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Population dynamics of *Heterodera schachtii* Schm. and yield response of susceptible and resistant sugar beet (*Beta vulgaris* L.) after cultivation of susceptible and resistant oilseed radish (*Raphanus sativus* L.)

Populationsdynamik von *Heterodera schachtii* Schm. und Ertragsreaktion von anfälligen und resistenten Zuckerrüben (*Beta vulgaris* L.) nach dem Anbau von anfälligem und resistentem Ölrettich (*Raphanus sativus* L.)

Abstract

Heterodera schachtii is an important pest of sugar beet. Field trials to quantify yield responses of sugar beet varieties to *H. schachtii* or to assess the effect of variety on population dynamics of the nematode are difficult due to its patchy distribution in the field. The aim of the present study was to develop an experimental method to achieve a more homogeneous distribution of the nematode and to relate yield of susceptible and resistant sugar beet to population density of *H. schachtii*. From 2002 to 2005, thirteen field trials were conducted in four regions of Germany. In the year prior to sugar beet cultivation, a susceptible and a resistant oilseed radish variety or a 50/50 mix of both were grown in strips to vary population densities of the nematode at each trial site. Significant differences in population densities after oilseed radish cultivation were obtained in six of the thirteen trials. The reproductive rates of *H. schachtii* were higher under the susceptible than under the resistant sugar beet variety in all trials and generally decreased with increasing initial population density (Pi). In both varieties, white sugar yields decreased with increasing Pi. This relation was not confirmed in all trials. Root quality was not related to Pi. It was concluded that the introduced methodology is too costly and not sufficiently reliable for extensive series of field trials.

Key words: Beet cyst nematode, population density, reproductive rate, sugar beet varieties, white sugar yield, field trials, experimental methodology

Zusammenfassung

Heterodera schachtii zählt zu den wichtigsten Schädlingen der Zuckerrübe. Feldversuche mit dem Ziel, die Ertragsreaktion von Zuckerrübensorten auf Befall mit *H. schachtii* zu quantifizieren oder den Einfluss der Sorte auf die Populationsdynamik der Nematoden zu beschreiben, werden durch das nesterweise Auftreten im Feld erschwert. Ziel der Untersuchung war es, ein Verfahren zu entwickeln, mit dem eine homogenere Verteilung der Nematoden im Feld erreicht werden kann, sowie den Einfluss unterschiedlicher Populationsdichten von *H. schachtii* auf den Ertrag einer anfälligen und einer resistenten Zuckerrübensorte zu untersuchen. In den Jahren 2002 bis 2005 wurden 13 Feldversuche in vier Anbauregionen in Deutschland durchgeführt. Im Jahr vor dem Anbau der Zuckerrüben wurden eine anfällige und eine resistente Ölrettichsorte oder eine 50/50-Mischung aus beiden in Streifen angebaut, um die Populationsdichte der Nematoden an jedem der Standorte zu variieren. Signifikante Unterschiede in der Populationsdichte wurden dabei an

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sechs der 13 Standorte erzielt. Die anfällige Zuckerrübensorte hatte in allen Versuchen höhere Vermehrungsraten als die resistente, generell nahm die Vermehrungsrate mit steigender Ausgangsdichte der Nematoden (Pi) ab. Mit steigendem Pi-Wert ging der bereinigte Zuckerertrag beider Sorten zurück. Diese Beziehung wurde allerdings nicht an allen Standorten bestätigt. Die Qualität der Rüben wurde nicht durch den Pi-Wert beeinflusst. Insgesamt erscheint die vorgestellte Methodik als zu aufwendig und nicht ausreichend verlässlich für umfangreiche Feldversuchsserien.

