RESISTANCE OF POTATO CULTIVARS TO *DITYLENCHUS DIPSACI* AND *DITYLENCHUS DESTRUCTOR*

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ABSTRACT

Ditylenchus dipsaci Kuhn and *Ditylenchus destructor* Thorne are economically important plant-parasitic nematodes, affecting potato production. Limited information exists on the resistance/susceptibility of currently cultivated potatoes to *D. dipsaci* and *D. destructor*. The greenhouse experiments were conducted to screen 10 potato cultivars for resistance/susceptibility for *D. dipsaci* and *D. destructor* infections. Reproduction factor (Rf) and relative susceptibility (RS) were used to evaluate resistance. Based on the Rf, 8 potato cultivars were evaluated as susceptible (S) while 2 cultivars (Spunta and Innovator) were evaluated as resistant (R) to *D. dipsaci*. Cultivars Sante and Orfei were resistant to *D. destructor* based on Rf. Potato cultivar Désirée was observed to be highly susceptible to *D. dipsaci* and *D. destructor* in both experiments and was used as the standard susceptible control cultivars for the calculation of RS.

Key words: *Solanum tuberosum, damage, Ditylenchus spp, screening, reproduction factor, relative susceptibility*

1. Introduction

Ditylenchus dipsaci (Filipjev) Kuhn and *Ditylenchus destructor* Thorne are economically important plant-parasitic nematodes, affecting potato (*Solanum tuberosum* L.) production (Plowright *et al.*, 2002; Samaliev and Stoyanov, 2008). The both nematodes cause qualitative damage to potato tubers by producing conical pits, often accompanied by skin splitting and rotting due to secondary invasion by bacteria and fungi (Jenkins and Taylor, 1967; Southey, 1971; Mai *et al.*, 1981; Cotton *et al.*, 1992). In Bulgaria first reports of *D. destructor* on potato are made by Kovachevski (1942) and *D. destructor* and *D. dipsaci* by Stoyanov (1980). Samaliev (2011) found *D. destructor and D. dipsaci* in four potato growing regions in Bulgaria (Smloyan, Pazardjik, Plovdiv and Samokov) with an overall frequency of 7 and 1% and density of 3 to 19 and 2 to 11 nematodes per 100 cm^3 soil, respectively.

Management through crop rotation is not feasible due to their wide host range (Samaliev and Stoyanov, 2008; EPPO, 2008). These nematodes are listed as quarantine pests in many countries. Cultivation of resistant cultivars often provides an effective alternative method for management of various plant parasitic nematodes (Cook and Starr, 2006; Samaliev and Stoyanov, 2008). Although earlier reports documented presence of resistant or tolerant potato cultivars against *D. destructor* (Kostina and Zholudeva, 1974; Ponin *et al.*, 1983) and *D. dipsaci* (Nikulina, 1970; Shepshelev and Chernikova, 1971), most of these cultivars are no longer available.

The aims of the present study are to investigate to assess resistance and tolerance of currently potato cultivars against *D. dipsaci* and *D. destructor*.

2. Materials and methods

Tubers from 10 potato cultivars were pre-germinated in the dark at 19±2^{o}C until sprouts were observed after which they were placed in the light to harden the sprouts. Tubers/tuber pieces weighing ~ 16 g each and bearing a single sprout (about 1 cm long) with were planted in 16 cm/diameter plastic pots filled with 800 ml steam sterilized sandy loam soil. One tuber/tuber pieces were planted per pot. Each pot was regularly irrigated with a hydroponic nutrient (N, P and K - 6.5, 2.7 and 13%, respectively) dissolved in tap water. Pots were maintained in a greenhouse at 16-23^{o}C.

Ditylenchus dipsaci and *D. destructor* populations used in this study were originally isolated from potato plants (from Plovdiv and Smolyan potato producing regions, respectively). Axenic cultures of these populations were maintained and multiplied on carrot discs in Petri dishes for 8 weeks by a modified method of Speijer and De Waele (Mwaura et al., 2015).

