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Effects of host plant resistance and fungicide application on phoma stem canker, growth parameters and yield of winter oilseed rape

Nazanin Zamani-Noor[a,*], Jessica Knüfer[b]

[a] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11-12, D-38104, Braunschweig, Germany
[b] Lindener Str. 9, 38300 Wolfenbüttel, Germany

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ABSTRACT

Multifactorial field trials were conducted over three cropping seasons (2012–2015) to assess the effects of selection of triazole fungicides when applied either alone or in combination on growth of winter oilseed rape, development of phoma stem canker, crop wintering and crop yield. The plots consisted of four winter oilseed rape cultivars differing in their levels of resistance to phoma stem canker and subplots of five fungicides with growth regulatory activity (prochloraz/tebuconazole, mepiquat/metconazole, tebuconazole, tebuconazole/prothioconazole and a mixture of fluxapyroxad/tebuconazole) which were applied twice; in autumn (BBCH 14–18) and in spring (BBCH 30–39). Visual scoring such as growth parameters like plants per m^2 and plant height, yield parameters like number of side branches, pods and seeds per plant, thousand grain weights and seed yield as well as evaluation of phoma leaf spots and basal stem canker were performed using standard procedures. Plant height was significantly affected by cultivars and fungicide treatments. In contrast, winter killing (%) was not reduced significantly. Application of mepiquat/metconazole reduced the winter killing below 10% compared to the untreated control. Development of phoma stem canker was affected by fungicides and cultivars. At the final rating date (BBCH 81), the moderately resistant cultivars Genie and Vitara exhibited less disease severity than the susceptible cultivars Elektra and PR 46W20 in untreated plots. Fungicide treatments were more effective in reducing the disease in moderately resistant cultivars than in susceptible ones. The lowest percentage of disease severity was achieved when fluxapyroxad/tebuconazole combination was applied to plants. To our knowledge, this study is the first to report multi-year efficacy of fluxapyroxad/tebuconazole against phoma stem canker in oilseed rape cultivations. Cultivars showed differences in all yield parameters. However, with the exception of seed yield the application of fungicides did not significantly impact the different yield parameters. Application of fluxapyroxad/tebuconazole was the most effective treatment in reducing yield losses.

1. Introduction

Phoma stem canker (blackleg) caused by *Leptosphaeria maculans* or *L. biglobosa* (anamorph: *Phoma lingam*), is one of the most damaging diseases of oilseed rape (*Brassica napus* L.) worldwide (West et al., 2001; Fitt et al., 2006). The pathogen attacks cotyledons, leaves, stems, roots and pods resulting in leaf spots, basal stem cankers, upper stem lesions and pod spots. In Germany, first symptoms appear during autumn/winter. Airborne ascospores that are released from infected debris infect leaves at the rosette stage and produce phoma leaf spots. As these lesions develop, the pathogen spreads down petioles to reach the stem base and causes stem cankers in spring/summer. In severe infections the disease girdle and weaken stems causing lodging and early senescence followed by premature ripening of the pods. The average yield losses in different countries vary from 30 to 50% depending on weather conditions, disease severity and oilseed rape cultivars (West et al., 2001; Fitt et al., 2006). Integrated control of phoma stem canker relies on the cultural management strategies, cultivar resistance and fungicide applications (Pilet et al., 1998; Aubertot et al., 2006; Eckert et al., 2010). Fungicide applications in autumn and spring seems to give the most effective control of the disease. In Germany, the triazole fungicides have been registered and applied because of their efficacy against causal agents of the disease as well as their relatively low cost compared to the alternatives. Amongst the triazoles, tebuconazole is the most commonly applied fungicides against basal stem canker. In addition to disease management, fungicides with plant growth regulator activity are also applied to German oilseed rape in autumn to improve crop winter hardiness and reducing crop lodging. Plant growth

* Corresponding author.
E-mail address: nazanin.zamani-noor@julius-kuehn.de (N. Zamani-Noor).

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regulators have previously been studied to improve growth and oil and protein content of oilseed rape. Mepiquat chloride is one of the most applied growth regulators in agriculture, applied either individually or together with other pesticides, which improves resistance to lodging and increasing crop yields both quantitatively and qualitatively (Hoffmann, 1992; Rademacher, 2000; Riediker et al., 2002). Triazole compounds, including metconazole and tebuconazole, are currently used in oilseed rape cultivation in Europe for both their fungicidal and growth regulatory properties (Davis et al., 1988; Fletcher et al., 1986). Many triazoles appear in distinct isomeric forms, which may vary in their fungicidal and growth regulatory activities (Davis et al., 1988; Berry and Spink, 2009). The fungicidal effect of the triazoles is caused by the interference into the fungal ergosterol biosynthesis by the inhibition of the C14 demethylase (Davis et al., 1988) which influence the sterol biosynthesis (classified as SBI = Sterol biosynthesis Inhibitor). Triazoles represent the largest and most important group of systemic compounds used for controlling plant diseases. On the other hand, the growth regulatory effect of triazoles is attributed to the inhibition of mono-oxygenases, which oxidize in three steps ent-kaurene to ent-kaurenoic acid, an early reaction in gibberellin biosynthesis (Child et al., 1993; Hedden and Kamiya, 1997; Rademacher, 2000). They induce various physiological and morphological changes in the plant such as shortening of shoots by inhibition of cell elongation, inhibition of ethylene synthesis, retardation of senescence (greening effect), promotion of chlorophyll synthesis, reduction of photosynthesis by decreasing stomatal conductance, improvement of resistance to abiotic stress and increase of root-shoot ratio (Kuchenbuch and Jung, 1988; Sankhla et al., 1985; Grossmann, 1990). In winter oilseed rape the inhibition of stem elongation by plant growth regulators can lead to an alteration in canopy architecture and may improve yield parameters (Child et al., 1993).