Stichwörter: Rübenzystennematoden, Populationsdichte, Reproduktionsrate, Zuckerrübensorten, bereinigter Zuckerertrag, Feldversuche, Versuchsmethodik

Introduction

The beet cyst nematode (*Heterodera schachtii* Schm.) is the most important pest of sugar beet in Central Europe (Müller, 1999). Under German conditions, three to four generations can develop between sowing and harvest of sugar beet (Daub and Westphal, 2012). High population densities may cause substantial yield losses of up to 50% and more (Heinrichs, 2000; Steudel and Thielemann, 1979). Thus, a management system to control *H. schachtii* is of high relevance for the economic efficiency of beet production. Nematode population density can be effectively suppressed by a more than 3-year cropping interval between susceptible sugar beet crops or by growing resistant cover crops, e.g. oilseed radish or white mustard (Müller, 1999). Whereas longer crop rotations are uneconomical, resistant cover crops are well-established in commercial sugar beet farming (Buhre et al., 2014). For effective nematode suppression, however, resistant cover crops need to be sown sufficiently early, i.e. in late July to mid-August (Koch and Gray, 1997). This is not always feasible depending on the pre-crop and the year. In some beet producing areas, cover crop cultivation is even principally restricted by limited water supply. In these cases, resistant sugar beet varieties may contribute to the system of biological nematode suppression.

In Germany, the first sugar beet variety resistant to *H. schachtii* was released in 1998, followed by the first tolerant variety in 2005 (Bundessortenamt, 2013). Resistance and tolerance are independent traits. Varieties classified as resistant suppress nematode multiplication, they may also carry some tolerance. Varieties classified as tolerant suffer less from nematode attack and produce higher yields in infested soil than comparable sensitive varieties (Müller, 1998). Tolerant varieties are occasionally indicated as partly resistant due to their genetic background originating from *Beta maritima*, but they cannot reduce population density of *H. schachtii* (Daub and Westphal, 2012; Niere, 2009). Reproductive rates of the nematode strongly depend on its initial population density (Pi) in the soil; at very low Pi, population can increase even in resistant varieties (Heijbroek et al., 2002; Smith et al., 2004). The Pi at which the reproductive rate = 1 is called equilibrium density (Seinhorst, 1966).

For the sugar beet producer, both reduction of nematode abundance and yield performance are highly important aspects of variety choice. Reliable testing methods of these parameters are required for registration purposes and for agricultural extension. Resistance is usually tested in the greenhouse according to Müller and Rumpenhorst (2000), whereas tolerance is tested under natural field conditions to gain information on yield performance. Based on the biology of *H. schachtii*, a number of challenges in performing field trials occur. A sufficient number of trials needs to be conducted at meaningful infestation levels of the nematode to obtain representative results. In some years, this latter requirement is difficult to achieve when only fields with too low population densities to measure yield effects are available. The main problem is the inhomogeneous, patchy distribution of the nematodes in any given field (Balke, 2001; Seinhorst, 1982). When measured in 10 m^2 plots, the standard size for variety testing in Germany, yield response of sugar beet to *H. schachtii* was inconsistent and tolerance could not be reliably tested in field trials in the early 2000s. These problems may be overcome if more homogeneous population densities in the field could be obtained as described by Schlang and Müller (1996). These authors established varying population densities of *H. schachtii* at the same location by growing diverse intercrops with different resistance levels.

The objectives of the present study were (I) to evaluate whether population density of *H. schachtii* can be systematically varied to measure yield response and thus tolerance of sugar beet to *H. schachtii*, and (II) to determine whether yield and quality of susceptible and resistant sugar beet are related to nematode population density. From 2002 to 2005, thirteen field trials were conducted with one susceptible and one resistant sugar beet variety. In the year prior to sugar beet cultivation, one susceptible and one resistant oilseed radish variety or a 50/50 mix of both were grown in strips to vary nematode population densities within the given trial sites.

Materials and Methods

Trial sites and experimental design

Field trials were carried out in four typical sugar beet producing areas in Germany at sites with different soil and climatic conditions from 2001/02 to 2004/05 (Tab. 1). The design of the field trials was a two factorial strip-plot (factor 1: cover crop, factor 2: sugar beet variety) with two true replications (Fig. 1). The planting strips of the oilseed radish were re-randomised at each location. The factor sugar beet variety was established by splitting the cover crop strips in four 6-row plots per block. In 2005, the number of replications had to be reduced from eight to five.