In the experiment, ten cultivars were screened. Cultivars were screened for resistance and tolerance against *D. dipsaci* and *D. destructor*. Each treatment consisted of a single species of nematode replicated 8 times (4 control pots and 4 nematode treated pots per cultivar). Two weeks after planting, each potato cultivar and of the control were inoculated with 2 000 nematodes of mixed life stages, by pipetting nematodes in 4 ml aqueous suspensions into 8 holes in the soil around the roots. Control pots were not infested with nematodes. The potato tubers were assessed 15 weeks after infestation with nematodes, giving a total duration of 17 weeks for the experiment from planting to harvest.

Potato tubers were harvested by passing the growing medium from each pot through a sieve. Adhering growing medium was washed using tap water and number of tubers and tuber weight was recorded. External and internal damage were recorded prior to nematode extraction from tuber tissues as explained later in evaluation for tolerance. Potatoes from each replicate were completely peeled using a knife. Peels were of approximately 2 mm in thickness and 47 approximately 22% of tuber weight. From the total tuber peels per replicate, a composite 10 g of potato tuber peels was obtained and chopped into fine pieces and used for nematode extraction. Nematodes were extracted using the modified Baermann funnel method for 12 hours (Hooper, 1990). The nematodes extracted were used to determine nematode numbers and developmental stages.

The soil from each pot was thoroughly mixed and a subsample of 300 ml collected, and packed in polythene bags and stored at 6°C until further use. Nematodes were extracted from 250 ml of the growing medium subsample for 24 hour using an Oostenbrink dish with 24 cm inner diameter (Oostenbrink, 1960) and extrapolated to the total growing medium volume per replicate (800 ml). Nematode numbers (all developmental stages) from both total tuber peels and growing medium were counted.

Two methods were used to evaluate resistance of potato cultivars to *D. dipsaci* and *D. destructor*. These methods were: a) Reproduction factor (RF) Pf (final nematode population)/Pi (Initial population density) (Oostenbrink, 1966). A cultivar was considered to be resistant (R) when the ratio was lower that initial population density (Pf/Pi < 1); b) Relative susceptibility (RS) of the potato cultivars to *D. dipsaci* and *D. destructor* was calculated using the RS formula (EPPO, 2006): Pf test cultivar / Pf standard susceptible control cultivar x 100, where - Pf standard susceptible control cultivar was that of Désirée cultivar. A score scale between 1 and 9 was adopted from the EPPO protocol to classify the potato cultivars for resistance to *Globodera* spp. into different levels of RS (EPPO, 2006). The score scale and its corresponding RS scores in brackets are as follows: 1 (> 100), 2 (50.1-100%), 3 (25.1-50%), 4 (15.1-25%), 5 (10.1-15%), 6 (5.1-10%), 7 (3.1-5%), 8 (1.1-3%), 9 (<1%). The scores of 1 and 9, respectively, indicate the lowest and highest levels of resistance respectively.

Data were analyzed by analysis of variance, using procedures of the SPSS-12 programme, significance being determined at $P_{0.05}$.

3. Results and discussion

3.1. Resistance of potato varieties to D. dipsaci and D. destructor

The RF for *D. dipsaci* in soil and tuber peels was significantly ($P_{0.05}$) different among the ten potato cultivars screened during experiment (Table 1, $P_{0.05}$). Cultivar Spunta significantly differed from Désirée in its RF. Since cultivar Innovator had RF less than 1 and was classified as resistant (R), while the rest of the cultivars in experiment two were classified as susceptible to *D. dipsaci* since the Pf/Pi ratio was > 1.

Highest RF of *D. destructor* was obtained from Désirée, which differed significantly ($P_{0.05}$) from all other cultivars apart from Provento. Overall, 8 cultivars (including Armada) were evaluated as susceptible (S) because Pf/Pi ratio was >1 while 2 cultivars (Orfei and Sante) were evaluated as resistant (R) to *D. destructor* (Pf/Pi was < 1). (Table 1, $P_{0.05}$).