The present study was conducted to evaluate the effect of selected triazole fungicides with plant growth regulatory activity (alone or in combination) on growth parameters of oilseed rape, fungal disease development, plant wintering and plant yield. As cultivar resistance is essential to the integrated control of phoma leaf spot and stem cancker, the interaction of these fungicides with cultivars differing in resistance to Phoma was also investigated. The influence of weather conditions on oilseed rape growth development also is taken into account at the same time.

2. Materials and methods

2.1. Field trial location and treatments

Field trials were carried out over three consecutive years in Ahlum, Lower Saxony, Germany in 2012/2013, 2013/2014 and 2014/2015. Four winter oilseed rape cultivars Elektra, Genie, PR 46W20 and Vitara were chosen from the German Plant Variety Catalogue in 2012 due to their varying levels of resistance to phoma stem canker (Suppl. Table 1).

Seeds were drilled in 22.5 cm rows on 29 August 2012, 26 August 2013 and 27 August 2014, respectively, at a seeding rate of 50 seeds per m^2 for hybrid cultivars (Elektra, Genie, PR 46W20) and 60 seeds per m^2 for the conventional cultivar Vitara. The experiments were conducted in a complete randomized block design with four replications. The plot size was 11 × 1.8 m. Different fungicide treatments were applied as foliar spray twice, first before winter at growth stage 14–18 (BBCH monograph, Bleiholder et al., 2001) and again after winter at BBCH 30–39. The complete treatment schedule and detailed information about fungicides are summarized in Table 1.

All fungicides were applied at 75% recommended dose with a four-nozzle precision sprayer (Air-Injector IDK-02, 1.5–3 bar, Lechler GmbH, Metzingen, Germany) using a fine mist at a slow walk to ensure an effective coverage. The delivery pressure at the nozzle was two bars. The untreated control plants were sprayed with the corresponding amount of water.

Weather data associated to the field location (monthly mean air temperature (°C), precipitation (mm) and relative humidity (%)) was obtained from by the German National Weather Service (Deutscher Wetterdienst: DWD) between 2012 and 2015.

2.2. Growth and yield parameters

Observations of growth was conducted on 25 randomly chosen plants within each plot and consisted of measurements of root neck diameter (mm) and plant height (cm) before and three to four weeks after 1st application in autumn. Number of plants per m^2 was recorded once in mid- November (before winter) and once in mid-March (after winter) and winter killing (%) was calculated in all treated and untreated plots. Height of the canopy (cm) was measured at the beginning and at the end of flowering stage (BBCH 60 and BBCH 70). Yield parameters including number of side branches and pods per plant as well as seeds per pod and per plant were recorded on 10 randomly chosen plants at maturity stage (BBCH 83–85). The plots were harvested on 12 July 2013, 17 July 2014 and 14 July 2015, respectively, with a Haldrup-C58 plot combine-harvester (Haldrup GmbH, Ilshofen, Germany) and the seed yield was determined at water content of 9%. Subsequently, a homogenous 500 g sample of seeds was taken from each plot, and the weight (g) of thousand grains (TGW) was recorded.

2.3. Assessment of oilseed rape fungal diseases

Oilseed rape plants were evaluated visually within each plot for phoma leaf spot severity (expressed as percent of affected leaf area), at BBCH 14 (before 1st application) and at BBCH 18 (three to four weeks after 1st application) as defined in Steed et al. (2007). The severity of basal stem canker was assessed once at BBCH 81 on 25 randomly collected plants from each plot. The severity of basal stem canker was evaluated by cutting the stem at the base of each collected plant followed by the scoring of the cross-sectional area of the necrotic tissue according to a 1 to 9 scale (Krüger, 1982). The infection by other fungal pathogens (e.g. *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Alternaria* spp.) was recorded as percentage by visual plot evaluation at BBCH 83–85.

2.4. Statistical analysis

1) Data collected from the different traits (growing parameters, yield parameters and phoma stem canker) at each treatment and the three growing seasons 2013, 2014 and 2015 were first analyzed separately in the ANOVA to show the influence of the different factors. The following linear mixed model was used:

$$Y_{ijkm} = \mu + c_i + r_j + t_k + y_m + (cy)_{im} + (ct)_{ik} + (cyt)_{ikm} + e_{ijkm}$$

Where Y_{ijkm} is the phenotypic observation with μ as the general mean, c_i the effect of the *i*th cultivar, r_j the effect of the *j*th replication, t_k the effect of the *k*th treatment, y_m the effect of the *m*th year. cy was the interaction between the cultivar and year effects of the *i*th cultivar and *m*th year. ct was the interaction between the cultivar and treatment of the *i*th cultivar and *k*th treatment followed by the cyt interaction of cultivar, year and treatment effects and the corresponding *i*th cultivar, *k*th treatment and *m*th year. e_{ijkm} was the designated residual error term. For the ANOVA calculation all effects except replications were considered as fixed.