# Variation in fungicide sensitivity among *Rhizoctonia* isolates recovered from potatoes in South Africa

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## Abstract

*Rhizoctonia* is a major pathogen of potato causing substantial yield losses worldwide. Control of *Rhizoctonia* diseases is based predominantly on the application of fungicides. However, little is known about the fungicide response variability of different *Rhizoctonia* anastomosis groups associated with potato diseases in South Africa. A total of 131 *Rhizoctonia* isolates were obtained from potato growing regions of South Africa from 2012 to 2014 and evaluated for sensitivity to fungicides *in vitro* and *in vivo*. The fungicides comprised six chemical formulations and one bio-fungicide representing seven Fungicide Resistance Action Committee groups. All *Rhizoctonia* anastomosis groups were sensitive to tolclofos-methyl ($EC_{50}$: 0.001-0.098 µg a.i $ml^{-1}$) and fludioxonil ($EC_{50}$: 0.06-0.09 µg a.i $ml^{-1}$) and showed variation in sensitivity to pencycuron, iprodione, benomyl and *Bacillus subtilis* QST 713. However, for azoxystrobin, *Rhizoctonia* isolates exhibited variable sensitivity ranging from sensitivity ($EC_{50}$: < 0.09 µg a.i $ml^{-1}$) to insensitivity with $EC_{50}$ values exceeding 5 µg a.i $ml^{-1}$. In greenhouse and field trials, tolclofos-methyl and fludioxonil exhibited significantly greater control of stem and black scurf whereas azoxystrobin was the least effective. This work demonstrated variable sensitivity within and among anastomosis groups of *R. solani* and binucleate *Rhizoctonia* to different fungicides. Information on fungicide sensitivity of *Rhizoctonia* isolates is crucial in the development of effective *Rhizoctonia* control strategies and facilitates monitoring of fungicide insensitive isolates in the pathogen population.


*Keywords: Rhizoctonia*, disease management, fungicide sensitivity, potato, $EC_{50}$

The basidiomycete fungus *Rhizoctonia* is an economically important pathogen of potato that lowers the yield and quality of potatoes in South Africa and other potato growing areas worldwide (Banville 1989). *Rhizoctonia* spp. confirmed to be associated with potato diseases in South Africa and elsewhere include the multinucleate *Rhizoctonia solani* and binucleate *Rhizoctonia* (Muzhinji et al. 2015; Woodhall et al. 2008). Presently, multinucleate *R. solani* contains 13 anastomosis groups (AG) whereas binucleate *Rhizoctonia* (BNR) is composed of 18 known AG groups (Sharon et al. 2008; Yang et al. 2015). Anastomosis groups vary greatly in pathogenicity, host preference and fungicide sensitivity (Tsror 2010).

In potato production, *Rhizoctonia* infection causes stem and stolon canker that result in quantitative losses through growth and yield suppression (Banville 1989; Woodhall et al. 2008). The pathogen also causes blemishes such as black scurf, elephant hide and growth cracks on potato tubers leading to qualitative yield losses (Campion et al. 2003). The *Rhizoctonia* fungus overwinters in propagules and in the soil as mycelia and/or sclerotia which serve as inoculum for the subsequent crop (Tsror 2010). When *Rhizoctonia* sclerotia and/or mycelia are present on seed tubers, they serve as a primary inoculum source for disease epidemics and act as a mechanism for long-distance dispersal of the pathogen to other potato growing regions (Tsror 2010).

Although potato growers control *Rhizoctonia* through crop rotation, use of biocontrol agents and certified pathogen-free seed tubers; the application of fungicide remains the most commonly employed strategy for management of *Rhizoctonia* in South Africa and elsewhere (Nel et al. 2003; Tsror (Lahkim) and Peretz-Alon 2005). Fungicide applications are made to seed tubers and/or in-furrow prior to or during planting for controlling seed tuber- and soil-borne inoculum, respectively. A wide range of site-specific fungicides, have been extensively used for controlling *Rhizoctonia* in South Africa. Some of the site-specific fungicides currently registered for the control of *Rhizoctonia* in South Africa, include azoxystrobin

registered for control of stem canker and black scurf; tolclofos-methyl for in-furrow and seed treatment applications, pencycuron registered for seed tuber treatment and fludioxonil registered for stem canker and black scurf treatment (Nel et al. 2003).

The application of fungicides for control of disease epidemics is however, not considered a long-term solution because of their potential harmful effects on the environment (Avenot et al. 2009). It is also increasingly common to find isolates that are less sensitive or resistant to fungicides, as has been reported for *R. solani* AG 1-IA in rice fields, in Louisiana (Olaya et al. 2013) and Henan Province, China (Chen et al. 2012). In addition, *Rhizoctonia* AGs have been reported to show variable sensitivity to fungicides in both *in vitro* and *in vivo* experiments in Virginia and Maryland (Amaradasa et al. 2014), France (Campion et al. 2003); North Carolina (Martin et al. 1984) and Mexico (Virgen-Calleros et al. 2000). Campion et al. (2003) reported moderate sensitivity of AG 5 as well as variable sensitivity of AG 2-1 isolates to pencycuron. Pencycuron was found to be effective against AG 3 and AG 2-1, whereas it had little effect on AG 5 and AG 8 (Kataria and Gisi 1999).

The response profiles of *Rhizoctonia* AGs to fungicides used by potato growers in South Africa have not yet been explored. The Fungicide Resistance Action Committee (FRAC 2015) reported high risk of insensitivity of pathogens to fungicides with a single mode of action with repeated use. Continued monitoring of fungicide sensitivity will provide an early warning if the population becomes insensitive to a fungicide.

Therefore, this study sought to evaluate the response profiles of *Rhizoctonia* AG populations to different classes of fungicides and a biocontrol agent currently registered and used by potato growers in South Africa to control *Rhizoctonia* diseases. The information obtained from this study will be useful in establishing sensitivity baselines of *Rhizoctonia* isolates from potatoes to different fungicides. Established sensitivity baselines of these

fungicides will help monitor and track fungicide sensitivity shifts in the field and to make effective fungicide recommendations for management of *Rhizoctonia* on potato.

