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Tuber Yield, Quality and Infestation Levels of Potato Genotypes, Resistant to the Root-Knot Nematode, *Meloidogyne chitwoodi*

Misghina G. Teklu[1,2] · Corrie H. Schomaker[1] · Thomas H. Been[1] · Leendert P.G. Molendijk[3]

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Abstract
As part of developing a routine potato cultivars resistance test to *Meloidogyne chitwoodi*, both the effect of nematode density (*P*i) and pot size on growth, tuber yield, quality and tuber infestation level were studied in glasshouse conditions. The study was carried out in four experiments using cv. Desiree as control and seven genotypes with a single resistance gene to *M. chitwoodi* and 1 genotype, with resistance to *Globodera pallida*. Plants were inoculated with ranges of *P*i from 0.0625 to 256 J2 (g dry soil)$^{-1}$ in log series. Haulm height, tuber yield, starch dry matter content (SDC) and tuber quality were recorded. Additionally, harvested tubers of experiment 2 were stored for 240–300 days to estimate actual tuber infestation at planting when used as seed in a subsequent season. Haulm height was positively affected with increasing *P*i's and negatively with decreasing pot size. The yield was not affected in four out of seven genotypes with resistance to *M. chitwoodi*; they can be considered as tolerant, having a relative minimum yield, $m = 1$. Three genotypes and cv. Desiree showed relative minimum yield, $m \leq 0.8$, the latter varying between 0.67 and 0.80 over experiments and pot sizes. The reduction of SDC equalled that of yield indicating that *M. chitwoodi* had no extra effect on starch content. Quality, expressed as tuber-knot index (TKI), used for accepting ware potatoes for processing, was below 10 for all genotypes, except for 2011M1. The TKI values of cv. Desiree and genotype MDG2 were > 20 and are not accepted for processing. The fraction of clean tubers of the resistant genotypes had significantly increased to 91% compared to < 8% for cv. Desiree and MDG2. Tuber infestation, expressed to number of juveniles per gram dry soil of cv. Desiree after storage, showed no regression with the *P*i and averaged 0.35 J2 (g dry soil)$^{-1}$, while all tested genotypes provided *ca*. 0.002 J2 (g dry soil)$^{-1}$.

Keywords Clean tubers · Modelling · Tolerance · Tuber-knot index and starch dry matter content

Extended author information available on the last page of the article

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Introduction

The root-knot nematodes, *Meloidogyne chitwoodi* and *Meloidogyne fallax* (Karssen 1995, 1996), are quarantine pests in the EPPO region and pose a serious threat to the seed potato production sector in The Netherlands (Been et al. 2007). Aiming at the same successful management approach used to control the potato cyst nematode (PCN) in The Netherlands, the use of resistant cultivars (Been et al. 1995) is envisioned, and Dutch breeders developed potato genotypes resistant to *M. chitwoodi*. These genotypes, with a single resistance gene, have been reported to possess a high level of resistance (Norshie et al. 2011; Teklu et al. 2016, 2017).

In addition to resistance, also the impact on yield—here fresh tuber weight (*FTW*)—, tuber quality and tuber infestation levels are of major interest when these resistant genotypes are grown in infested fields. The quantitative information available concerning the effect of *M. chitwoodi* on yield of potato cultivars is often contradicting. Both Griffin (1985) and Ingham et al. (2007) reported that *M. chitwoodi* did not affect yield significantly. Contrary, Pinkerton and Santo (1986) reported a yield reduction of up to 10-tonnes ha^{-1} in *M. chitwoodi*-infected plots, when compared to nematicide-treated plots. Viglierchio (1987) reported yield reduction by *M. chitwoodi*, but only at a very high (but unknown) initial population densities (*P*i). In general, no yield losses have been reported in The Netherlands caused by *M. chitwoodi* under field conditions, while in pot experiments, a reduction of 14%, of cv. Desiree, was reported by Norshie et al. (2011) at $Pi = 256$ J2 (g dry soil)$^{-1}$.

The most economically significant damage of *M. chitwoodi* is the quality loss inflicted to potato tubers and root vegetable crops such as carrots and black salsify (Santo et al. 1988; Wesemael and Moens 2008; Norshie et al. 2011; Heve et al. 2015). Generally, zero tolerance applies when *M. chitwoodi* is detected in seed potato lots (Ingham et al. 2007; EPPO/OEPP 2013; King and Taberna 2013). In Australia, up to 2% damage in seed potatoes caused by root-knot nematodes in general is tolerated (Anonymous 2007).

However, for industrial processing, there are acceptable limits even though they vary from country to country. In the USA, a potato lot will be rejected when 5–15% of the tubers is discarded due to quality deformations caused by *M. chitwoodi* (Ingham et al. 2000, 2007; King and Taberna 2013). In the Netherlands, infested tubers with a tuber-knot index (TKI) not exceeding 10 are accepted (Visser and Korthals 2004). This index is stretched up to a TKI of 20 in case of shortages of ware potatoes in the market.

Generally, yield reduction and quality associated with nematodes are mainly dependent on sensitivity of the host, and the initial population density (*P*i) at planting (Van Riel 1993; Wesemael and Moens 2008; Norshie et al. 2011). Therefore, any effect on yield or quality of the produce should be investigated using a range of population densities (Schomaker and Been 2013). This paper combines the data on haulm height, yield, quality and tuber infestation from Norshie et al. (2011) and three subsequent experiments carried out to develop a routine resistance test for potatoes resistant to *M. chitwoodi* (Teklu et al. 2016, 2017). Different pot sizes ranging from 10-kg (equivalent to volume used under field condition) and subsequently downscaling it to 5, 3 and 2-kg were used in the experiments with the objective of developing a robust and simple resistance/tolerance test for breeders.

The experiments include two starch and six ware potato genotypes with various sources of resistance to *M. chitwoodi*, except MDG2 having a resistance to *G. pallida*. The findings should provide some basic insights regarding the effect of *P*i on possible changes of the growth pattern, yield, quality and tuber infestation levels of the newly developed resistant genotypes to *M. chitwoodi*. This research was part of a larger research project (MeloResist), a cooperation between Wageningen University and Research and Dutch potato breeders.

Materials and Methods

Experimental Designs and Glasshouse Conditions

The resistance of eight potato genotypes were compared to the susceptible cv. Desiree at ranges of 11–13 *P*i's. Greenhouse conditions were 18 to 20°C during the day, 16°C during the night and 16 h of day light provided by six 400 W, 58,500 lumen lamps. Humidity was kept at 60 to 70%, slightly decreasing towards harvesting time. An overview of the experimental design, with particulars about the tested genotypes, sources of resistance, *P*i series, pot sizes, etc., is presented in Table 1.

Soil Mixtures Used

A soil mixture free of any other pathogens was prepared by combining silver sand (60%), hydro-grains (30%) and clay powder (10 %), to which 1-g NPK (12:10:18) fertiliser per kg was added. Steiner's nutrient solution (Steiner 1968) was added, to supplement micro-nutrients. The soil components were manually mixed 4 times to obtain homogeneity of the mixture while adding 38-l of water per ton of a mixture. After mixing, sub-samples were collected, which were oven-dried over night at 105°C, to estimate the moisture content.

Preparation of the Pots

Pots with perforated base were used in all the experiments. Before filling the pots, the perforations were closed by Ederol filter paper number 261, 40-g/m^2 (J.H.

Table 1 Overview of experimental set up used, potato genotypes tested, source of resistance, parent material and the breeding company. Exp. 1 by Norshie et al. (2011). Exp. 2 to 4 by Teklu et al. (2016). The susceptible cv. Desiree was used as a control in all experiments

Exp.	Year	Pot size (kg)	Replications	Densities (*P*i)	Genotypes	Source of resistance	Resistant parent	Breeding company
1	2010	5	5	11	AR04-4096	*S. bulbocastanum*	BLBC 398.89	Agrico
					AR04-4098	*S. bulbocastanum*	BLBC 398.89	Agrico
				0.5, .., 256	AR04-4107	*S. hougasii*	HOU M 94-110-2	Agrico
2	2011	10	4	12	AR04-4096	*S. bulbocastanum*	BLBC 398.89	Agrico
					AR05-4044	*S. hougasii*	HOU M 94-110-2	Agrico
					Ka-2006/2217	*S. hougasii*	M 94-110-2	Averis
				0.125, .., 128	Ka-2007/1312	*S. fendleri*	M 94-125-1	Averis
3	2012	10, 5, 2	4, 4, 5	12	2011M1	*S. fendleri*	M 94-144-1	HZPC
				0.125, .., 128	MDG2	*S. vernei*	AM 78-3778	KWS
4	2014	3	5	13	AR04-4096	*S. bulbocastanum*	BLBC 398.89	Agrico
				0.0625, ..,128	2011M1	*S. fendleri*	M 94-144-1	HZPC

Agrico, PB 70, 8300 AB Emmeloord, The Netherlands; Averis Seeds B.V., Kweekinstituut Karna, Valtherblokken Zuid 40, 7876TC, Valthermond, The Netherlands; HZPC Holland B.V., Research & Development, PB 2, 9123ZR Metslawier, The Netherlands; KWS POTATO B.V., Johannes Postweg 8, 8308 PB Nagele, The Netherlands

Ritmeester B.V., Utrecht, The Netherlands). The soil mixture, adjusted for dry weight, was then filled to the pots in four steps by gently compressing. These steps were followed to prevent compaction of the soil in the pots.

