | – |
| Za | 1 | 1 l | 61–63 | 4.40* | 4.95* | 3.76* | 4.62* | 4.43 | 103 | 113 | 123 | 110 | 112 | – |
| – | – | – | – | – | – | – | – | – | – | – | – | – | – | BBCH 65–67 |
| AX | 1 | 1 l | 65–67 | 4.28 | 5.19* | 3.62* | 4.82* | 4.48 | 103 | 118 | 119 | 115 | 114 | – |
| Pic | 1 | 0.5 l | 65–67 | 4.54* | 5.09* | 3.57* | 4.65* | 4.46 | 109 | 116 | 117 | 111 | 113 | – |
| Pro | 1 | 1 l | 65–67 | 4.31 | 5.16* | 3.29 | 4.85* | 4.40 | 104 | 118 | 108 | 116 | 111 | 112 |
| AcP | 1 | 1 l | 65–67 | 4.43* | 5.03* | 3.58* | 4.87* | 4.48 | 107 | 115 | 118 | 116 | 114 | – |
| Za | 1 | 1 l | 65–67 | 4.22 | 4.97* | 3.62* | 4.51* | 4.33 | 99 | 113 | 119 | 108 | 110 | – |
| Pic | 2 | 0.25 l | 61–63 + 65–67 | 4.38 | 5.42* | 3.31 | 4.65* | 4.44 | 102 | 124 | 109 | 111 | 111 | – |
| Pic | 2 | 0.5 l | 61–63 + 65–67 | 4.87* | 5.42* | 3.69* | 4.87* | 4.71 | 114 | 124 | 121 | 116 | 119 | – |
| AX | 2 | 0.5 l | 61–63 + 65–67 | 4.67* | 5.33* | 3.20 | 4.99* | 4.55 | 109 | 122 | 105 | 119 | 114 | – |
| AX | 2 | 1 l | 61–63 + 65–67 | 4.55* | 5.33* | 3.88* | 4.65* | 4.60 | 106 | 122 | 127 | 111 | 117 | – |

*AX* Amistar Xtra, *Pic* Pictor, *Pro* Propulse, *Ac* Acanto Plus, *Za* Zamir40EW
*Significant at 0.05 probability level

**Table 3** Influence of application timing on *S. sclerotiorum* infection

| Treatment | Number of applications | Dose Per ha | Applications at BBCH | Infection rate of S. *sclerotiorum* (%) 2013 | 2014 | 2015 | 2016 | Mean | Effectiveness according to Abbot 2013 | 2014 | 2015 | 2016 | Mean | Mean BBCH 61–63 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | – | – | – | 19.4 | 36.1 | 47.5 | 50.0 | 38.25 | 0 | 0 | 0 | 0 | – | – |
| AX | 1 | 1 l | 61–63 | 16.9 | 10.6* | 25.6* | 41.9 | 23.75 | 15 | 71 | 46 | 16 | 37 | – |
| Pic | 1 | 0.5 l | 61–63 | 3.1* | 20.3 | 33.8 | 19.4* | 19.13 | 84 | 44 | 29 | 61 | 55 | – |
| Pro | 1 | 1 l | 61–63 | 8.8* | 5.1* | 23.1 | 18.8* | 13.94 | 55 | 86 | 51 | 62 | 64 | 48 |
| AcP | 1 | 1 l | 61–63 | 12.5 | 15.5* | 19.4* | 34.4* | 20.44 | 36 | 58 | 59 | 31 | 46 | – |
| Za | 1 | 1 l | 61–63 | 19.4 | 8.4* | 28.1 | 28.8* | 21.16 | 0 | 77 | 41 | 42 | 40 | – |
| – | – | – | – | – | – | – | – | – | – | – | – | – | – | BBCH 65–67 |
| AX | 1 | 1 l | 65–67 | 3.8* | 27.4 | 39.4 | 29.4* | 24.97 | 80 | 24 | 17 | 41 | 41 | – |
| Pic | 1 | 0.5 l | 65–67 | 0.0* | 16.5* | 26.3 | 5.0* | 11.94 | 100 | 54 | 45 | 90 | 72 | – |
| Pro | 1 | 1 l | 65–67 | 0.0* | 18.4* | 37.5 | 8.8* | 16.16 | 100 | 49 | 21 | 82 | 63 | 52 |
| AcP | 1 | 1 l | 65–67 | 1.9* | 23.1 | 38.8 | 19.4* | 20.78 | 92 | 36 | 18 | 61 | 52 | – |
| Za | 1 | 1 l | 65–67 | 4.4* | 27.1 | 32.5 | 50.0 | 28.50 | 77 | 25 | 32 | 0 | 34 | – |
| Pic | 2 | 0.25 l | 61–63 + 65–67 | 0.0* | 7.8* | 11.9* | 5.0* | 6.16 | 100 | 78 | 75 | 90 | 86 | – |
| Pic | 2 | 0.5 l | 61–63 + 65–67 | 0.0* | 23.1 | 13.1* | 2.6* | 9.71 | 100 | 36 | 72 | 95 | 76 | – |
| AX | 2 | 0.5 l | 61–63 + 65–67 | 5.0* | 19.6 | 11.9* | 22.5* | 14.75 | 74 | 46 | 75 | 55 | 63 | – |
| AX | 2 | 1 l | 61–63 + 65–67 | 0.0* | 11.3* | 16.9* | 9.4* | 9.38 | 100 | 69 | 64 | 81 | 79 | – |