## Materials and Methods

**Origin of *Rhizoctonia* isolates.** *Rhizoctonia* AGs used in this study were from previously collected isolates that were obtained from potatoes sampled from different potato growing regions in South Africa from 2012 to 2014 (Muzhinji et al. 2015). The AG identity of the 131 isolates was verified using the internal transcribed spacer (ITS) sequencing. *Rhizoctonia* isolates consisted of 112 *R. solani* (97 AG 3-PT, 8 AG 2-2IIB, 1 AG 5, 3 AG 4HG-I, and 3 AG 4HG-III) and 19 binucleate *Rhizoctonia* (16 AG A and 3 AG R). Each isolate was stored on barley grains at -20°C before it was grown on potato dextrose agar (PDA) for use in this study.

**Fungicides used in the study.** Six chemical fungicides (tolclofos-methyl, pencycuron, iprodione, azoxystrobin, benomyl and fludioxinonil) and a biofungicide (*Bacillus subtillis* QST 713) were evaluated for efficacy against *R. solani* and BNR AGs *in vitro* and *in vivo*. The fungicides were selected based on their current use in South Africa and were obtained from local agro-chemical suppliers (Table 1). The fungicide's effective concentration that reduces 50% of the mycelial growth ($EC_{50}$ values) was determined *in vitro* using the poison-agar method with plates amended with different concentrations of fungicide (Everett et al. 2005). The six chemical fungicides and biofungicide were also evaluated for efficacy *in vivo*, under controlled greenhouse conditions and field experiments. The fungicides were prepared by diluting the stock solution (100 mg a.i $ml^{-1}$) in water and stored in the dark at $4^{o}$C for no longer than 2 weeks before being used for the experiment. Iprodione (Prodione, Plaaskem) and azoxystrobin (Amistar, Syngenta) were dissolved in methanol to 100 mg a.i $ml^{-1}$ for the stock solutions. For azoxystrobin, salicylhydroxamic acid (SHAM,

Sigma-Aldrich, St. Louis, MO) was added to media to a final concentration of 100 mg $ml^{-1}$ to block the use of an alternative pathway for cellular respiration (Amiri et al. 2010). Control plates for azoxystrobin were amended with methanol and SHAM.

***In vitro* assessment of fungicide sensitivity.** The sensitivity of 131 *Rhizoctonia* isolates to six chemical commercial fungicides and one biofungicide was evaluated in mycelial growth assays. The fungicides were added at different concentrations (microgram of active ingredient per milliliter: µg a.i $ml^{-1}$) to autoclaved PDA medium to produce a concentration series of 0, 0.01, 0.1, 1, 10 and 100 µg a.i $ml^{-1}$ for each fungicide. The fungicide working concentrations were selected based on previous published studies and recommended application rates (Amaradasa et al. 2014; Olaya et al. 1994). For, *Bacillus subtilis* QST 713 (Serenade, Bayer; 1 x $10^9$ colony forming units (cfu) $ml^{-1}$) *in vitro* evaluations were carried out by diluting formulated Serenade with sterile water to give concentrations of 1 x $10^2$, 1 x $10^4$, 1 x $10^6$, 1 x $10^8$ cfu $ml^{-1}$ of *B. subtilis* QST 713. Mycelial plug (5 mm) of each *Rhizoctonia* isolate was punched from the margin of an actively growing colony of a 5-day old culture with a sterile cork-borer and placed mycelia-side down at the center of a 90 mm PDA plate amended with chemical fungicide or biofungicide and also on non-amended PDA plates. Plates were incubated at 25 °C for 5 days. Each concentration was replicated three times. After 6 days of incubation, the diameter of the fungal colony of each plate was measured at perpendicular angles and the average of the two measurements minus the original mycelia plug diameter (5 mm) was used for data analysis. The experiment was repeated once.

Percent inhibition was determined using the formula: % inhibition = [(mean non-amended PDA - mean fungicide amended PDA) / mean non-amended PDA] x 100. For each isolate, the effective fungicide concentration that inhibited 50% of mycelial growth ($EC_{50}$) was calculated by linear regression of the probit of the percent of inhibition of mycelial

growth versus the log10 transformation of the four concentrations of fungicide using the XLSTAT® (Addinsoft, www.xlstat.com). Probit analysis transforms the sigmoid dose-response curve to a straight line that can be analyzed by regression through maximum likelihood or least squares (Postelnicu 2014). Frequency distributions of $EC_{50}$ values were established for each fungicide using GraphPad Prism ver. 6.01 (Liang et al. 2015). For all assays, isolates were considered sensitive if the $EC_{50}$ values were less than 0.1 µg a.i $ml^{-1}$, isolates with $EC_{50}$ values ranging between 0.1 - 1 µg a.i $ml^{-1}$ were considered intermediately sensitive, and isolates were considered less sensitive if the $EC_{50}$ values exceeded > 5 µg a.i $ml^{-1}$ (Martin et al. 1984).

**Greenhouse experiment.** A study was conducted to compare the efficacy of fungicides against *Rhizoctonia* isolates on potato plants. Maize meal-sand mixtures (3:1) each colonized by a different isolate, viz. Rh68 (AG 3-PT); Rh96 (AG 4HG-I); Rh83 (AG 4HG-III); Rh30 (AG 5); Rh97 (AG 2-2IIIB); Rh26 (AG A) and Rh113 (AG R), were used as inoculum source (Table S1). Fifty grams of fully colonized maize meal-sand inoculum were mixed with 2 kg soil in 2 l pots, 2 days before planting. Soil in 2 l greenhouse pots was drenched with fungicide of desired concentration according to the manufacturer's in-furrow recommendation rates (Table 1). A single seed tuber, cultivar (cv.) Mondial, was planted in each pot. Plants were planted on 14 April 2015. Pots drenched with sterile water served as control. There were four replications for each treatment and 10 plants in each replication. After inoculation, the plants were grown in the greenhouse at 22 ± 2°C with a 12-hr photoperiod. Pots were watered every two days with 200 mls of sterile water. The experiment was laid out in a randomised complete block design. The experiment was repeated once.

**Field experiment: In-furrow application.** Field experiments were conducted at the University of Pretoria Experimental Farm in 2014. The plots were first fumigated with formalin solution three weeks before the experiment to eliminate soil-borne pathogens

including *Rhizoctonia*. Representative isolates from each AG group that showed intermediate sensitivity to each fungicide were selected and used in this experiment. Briefly, soil inoculum was prepared by growing representative AG isolates on PDA. *Rhizoctonia* cultures were blended with water into slurry, mixed with vermiculite to create soil inoculum and incubated for 21 days at 25$^{o}$C. Soil was inoculated by applying colonized vermiculite by hand in-furrows 2 h before planting. Fungicides were applied in-furrow at planting following the application rates recommended by the manufacturer (Table 1). The trials were laid out in a randomized complete block design with four treatments and four replications. Experimental plots were 4.5 m x 1.5 m, and each plot consisted of four rows with 18 potato plants per row. Disease-free certified tubers of cv. Mondial were planted by hand 25 cm apart. The experiment was repeated once.

