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Influence of selected *Rhizoctonia solani* isolates on sugar beet seedlings

Paweł Skonieczek[1]*, *Mirosław Nowakowski*[1], *Jacek Piszczek*[2], *Marcin Żurek*[1], *Łukasz Matyka*[1]

[1] Plant Breeding and Acclimatization Institute – National Research Institute, Research Division in Bydgoszcz, Powstańców Wlkp. 10, 85-090 Bydgoszcz, Poland

[2] Institute of Plant Protection – National Research Institute, Regional Experimental Station Toruń, Pigwowa 16, 87-100 Toruń, Poland

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Abstract: From 2008 to 2010 the levels of sugar beet seedlings infection caused by *Rhizoctonia solani* were compared in laboratory tests. Seven sugar beet lines were tested: H56, H66, S2, S3, S4, S5 and S6 as well as three control cultivars: Carlos, Esperanza and Janosik. Sugar beet lines with tolerance to rhizoctoniosis and cultivars without tolerance were infected artificially by *R. solani* isolates: R1, R28a and R28b. These isolates belong to the second anastomosis group (AG), which is usually highly pathogenic to beet roots. The aim of the experiment was to test whether the tolerance of sugar beet genotypes to *R. solani* AG 2 prevents both root rot, and damping-off of seedlings, induced by the pathogen. Sugar beet lines tolerant to brown root rot in laboratory tests were significantly less sensitive to infection of the seedlings by *R. solani* AG 2 isolates in comparison to control cultivars. *Rhizoctonia solani* AG 2 isolates demonstrated considerable differences in pathogenicity against seedlings of sugar beet lines and cultivars. The strongest infection of sugar beet seedlings occurred with the isolate R28b. The greatest tolerance to infection by AG 2 isolates was found for the S5 and S3 breeding lines.

Key words: health of seedlings, *Rhizoctonia solani,* sugar beet

Introduction

Intensification and concentration of plant production, especially sugar beets leads to unfavorable shortening of crop rotations. This way of cultivation has ad hoc positive effects, however, it also causes many problems associated with increased growth of weeds, diseases and pests (Wesołowski *et al*. 2005; Szymczak-Nowak *et al*. 2007; Górski and Piszczek 2008). In addition to many previously known diseases of sugar beet leaves and roots, increasingly sugar beet plantations have symptoms of *R. solani* (Piszczek *et al*. 2012; Skonieczek and Nowakowski 2013).

Worldwide fungus *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris*) is a common pathogenic parasite in potato, rapeseed, corn, cereal crops, vegetable and ornamental plants (Sneh *et al*. 1994; Engelkes and Windels 1996; Führer Ithurrart *et al*. 2004). Losses due to this pathogen are estimated at 5–20% yield in crops in nearly 200 plant species (Ogoshi 1987). Many weeds provide an alternative host for *R. solani,* adding to the threat.

Due to a high variability of the pathogen, it is subjected to classification with the use of anastomosis groups (AG) form AG 1 to AG 13 and AG-BI, which have been determined taking into account the ability to link fungal hyphae (Carling 1996; Coosemans *et al.* 2001; Carling *et al*. 2002). Particular anastomosis isolate groups vary in pathogenicity compared to their hosts, and sometimes even in sugar beets, cause various diseases – seedling blight and brown root rot (Führer Ithurrart 2003; Buhre 2008). Infection by *R. solani* is conducive at high temperatures and humidity, as well as acidic and excessive compaction of the soil and subsoil (Büttner *et al*. 2002; Buhre 2008). Favorable conditions for the development of the disease also occur during crop rotation with a large share of corn, oilseed rape and sugar beet (Rush *et al*. 1994; Buhre *et al*. 2009).

The fungus is not sensitive to fungicides. Therefore a significant reduction in emergence, plant density and root yield, sometimes even more than 50% occurs (Allen *et al*. 1985). First the external vertical beet leaves wilt and turn yellow and then completely die as a result of infection. A clearly visible boundary on rootsinfested by *R. solani* between healthy and diseased tissue can be observed (Windels and Lamey 1998; Borodynko *et al*. 2011). An early and intensive infection leads to cracking of the roots and secondary infections. The pathogen also inhibits its plant growth, through secreted toxins (Moliszewska 2009). The yield from sugar beets which survived infection is usually lower and has poorer juice quality. Roots are also very susceptible to decay during storage.