In previous our experiments potato cultivar Armada tested to *D. destructor,* population isolated from carrot in Bulgaria, were poor hosts and no damage was caused to the potato tubers (Samaliev, 2012), until in this experiment the same cultivar was evaluated as susceptible. Potato cultivars should be distributed after preliminary biological testing with most aggressive populations of *D. destructor* found in our country.

Table 1. Reproduction factor (RF) of *D. dipsaci and D. destructor* calculated from total growing medium and tuber peels per replicate obtained during experiment.

Culrivar	*D. dipsaci*		*D. destructor*	
	Reproduction factor	Resistance	Reproduction factor	Resistance
Désirée	9.5 ± 4.9a	S	19.9 ± 6.3a	S
Provento	8.5 ± 2.1b	S	11.7 ± 3.6a	S
Arizona	6.5 ± 2.3 b	S	8.9 ± 4.1b	S
Almera	4,5 ± 1.6 b	S	2.4 ± 0.6bc	S
Agria	1.4 ± 1.1 bc	S	4.1 ± 2.0bc	S
Innovator	0.9 ± 0.3 d	R	2.8 ± 0.7bc	S
Armada	1.1 ± 0.3 cd	S	9.3 ± 3.2c	S
Spunta	0.0 ± 0.0d	R	1.1 ± 0.7c	S
Orfei	1.2 ± 0.5bc	S	0.7 ± 0.2d	R
Sante	1.1± 0.4cd	S	0.4 ± 0.0d	R

Reproduction factors are means of ten replicates followed by ± standard error. Means separated by the same letter are not significantly different at $P_{0.05}$ according to Duncan's multiple comparison test. A variety was considered resistant (R) when the reproduction factor (RF) was lower than 1 and susceptible (S) when the ratio was higher than 1.

3.2. Relative susceptibility of potato varieties to D. dipsaci and D. destructor

D. dipsaci: The RS of nine potato cultivars significantly differed from that of the standard susceptible control cultivar Désirée. Spunta had the highest resistance index to *D. dipsaci* (Table 2, $P_{0.05}$).

Table 2. Final nematode population densities and relative susceptibility (RS) of potato culrivars to *D. dipsaci* and *D. destructor*

Culrivar	*D. dipsaci*			*D. destructor*		
	Final nematode population (Pf)	Relative susceptibility	Relative suscep-tibility Score	Final nematode population (Pf)	Relative susceptibility	Relative suscep-tibility Score
Désirée	19111 ± 1612a	100.0	1	39900 ± 7870a	100.0	1
Provento	16899 ± 2999ab	88.4	2	23470 ± 1411bc	58.8	2
Arizona	13070 ± 5912b	68.3	2	15988 ± 2521c	40.1	3
Almera	8915 ± 2789bc	46.6	3	4748 ± 2344d	11.3*	5
Agria	2824 ± 199c	14.7*	5	8198 ± 4831cd	19.1*	4
Innovator	1790 ± 749c	9.4*	6	5676 ± 1587d	11.9*	5
Armada	2103 ± 111cd	11.0*	5	18511 ± 997bc	46.4	3
Spunta	0 ± 0.0e	0.0*	9	2056 ± 811d	5.1*	6
Orfei	2314± 122cd	12.1*	5	1490de	3.7*	7
Sante	2094± 101d	10.1*	5	817e	2.0*	8

Final population densities followed by ± standard error and relative susceptibility means were obtained from ten replicates. Final nematodes population means separated by the same letter are not significantly different at $P_{0.05}$ according to Duncan's multiple comparison test. Relative susceptibility significant differences with the control (Désirée) using Dunnett test are indicated by asterisks (*).

D. destructor: Significant differences in RS to *D. destructor* among the ten potato cultivars were observed during in the experiment (Table 2, $P_{0.05}$). The highest RS of 100% was recorded from cultivar Désirée. Relative susceptibility of Provento, Armada, Arizona, Agria, Innovator, Almera, Spunta and Orfei was lower compared to the standard susceptible control variety Désirée. Cultivar Sante had a score of 8, which indicated the highest level of resistance to *D. destructor*.