**Seed tuber treatment.** *R. solani* AG 3-PT (Rh68), the predominant AG in potato production systems (Muzhinji et al. 2015; Woodhall et al. 2008) was chosen for this experiment. Six chemical fungicides and one commercially available biocontrol agent were evaluated for efficacy in controlling tuber-borne inoculum of *R. solani* AG 3-PT. Seed tubers, cv. Harmony with black scurf (>20%) harvested from a companion field experiment previously inoculated with *R. solani* AG 3-PT (Rh68), were used as seed tubers in this experiment (Muzhinji et al. 2018). The chemical fungicides and a biofungicide were applied as seed treatments following manufacturer's recommended rates before planting (Table 1). Sowing and management of the plots were as previously described for in-furrow treatment. Control tubers were not treated with fungicide. The seed tubers were planted by hand in a randomized complete block design with four replications. The trial was repeated once.

**Disease assessment and statistical evaluation.** For all the field experiments, potato plant samples were taken from the two centre rows 45 days after planting, during flowering stage, for stem canker scoring. For the greenhouse experiment, six plants from each

replication were destructively sampled 47 days after planting for stem canker assessment. Plants were visually rated using an assessment key described by Woodhall et al. (2008). Disease index was calculated by the formula adapted from Lootsma and Scholte (1996), where: DI= $\sum$ [0(n0) + 0.2(n1) + 0.4(n2) + 0.6(n3) +0.8(n4) +1(n5)] x 100/ ($N_{total}$) where $n_x$ = number of stems in the x rating class and $N_{total}$ = total number of stems assessed in each category. After 120 days, the remaining plants were removed and assessed for black scurf symptoms on progeny tubers using the following scale: 0 = no sclerotia present; 1 = less than 1 % of the tuber surface area covered in sclerotia; 2 = 1 to 10 % of the tuber surface area covered in sclerotia; 3 = 11 to 20 %; 4 = 21 to 50 %; 5 = $\geq$ 51 % tuber surface area covered in sclerotia. The disease index was calculated as: DI= $\sum$ [0(n0) + 0.25(n1) + 0.5(n2) + 0.75(n3) +1(n4)] x 100/ ($N_{total}$), where $n_x$ = number of tubers in the x rating class and $N_{total}$= total number of tubers in each category (Lootsma and Scholte 1996). The stolon, stem and black scurf index were analysed using non-parametric Kruskal-Wallis test at the 5% significance level implemented in SAS version 9.2 (SAS Institute Inc., Cary, NC). The repeated trials for each experiment were combined based on Levene's homogeneity of variance (Gastwirth et al. 2009).

## Results

***In vitro*** **assessment of fungicide sensitivity.** All AGs were able to grow on fungicide-amended PDA but with varying degrees of inhibition (Fig.1a - 1g). *Rhizoctonia* AGs tested were found to be sensitive to tolclofos-methyl with $EC_{50}$ values less than 0.1 µg a.i $ml^{-1}$. Most isolates had mean $EC_{50}$ values ranging from 0.001 to 0.002 µg a.i $ml^{-1}$ (Fig. 1a). Similar results were obtained for isolates evaluated against fludioxonil (Celest; Syngenta) with $EC_{50}$ values of less than 0.099 µg a.i $ml^{-1}$ (Fig. 1b). For azoxystrobin, AG 4HG-I, AG 4HG-III and AG 5 were the most sensitive with $EC_{50}$ values ranging from 0.07 to 0.89 µg a.i

$ml^{-1}$. Intermediate sensitivity was observed for AG A and AG 2-2IIIB. However, the highest $EC_{50}$ value of 8.18 µg a.i $ml^{-1}$ was obtained for an isolate of AG 3-PT (Fig. 1c). With iprodione, AG 4HG-III and AG 5 were most sensitive with mean $EC_{50}$ values of 0.015 and 0.021 µg a.i $ml^{-1}$, respectively. BNR AGs A and R had the highest average $EC_{50}$ values of 4.4 and 5.8 µg a.i $ml^{-1}$, respectively (Fig. 1d).

For pencycuron, anastomosis groups AG 3-PT and AG 4HG-I isolates exhibited sensitivity whereas AG 5, AG 4HG-I and AG 4HG-III isolates were less sensitive (Fig. 1e). With benomyl (Benomil; Dow AgroSciences), AG 2-2IIIB isolates were the most sensitive with a mean $EC_{50}$ value of 0.02 µg a.i $ml^{-1}$, while AG A and AG 3-PT isolates were the least sensitive with a mean $EC_{50}$ value of 0.4 and 0.72 µg a.i $ml^{-1}$, respectively (Fig. 1f). All the *R. solani* and BNR AGs were sensitivity to the biofungicide *B. subtilis* QST 713. Among them, 65% had $EC_{50}$ values of less than 0.1 µg a.i.$ml^{-1}$, whereas 30% had $EC_{50}$ values of between 0.1 and 1 µg a.i $ml^{-1}$ (Fig. 1g).

**Greenhouse experiment.** The fungicide applications significantly reduced the disease indices of stem canker and black scurf under controlled conditions in the greenhouse. There were highly significant differences ($P \leq 0.05$) in stem canker and black scurf disease indices among the fungicide treatments compared with the control (Table 2). Tolclofos-methyl and fludioxonil were the most effective since they completely prevented development of stem canker and black scurf. In comparison, a significantly lower level of effectiveness was observed in pots infested with the same pathogens and treated with azoxystrobin as compared to other fungicides. Iprodione, benomyl and *B. subtilis* QST 713 were effective in reducing stem canker and black scurf in pot trials. Pencycuron showed poor control of AG 4HG-III with a stem canker disease index of 21%, although it was effective against other AGs.

**Field experiments: In–furrow treatment.** The results from the field experiments are summarized in Tables 3 to 4. All fungicide treatments had significantly lower stem canker and black scurf disease indices than the untreated control. In plots infested with AG 3-PT, tolclofos-methyl and fludioxonil provided the highest level of stem canker and black scurf disease control as indicated by low disease indices for all AGs compared to the control. In comparison, a significantly lower level ($P \leq 0.05$) of performance was observed for azoxystrobin against AG 3-PT as the stem canker (29%) and black scurf (28%) disease index values differed significantly ($P \leq 0.05$) from those for other fungicides. The stem canker disease index values for pencycuron (10%), iprodione (10%), benomyl (19%) and *B. subtilis* QST 713 (20%) were not significantly different at $P \leq 0.05$ but were significantly different from the control (54%), suggesting efficacy against *Rhizoctonia* in the field (Table 3).