Source and Preparation of Inoculum

The "Smakt" population (Mc-31) of *M. chitwoodi*, which is the test population used in the European DREAM project (EU QLRT-1999-1462) and chosen as the Dutch standard test population so far, was used in this research. The population was multiplied in 5-kg pots, on tomato (cv. Moneymaker) for about 3 to 5 months. The multiplication rate, Pf/Pi, on Moneymaker at Pi = 4 J2 (g dry soil)$^{-1}$ was 45. On average, 9×10^5 J2 per pot could be retrieved.

After multiplication, the soil was sieved through a 10-mm mesh sieve to collect the tomato roots, which were then stored in plastic bags at 4°C until further processing. One week before the tubers were planted, the tomato roots were chopped into 1-cm pieces and placed in 20-cm diam., 150-μm, extraction sieves. The sieves were put on 25-cm diam. extraction dishes which were then transferred to a Seinhorst mist-chamber (Seinhorst 1988). Suspensions of hatched juveniles were tapped and counted daily until a required number of inoculum was harvested. This process took 5 days at maximum. The suspensions were stored at 4°C, aerated using aquarium pumps and eventually combined into one stock suspension which was used to prepare all required nematode densities. A 2-log series of nematode densities were prepared ranging from 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 J2 (g dry soil)$^{-1}$ in pilot Exp. 1, from 0, 0.125 to 128 in Exp. 2 and 3 and from 0, 0.0625 to 128 J2 (g dry soil)$^{-1}$ in Exp. 4. Each density had either 4 or 5 replicates.

Inoculation and Planting

The pots were inoculated by injecting J2 suspensions in uniformly distributed channels, which extended from the top to the bottom of the pots using inoculation needles. Four up to 20 channels were needed depending on pot size to provide a clustered distribution of the infective juveniles (J2). A cylindrical piece of tuber weighing 12-g and 3-cm long with a single sprout was cut using a cork borer of 15-mm diam. and planted at 6-cm depth in a hole at the centre of the pot made by a cork borer of 18.5-mm in diam. Side branches were regularly removed to keep a single stem potato plant. The experiments lasted 13–16 weeks.

Watering and Maintaining the Potato Plants

Pots were watered twice and rotated once every week, the latter to diminish positional effects in the glasshouse. Moisture content of the soil was maintained at 12–15% after weighing each pot. Additional, in the period of exponential plant growth, 10 pots were weighed randomly, and an equal amount of water was added to each pot to compensate evaporation (Teklu et al. 2016). Just before flowering, an additional fertilisation was carried out to help in tuber setting.

Measurements and Observations

Haulm Height

Height of plants was measured every week until growth stopped and at least three consecutive measurements produced the same height. Measurements were taken

Table 2 Glossary of terms, their description and dimension used

Term	Description	Dimension
$h(t)$	Logistic model, haulm height in time, t	cm
α	Time for the plants to reach $0.5 \times \lambda$	days
β	The relative rate of growth	-
t	Time	days
y	Fresh tuber yield	g
Y_{max}	Maximum fresh tuber weight at $P\mathrm{i} = 0$	g
m	The relative minimum yield when $P\mathrm{i} \rightarrow \infty$	-
T	Tolerance limit when yield started to decline	J2 (g dry soil)$^{-1}$
SDC	Starch dry matter content	%
$Ymax_{SDC}$	Maximum starch dry matter content at $P\mathrm{i} = 0$	g
m_{SDC}	The relative minimum starch dry matter content when $P\mathrm{i} \rightarrow \infty$	-
T_{SDC}	Tolerance limit when starch dry matter content started to decline	J2 (g dry soil)$^{-1}$
TSW	Tuber specific weight	g/cm^3
FTW	Fresh tuber weight	g
$FTWu$	Fresh tuber weight under water	g
TKI	Tuber knot index	-
T_{qual}	Tolerance limit when fraction of clean tubers ($F0$) starts to decline	J2 (g dry soil)$^{-1}$
$F0$	Proportion of tubers without internal or external symptoms	-
$F1$	Proportion of tubers with internal but without external symptoms	-
z	Fraction of clean tubers at $P\mathrm{i} = 1$	-
$P\mathrm{i}$	Initial population density	J2 (g dry soil)$^{-1}$
$P\mathrm{f}$	Final population density from the roots	J2 (g dry soil)$^{-1}$
$P\mathrm{f}_{\mathrm{tubers}}$	Final population density from tuber	J2 (g dry soil)$^{-1}$
$P\mathrm{f}_{\mathrm{tubers}}(t)$	Logistic model, nematode densities in tubers in time, t expressed as J2 per gram dry soil	J2 (g dry soil)$^{-1}$
C	Maximum of $P\mathrm{f}_{\mathrm{tubers}}$	J2 (g dry soil)$^{-1}$
A	Time when half of the C is reached	days
B	The relative rate of hatching in $P\mathrm{f}_{\mathrm{tubers}}$	-

from a fixed point of an inserted plastic peg in the pot up to the tip of the potato stem. The description of all the parameters and variables along with their units of measurement can be found in Table 2.

Yield (Fresh Tuber Weight)

After harvest, the shoot was removed, roots were retrieved and tubers were separated and collected. Harvested tubers were placed in meshed bags, rinsed with a thin film of water to remove any adhering soil and could dry overnight in the glasshouse at 20°C; after which, the fresh tuber weight (*FTW*) per pot was measured.

Quality of Tubers

Cleaned tubers were scored according to the classes listed in Table 3 and the tuber-knot index (TKI) was calculated according to (Eq. 1), which is used by the Dutch potato processing industry to decide the suitability of infected tubers for industrial processing (Visser and Korthals (2004).

$$\mathrm{TKI} = \frac{0 \bullet \left(\mathrm{cl}_0 + \mathrm{cl}_1\right) + 10 \bullet \mathrm{cl}_2 + 33 \bullet \mathrm{cl}_3 + 100 \bullet \mathrm{cl}_4}{\Sigma\left(\mathrm{cl}_0 \ldots \mathrm{cl}_4\right)} \tag{1}$$

As zero tolerance applies for seed potatoes, the proportion of clean tubers (class zero) was also estimated by partially or completely peeling of the tubers without external symptoms to detect any egg masses under the tuber skin. The first 5-mm of the skin, where 96% of the nematodes are located (Viaene et al. 2007), was peeled and investigated. Partial peeling was only done for Exp. 2 at three suspected places with visible knots. Out of the total tubers harvested per pot, only those ≥ 25-mm, except Exp. 4 (≥ 15-mm), diam. was scored to avoid the scoring of relatively few tubers harvested from smaller pots. Tuber quality for seed requires either the $F0$ tubers (without internal or external symptoms) which is scientifically sound or the $F1$ tubers (with internal but without external symptoms) which is commonly used. They were estimated using Eqs. 2 and 3, respectively.

Table 3 Different classes of tubers infected with *M. chitwoodi* after partial and complete peeling based on visual observation (adopted from Wageningen University and Research, Field crops, The Netherlands for estimation of the tuber-knot indexes in Eq. 1)

Class	Symptoms on the skin	Egg mass under the skin
0	None	None
1	None	Yes
2	< 30% tuber surface affected	Yes
3	30–100% tuber surface affected	Yes
4	Heavily deformed	Yes

$$F0 = \frac{\mathrm{cl}_0}{\Sigma\left(\mathrm{cl}_0 \ldots \mathrm{cl}_4\right)} \tag{2}$$

$$F1 = \frac{\mathrm{cl}_1}{\Sigma\left(\mathrm{cl}_0 \ldots \mathrm{cl}_4\right)} \tag{3}$$

cl_n = number of tubers in class n; $0 \le n \le 4$

Tuber Infestation Levels After Storage (Pf_{tubers})

Harvested tubers from Exp. 2 were stored to estimate actual tuber infestation at planting of the next season. Throughout the storage period, a temperature of 7°C and a humidity of 99% were maintained. After storage, tubers were exposed to 20°C for 2 weeks to harden their skin. Potato tubers were peeled 5-mm deep (Viaene et al. 2007). The peel was then cut into 1-cm^2 pieces and placed on 20-cm diameter, 425-µm extraction sieves. The sieves were put on 25-cm diameter extraction dishes and kept in the spray-mist chamber for 7 weeks, while hatched J2 were collected and counted every 7 days. The chosen mesh size for tuber peel was larger than that used for extraction of nematodes from root to avoid clogging of the sieves by starch. The logistic hatching curve of J2 of the susceptible cv. Desiree was used as a reference for the genotypes to monitor the hatching process and to determine the time to terminate the hatching test. Due to limited capacity of the mist-chamber, replications 1 and 2 from each density used were processed after 240 days and replications 3 and 4 after 300 days.

Data Analysis and Modelling

Scripts for data analysis and modelling were written in R using RStudio version 1.3.959 and run using the R console version 4.0.2 (Venables et al. 2022). Nonlinear regression analysis using (ordinary least squares) was carried out when necessary to describe any relationship between the independent variable *P*i and a measured response variable (Haulm height, yield, quality of tubers). Starting parameters for the nonlinear regression analysis were estimated directly from the data. The standard errors were estimated using the inverse of the Hessian matrix. If relevant, estimated parameters of cultivars were compared and tested at a 5% level of uncertainty using the least significant difference (LSD) method.