Integrated sugar beet protection against *R. solani* can be achieved with proper sanitary crop rotation, maintainance of good soil structure and the use of varieties resistant to the pathogen (Büttner *et al*. 2002; Buddemeyer and Märländer 2004).

*Corresponding address:
p.skonieczek@ihar.bydgoszcz.pl

However, the high ability of *R. solani* to survive in the soil and on a large number of host plants (Ruppel and Hecker 1994; Buhre 2008; Nowakowski *et al.* 2014) makes it difficult to develop effective strategies of protection against the fungus.

This study was designed to evaluate the sensitivity in laboratory conditions of some breeding line cultivars of sugar beet to infection by three isolates of *R. solani* belonging to the second anastomosis group.

Materials and Methods

The research was conducted from 2008 to 2010 in the microbiology laboratory of the Bydgoszcz Branch of the Institute of Plant Breeding and Acclimatization – National Research Institute (IHAR – PIB) in collaboration with the Regional Experimental Station of the Institute of Plant Protection – National Research Institute, in Toruń. The testing of sugar beet genotypes' susceptibility to seedling damping-off caused by *R. solani* was tested with the use of R1, R28a and R28b isolates, which are part of the second anastomosis group from the IHAR – PIB, Bydgoszcz. Determining the anastomosis group of *R. solani* was carried out by combining investigated isolates and isolates-testers with a known AG previously identified by the method of Kronland and Stanghellini (1988). The R28a and R28b were isolated from sugar beet seedlings from the Lubelskie province and R1 from the Kujawsko-Pomorskie province. The isolates were stored on a standard Potato Dextrose Agar (PDA) medium at 4°C. Plastic cuvettes (46 × 36 × 8 cm) were filled with sterile typical lessive soil and it was maintained at 65% of maximum water-holding capacity of soil. No pelleted seeds of the breeding lines H56, H66 (H – KWS), S2, S3, S4, S5 and S6 (S – Syngenta), with tolerance to rhizoctoniosis, and three control varieties: Carlos (Strube), Esperanza (KWS) and Janosik (KHBC), without this tolerance, were sown into this soil. According to information forwarded from breeding companies, the tested lines have increased resistance to brown root rot caused by *R. solani* AG 2. Seeds were sown in rows and infected with an isolate of *R. solani*. In each of the three experiments, the same methodology and one of the aforementioned isolates were used. The mycelia of individual isolates were grown for three weeks in 250 cm^3 flasks on 100 cm^3 of sand-maize medium (Garrett 1970; Moliszewska 2009). The medium was inoculated with five 0.5-cm-diameter discs with one-week old mycelium of a particular isolate grown on a PDA medium. For all replicates 40 g of medium per cuvette was used. The level of the inoculum density was 48–54 cfu · g^{-1} calculated per 1 g of the sand-maize.

The experiments were performed in three replications (in three cuvettes), by sowing 100 seeds into each of them. The cuvettes were placed in a vegetation chamber with temperature, humidity and light exposure control. The daily cycle was 16 h of light and 8 h of darkness, at 24°C.

To determine the level of infestation, the roots of six-week old seedlings were evaluated according to a two degree scale:

0 – healthy seedlings,

1 – seedlings affected by *R. solani* in varying severity (tiny spots and places of rotting on the root surface).

Plants with symptoms of infection by *R. solani* are defined as infected plants in the accompanying graphs (Figs. 1, 2, 3 and 4).

The test results were statistically analyzed by the analysis of variance and the differences were verified by the Student's t-test, at a significance level of $p = 0.05$.