**Seed tuber treatment.** Tolclofos-methyl and fludioxonil were effective in reducing the incidence and severity of stem canker and black scurf disease on potato when used to treat *Rhizoctonia* infected seed tubers (Table 4). Pencycuron and benomyl were also more effective in reducing the severity of black scurf and stem canker than iprodione and *B. subtilis* QST 713. Azoxystrobin was the least effective in reducing black scurf and stem canker (Table 4). Tolclofos-methyl and fludioxonil showed more effectiveness in reducing stem canker and black scurf to all AGs compared to other fungicides tested. Iprodione and pencycuron were most effective in controlling AG 4HG-I and AG 2-2IIIB, respectively. *B. subtilis* QST 713 showed the highest efficacy against AG 2-2IIIB and AG 4HG-I, while benomyl showed efficacy against AG 4HG-I. For AG A no stem canker or black scurf symptoms were observed in any of the treatments including the control.

## Discussion

The application of fungicides is the most common strategy for controlling *Rhizoctonia* on potato. In the present study, six chemical fungicides with diverse modes of action, and one biofungicide were evaluated for their efficacy to control various *Rhizoctonia* AGs obtained from different potato growing regions in South Africa.

*In vitro* sensitivity of the fungicides to *Rhizoctonia* AGs showed that some AGs were highly sensitive whereas others were less sensitive to certain fungicides based on the classification given by Martin et al. (1984) (Fig. 1a-1b). This corroborates well with previous studies, which showed the existence of differential sensitivity of AGs to fungicides (Campion et al. 2003; Kataria et al.1991).

Generally, tolclofos-methyl and fludioxonil were the most effective fungicides against all the *R. solani* and BNR AGs in *in-vitro*, greenhouse and field experiments. This is in agreement with other studies that showed the efficacy of tolclofos-methyl and fludioxonil against a wide range of *Rhizoctonia* AGs from different crops (Kataria et al. 1991; Olaya et al. 1994; Virgen-Calleros et al. 2000). Inter AG variation was noted for pencycuron with AG 3-PT being the most sensitive followed by AG 2-2IIIB and AG A. Anastomosis group 4, subgroups HG-I and HG-III exhibited an intermediate to less sensitive response to pencycuron, as did an isolate of AG 5. Other studies on sugar beet and potato found isolates of AG 4 and AG 5 to be insensitive to pencycuron (Campion et al. 2003; Olaya et al. 1994). Ueyama et al. (1993) reported that isolates of AG 4 are able to metabolize pencycuron and are therefore less sensitive, whereas isolates of AG 3 cannot metabolize this fungicide and are sensitive.

Iprodione, benomyl and *B. subtilis* QST 713, a biofungicide, were also effective at inhibiting mycelial growth of all *R solani* and BNR AGs. The data from this study is consistent with the range of $EC_{50}$ values previously reported for fungicides containing iprodione against *R. solani* and BNR isolated from potato in France (Campion et al. 2003);

and North Carolina (Carling et al. 1990). *Rhizoctonia solani* and BNR AGs showed differential sensitivity to azoxystrobin, a strobilurin-containing fungicide. The variability in sensitivity of different AGs to azoxystrobin reported in this study and in Tunisia (Djébali et al. 2014) suggested that there may be potential for fungicide-insensitive field isolates of *R. solani* to emerge through continued use of the same fungicide, leading to fitness penalty (Ma and Michailides 2005). Repeated application of fungicides has been reported to increase the selection pressure for fungicide insensitivity (Ma and Michailedes 2005). In Tunisia, Djébali et al. (2014) found resistance of *R. solani* isolates to azoxystrobin with $EC_{50}$ values exceeding 30 µg a.i $ml^{-1}$. This fungicide probably should be used with caution for controlling *Rhizoctonia* on potato in South Africa.

All six tested fungicides and the biofungicide treatment were effective in controlling Rhizoctonia diseases on potato *in vivo*. The results of the field experiments showed that the application of pencycuron, tolclofos-methyl and fludioxonil on seed potato tubers and in-furrow had a superior effect compared to other fungicides in reducing stem canker and black scurf disease indices when used at recommended application rates. The results corroborate with previous studies that showed the efficacy of fungicides against *Rhizoctonia* in a variety of crops (Kataria and Verma 1989; Sundravadana et al. 2007). In America, Hide and Read (1991) showed that treating seed tubers with tolclofos-methyl decreased the severity of black scurf. Recently, Ozer and Bayraktar (2015) showed that fludioxonil provided excellent control of AG 5, AG 2-1 and AG K in field experiments.

The performance of *B. subtilis* QST 713 in a field experiment against all AGs was comparable to that of iprodione and benomyl, suggesting that it has great potential as an alternative to chemical control of *Rhizoctonia* AGs on potato. Ozer and Bayraktar (2015) reported that *B. subtilis* QST 713 control of stem canker and black scurf disease was comparable to that of pencycuron under greenhouse conditions in Turkey.

Results from fungicide sensitivity studies are of great interest to plant pathologists, extension agents, and the industry sector. Identification of insensitive isolates in some of the AGs in this study is a cause for concern, given that *Rhizoctonia* has been reported to possess a mixed reproductive mode of recombination that includes clonality and recombination (Muzhinji et al. 2016). It is therefore, of paramount importance to institute monitoring programs to understand the population dynamics of *Rhizoctonia* populations exposed to fungicides to ensure continued effectiveness of fungicide control programs.

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## Literature Cited

Amaradasa, B. S., Lakshman, D. K., McCall, D., and Horvath, B. J. 2014. *In vitro* fungicide sensitivity of *Rhizoctonia* and *Waitea* isolates collected from turfgrasses. J. Environ. Hortic. 32:126-132.

Amiri, A., Brannen, P. M., and Schnabel, G. 2010. Reduced sensitivity in *Monilinia fructicola* field isolates from South Carolina and Georgia to respiration inhibitor fungicides. Plant Dis. 94:737-743.

Avenot, H., Sellam, A., and Michailides, T. 2009. Characterization of mutations in the membrane-anchored subunits AaSDHC and AaSDHD of succinate dehydrogenase from *Alternaria alternata* isolates conferring field resistance to the fungicide boscalid. Plant Pathol. 58:1134-1143.