Haulm Height

Haulm heights per replications per *P*i and per date measured were averaged and nonlinear regression was carried out to describe the relationship between height and growth period (days) using the logistic model (Eq. 4):

$$h(t) = \frac{\lambda}{1 + \exp(-\beta(t - \alpha))} \tag{4}$$

where:

λ the maximum haulm height (cm)

α time for the plants to reach $0.5 \times \lambda$ in days

β the relative maximum growth rate

t time/growth period (days)

Yield (Fresh Tuber Weight)

Seinhorst's (1965, 1998) yield loss model was used to describe the relationship between Pi and yield. Fresh tuber weight of the replications per density was first averaged. In nonlinear regression analysis, the yield loss parameters were estimated using Eq. 5.

$$y = Ymax * \left(m + (1 - m) * 0.95^{((Pi/T)-1)}\right) \tag{5}$$

where:

y fresh tuber yield.

Y_{max} yield when $P\text{i} \rightarrow 0$.

m relative minimum yield when $P\text{i} \rightarrow \infty$.

T tolerance limit, the density above which yield starts to decline and is estimated when $P\text{i} \rightarrow 0$.

Starch Dry Matter Content

In Exp. 2, in addition to the yield, the underwater weight ($FTWu$) was recorded to calculate the tuber-specific gravity using Eq. 6, which was required to estimate the SDC Eq. (7) (www.starch.dk/isi/methods/starchct.htm). The relation between Pi and SDC was described using Eq. 8.

$$TSW = \frac{FTW}{FTW - FTWu} \tag{6}$$

$$SDC = \frac{TSW \quad - 1.01506}{0.0046051}\% \tag{7}$$

$$SDC = Ymax_{SDC} * \left(m_{SDC} + \left(1 - m_{SDC}\right) * 0.95^{((Pi/T_{SDC})-1)}\right) \tag{8}$$

where:

TSW tuber-specific weight or density of potato tubers (g/ml)

FTW fresh tuber weight (g)

FTW_u under water fresh tuber weight (g)

SDC starch dry matter content

T_{SDC} tolerance limit, the density above which *SDC* starts to decline measured in J2 (g dry soil)$^{-1}$

Tuber Quality (*F*0)

The model developed for quality damage of stem nematodes in onions Eq. 9 was used for describing the relation between *P*i and the fraction of clean tubers (*F*0) (Seinhorst 1965).

$$F0 = z^{Pi} \tag{9}$$

where:

*F*0 fraction of clean tubers

z fraction of clean tubers when *P*i = 1

To quantify the tolerance, T_{qual}, of the genotypes for quality damage, the maximum P_i was estimated where *F*0, the fraction of tubers in class 0, was larger than 0.90 according to Eq. 10. Class 0 comprises the tubers without external and internal symptoms.

$$T_{qual} = \max\left(Pi[F0 > 0.90]\right) \tag{10}$$

Tuber Infestation Levels Pf_{tubers}

To estimate Pf_{tuber} per genotype/cultivar and Pi, replicates were first log transformed, averaged per density and then back transformed. Average number of juveniles were plotted against ranges of Pi to study the pattern and choose the best model.

The logistic model, Eq. 11, was fitted to the cumulative number of hatched J2 from the tuber peel and the three parameters (C, B, A) of the hatching curves were estimated

$$Pf_{tubers}(t) = \frac{C}{1 + \exp(-B(t - A))} \tag{11}$$

where:

C the maximum cumulative nematode estimates Pf_{tubers} J2 (g dry soil)$^{-1}$. The total number of juveniles obtained from the whole tubers per pot was divided per volume of soil and is expressed per gram of dry soil.

A time t when the cumulative nematode numbers equal to $0.5 \times C$ (days)

B the relative maximum hatching rate

t hatching time (days)

Results

Haulm Height as Growth Indicator

The growing period of the potato plants varied from 13 to 16 weeks between experiments. The logistic model (Eq. 4) fitted well to the height data of the haulm of all experiments and all Pi's used, with $0.911 < R^2 < 0.999$. As an example, parameter values of Exp. 2—different Pi's—and Exp. 3—different pot sizes—are presented in Tables 4 and 5, respectively.

The Maximum Height Reached, Parameter λ

In 68% of the fitted lines, the maximum plant height λ at $P\text{i} \geq 32$ J2 (g dry soil)$^{-1}$ was significantly higher compared to that at $P\text{i} = 0$ (Fig. 1). Plant height also increased with pot size as depicted in Table 5 for the control pots.

Table 4 Parameter estimates of the logistic function Eq. 4; $h\,(t) = \frac{\lambda}{1+\exp\left(-\beta(t-\alpha)\right)}$ fitted to the data of haulm height of Exp. 2 and displayed in Fig. 1. Potato haulm height at Pi = 0 was compared to that of haulm height at $P\mathrm{i} \geq 32$ J2 (g dry soil)$^{-1}$, where λ = maximum height in cm; α = time to reach $0.5 \times \lambda$ in days; β = relative maximum growth rate; Se = standard error of the parameters; R^2 = coefficient of determination; and df = degrees of freedom. LSD least significant difference

Genotype/cv	Pot size (kg)	Pi	λ	α	β	Se_{λ}	Se_{α}	Se$\boldsymbol{\beta}$	R^2	df	LSD_{λ}	LSD_{α}	LSD_{β}
AR04-4096	10	0	24.6	0.14	81.9	0.60	0.01168	0.58	0.997	9			
AR04-4096	10	32	44.7	0.11	121.7	0.34	0.004	0.91	0.999	9	2.28*	1.46*	0.026*
AR04-4096	10	64	45.7	0.12	121.9	0.35	0.005	0.98	0.999	9	2.41*	1.47*	0.027
AR04-4096	10	128	44.5	0.12	123.5	0.33	0.004	0.92	0.999	9	2.3*	1.45*	0.026*
AR05-4044	10	0	28.9	0.12	113.2	0.59	0.0082	1.05	0.997	9			
AR05-4044	10	32	55.0	0.08	148.8	0.55	0.003	1.94	0.999	9	4.66*	1.71*	0.019*
AR05-4044	10	64	48.3	0.08	138.0	0.71	0.005	2.16	0.998	9	5.08*	1.96*	0.020*
AR05-4044	10	128	54.7	0.08	132.1	0.90	0.004	2.74	0.998	9	6.21*	2.28*	0.020*
Ka-2006/2217	10	0	35.8	0.08	134.1	0.86	0.00608	2.17	0.995	9			
Ka-2006/2217	10	32	55.5	0.07	156.6	1.96	0.00604	6.62	0.994	9	14.8*	4.54*	0.018
Ka-2006/2217	10	64	49.0	0.08	161.2	0.54	0.003	1.91	0.999	9	6.12*	2.15*	0.015
Ka-2006/2217	10	128	64.7	0.07	135.8	0.82	0.002	2.67	0.999	9	7.30*	2.52*	0.014
Ka-2007/1312	10	0	28.6	0.11	106.4	1.42	0.01686	2.33	0.983	9			
Ka-2007/1312	10	32	53.3	0.08	152.6	0.59	0.003	2.06	0.999	9	6.60*	3.26*	0.036
Ka-2007/1312	10	64	44.0	0.10	151.0	0.33	0.003	1.08	0.999	9	5.44*	3.09*	0.036
Ka-2007/1312	10	128	49.6	0.09	148.9	0.33	0.003	1.11	0.999	9	5.48*	3.09*	0.036
Desiree	10	0	32.6	0.10	113.5	0.74	0.00777	1.46	0.995	9			
Desiree	10	32	67.2	0.07	160.4	1.55	0.005	6.33	0.997	9	13.8*	3.64*	0.020*
Desiree	10	64	52.8	0.07	145.0	1.17	0.005	3.71	0.997	9	8.46*	2.93*	0.019*
Desiree	10	128	52.1	0.08	144.8	0.89	0.005	2.93	0.997	9	6.94*	2.45*	0.019

*Significantly different parameter values at $P = 0.05$

Table 5 Parameter estimates of the logistic function Eq. 4; $h(t) = \frac{\lambda}{1+\exp(-\beta(t-\alpha))}$ fitted to the data of haulm height of Exp. 3,with three different pot sizes (2, 5 and 10-kg) at *P*i = 0 J2 (g dry soil)$^{-1}$ of *M. chitwoodi*, where λ = maximum height in cm; α = time to reach 0.5 × λ in days; β = relative maximum growth rate; se = standard error of the parameters; R^2 = coefficient of determination; and df = degrees of freedom

Genotype/cv	Pot size (kg)	*P*i	λ	α	β	Se_λ	Se_α	se_β	R^2	df
2011M1	2	0	83.4	23.04	0.173	0.56	0.3096	0.0095	0.999	7
2011M1	5	0	104.8*	30.99*	0.125*	4.32	1.9123	0.0369	0.979	7
2011M1	10	0	122.7*	34.56*	0.088	5.17	2.0988	0.0146	0.986	7
Desiree	2	0	84.3	28.64	0.097	2.82	1.6902	0.0171	0.984	7
Desiree	5	0	109.8*	36.45*	0.086	7.12	3.2170	0.0195	0.973	7
Desiree	10	0	147.4*	42.18*	0.067	13.66	4.5424	0.0140	0.973	7
MDG2	2	0	83.5	26.30	0.161	0.99	0.5148	0.0151	0.996	7
MDG2	5	0	95.5*	29.28*	0.141*	1.69	0.7860	0.0184	0.994	7
MDG2	10	0	129.0*	35.94*	0.092*	4.97	1.8987	0.0136	0.988	7

*Significant difference with previous data point at $P = 0.05$

The Half Time to Reach *λ*, Parameter *α*

The half time needed to reach λ, parameter α, behaved in a similar way as that of parameter λ. Out of the total α specified at the highest densities used, *P*i ≥ 32 J2 (g dry soil)$^{-1}$, 60% required a statistically higher extra time *D*, in total 2 * (α + *D*), to reach their maximum height λ compared to plants at *P*i = 0 (Tables 4 and 5). Over all experiments, *D* varied between 10 and 50 days. In experiment 2, *D* was the largest and varied between 40 and 50 days (Fig. 1). The parameter α is also positively influenced by pot size.