All trials were conducted on fields with 3-year rotation of sugar beet. Following winter barley (*Hordeum vulgare* L.) or winter wheat (*Triticum aestivum* L.; only at Borsum

Tab. 1. Trial sites, soil parameters and sowing dates of cover crop (oilseed radish) and sugar beet, Germany 2001–2005

Year	Region	Site	Soil parameters				Tempe-rature[2] (°C)	Rain-fall[3] (mm)	Sowing date	
			Tex-ture[1]	P mg 100 g⁻¹	K mg 100 g⁻¹	pH			Oilseed radish	Sugar beet
2001/2002	1 Hildesheim Plain	Ottbergen	L	3.2	6.4	7.0	14.3	546	2001–08–28	2002–04–04
	2 South Lower Saxony	Niedernjesa	CL	4.6	7.1	7.3	14.0	588	2001–08–09	2002–04–02
	3 Rhineland	Koslar	SI	4.4	7.9	6.8	15.6	440	2001–07–25	2002–03–28
	4 Franconia	Geroldshausen	SIL	4.0	13.0	7.3	15.0	472	2001–08–08	2002–04–03
2002/2003	1 Hildesheim Plain	Ottbergen	L	2.2	6.6	6.8	14.6	303	2002–08–28	2003–03–28
	2 South Lower Saxony	Niedernjesa	CL	3.5	8.0	ND	14.5	297	2002–07–29	2003–03–25
	3 Rhineland	Koslar	SI	2.2	9.5	6.3	15.6	288	2002–07–23	2003–04–28
	4 Franconia	Geroldshausen	SIL	15.3	8.3	7.5	15.7	251	2002–08–21	2003–03–25
2003/2004	1 Hildesheim Plain	Ottbergen	CL	6.1	13.3	7.4	14.2	416	2003–07–14	cancelled
	2 South Lower Saxony	Niedernjesa	CL	6.1	14.9	7.2	13.7	476	2003–07–02	cancelled
	3 Rhineland	Niederembt	SI	6.8	14.1	7.2	15.5	403	2003–07–11	2004–04–02
	4 Franconia	Geroldshausen	SIL	4.3	16.0	7.0	13.4	404	2003–07–29	cancelled
2004/2005	1 Hildesheim Plain	Borsum	L	4.8	14.9	7.2	14.4	331	2004–05–19	2005–04–04
	2 South Lower Saxony	Obernjesa	SIL	2.4	4.6	6.9	14.0	422	2004–05–05	2005–04–04
	3 Rhineland	Kelz	SIL	0.9	4.6	7.0	15.4	399	2004–07–13	2005–04–04
	4 Franconia	Geroldshausen	SIL	3.8	13.0	7.2	14.5	392	2004–08–17	2005–04–05

[1] L: loam, CL: clayey loam, SI: silt, SIL: silty loam
[2] mean April–October (sugar beet season)
[3] sum April–October (sugar beet season)
ND: not determined

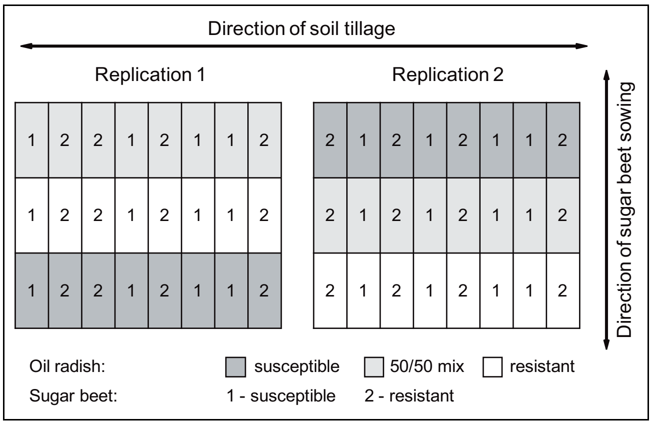

Fig. 1. **Experimental design of the field trials, 2001–2005. Planting strips of the oilseed radish were re-randomised at each location.**

2005 and at Kelz 2005), oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) 'Adagio' (nematode-resistant), 'Siletta Nova' (susceptible) or a 50/50 mix of both were grown as a cover crop in the year prior to sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Doell).