Banville, G. J. 1989. Yield losses and damage to potato plants caused by *Rhizoctonia solani* Kühn. Am. Potato J. 66:821-834.

Campion, C., Chatot, C., Peraton, B., and Andrivon, D. 2003. Anastomosis groups, pathogenicity, and sensitivity to fungicides of *Rhizoctonia* isolates collected in potato fields in France. Eur. J. Plant Pathol. 109:983-992.

Carling, D. E., Helm, D. J., and Leiner, R. H. 1990. *In vitro* sensitivity of *Rhizoctonia solani* and other multinucleate and binucleate *Rhizoctonia* to selected fungicides. Plant Dis. 74:860-863.

Chen, Y., Zhang, A. F., Wang, W. W., Zhang, Y., and Gao, T. C. 2012. Baseline sensitivity and efficacy of thifluzamide in *Rhizoctonia solani*. Ann. Appl. Biol. 161:247–254.

Djébali, N., Elkahoui, S., Taamalli, W., Hessini, K., Tarhouni, B., and Moncef, M. 2014. Tunisian *Rhizoctonia solani* AG 3 strains affect potato shoot macronutrients content, infect faba bean plants and show in vitro resistance to azoxystrobin. Australas. Plant Pathol. 43:347-358.

Everett, K. R., Owen, S. G., and Cutting, J. G. M. 2005. Testing efficacy of fungicides against postharvest pathogens of avocado (*Persea americana* cv. Hass). *N. Z. Plant Prot*: 89-95.

FRAC. 2015. FRAC (Fungicide Resistance Action Committee) Code List: Fungicides Sorted by Modes of Action Available from: www.frac.info.

Gastwirth, J. L; Gel, Y. R., and Miao, W 2009.The impact of Levene's Test of equality of variances on statistical theory and practice. Stat Sci. 24:343-360.

Hide, G. A., and Read, P. J. 1991. Effects of rotation length, fungicide treatment of seed tubers and nematicide on diseases and the quality of potato tubers. Ann. Appl. Biol. 119:77-87.

Kataria, H. R., and Verma, P. R. 1989.Variations in the sensitivity to fungicides among different anastomosis groups of *Rhizoctonia solani*. Can. J. Plant Pathol. 11:192.

Kataria, H. R., Hugelshofer, U., and Gisi, U. 1991. Sensitivity of *Rhizoctonia* species to different fungicides. Plant Pathol. 40:203-211.

Kataria, H. R., and Gisi, U. 1999. Selectivity of fungicides within the genus *Rhizoctonia*. Pages 421-429 in: Modern Fungicides and Antifungal Compounds H. Lyr, P. E.Russell, H-W.Dehne, H. D. Sisler, eds. Andover, UK: Intercept.

Liang, H., Li, J., Di, Y., Zhang, A., and Zhu, F. 2015. Logarithmic transformation is essential for statistical analysis of fungicide $EC_{50}$ values. J Phytopathol. 163:456-464.

Lootsma, M., and Scholte, K. 1996. Effects of soil disinfection and potato harvesting methods on stem infection by *Rhizoctonia solani* Kühn in the following year. Potato Res. 39:15-22.

Ma, Z., and Michailides, T. J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24:853-863.

Martin, S. B., Lucas, L. T., and Campbell, C. 1984. Comparative sensitivity of *Rhizoctonia solani* and *Rhizoctonia*-like fungi to selected fungicides *in vitro*. Phytopathology 74:778-781.

Muzhinji, N., Woodhall, J. W., Truter, M., and van der Waals, J. E. 2018. Relative contribution of seed tuber- and soilborne inoculum to potato disease development and changes in the population genetic structure of *Rhizoctonia solani* AG 3-PT under field conditions in South Africa. Plant Dis.102:60-66

Muzhinji, N., Truter, M., Woodhall, J. W., and van der Waals, J. E. 2015.Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* from potatoes in South Africa. Plant Dis. 99:1170-1890.

Muzhinji, N., Truter, M., Woodhall, J. W., and van der Waals, J. E. 2016. Population genetic structure of *Rhizoctonia solani* AG 3-PT from potatoes in South Africa. Fungal Biol. 120:701-710.

Nel, A., Krause, M., and Khelawanlall, N. 2003. A guide for the control of plant diseases. Department of Agriculture. Pretoria.

Olaya, G., Abawi, G. S., and Barnard, J. 1994. Response of *Rhizoctonia solani* and binuceate *Rhizoctonia* to five fungicides and control of pocket rot of table beets with foliar sprays. Plant Dis. 78:1033-1037.

Olaya, G., Sarmiento, L., Edlebeck, K., Buitrago, C., Sierotzki, H., Zaunbrecher, J., and Tally, A. 2013. Azoxystrobin (QOI) resistance monitoring of *Rhizoctonia solani* isolates causing rice sheath blight in Louisiana. Abstract APS.MSA Joint Meeting 11-14, Austin Texas.

Ozer, G., and Bayraktar, H. 2015.Characterisation and sensitivity to fungicides of *Rhizoctonia* spp recovered from potato plants in Bolu, Turkey. J. Phytopathol 43:11-18.

Postelbicu, 2014. Probit analysis. Pages 1128-1131 in: International Encyclopedia of Statistical Science. L. Miodrag. eds. Springer-Verlag Berlin Heidelberg, Berlin

Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., and Sneh, B. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience 49:93-114.

Sundravadana, S., Alice, D., Kuttalam, S., and Samiyappan, R. 2007. Azoxystrobin activity on *Rhizoctonia solani* and its efficacy against rice sheath blight. Tunis J Plant Prot 2:79-84.

Tsror (Lahkim), L., and Peretz-Alon, I. 2005. The influence of the inoculum source of *Rhizoctonia solani* on development of black scurf on potato. J. Phytopathol 153:240-244.

Tsror, L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on potato. J. Phytopathol 158:649-658.

Ueyama, I., Arald, Y., Kurogoshi, S., and Yanmguchi, I. 1993. Metabolism of the phenylurea fungicide, pencycuron, in sensitive and tolerant strains of *Rhizoctonia solani.* Pestic Sci.18:109-117.

Virgen-Calleros, G., Olalde-Portugal, V., and Carling, D. E. 2000. Anastomosis groups of *Rhizoctonia solani* on potato in Central Mexico and potential for biological and chemical control. Am. J. Potato Res.77: 219-224.