Results and Discussion

Selected sugar beet genotypes exhibited varying susceptibility to artificial infection caused by tested isolates of *R. solani*, belonging to the second anastomosis group. A low number of plants infected by isolate R1 was found in lines S5, S4 and H56 (15.3%, 26.4%, 30.6%, respectively) (Fig. 1). The lines S3, S5, and H56 showed high resistance to the pathogen in the case of infection caused by isolate R28a (28.6%, 48.1% and 48.1% of the infested plants, respectively) (Fig. 2), and the lines S5, S3 and S6 to isolate R28b (44.7%, 59.8% and 64.5% of infected plants, respectively) (Fig. 3). Seedlings of each of the three control sugar beet varieties were strongly affected by the tested isolates of *R. solani* in comparison to the tested lines.

Tested lines and beet varieties were the most susceptible to isolate R28b, which caused the severest infestations of sugar beet seedlings (Fig. 3). The greatest diversity of infestation in both lines and varieties, and the smallest susceptibility of lines were observed in beet seedling infection by isolate R1. The level of infestation ranged from 15.3% for line S5, to 98.6% for the Janosik variety (Fig. 1).

Analyzing the average infestation in three tests it was found that the least infected lines were S5 (36.0%), S3 (44.3%) and H56 (50.4%) (Fig. 4). Comparative varieties were characterized by significantly higher levels of infestation, and the Janosik variety was the most sensitive to *R. solani* isolates (average 98.2%).

Sugar beet lines, defined by breeding companies as resistant to brown root rot caused by *R. solani* (usually AG 2), stood out significantly with a lower susceptibility to seedling blight caused by *R. solani* isolates from AG 2 with respect to not resistant standard varieties.

The described relationship has not been confirmed when the seedlings of sugar beet resistant lines were tested for infection by *R. solani* from the fourth anastomosis group (Skonieczek *et al*. 2014; Nowakowski *et al*. 2014). That group has previously been identified in the damping-off process in sugar beet cultivation (Windels and Nabben 1989; Rush *et al*. 1994; *et al*. 2008). Perpetrators of the root rot and damping-off of beet seedling are also representatives of the AG 3 and AG 5 *R. solani* (Rush *et al*. 1994). The AG 2 isolates demonstrate high volatility in their pathogenicity and are particularly dangerous for beets in their later vegetation period (Engelkes and Windels 1996), while the AG 4 isolates heavily infect the seedlings (Herr and Roberts 1980).

The study shows that resistance to sugar beet brown root rot may overlap with resistance to seedling blight caused by *R. solani* only if the agent of both diseases belongs to the second anastomosis group.

Different seedling infection by *R. solani* is dependent on the variety of the sugar beet as well as the pathogen isolate and if it belongs to the AG. This has been de-

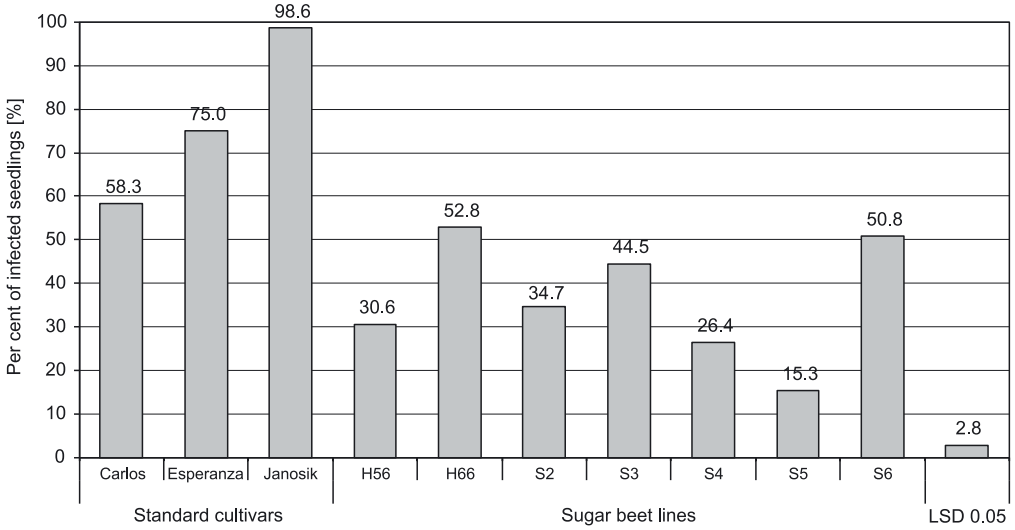

Fig. 1. Per cent of infected seedlings of selected sugar beet lines and cultivars as a result of artificial infection by isolate R1 of *Rhizoctonia solani*