Oilseed radish was planted in July/August (Tab. 1) at 25–30 kg ha⁻¹ after site-specific tillage and seedbed preparation and application of quick-acting N-fertiliser (calcium ammonium nitrate or urea-ammonium nitrate solution, 50–60 kg N ha⁻¹). The oilseed radish stands were cut once or twice during the vegetation period and finally ploughed down. A drought period in June 2003 caused low emergence and poor establishment of the plant stands at Ottbergen, Niedernjesa and Geroldshausen. Therefore, the 2004 sugar beet trials at these sites were cancelled, and oilseed radish was sown in May 2004 at Obernjesa and Borsum. At the other sites, oilseed radish development was good or adequate.

Two sugar beet varieties were grown, one susceptible ('Macarena') and one resistant to *H. schachtii* ('Paulina'). For all trials in 2002–05, sugar beet seeds from the same seed lots were freshly coated every year and treated with standard amounts of the fungicides thiram and hymexazol and the insecticides imidacloprid and tefluthrin.

In all trials, sugar beets were sown after site-specific seedbed preparation and across the tillage direction. Seeding distance was 6–11 cm within and 45 cm between the rows. The stands were thinned to a plant distance of 21–25 cm as early as possible to avoid a trap effect of the sugar beet plants. Crop protection was carried out according to best local practice. In all 6-row plots, three of the four central rows were harvested (5.40–11.25 m^2) with on-site available machinery. Root yield and concentrations of sucrose, K, Na and amino-N were determined in tarehouses in the particular growing regions or at the Institute of Sugar Beet Research Göttingen. The beets were washed, weighed and brei samples were prepared. Quality analysis was carried out using an automatic beet laboratory system (Venema, Groningen, NL) according to standard procedures (ICUMSA, 1994; Kubadinow and Wieninger, 1972; Burba and Georgi, 1975, 1976). White sugar yields were calculated according to the standard equations of quality assessment in Germany (Buchholz et al., 1995; Märländer et al., 2003).

The sugar beet at Geroldshausen 2003 were frost-damaged in mid April (BBCH 10–12) followed by drought later in the season. Consequently, yield was far below average and the data were excluded from overall yield analysis (see below). In Koslar 2003, beets had to be re-sown in late April due to technical problems caused by cover crop residues on the soil surface and a patchy crop stand after sowing on 24 March.

Determination of nematode population density

All soils were checked for presence of *Heterodera avenae* in a greenhouse test at the former German Federal Biological Research Centre for Agriculture and Forestry (BBA) in Münster because mixed populations with *H. avenae* complicate analysis of *H. schachtii* (Hallmann et al., 2009). No additional cyst nematode species to *H. schachtii* were detected in any sample.

The heterogeneous distribution of cyst nematodes in the field requires a high number of soil samples and an adequate amount of soil to assess the actual nematode density (Müller, 1983a, b). Before oilseed radish sowing, the initial population density of *H. schachtii* (Pi_{OR}) was determined from one mixed soil sample (20–24 cores) per experimental field. The initial population density in sugar beet (Pi_{SB}) was measured plotwise and also regarded as the final population density under oilseed radish (Pf_{OR}). Soil samples were taken within 8–10 days after sugar beet sowing to prevent an earlier hatching of the juveniles. The final nematode population in sugar beet (Pf_{SB}) was determined in soil samples taken shortly before harvest. At each sampling date, four cores were taken from each row (i.e. 24 samples per plot) in 10 cm distance to the beet to a depth of 30 cm. Each core contained ca. 250 g of soil, amounting to a composite sample of ca. 6 kg soil per plot. The composite samples were mixed and stored in plastic bags at 5°C until analysis. Three aliquots were processed for each plot (see below). Reproductive rates (r) were calculated plot-wise for sugar beet ($r_{SB} = Pf_{SB}/Pi_{SB}$) and trial-wise for oilseed radish ($r_{OR} = Pf_{OR}/Pi_{OR}$; identical reference area).

The number of eggs and juveniles (E + J) in soil was determined by a standardised procedure in the laboratories of the former BBA at Münster (for trials in South Lower Saxony) and Elsdorf (trials in Rhineland), at Bavarian State Research Centre for Agriculture (LfL) at Freising (trials in Franconia) and Plant Protection Office (PSA) Hanover (trials in the Hildesheim Plain). In an annual round robin test, systematic differences between the four laboratories were detected (data not shown). In the overall analysis of white sugar yield and Pi-levels, values were adjusted using laboratory specific correction factors. These were calculated for each laboratory and year as the deviation from the mean value by all laboratories in the round robin test.