The Relative Growth Rate, Parameter *β*

The relative growth rate β behaved opposite to λ and α and declined with increasing *P*i. In general, at the highest densities used, *P*i ≥ 32 J2 (g dry soil)$^{-1}$, a 98% decrease in the relative growth rate β, compared to *P*i = 0, for all genotypes was observed. Out of this, only 30% of the total parameter values β at the highest densities specified were statistically lower than that of *P*i = 0 (Tables 4 and 5). The parameter β was inversely related to pot size. In the 10-kg pots, the relative maximum growth rate (β) was smaller than that in the 2-kg pots.

Fresh Tuber Weight and Starch Dry Matter Content

In 10 out of 21 analysis, no regression was found between *P*i and yield (Table 6A). Over all pot sizes and experiments, an average minimum yield (*m*) of 0.74 and 0.84 was obtained for cv. Desiree and genotype MDG2, respectively, which lacked any resistance to *M. chitwoodi*. Genotype AR04-4096 and 2011M1, at different pot

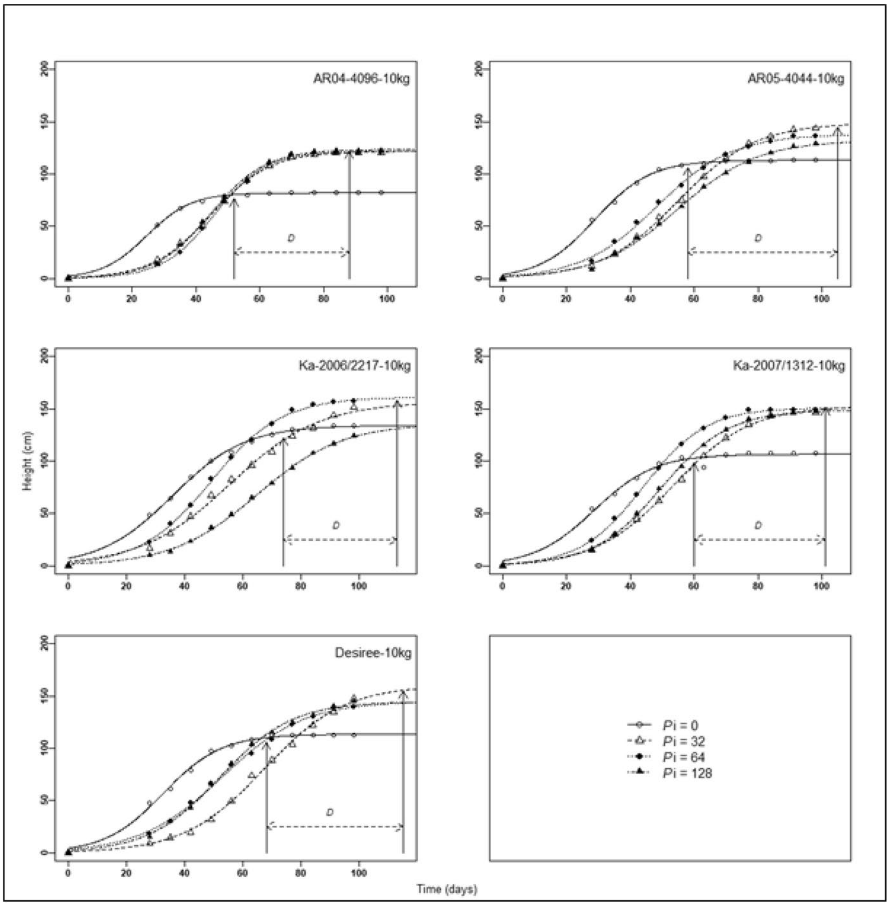

Fig. 1 The logistic growth model Eq. 4: $h(t) = \frac{\lambda}{1+\exp(-\beta(t-\alpha))}$ fitted to the haulm height of potato genotypes and cv. Desiree from Exp. 2. Haulm height at *P*i = 0 is compared to that of *P*i = 32, 64 and 128 J2 (g dry soil)$^{-1}$ of *M. chitwoodi* in time. *D* = extra time needed to reach maximum height (*λ*)

sizes, showed no regression with *P*i where (m = 1). For genotypes AR05-4044, Ka-2006/2217 and Ka-2007/1312 grown in 10 kg pots (Exp. 2), *m* decreased as *P*i increased (Fig. 2). All remaining genotypes showed no decline in yield at increasing *P*i values. Generally, the absolute yield harvested was directly proportional to pot size and a linear correlation was established (Fig. 3): $Y_{\max} = 15.23 + 28.3 \times$ pot size ($R^2 = 0.99$).

Starch dry matter (SDC) estimated for the genotypes of Exp. 2 showed a similar pattern as that of fresh tuber weight (Fig. 4) of these genotypes. Starch content of AR04-4096 was not affected with $m_{SDC} = 1$, with an increasing *P*i. The actual starch potato genotypes Ka-2007/1312, and Ka-2006/2217, had the highest starch dry matter content of 17 and 19%, respectively, compared to 13–14% for cv. Desiree, AR04-4096 and AR05-4044, respectively (Table 6B).

Table 6 (A) Parameter estimates for the relation between Pi of *M. chitwoodi* and yield according to Eq. 5: $y = Ymax * \left(m + (1 - m) * 0.95^{((Pi/T)-1)}\right)$; T = tolerance limit J2 (g dry soil)$^{-1}$, m = minimum yield, Y_{max} = maximum yield in g at $Pi = 0$, Se = standard error of the parameters, R^2 = coefficient of determination. Reg.: no regression found "–", regression found "+." (B) Parameter estimates for the relation between Pi of *M. chitwoodi* and starch dry matter content according to Eq. 8: $SDC = Ymax_{SDC} * \left(m_{SDC} + \left(1 - m_{SDC}\right) * 0.95^{((Pi/T_{SDC})-1)}\right)$; T_{SDC}= tolerance limit J2 (g dry soil)$^{-1}$, m_{SDC} = minimum yield for starch dry matter content, Y_{maxSDC} = maximum starch dry matter content (SDC) in % at $Pi = 0$, Se = standard error, R^2 = coefficient of determination

A. Parameter values of fresh tuber weight

Genotype/Cult	Exp	Pot size (kg)	T	m	Y_{max}	Se_T	Se_m	Se_{Ymax}	R^2	df	Reg
AR04-4107	1	5	NA	1.00	158.8	NA	0.028	4.42	NA	9	-
AR04-4098	1	5	NA	1.00	168.8	NA	0.029	4.97	NA	9	-
AR04-4096	1	5	NA	1.00	166.6	NA	0.031	5.15	NA	9	-
Desiree	1	5	3.98	0.72	161.0	7.30	0.192	10.13	0.35	8	+
AR04-4096	2	10	NA	1.00	288.5	NA	0.024	6.97	NA	10	-
AR05-4044	2	10	1.20	0.51	309.0	0.69	0.091	12.70	0.84	9	+
Ka-2006/2217	2	10	3.35	0.40	241.0	NA	0.400	13.91	0.66	9	+
Ka-2007/1312	2	10	0.56	0.86	296.0	NA	0.040	7.23	0.58	9	+
Desiree	2	10	1.07	0.67	326.6	0.59	0.064	10.32	0.79	9	+
2011M1	3	10	NA	1.00	275.1	NA	0.026	7.12	NA	10	-
MDG2	3	10	0.027	0.74	328.0	0.017	0.044	18.01	0.77	9	+
Desiree	3	10	0.015	0.70	319.0	0.010	0.062	25.73	0.66	9	+
2011M1	3	5	NA	1.00	148.1	NA	0.026	3.87	NA	10	-
MDG2	3	5	NA	1.00	146.5	0.107	0.043	6.30	0.23	10	-
Desiree	3	5	0.003	0.80	154.0	0.006	0.098	17.45	0.16	9	+
2011M1	3	2	NA	1.00	64.9	NA	0.026	1.71	NA	10	-
MDG2	3	2	0.112	0.78	74.4	0.115	0.051	4.01	0.65	9	+
Desiree	3	2	0.195	0.79	64.0	0.150	0.051	2.77	0.64	9	+
AR04-4096	4	3	NA	1.00	93.1	2.660	0.027	2.55	NA	11	-
2011M1	4	3	NA	1.00	119.2	NA	0.015	1.84	NA	11	-
Desiree	4	3	0.013	0.78	101.2	0.010	0.048	5.70	0.71	10	+

Table 6 (continued)

B. Starch dry matter conent

Genotype/Cult	Exp	Pot size (kg)	T_{SDC}	m_{SDC}	Y_{maxSDC}	Se_{TSDC}	Se_{mSDC}	$Se_{YmaxSDC}$	R^2	df	Reg
AR04-4096	2	10	NA	1.00	13	0.02	0.12	1.32	0.30	9	+
AR05-4044	2	10	1.48	0.54	14	2.29	0.20	0.91	0.71	9	+
Ka-2006/2217	2	10	5.53	0.06	19	5.41	0.62	0.91	0.78	9	+
Ka-2007/1312	2	10	0.64	0.82	17	0.43	0.05	0.52	0.61	9	+
Desiree	2	10	0.06	0.54	13	0.01	0.10	1.37	0.56	9	+