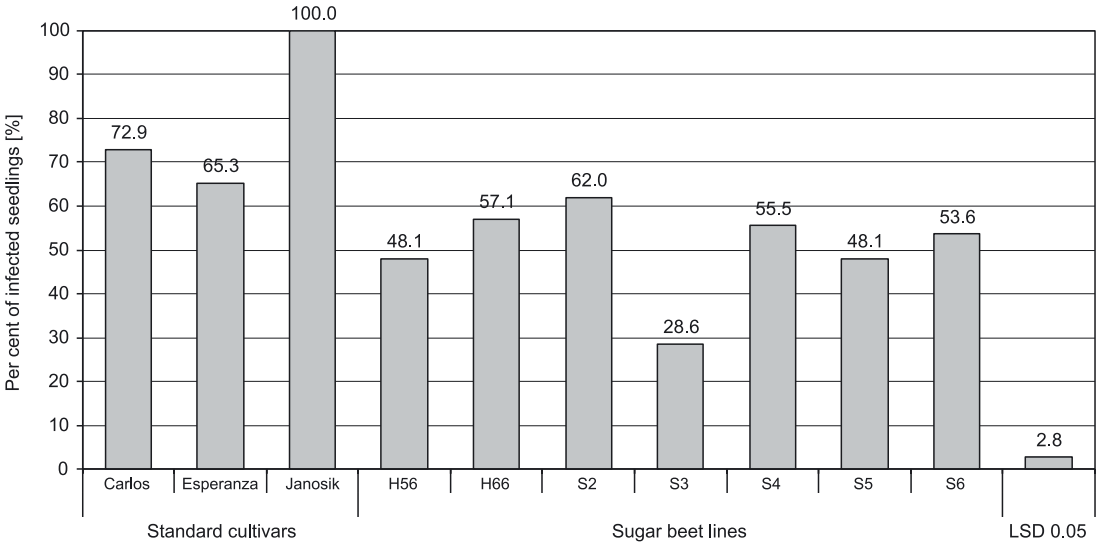

Fig. 2. Per cent of infected seedlings of selected sugar beet lines and cultivars as a result of artificial infection by isolate R28a of *Rhizoctonia solani*

scribed in several studies conducted in Poland. The authors confirmed the presence of *R. solani* as the agent of damping-off on the sugar beet plantations in the country (Moliszewska 2000; Szymczak-Nowak *et al*. 2001, 2002; Moliszewska and Burgieł 2002; Szymczak-Nowak 2005).

Seedling infection of tested sugar beet lines and varieties by *R. solani* AG 2 confirm a significant danger of this anastomosis group to the young sugar beet plants and not only by AG 4 isolates, as was previously thought (Moliszewska and Schneider 2002; Führer Ithurrart 2003; Buhre 2008; Moliszewska 2009). It is important to search for new methods of protecting sugar beet seedlings against this fungal pathogen, which shows low sensitivity to fungicides and has a very high number of host plants currently occurring in crop rotation and fields. Unfortunately, there are no effective chemical methods of protection against damping-off of sugar beet caused by *R. solani*.

Conclusions

1. Sugar beet genotypes identified as resistant to brown root rot caused by *R. solani* in laboratory tests show significantly less susceptibility to seedling infection by the *R. solani* isolates belonging to AG 2 in comparison with not resistant control cultivars.
2. The tested *R. solani* AG 2 isolates significantly differ in pathogenicity to seedlings of sugar beet varieties and breeding lines. Severe pathogenicity was found for isolate R28b. Lines S5 and S3 were found to be highly resistant to *R. solani*, and the Janosik variety was the most sensitive to infection.
3. Strong infection of the seedlings of control sugar beet varieties by *R. solani* AG 2 shows a considerable menace of the pathogen to the emerging plants. The large differences among the effects of infections caused by used isolates show diversity within the AG 2 of the fungus *R. solani*.