According to the different techniques of the respective laboratories, cysts were either extracted by density centrifugation or a modified Oostenbrink elutriator (EPPO, 2013). Cysts were extracted from a defined quantity of soil (depending on the laboratory 200 to 300 g). After cysts were crashed by a modified revolving grinding mill, the number of eggs + juveniles was determined at minimum dilution of 30 mL and at higher dilutions if the sample exceeded 20 cysts. The viable eggs and juveniles were counted under a microscope in calibrated counting chambers with 1 mL volume. The results were converted to counts per 100 g of soil.

Statistical analysis

The statistical analysis was carried out with SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). Data were subject to analysis of variance using the *proc mixed* procedure. The developed model (Ladewig and Lukashyk, 2007) was also used to estimate white sugar yield at given Pi-levels by regression. To compare parameter means, a multiple post-hoc Tukey test ($p < 0.05$) was applied. The Pi- and Pf-values were log_{10}-transformed to obtain normal distribution ($log_{10}(x + 1)$).

Results

Population density of Heterodera schachtii

The trial sites had different levels of nematode infestation before cultivation of oilseed radish ranging from 116 to 1783 E + J · 100 g^{-1} soil (Tab. 2). Population densities increased under susceptible oilseed radish and the 50/50 mix of the susceptible and resistant varieties at six out of thirteen environments (site × year). At seven environments, population densities decreased. Under resistant oilseed radish, population densities decreased in twelve environments. Consequently, Pi_{SB} was highest after susceptible and lowest following resistant oilseed radish, the 50/50 mix was intermediate (except for Ottbergen 2001/02 and 2002/03). Differences between oilseed radish treatments were significant at those five environments with maximum Pi_{SB} of 1500 E + J · 100 g^{-1} soil or higher and at Obernjesa 2005 with max. 615 E + J · 100 g^{-1} soil. Mean reproductive rate (r_{OR}) was 1.59, 1.11 and 0.45 for susceptible oilseed radish, 50/50 mix and resistant oilseed radish, respectively. In general, the variability of nematode population densities was very high in all treatments (data not shown).

The reproductive rate in sugar beet (r_{SB}) was higher in the susceptible variety than in the resistant one at all locations (Tab. 3, Fig. 2) and highest when Pi_{SB} was low (Fig. 3). Varietal differences in r_{SB} were greater after resistant oilseed radish (i.e. at low Pi_{SB}) than after susceptible oilseed radish (i.e. at high Pi_{SB}) (Tab. 3). In the

Tab. 2. Population density of *Heterodera schachtii* (Pi_{SB}) shortly after sowing of a susceptible and a resistant sugar beet variety following susceptible (S) or resistant (R) oilseed radish or a 50/50 mix of both as cover crops, and population density before sowing of oilseed radish cover crop in the preceding year (Pi_{OR}; n = 1); Germany 2001–2005. Pi_{SB} with the same letter within a row were not significantly different (Tukey, $p \leq 0.05$), n = 5–8

			Pi_{OR}	Pi_{SB} (Number of eggs and juveniles · 100 g^{-1} soil)					
				Susceptible sugar beet			Resistant sugar beet		
				Oilseed radish (cover crop)					
Year		Site		S	50/50	R	S	50/50	R
2001/2002	1	Ottbergen	990	850 a	939 a	894 a	766 a	822 a	1043 a
	2	Niedernjesa	1090	145 a	159 a	88 a	408 a	90 a	86 a
	3	Koslar	806	3583 c	1811 b	306 a	3675 c	1656 b	344 a
	4	Geroldshausen	512	238 a	172 a	178 a	214 a	234 a	171 a
2002/2003	1	Ottbergen	807	371 a	483 a	363 a	362 a	514 a	360 a
	2	Niedernjesa	1783	6945 b	4408 b	391 a	6989 b	4180 b	368 a
	3	Koslar	1118	2782 b	2371 b	178 a	2728 b	3011 b	185 a
	4	Geroldshausen	825	1530 b	1068 b	512 a	1456 b	985 b	405 a
2003/2004	3	Niederembt	979	2318 c	1122 b	526 a	2120 c	1052 b	427 a
2004/2005	1	Borsum	694	125 a	99 a	71 a	104 a	94 a	71 a
	2	Obernjesa	838	615 b	344 b	61 a	576 b	296 b	73 a
	3	Kelz	1097	700 a	405 a	235 a	587 a	428 a	193 a
	4	Geroldshausen	116	282 a	265 a	213 a	246 a	240 a	218 a
		Mean	897	1576 b	1050 b	309 a	1556 b	1046 b	303 a