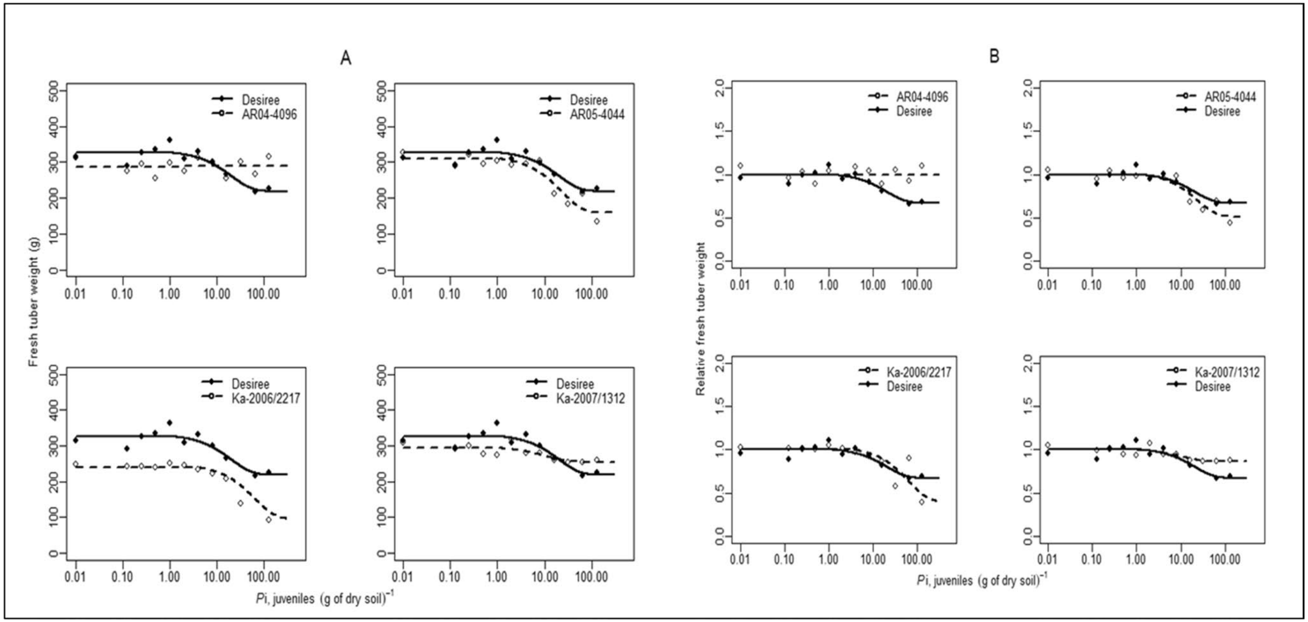

Fig. 2 Seinhorst's model for yield loss Eq. 5: $y = Ymax * (m + (1 - m) * 0.95^{((Pi/T) - 1)})$ was fitted to describe the relation between (**A**) initial population density (*P*i) of *M. chitwoodi* and absolute yield and (**B**) *P*i and relative yield at harvest for four genotypes and cv. Desiree from Exp. 2

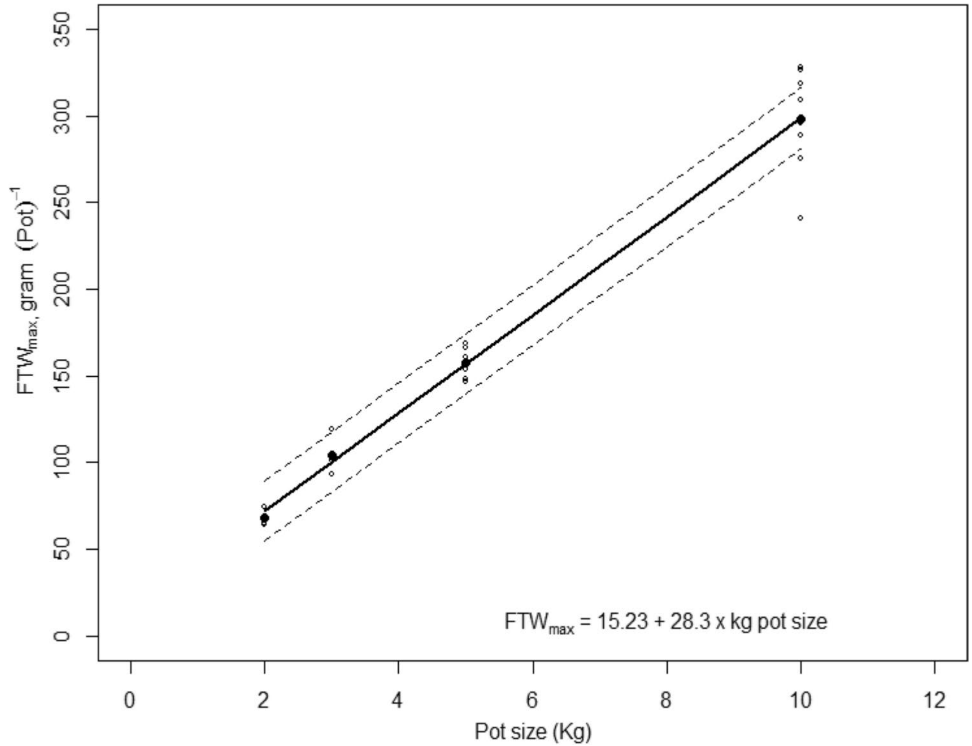

Fig. 3 Linear relation between pot size and yield at $P\mathrm{i} = 0$ J2 (g dry soil)$^{-1}$ of *M. chitwoodi*, irrespective of genotype and cv Desiree. $R^2 = 0.99$. Open dots (○) data points, closed dots (●) mean Y_{max}. Broken lines are the 2.5 and 97.5 quantile of the regression line

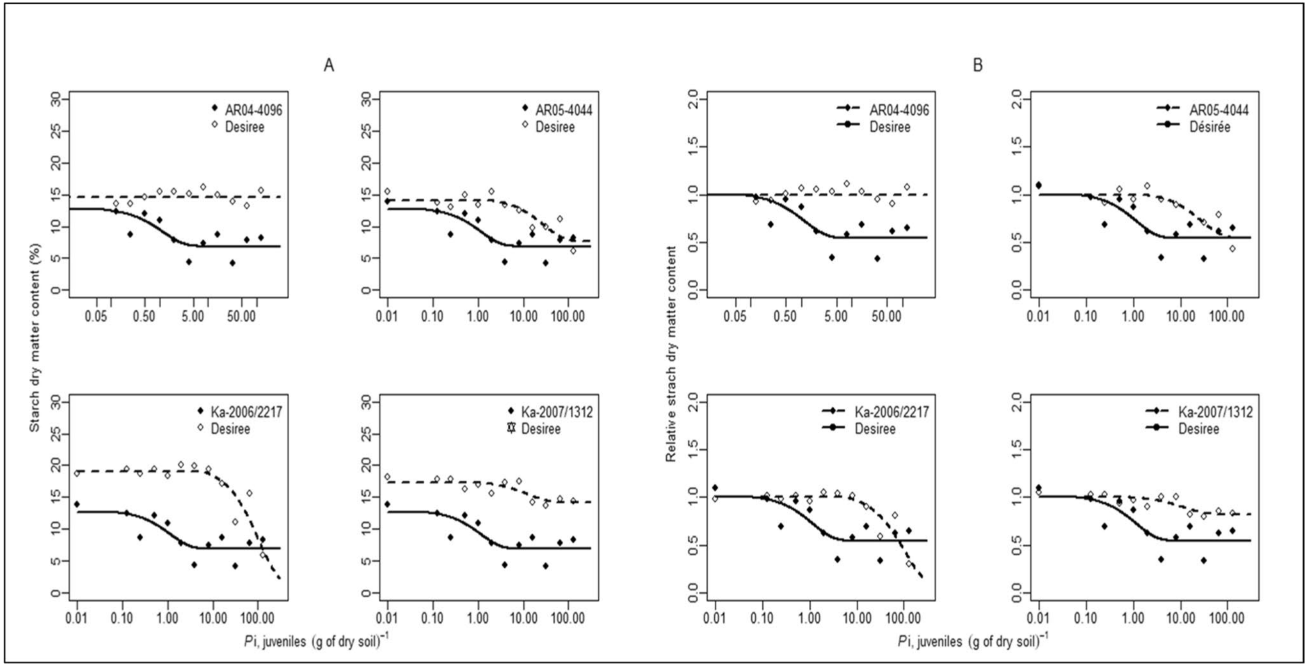

Fig. 4 Seinhorst's model for yield loss Eq. 8: $SDC = Ymax_{SDC} * \left(m_{SDC} + \left(1 - m_{SDC}\right) * 0.95^{((Pi/T_{SDC})-1)}\right)$ was fitted to describe the relation between (**A**) initial population density (*P*i) of *M. chitwoodi* and starch dry matter content and (**B**) *P*i and relative starch dry matter content (SDC) at harvest for four genotypes and cv. Desiree from Exp. 2