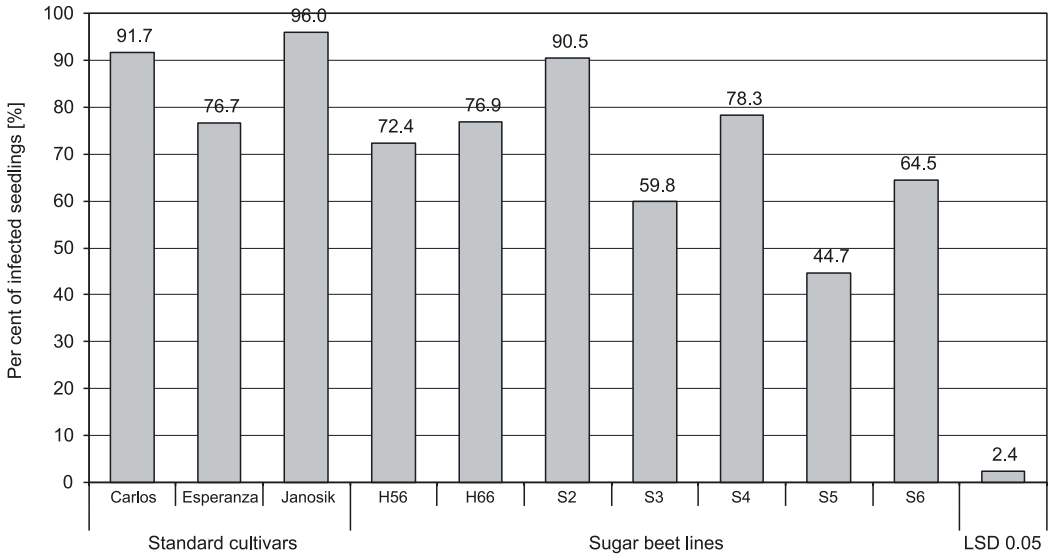

Fig. 3. Per cent of infected seedlings of selected sugar beet lines and cultivars as a result of artificial infection by isolate R28b of *Rhizoctonia solani*

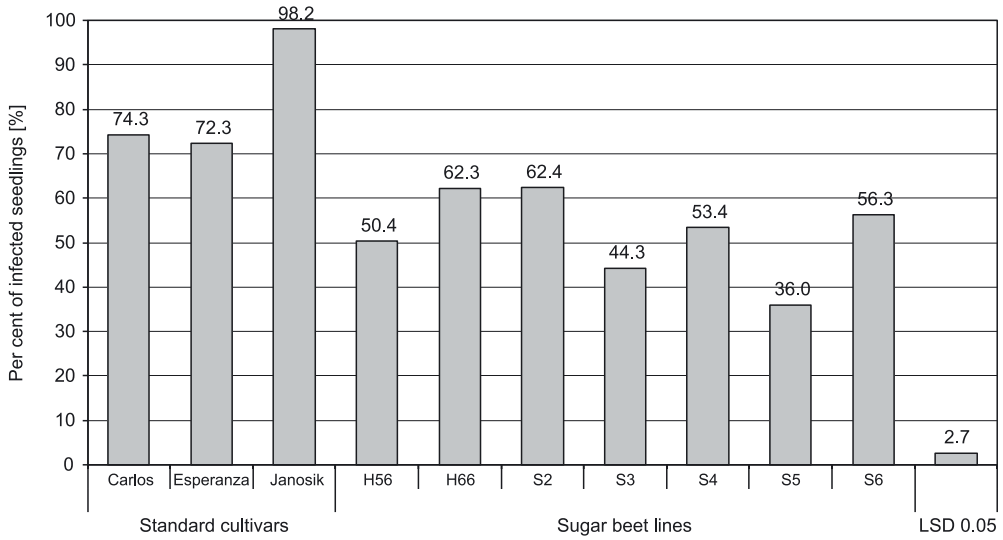

Fig. 4. Per cent of infected seedlings of selected sugar beet lines and cultivars as a result of artificial infection by *Rhizoctonia solani* isolates: R1, R28a and R28b; average value from three tests

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