Tab. 3. Reproductive rates of *Heterodera schachtii* in a susceptible and a resistant sugar beet variety (r_{SB}) following susceptible (S) or resistant (R) oilseed radish or a 50/50 mix of both as cover crops, Germany 2002–2005. Treatments with the same letter within a row were not significantly different (Tukey, $p \leq 0.05$), n = 5–8

			Reproductive rate (r_{SB})					
			Susceptible sugar beet			Resistant sugar beet		
			Oilseed radish (cover crop)					
Year		Site	S	50/50	R	S	50/50	R
2002	1	Ottbergen	0.27 a	0.35 a	0.32 a	0.21 a	0.24 a	0.16 a
	2	Niedernjesa	7.83 b	39.52 b	11.92 b	1.01 a	1.72 a	2.11 a
	3	Koslar	0.48 bc	1.28 d	10.02 e	0.18 a	0.23 ab	0.73 cd
	4	Geroldshausen	5.63 b	6.29 b	5.31 b	0.39 a	0.72 a	0.72 a
2003	1	Ottbergen	1.16 bc	0.95 ac	1.57 c	0.46 a	0.57 ab	0.55 ab
	2	Niedernjesa	0.39 bc	0.62 cd	1.27 e	0.19 a	0.25 ab	1.03 de
	3	Koslar	3.99 bc	3.69 bc	12.13 c	0.22 a	0.20 a	1.30 ab
	4	Geroldshausen	0.75 ac	0.88 bc	1.71 d	0.42 a	0.48 a	1.09 cd
2004	3	Niederembt	2.60 b	6.98 bc	13.72 c	0.47 a	1.03 a	4.21 b
2005	1	Borsum	1.29 ac	1.98 bc	2.21 c	0.63 a	0.76 ab	1.00 ac
	2	Obernjesa	3.20 ac	2.59 ac	8.82 c	1.02 a	1.08 ab	5.91 bc
	3	Kelz	5.00 bc	8.17 c	27.07 d	2.11 a	2.58 ab	4.70 ac
	4	Geroldshausen	8.87 cde	8.60 e	6.33 be	1.34 ab	2.26 abc	1.51 ad
		Mean	3.19 cd	6.30 de	7.88 e	0.67 a	0.93 ab	1.92 bc