Woodhall, J. W., Lees, A. K., Edwards, S. G., and Jenkinson, P. 2008. Infection of potato by *Rhizoctonia solani*: Effect of anastomosis group. Plant Pathol. 57:897-905.

Yang, Y., Zhao, C., Guo, Z., and Wu, X. 2015. Characterization of a new anastomosis group (AG-W) of binucleate *Rhizoctonia*, causal agent for potato stem canker. Plant Dis. 99:1757-1763.

**Table 1.** Fungicides used in the study and their recommended application rates.

| Active ingredient (a.i.) | Commercial name | Chemical family | FRAC group | Application rate | |
|---|---|---|---|---|---|
| | | | | In-furrow | Seed treatment |
| Tolclofos-methyl | Rizolex 50WP | Aromatic hydrocarbons | 14 | 2 g a.i $m^{-1}$ | 2 g a.i $kg^{-1}$ |
| Pencycuron | Monceren® 250 SC | Phenylurea | 20 | 0.125 g a.i $m^{-1}$ | 18.75 g a.i10 $^{-1}$ |
| Iprodione | Prodione 500 SC | Dicarboximide | 2 | 20 g a.i 100 $m^{-2}$ | 20 g a.i 100 $kg^{-1}$ |
| Benomyl | Benomyl 500WP | Benzimidazole | 1 | 25 g a.i 100 $l^{-1}$ | 0.75 g a.i $kg^{-1}$ |
| Azoxystrobin | Amistar® 250FC | Strobilurin | 11 | 25 g a.i $ha^{-1}$ | 20 ml a.i100 $kg^{-1}$ |
| Fludioxonil | Celest 100 FS | Phenlypyrrole | 12 | 0.67 g a.i 15 $l^{-1}$ | 25 g a.i1000 $kg^{-1}$ |
| *Bacillus subtilis* QST 713 | Serenade ASO 1.34SC | Biofungicide (*Bacillus* spp.) | 44 | 14 l $ha^{-1}$ | 2 l 1000 $kg^{-1}$ |

SC- Suspension concentrate

WP-Wettable powder

**Table 2.** Black scurf and stem canker disease indices of progeny tubers planted in soil artificially inoculated with different *Rhizoctonia* AGs and treated with different fungicides under controlled conditions

| | Disease Index | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Black scurf | | | | Stem canker | | | | | | |
| | *R. solani* | | BNR[x] | | *R. solani* | | | | BNR | | |
| Fungicide | AG 3-PT | AG 5 | AG R | AG A | AG 3-PT | AG 2-2 IIIB | AG 5 | AG 4HG-I | AG 4HG-III | AG R | AG A |
| Azoxystrobin | 6b[y] | 3c | 7b | 2bc | 14.5b | 20b | 14b | 18b | 12c | 15.1b | 5.3b |
| Tolclofos-methyl | -[z] | - | - | - | - | - | - | - | - | 10 | - |
| Pencycuron | - | 9b | 3c | - | 5.3de | 7.2d | 12.5b | 8.6c | 21b | 9c | - |
| Prodione | 3c | 7.6b | - | - | 6.7d | 11c | 8.5c | - | 10c | 11c | - |
| Fludioxoni | - | - | - | - | - | 5de | - | - | - | - | - |
| Benomyl | 4c | - | 8b | 4.6b | 8d | 11c | 5.5d | - | - | 12.5c | 4.2b |
| *Bacillus subtilis* | 4c | 8b | 6b | 3b | 11c | 10.6c | 8.5c | 4.2d | 12c | 14.5b | 3cb |
| Control | 56a | 18a | 15a | 8a | 56.5a | 30a | 28a | 45a | 45a | 51a | 12a |

[x]BNR

[y]Means followed by the same letter within a column are not significantly different according to the Duncan's Multiple Range Test ($P \leq 0.05$)

[z]No disease symptoms observed

**Table 3.** Stem canker and black surf disease indices of progeny tubers planted in soil artificially inoculated with different AGs and treated with different fungicides under field conditions.

| | Disease Index | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stem canker | | | | | | Black scurf | | |
| | *R. solani* | | | | | BNR[x] | *R. solani* | | BNR |
| Fungicide | AG 3-PT | AG 2-2IIIB | AG 5 | AG 4HG-I | AG 4HG-III | AG R | AG 3-PT | AG 5 | AG R |
| Azoxystrobin | 29b[y] | 16.5c | 15c | 15 c | 17.5b | 17b | 28c | 10b | 18ab |
| Tolclofos-methyl | -[z] | - | - | - | - | - | - | - | - |
| Pencycuron | 10c | 10bc | 17c | 11c | 18b | 13c | 10bc | 7.5b | 11c |
| Prodione | 10c | - | 8b | 11c | 12b | 10c | 15bc | 10ab | 10bc |
| Fludioxoni | - | - | - | - | - | 12c | - | - | 10bc |
| Benomyl | 19bc | 10bc | 10bc | 12c | - | 17.5b | 15bc | - | 13.8bc |
| *Bacillus subtilis* | 20bc | 10bc | 12bc | 10c | 15b | 13c | 20bc | - | 18c |
| Control | 53a | 32a | 35a | 46a | 28a | 55a | 63a | 25a | 44a |

[x]BNR = binucleate *Rhizoctonia*

[y]Means followed by the same letter within a column are not significantly different according to the Duncan's Multiple Range Test ($P \leq 0.05$)

[z]No disease symptoms observed

**Table 4.** Stem canker and black scurf disease indices of plants showing the effect of different fungicide potato seed treatments on different *Rhizoctonia* AGs under field conditions.

| | Disease Index | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stem canker | | | | | | | Black scurf | | | |
| | *R. solani* | | | | | BNR[x] | | *R. solani* | | BNR | |
| Fungicide | AG 3-PT | AG 2-2IIIB | AG 5 | AG 4HG-I | AG 4HG-III | AG R | AG A | AG 3-PT | AG 5 | AG R | AG A |
| Azoxystrobin | 21b[y] | 14b | 17b | 8b | 26b | 12b | 4 b | 35b | 3b | 18b | - |
| Tolclofos-methyl | -[z] | - | 10c | 9b | - | - | - | - | 12c | - | - |
| Pencycuron | 6e | - | 15bc | 9b | 14c | - | - | 10c | 8.9c | 15b | - |
| Iprodione | 12d | 8.9c | - | 5c | - | - | 6b | 14c | 12.5c | - | - |
| Fludioxoni | 4.8ef | - | - | - | - | 10c | - | - | - | 15b | - |
| Benomyl | 10d | 10c | - | 7.8b | 8d | - | - | 10c | - | - | 5.5b |
| *Bacillus subtilis* | 16 c | 14c | 14b | 5c | 12c | 12b | 4.8b | 8c | 13c | 14.5b | 4.6b |
| Control | 63a | 42a | 57a | 33a | 42a | 51a | 8a | 42a | 57a | 51a | 10a |