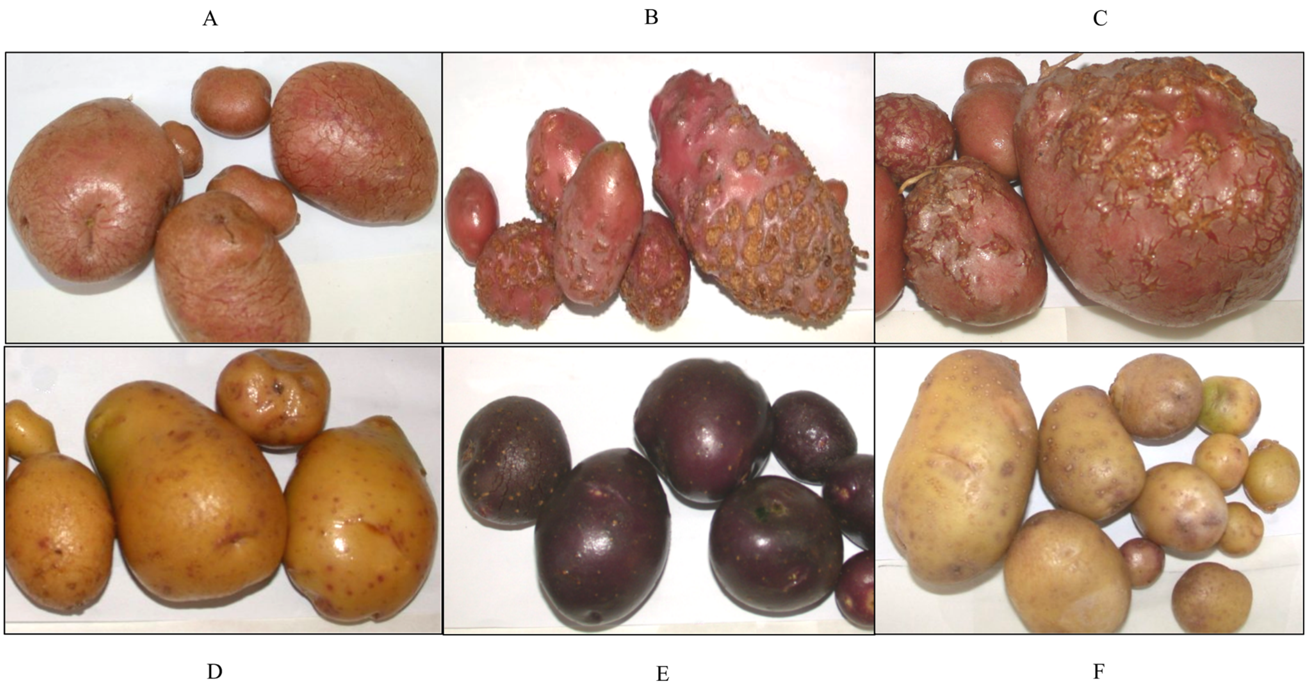

Fig. 5 Quality damage of *M. chitwoodi* of the susceptible cv. Desiree (top row) and partially resistant genotypes of Exp. 2 (bottom row). **A**, **B**, **C** cv. Desiree at Pi = 0, 0.25 and 0.5 J2 (g dry soil)$^{-1}$, respectively. **D**, **E**, **F** Genotypes AR04-4096, AR05-4044 and Ka-2007/1312, respectively, at Pi = 128 J2 (g dry soil)$^{-1}$

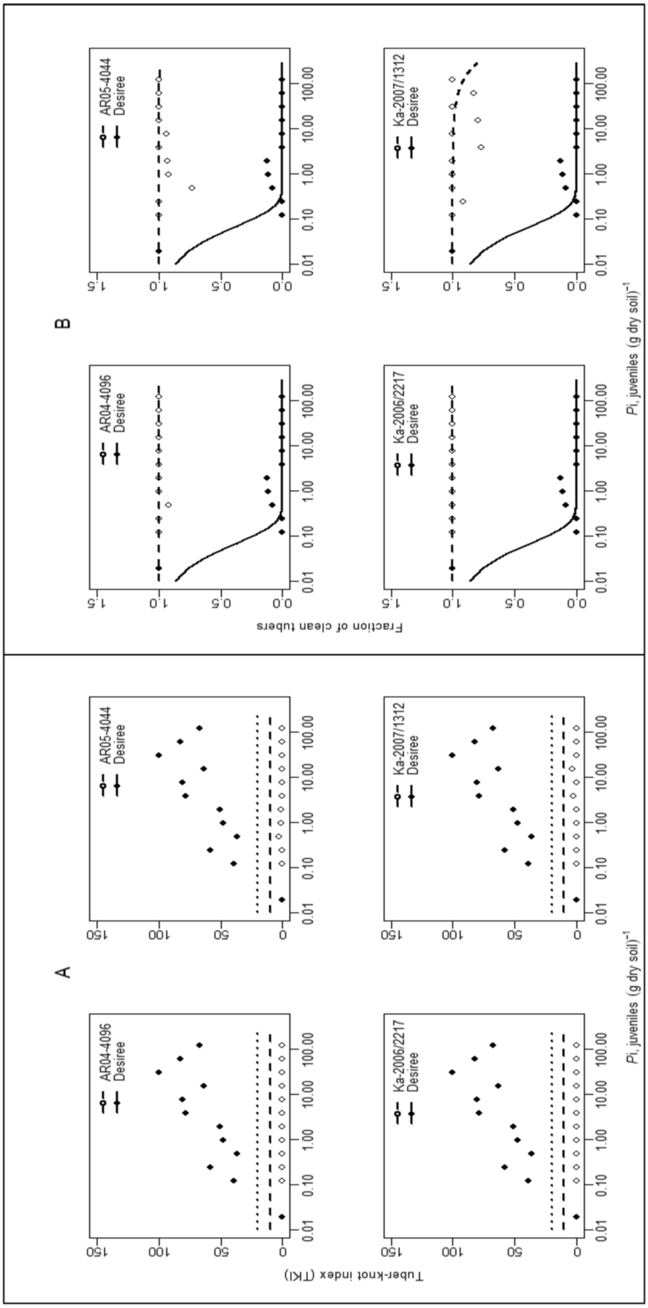

Fig. 6 Tuber quality of four genotypes and cv. Desiree in Exp 2. **A** Equation 1 was used to describe the relation between the initial population density (Pi) of *M. chitwoodi* and the tuber-knot index (TKI) and (**B**) the relation between Pi and $F0$ (no eternal or internal symptoms) according to Eq. 9. $F0 = z^{Pi}$

Table 7 The tuber knot index according to Eq. 1 and the proportion of clean tubers ($F0$) with no external or internal symptoms, ($F1$) no external symptoms and ($F0 + F1$) combined according to Eqs. 2 and 3, per genotype averaged over all Pi densities. T_{qual} = max (Pi[$F0 > 0.90$]) according to Eq. 10

Genotype/cv	Exp	Pot size (kg)	TKI	T_{qual}	Proportion of clean tubers		
					$F0$	$F1$	$F0 + F1$
AR04-4107	1	5	1.33	32	0.88	0.08	0.92
AR04-4098	1	5	0.27	32	0.96	0.02	0.97
AR04-4096	1	5	1.57	16	0.87	0.07	0.95
Desiree	1	5	70.21	≤0.5	0.09	0.00	0.18
AR04-4096	2	10	0.38	128	0.99	0.00	0.96
AR05-4044	2	10	0.71	0.25	0.96	0.00	0.93
Ka-2006/2217	2	10	0.00	>128	1.00	0.00	1.00
Ka-2007/1312	2	10	1.42	2	0.94	0.00	0.91
Desiree	2	10	57.18	≤0.125	0.11	0.00	0.16
2011M1	3	10	9.16	0.25	0.48	0.02	0.51
MDG2	3	10	67.30	≤0.125	0.11	0.00	0.12
Desiree	3	10	63.62	≤0.125	0.14	0.02	0.18
2011M1	3	5	16.62	0.125	0.46	0.00	0.49
MDG2	3	5	61.38	≤0.125	0.09	0.00	0.14
Desiree	3	5	66.83	≤0.125	0.11	0.00	0.14
2011M1	3	2	12.19	0.5	0.50	0.00	0.52
MDG2	3	2	69.51	≤0.125	0.08	0.00	0.08
Desiree	3	2	76.61	≤0.125	0.08	0.00	0.09
AR04-4096	4	3	0.00	NA	1.00	0.00	1.00
2011M1	4	3	0.66	NA	0.98	0.00	0.95

Tuber Quality

In general, external symptoms were drastically reduced as is shown in Fig. 5, where tubers from genotypes AR04-4096, AR05-4044 and Ka-2007/1312 from experiment 2, all originating from the highest Pi of 128 J2 (g dry soil)$^{-1}$, are compared to tubers from cv. Desiree at 0, 0.25 and 0.5 J2 (g dry soil)$^{-1}$. All resistant genotypes had TKI scores < 10 at all initial population densities except 20211M1 at few higher densities. Both cv. Desiree and genotype MDG2 produced TKI values exceeding 20 at all Pi values and example is provided from Exp. 2 (Fig. 6A).