This study supports the fact that *D. dipsaci* is as viable on the potatoes as *D. destructor*. Cultivar Désirée was also susceptible to *D. dipsaci* as *D. destructor*. Cultivar Innovator and Spunta were resistant to *D. dipsaci* while Orfei and Sante to *D. destructor*.

Ditylenchus dipsaci is among harmful plant parasitic nematodes listed in Annex of European Council Directive 2000/29/EC. This means that this species must not be present on seeds, bulbs and corms intended for planting (Samaliev and Stoyanov, 2008). Whereas *D. destructor* is regulated on potato, *D. dipsaci* is not. The findings from the current experiments demonstrate the importance of *D. dipsaci* on potato. This finding confirms observations by Seinhorst (1957) who considered it a serious pest of potato in Germany and the Netherlands. Recently, concerns over *D. dipsaci* re-emerging as a major threat to other crops in Europe including our country has been raised (Mouttet et al., 2014). Our study offers information which may be important in regulating pathways for *D. dipsaci*. Whereas some of the cultivars studied were highly susceptible to *D. dipsaci* and *D. destructor*, some (Spunta and Innovator, and Sante and Orfei, respectively) were moderately resistant to highly resistant. In other our experiments cultivar Spunta was resistant to *Meloidogyne arenaria* and *M. incognita* and cultivars Orfei and Sante were moderately resistant to highly resistant to *Globodera rostochiensis* and *G. pallida* (Samaliev et al., 1998, Markova et al., 2011, Samaliev and Nacheva, 2012). This offers new control options in cases where either some of these nematodes species may be present in the field.

3.2. Tolerance of potato varieties to D. dipsaci and *D. destructor*

Damage of potato cultivars due to *D. dipsaci* differed significantly among the cultivars (Fig. 1, $P_{0.05}$). Arizona was the most externally damaged at 58.5%, differing significantly ($P_{0.05}$) from Désirée and Spunta, Orfei and Sante did not suffer any external and internal tuber damages.

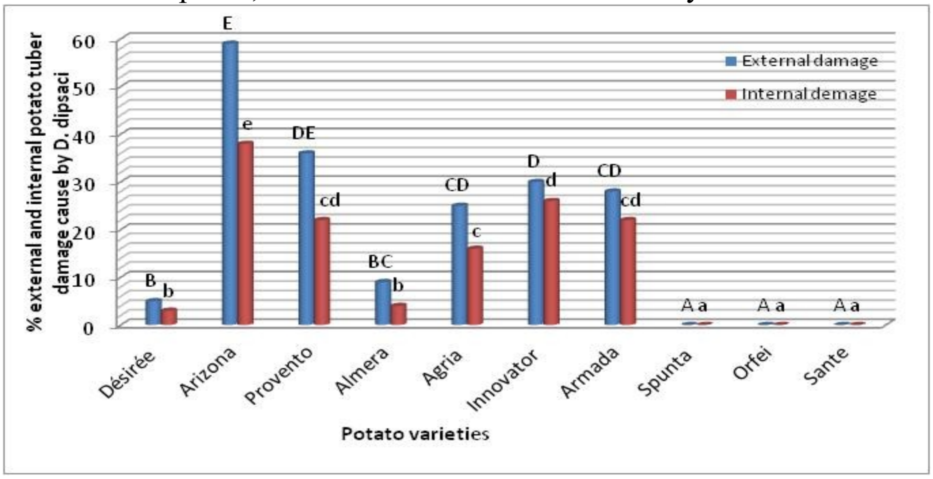

Fig. 1. Mean external and internal potato tuber damage caused by *Ditylenchus dipsaci* on ten potato cultivars (expressed as percentage of means of all tubers per replicate).
*Means separated by the same letter are not significantly different at $P_{0.05}$ according to Duncan's multiple comparison test

Ditylenchus destructor caused significant potato tuber damage, both externally and internally during experiment (Fig. 2, $P_{0.05}$). Percentage external and internal damage ranged between 0 – 38.5% and 0 - 19%. The most severely damaged potato cultivars were Provento and Innovator while Orfei and Sante were the least damaged cultivars. Internal potato tuber damage cultivar significantly among 10 cultivars. Cultivar Spunta was observed as symptomless after damage evaluation (Fig. 2).