[x]BNR = binucleate *Rhizoctonia*

[y]Means followed by the same letter within a column are not significantly different according to the Duncan's Multiple Range Test ($P \leq 0.05$)

[z]No disease symptoms observed

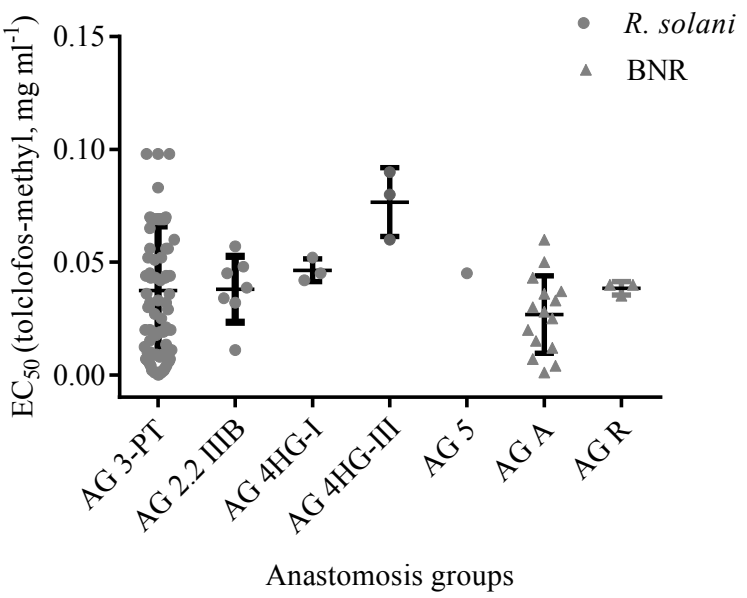


**Fig. 1a.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to tolclofos-methyl.

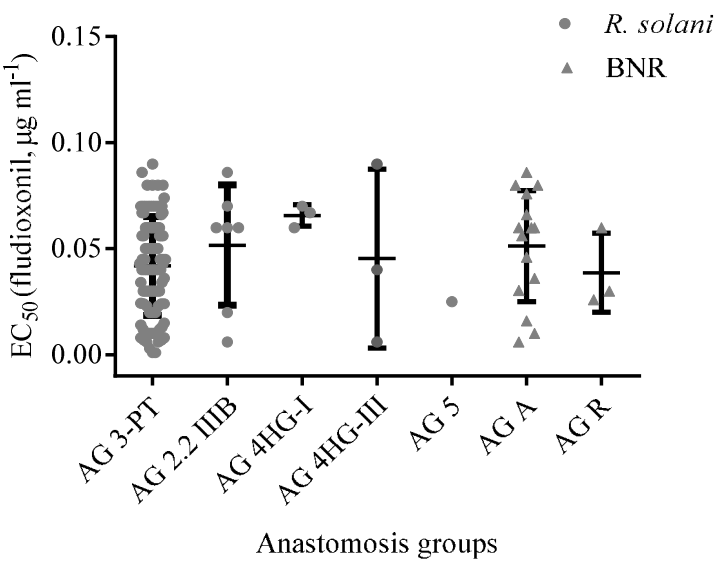


**Fig. 1b.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to fludioxonil.

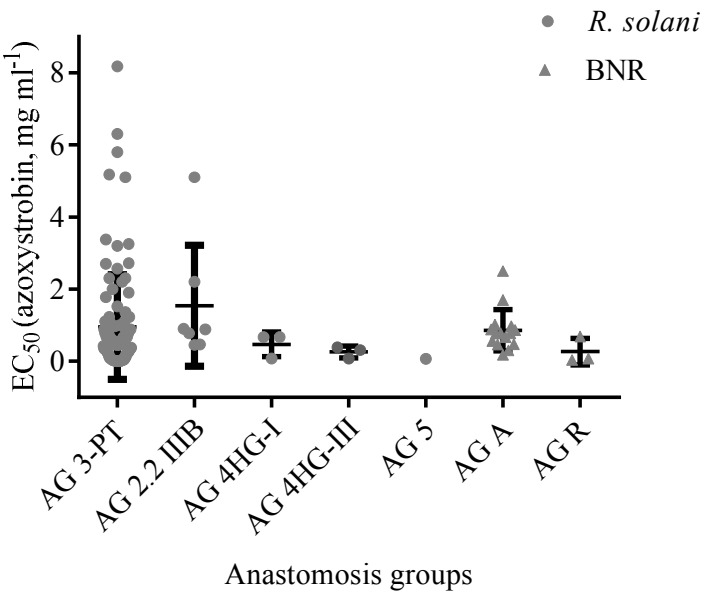


**Fig. 1c.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to azoxystrobin.

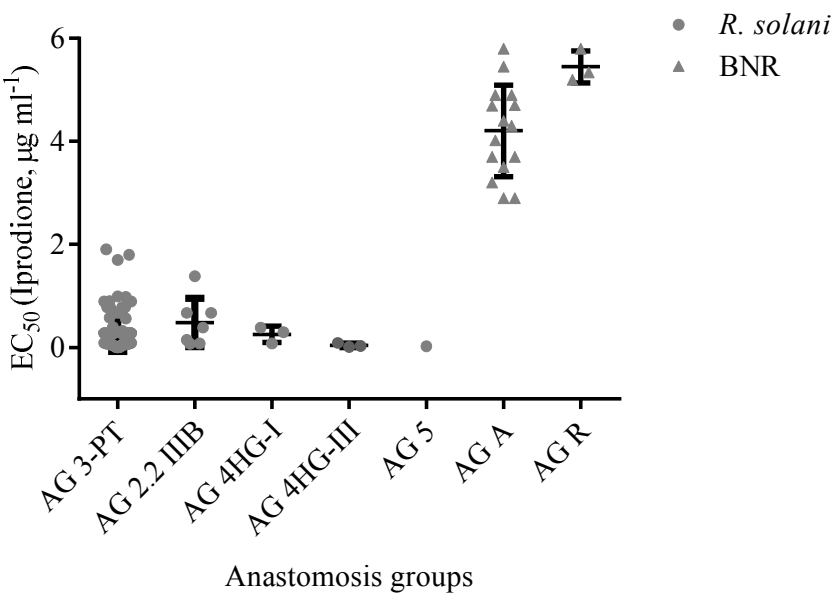


**Fig. 1d.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to iprodione.

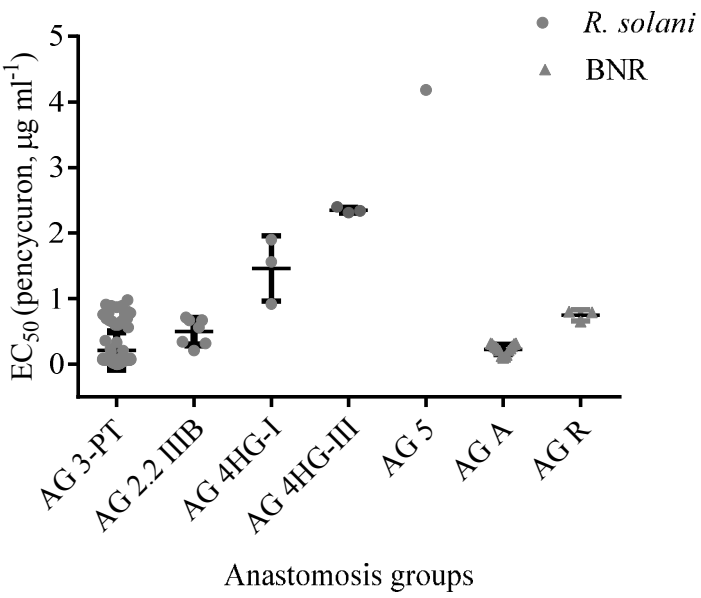


**Fig. 1e.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to pencycuron.

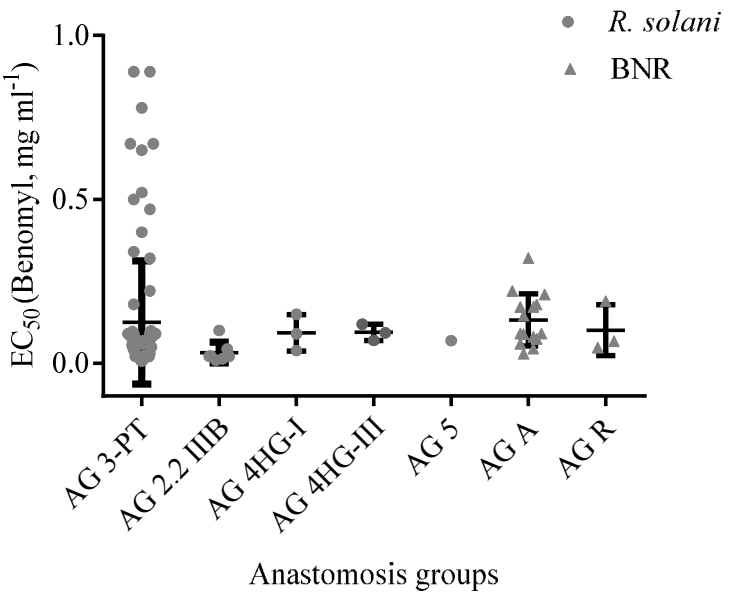


**Fig. 1f.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to benomyl.

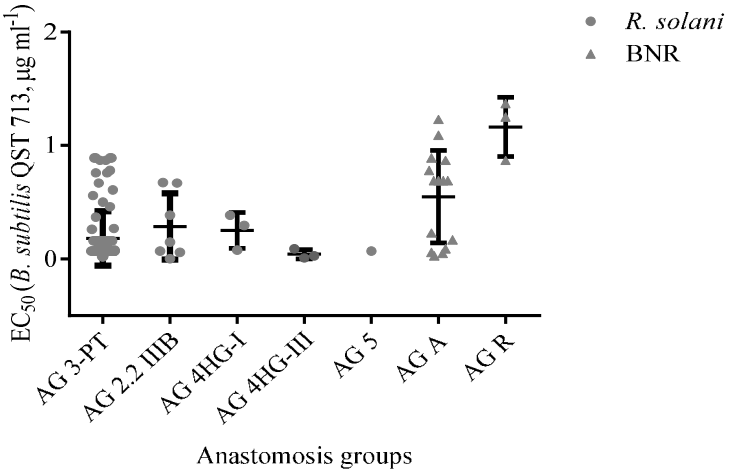


**Fig.1g.** Frequency distribution of *in vitro* response ($EC_{50}$) of *Rhizoctonia* isolates to biofungicide *Bacillus subtilis* QST 713.

**Table S1.** *Rhizoctonia* isolates used in greenhouse and field trials

| | | | | | Experiment used | |
|---|---|---|---|---|---|---|