Quality was affected at the lowest Pi used in each experiment for cv Desiree and MDG2 (Table 7), the latter having resistance against *G. pallida*, see example for Exp 2 (Fig. 6B). All resistant genotypes performed better, but presence of *M. chitwoodi* in the produced daughter tubers was still demonstrated. The proportion of clean tubers ($F0$) was 91% for all resistant genotypes, except for 2011M1 with 53%. Desiree and MDG2 yielded no clean tubers, except some rare exceptions where potatoes surfaced above the topsoil and were not infected. The lowest tolerance limit observed was 16 J2 (g dry soil)$^{-1}$ for $F0$. The proportion of $F1$ tubers was ≤ 0.08 for

Table 8 Cumulative hatch parameter (C) according to Eq. 11; $Pf_{\text{tubers}}(t) = \frac{C}{1+\exp(-B(t-A))}$ of *M. chitwoodi* expressed as J2 (g dry soil)$^{-1}$ after 7 weeks of hatching in the mist-chamber, from tubers of cv. Desiree and the resistant potato genotypes in Exp. 2 after 240–300 days of storage. Mean* - back transformed average of log-transformed values of parameter C, over all Pi's. Pi initial population density J2 (g dry soil)$^{-1}$

Cumulative hatch C, J2 (g dry soil)$^{-1}$					
Pi	Desiree	AR04-4096	AR05-4044	Ka-2006/2217	Ka-2007/1312
0.125	0.116	0.0050	0.0000	0.0000	0.0000
0.25	0.226	0.0043	0.0000	0.0005	0.0017
0.5	0.184	0.0000	0.0000	0.0010	0.0008
1	0.108	0.0000	0.0000	0.0021	0.0026
2	0.254	0.0000	0.0000	0.0000	0.0023
4	0.273	0.0018	0.0000	0.0000	0.0026
8	0.164	0.0000	0.0011	0.0032	0.0007
16	0.273	0.0000	0.0018	0.0000	0.0030
32	0.087	0.0033	0.0000	0.0019	0.1690
64	0.600	0.0007	0.0008	0.0035	0.0034
128	0.249	0.0011	0.0000	0.0000	0.0055

all genotypes and improved the quality when added to $F0 + F1$ (Table 7). Pot size affects the number and size of tubers produced and usually a care has to be taken when measuring quality damage at smaller pots due to variations encountered.

Pf in Tubers After Storage (Experiment 2)

The difference in storage time of 240 and 300 days needed to process all stored tubers did not cause any significant change in the number of nematodes hatched and, therefore, storage times were combined in the analysis. The logistic equation (Eq. 11) fitted well to the number of hatched J2 of *M. chitwoodi* from tubers of cv. Desiree ($R^2 > 0.98$) for all Pi's, but not to those of the four tested resistant genotypes tested in Exp. 2, due to the small numbers present in the tubers which made fitting the model impossible. The output of the logistic regression for cv. Desiree and the average of all resistant genotypes of Exp. 2 are presented in Table 8. The estimates of C of each Pi provide the Pf_{tuber} of cv. Desiree. As no regression was found between Pi and parameter C for cv. Desiree (Fig. 7), all back transformed means were pooled and the average Pf_{tuber} (g dry soil)$^{-1}$ on cv. Desiree was calculated using the Pf_{tuber} (g peel)$^{-1}$ × peel weight pot^{-1} × g dry soil^{-1} and estimated to be 0.35 J2 (g dry soil)$^{-1}$. This value equates to 1.9% of the total final population density (Pf) extracted from the roots, soil and tubers combined for cv. Desiree. The resistant genotypes were only marginally infested with a Pf_{tuber} averaging 0.002 J2 (g dry soil)$^{-1}$, 2.1% of their total Pf, the same percentage as on the tubers of cv. Desiree. Tuber infection of the resistant genotypes was less than 1%

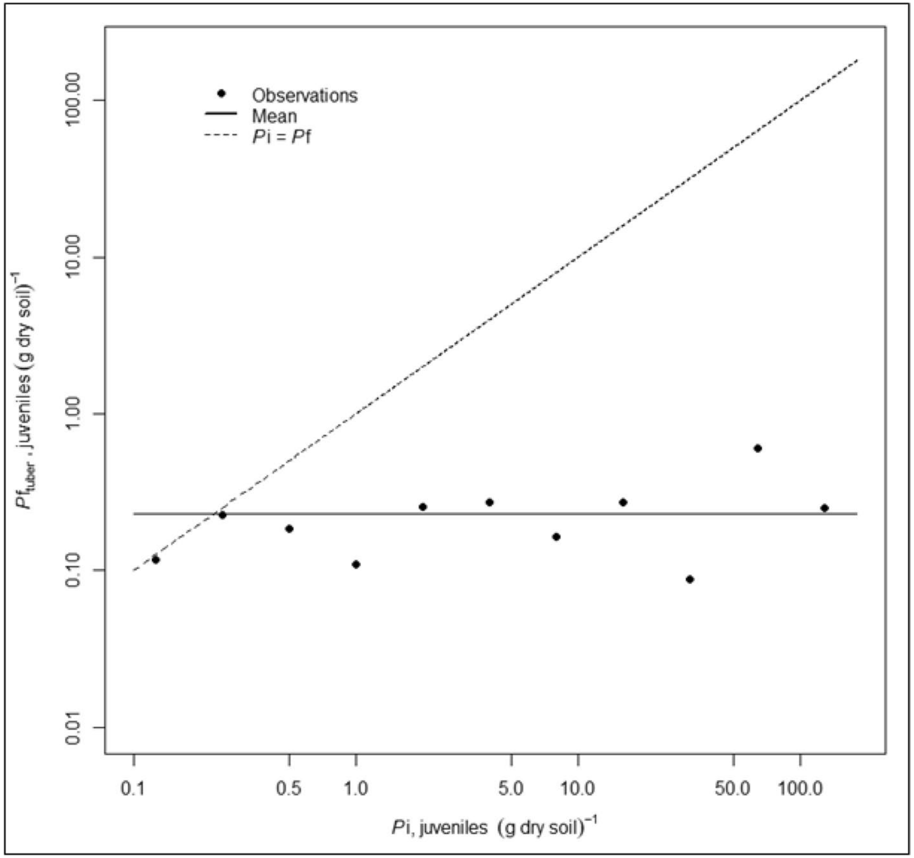

Fig. 7 The relation between *P*i of *M. chitwoodi* and parameter *C*, the maximum cumulative hatch expressed here as ($Pf_{tubers)}$ of cv. Desiree resulted from Eq. 11. $Pf_{tubers}\ (t) = \frac{C}{1+\exp(-B(t-A))}$; The diagonal straight broken line is the equilibrium line (*P*f = *P*i); the solid fitted line is the mean of Pf_{tubers} over the different *P*i's

compared to cv. Desiree, except for Ka-2007/1312 with 7.6%, which was mainly caused by a single, deformed, tuber.

Discussion

Growth

No specific above ground symptoms were observed in any genotype tested, including cv. Desiree, in contrast to other crops (Wesemael and Moens 2008; EPPO/OEPP 2013). Growth defined by haulm height was logistic regardless of genotype, but differed in the maximum height reached (λ), per genotype and pot size. Thinner and longer stems, noticed at higher *P*i's ≥ 32 J2 (g dry soil)$^{-1}$, were also reported by Norshie et al. (2011) and Heve et al. (2015) in potato and carrots, respectively, infested with *M. chitwoodi*. Seinhorst and Den Ouden (1971) also reported taller plants at higher densities of *Globodera rostochiensis*, > 50 eggs (g dry soil)$^{-1}$ of potato varieties, Libertas and Multa. Increasing crop height with increasing pot size can be attributed to the increasing volume of soil accommodating larger plant roots, resulting also in higher fresh tuber yields.

Yield (Fresh Tuber Weight)

The yield of cv. Desiree and genotype MDG2 (both susceptible to *M. chitwoodi*) showed a reduction at the highest nematode densities in most experiments in which they were tested. In addition, resistant genotypes AR05-4044, Ka-2006/2217 and Ka-2007/1312 also showed yield reductions at densities ≥ 32 J2 (g dry soil)$^{-1}$. This proves that *M. chitwoodi* can also reduce the yield of potatoes and might support the findings of Pinkerton and Santo (1986), who also reported a yield reduction of up to 10 tons ha^{-1}, when control plots were compared to nematicide-treated plots. However, these plots would also have benefited from *ca*. 100-kg extra N due to soil fumigation and only part of the yield difference might be attributed to *M. chitwoodi*. Viglierchio (1987) reported tuber yield reduction by *M. chitwoodi* only at very high (but not specified) initial population densities. In the experiments reported here, yield reduction becomes only visible at densities ≥ 32 J2 (g dry soil)$^{-1}$. Generally, these high densities of *M. chitwoodi* are not available under normal field conditions in spring in the Netherlands. The highest population density so far recorded was about 5–10 J2 (g of dry soil)$^{-1}$ in The Netherlands (Molendijk, pers. comm). This might explain that under field conditions, the probability of encountering yield reduction is almost non-existing. No yield losses have been reported in The Netherlands so far under field situations. The relative minimum yield is stable in relation to pot size for those genotypes tested in 10, 5 and 2-kg cylindrical pots. The possibility of coupling resistance and tolerance test seem to be possible at smaller pots which is much simpler for breeder's test at *P*i ≥ 32 J2 (g dry soil)$^{-1}$ slightly higher than the test *P*i for resistance test 24 J2 (g dry soil)$^{-1}$ (Teklu et al. 2016).

Genotypes AR04-4096, 2011M1, AR04-4098 and AR04-4107, had a relative minimum yield, $m = 1$, and can be considered tolerant to *M. chitwoodi*. Tolerance in these genotypes also indicates that root growth was not reduced and that these resistant potato genotypes, when used in the field, are likely to provide a maximum effect in reducing population densities present. The findings also prove once more that resistance and yield loss are not linked.

Starch dry matter content reduction of the potato genotypes AR05-4044, Ka-2006/2217 and Ka-2007/1312 equalled the yield reduction of these genotypes and we can conclude that no additional effect on the starch production was noticeable.