Both *D. dipsaci* and *D. destructor* affect potato tubers by reducing their marketable quality. External and internal tuber damage was used to evaluate potato tolerance to these species. External damage was higher in all cases than the internal damage. Earlier reports documented that *D. dipsaci* caused higher internal damage than *D. destructor* (Seinhorst and Dunlop, 1945; Jenkins and Taylor, 1967). However, current experiments contradicted those finding and revealed that internal damage caused by *D. dipsaci* was lower than damage caused by *D. destructor* in the same potato cultivars.

Ditylenchus dipsaci and *D. destructor* have short life cycle and under optimal conditions, they can be able to complete several generation in one cropping season (Hooper, 1972; Hooper, 1973; Sturhan and Brzeski, 1991). As a result of short life cycles, the rapid population growth of these nematodes could have lead to severe potato tuber damage and higher nematodes numbers at week 15. We assume that in potato with longer vegetation the number of final population density of both species will be higher.

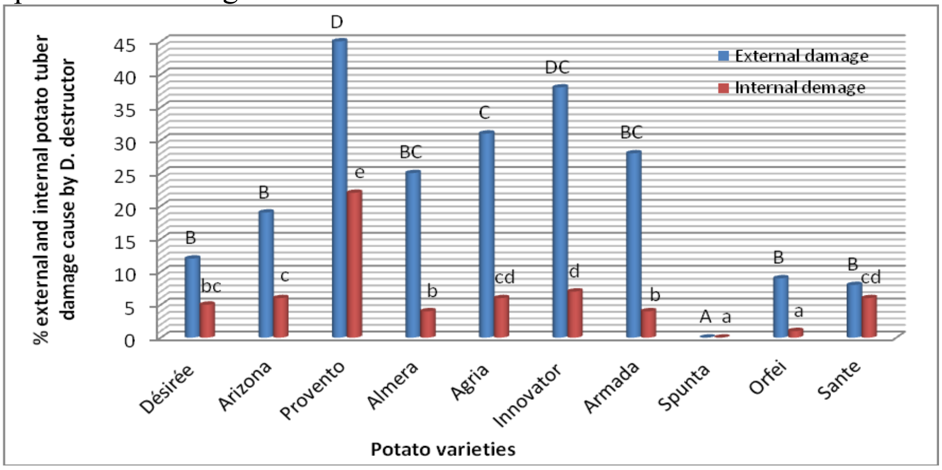

Fig. 2. Mean external and internal potato tuber damage caused by *Ditylenchus destructor* on ten potato varieties (expressed as percentage of means of all tubers per replicate).
*Means separated by the same letter are not significantly different at $P_{0.05}$ according to Duncan's multiple comparison test

4. Conclusion

This study provides information on resistance and tolerance of potato cultivars to *D. dipsaci* and *D. destructor*. Resistance was best evaluated using RS instead of the RF. The RS method was based on more classes which separated cultivars into more resistant classes. Additionally, inclusion of a susceptible cultivar as an internal standard in both screening experiments helped normalize variations in the screening conditions. External damage was found more suitable as a measure for tolerance than internal damage. Tuber damage was in most cases high in some cultivars. Although our experiments offer important information, future experiments in *D. dipsaci* and *D. destructor* infested micro-plots and fields are necessary to assess tolerance under outdoor conditions. The study also demonstrates the importance of *D. dipsaci* as a serious nematode pest of potato. Since both are regulated through phytosanitary measures, it may be important to regulate potato as a pathway for *D. dipsaci*.

5. References

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