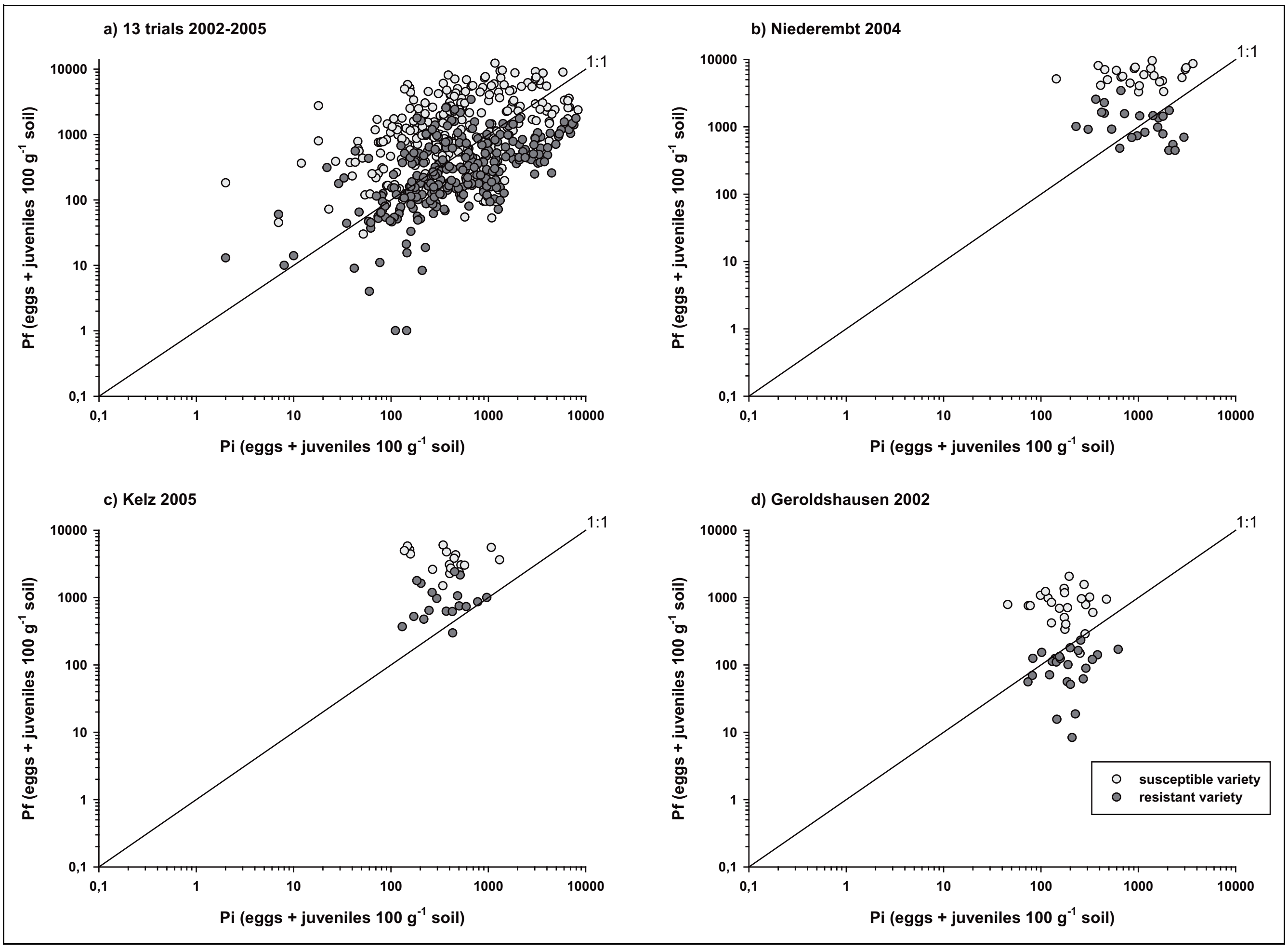

Fig. 2. Population density of eggs and juveniles of *Heterodera schachtii* shortly after sowing (Pi) and shortly before harvest (Pf) of a nematode-susceptible and a nematode-resistant sugar beet variety. Thirteen environments, Germany 2002–2005 (a), Geroldshausen 2002 (b), Niederembt 2004 (c) and Kelz 2005 (d); n = 5–8.