Quality Deterioration

Ware Potatoes for Industrial Processing

The tuber-knot index for all genotypes, except 2011M1, was below the minimum threshold level (10) at all initial population densities tested, and therefore, these potatoes were acceptable for industrial processing. The noticeable higher TKI of 2011M1 as compared to the rest of the genotypes most likely can be attributed to a slightly lower partial resistance (Teklu et al. 2016) and the effect of higher population densities at tuber formation (Teklu et al. 2017). The TKI values of 2011M1were only ≤ 10 at *P*i values < 2 J2 (g dry soil)$^{-1}$, but at *P*i values ≥ 2 J2 (g dry soil)$^{-1}$ TKI values ranged between 10 and 20, which indicated that tubers from 2011M1 would only be acceptable for processing during times of shortages when the TKI score is stretched to 20. The susceptible genotype MDG2 and cv. Desiree, which does not contain *M. chitwoodi* resistance, had TKI scores > 20 at almost all densities and would not be accepted for industrial processing. This confirms how a single resistance gene makes a big difference in improving quality.

Quality of Seed Tubers

Tuber infestation was observed on both cv. Desiree and MDG2 in all four experiments, even at the lowest *P*i used, 0.0625–0.125 J2 (g dry soil)$^{-1}$, respectively with > 92% of the tubers infected. It indicates that a tolerance limit for clean potatoes, if it exists, must be found at an even lower population density. A tolerance limit ≤ 0.004 J2 (g dry soil)$^{-1}$ for quality damage was reported (Ingham et al. 2000; Pinkerton and Santo 1986). This was mainly for cultivars which lack resistance for *M. chitwoodi*, equivalent to cv. Desiree and MDG2 in this research.

However, this was not the case with the resistant genotypes, with > 91% of the tuber free from infestation at all *P*i levels. As zero tolerance applies for *M. chitwoodi* in seed potatoes, the use of these resistant potatoes for seed, when grown on infected soil, should be carefully monitored. As, in the controlled environment of a glasshouse, the resistant genotype showed that a remarkable improvement in quality—at lower densities, quality loss was not always demonstrable—application in the field below a certain population threshold might be feasible option to produce healthy seed. In a first pilot experiment with a selection of these genotypes on a farmer's field, no quality loss was observed at all, while on a field where populations during winter were kept considerably higher to normal by growing a susceptible green manure crop, only a limited amount of quality damage was registered (Teklu et al. unpubl.).

In general, these findings make it clear that quality damage is not directly related to the initial population density, indicating that the nematodes present at planting are not the nematodes which will invade the tubers causing quality damage. The nematodes invading the tubers and causing quality damage belong to a new generation of *M. chitwoodi*. Is it a second or third generation? It is impossible to define as no separation in time between generations is possible. While the expanding root system of the potato still encounters first-generation J2 in the soil, the first J2 from the second-generation hatch from galls of first J2 generation, which entered the roots at planting. What is more important from this insight is the possibility to prevent quality damage of susceptible potatoes when the nematodes present at the time of tuber formation could be controlled, e.g., by using a systemic nematicide or any other agent that could inhibit J2 to move into the new developing tubers when susceptible cultivars are grown. Boydston et al. (2007) and Brown et al. (2009) suggest that it might be possible that resistance to *M. chitwoodi*, introgressed with a single gene, can have a resistance trait that is inherited independently in the roots and tubers, indicating that the roots might be resistant but not the tubers and that control might still be needed. The use of nematostatics to prevent quality damage is probably not needed when resistance is available in both roots and tubers as was demonstrated by Teklu et al. (2017).

Tuber Infestation Levels (Pf_{tubers})

Obviously, the J2 obtained from the tubers are part of the final population density that the potato plant produces. However, they do not remain in the soil, but are transported off-field with the harvested tubers. While tuber infestation of starch potatoes is ignored, and a certain level is tolerated in ware potatoes (TKI $\leq$ 10 or 20, depending on demand and supply), seed will be rejected when detection occurs. The question whether J2 are still alive after 6 months of storage (seeds for next season) is an important question. Juveniles of *M. chitwoodi* were found in peel of potato tubers of both cv. Desiree and the resistant genotypes after 240–300 days of storage, although in lower levels: on average 2%, compared to the numbers in the roots and soil directly after harvest of the potatoes. Compared to Pf_{tubers} of cv. Desiree, the Pf_{tubers} in the resistant potato genotypes were reduced by > 90% and were extremely low, Pf_{tuber} = 0.002 J2 (g dry soil)$^{-1}$, average back transformed mean over all the densities. It is not known whether these low numbers are the result of low densities at tuber formation or is related with a certain maximum carrying capacity of the tubers reduced by the single resistance gene introgressed.

In addition, only infestation levels after storage were estimated; due to the limited numbers of tubers available, we do not know whether *M. chitwoodi* numbers increased, declined or were stable during storage. However, according to Teklu et al. (2018), a storage temperature of 4°C reduces Pf_{tubers}, while at 8°C, population densities remain the same for 120 days and then start to decline. At 12°C, during the first 60 days, densities even increase, probably by further egg laying of the matured females; after which, numbers remain unchanged until 240 days, the maximum storage time used. The surviving J2 on cv. Desiree, independent on storage temperature, were as viable as freshly harvested ones (Teklu et al. 2018). The 7°C in this experiment suggest that there might be a slight reduction in numbers compared to harvest and it is likely that they will multiply under the next susceptible potato crop.

Phytosanitary Implication

The method of visual inspection of 200 tubers per 10-tonnes of potato (EPPO/OEPP 2006) seems not very effective to trace the infection levels obtained in the resistant genotypes. The recently introduced molecular techniques, with sensitivity of 1 matured female, might help (De Haan et al. 2014), but detection in susceptible potato cultivar lots currently is mostly based on only one potato tuber with a single female from the whole lot (Jan Luimes, pers. comm.). So, infected lots will slip through inspection and reach new fields.

When we try to estimate the risk of using infected susceptible tubers after storage as seed in the field situation, we can make the following assumptions:

i. Seed density is 4 tubers per m^2 (Van der Zaag 1992); seed tuber size is 35-45-mm long, oval shaped.
ii. Depth of tilth is 25-cm; consequently, a volume of 100*100*25-cm^3 per m^2 is available.
iii. 1-cm^3 soil is equivalent to 1.3-g soil.
iv. Based on four tubers, approx. 83-g $(m^2)^{-1}$ peel is available.

From earlier work on the effect of storage temperature on population dynamics of *M. chitwoodi* in infected tubers of cv. Desiree, we know the following: peel weight × the actual number of J2 (g peel)$^{-1}$ found after 240 days at storage temperatures of 4°C, 8°C and 12°C for cv. Desiree (Teklu et al. 2018) would provide 0.007, 0.022 and 0.204 J2 (g soil)$^{-1}$, respectively, in the tilth. These *P*i values are higher than the quality thresholds reported by Ingham et al. (2000): *ca*. 0.004 J2 (g soil)$^{-1}$, and close to those reported in the 4 experiments in this paper < 0.0625 J2 (g dry soil)$^{-1}$. Therefore, using susceptible potatoes after storage as seed in an uninfected area poses a risk, both for infecting a new site and for direct quality damage of the produce. This risk of quality damage can be avoided when resistant genotypes, e.g. AR04-4096, are used with a Pf_{tuber} of 0.002 J2 (g dry soil)$^{-1}$ after 240–300 days of storage at 7°C and ca. 0.0005–0.0008 J2 (g dry soil)$^{-1}$ when stored at 4°C for 240 days. Even then the cultivation of susceptible host should be avoided, and strict control of weeds should be applied while growing resistant crops.

Conclusion

Potato genotypes with resistance to *M. chitwoodi* follow a normal growth pattern. The introduction of these genotypes, when ready for practical use, will have no effect on the current potato growing conditions. Some genotypes showed yield reduction associated with delay of growth at high nematode densities ($P\mathrm{i} \geq 32$ J2 (g dry soil)$^{-1}$), which are not prevalent under normal growing conditions in spring. Tuber knot-index values are below the rejection level for industrial processing, both in the four glasshouse experiments as in two field experiments conducted so far (Teklu et al. unpubl.). Thus, the use of these genotypes provides a direct solution for ware potato growers. Although the number of clean tubers of the genotypes tested is increased and tuber infestation is significantly lower than those of susceptible cultivars, some tuber infestation still occurs in glasshouses where optimum conditions are maintained. The conditions in the field are less optimal for both the potato crop as the nematode and the actual pre-plant densities are relatively low compared to the extreme densities used in the pot experiments which might result in even better performance than in the glasshouse experiments. The performance of infected resistant tubers in clean soil is necessary to be assessed, to study the risk of these few nematodes on subsequent crops. Can these few number be stopped by the resistance in the root of the genotypes? Could the presence of weeds affect their re-distribution again all needs to be investigated? So far, these potato genotypes will be essential in the management of *M. chitwoodi*, especially when combined with other control measures in an integrated management approach such as a combination with resistant green manure crops, e.g. fodder radish followed by fallowing (Teklu et al. 2014).

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Declarations

Ethics Approval No ethics have been violated in compiling this article.

Conflict of Interest The authors declare no competing interests.

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Authors and Affiliations

Misghina G. Teklu[1,2] · Corrie H. Schomaker[1] · Thomas H. Been[1] · Leendert P.G. Molendijk[3]

✉ Misghina G. Teklu
Misghina.goitomteklu@wur.nl

1 Wageningen Plant Research, Wageningen University and Research, 6700 AP Wageningen, The Netherlands

2 Laboratory of Nematology, Wageningen University and Research, 6708 PD Wageningen, The Netherlands

3 Wageningen Plant Research, Wageningen University and Research, Open Fields, 8200 AK Lelystad, The Netherlands