| Isolate[a] | AG | Symptom of origin | Potato growing region | Potato variety | Greenhouse | Field trial |
| Rh1b | 3-PT | Elephant hide | Limpopo | BP1 | | • |
| Rh6b | 3-PT | Elephant hide | Mpumalanga | BP1 | | • |
| Rh68 | 3-PT | Black scurf | KwaZulu-Natal | Sifra | • | |
| Rh89 | 3-PT | Black scurf | Gauteng | BP1 | | |
| Rh20 | 2-2IIIB | Stem canker | North West | Mondial | | • |
| Rh97 | 2-2IIIB | Black scurf | Sandveld | BP1 | • | |
| Rh99 | 2-2IIIB | Stem canker | North West | BP1 | | • |
| Rh86 | 2-2IIIB | Stem canker | North West | Mondial | | |
| Rh88 | 4 HG-I | Stem canker | Gauteng | BP1 | | • |
| Rh91 | 4 HG-I | Stolon canker | Northern Cape | Nicola | | |
| Rh96 | 4 HG-I | Stem canker | Sandveld | BP1 | • | • |
| Rh81 | 4 HG-III | Corky spots | Limpopo | Mondial | | • |
| Rh83 | 4 HG-III | Stem canker | Limpopo | Mondial | • | • |
| Rh30 | 5 | Black scurf | Sandveld | Fianna | • | |
| Rh26 | A | Elephant hide | Eastern Free State | Mondial | • | |
| Rh112 | R | Black scurf | Sandveld | Nicola | | • |
| Rh113 | R | Black scurf | Sandveld | Nicola | • | • |

[a]Isolates with the same numerical value originated from the same tuber but from different blemish types

Isolates used either and/or in the greenhouse or field trial