AX Amistar Xtra, *Pic* Pictor, *Pro* Propulse, *AcP* Acanto Plus, *Za* Zamir40EW
*Significant at 0.05 probability level

## Discussion

It is apparent from the results obtained that the applied fungicides generally reduced the infection rate of *S. sclerotiorum* in winter oilseed rape and increased yields above the control in all study years. Similar results had been reported also by Brazauskiene et al. (2013), who monitored the sensitivity of winter oilseed rape to diseases commonly occurring in oilseed rape (phoma stem cancer, alternaria black spot, and sclerotinia stem rot) and their reaction to fungicide applications. They had concluded that fungicide applications in general significantly reduced incidence of the stated diseases and increased yields.

The results obtained by comparing the two fungicide application timings indicate that for new fungicides based on newly synthesized triazoles and strobilurins the application timing is no longer as important as was previously the case. Older fungicides based on triazoles and SBI inhibitors had a greater impact on *S. sclerotiorum* infection rates and yields if applied at the BBCH 65–69 stage (Spitzer et al. 2012). Del Rio et al. (2007) monitored the use of older types of fungicides against *S. sclerotiorum* in different concentrations and different time spans within the rape flowering period and determined that the *S. sclerotiorum* disease develops with various intensities depending on the application timing. In our study, on the other hand, the fungicide application term did not play a significant role. The timings for optimal use of new fungicides may therefore be expanded within the BBCH 61–67 rape development period without concern for loss of effectiveness or loss of yields.

The evaluation of fungicides' impact on effectiveness and yield nevertheless indicates that generally slightly higher fungicide effectiveness against *S. sclerotiorum* was recorded for the BBCH 65–67 application term, specifically by an insignificant 4%. This slightly higher effectiveness had no effect, however, on yield.

Bradley et al. (2006) had reported similar findings based on their results from experiments examining the effectiveness of fungicides on *S. sclerotiorum* and on yield. In their study, the pathogen's rate of occurrence differed by individual year and individual location. A generally marked decrease in infection was recorded for the active ingredients azoxystrobin, benzyl, boscalid, iprodione, protioconazole, tebuconazole, thiophanate-methyl, trifloxystrobin, and vinclozolin. Nevertheless, a significant decrease in *S. sclerotiorum* infection was not always reflected in the yield. Different results were observed at various application timings according to the stage of flowering and active ingredients used.

The best results, in terms of both effectiveness and increased yield, were recorded for the two fungicides most commonly used against *S. sclerotiorum* on winter oilseed rape in the Czech Republic when applied at the two different phases at either reduced or full doses. This split-application technique is not used in agricultural practice, however, primarily for technical and economic reasons because increasing the number of passes by machinery through an interconnected stand of winter oilseed rape causes a loss in yield.

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**Conflict of interest** T. Spitzer, J. Bílovský and J. Kazda declare that they have no competing interests.

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