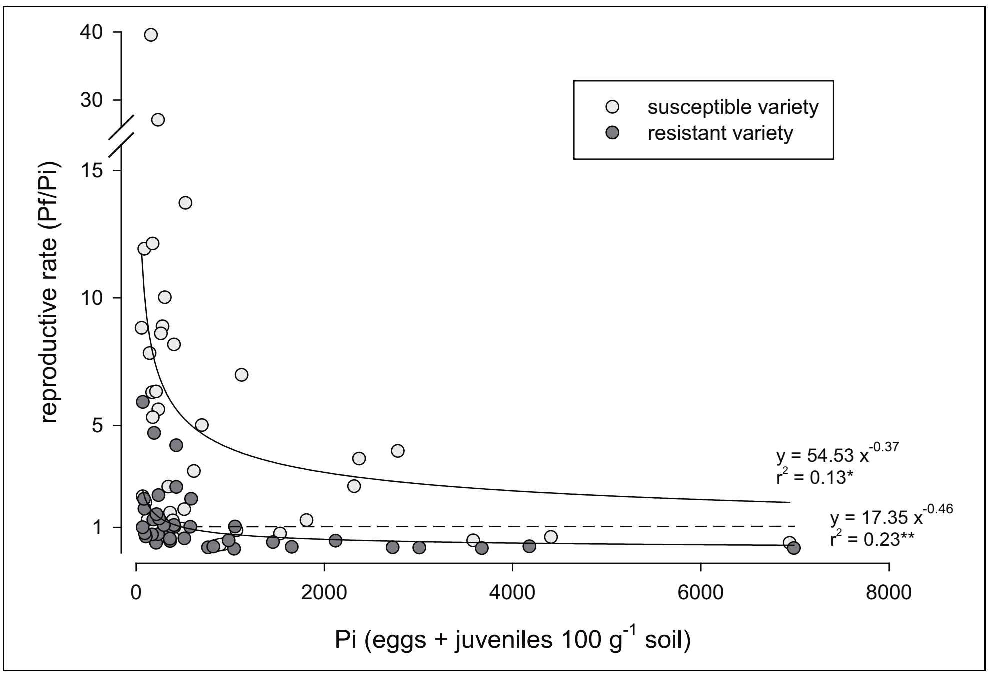

Fig. 3. Relation between initial population density (Pi) of *Heterodera schachtii* and reproductive rate in a susceptible and a resistant sugar beet variety. Thirteen environments, Germany 2002–2005. *, **: significant at $p \leq 0.05$ and $p \leq 0.01$; n = 5–8.

susceptible variety, reproductive rates > 1 were found at all environments except Ottbergen 2002, where Pi_{SB} was about 900 E + J · 100 g soil^{-1} (Tab. 2, Tab. 3). Contrastingly, a population increase was found at Koslar 2003 at Pi_{SB} beyond 2300 E + J · 100 g soil^{-1} resulting in a maximum Pf_{SB} of 7636 E + J · 100 g soil^{-1} in the susceptible variety (data not shown). In the resistant sugar beet variety, reproductive rates > 1 were found at eight environments in the resistant oilseed radish treatment, at four of these environments in the susceptible oilseed radish treatment as well (Tab. 3). Comparing all sites, equilibrium density of the population was at a maximum at Niederembt 2004 and at Kelz 2005 being close to 1000 E + J 100 g^{-1} soil (Fig. 2b and 2c) whereas reproductive rates were considerably lower at Niederembt 2004 than at Kelz 2005 (Tab. 3). At all other environments, population growth could be detected at Pi_{SB} below 400 E + J 100 g^{-1} soil and lower (Fig. 2d).

Yield and quality of sugar beet

Root yield was higher in the resistant variety Paulina (mean 74.1 t ha^{-1}) than in the susceptible Macarena (mean 68.9 t ha^{-1}) at all environments (Fig. 4). Mean sucrose concentration was lower in the resistant (17.1%) than in the susceptible (17.7%) variety whereas the mean concentrations of potassium, sodium and amino-N were higher in the resistant (44.1, 7.3 and 16.2 mmol 1000 g^{-1} beet) than in the susceptible (32.7, 5.9 and 9.6 mmol 1000 g^{-1} beet) variety. Pi_{SB} had no significant effect on any of the measured quality parameters.

Averaged over all trials, in plots following susceptible oilseed radish and the 50/50 mix, white sugar yield was higher in Paulina than in Macarena, whereas Macarena yielded higher than Paulina following resistant oilseed radish (Tab. 4). Differences between the sugar beet varieties were greater in the susceptible (significant at four locations) than in the resistant oilseed radish treatment (significant at two locations). Average yield gain following resistant compared to susceptible oilseed radish was 0.89 t white sugar ha^{-1} in Macarena and 0.36 t ha^{-1} in Paulina. The 50/50 mix of the oilseed radish varieties was intermediate. However, this effect was not consistent at all environments. Significantly higher yield in Paulina than in Macarena could be realised at low initial population densities of *H. schachtii* (Geroldshausen 2002, Ottbergen 2003; both sites without significant variation in Pi_{SB}). At other environments with high Pi_{SB}, Paulina yielded lower than Macarena in all oilseed radish treatments (Niedernjesa 2003 and Koslar 2003).

The effect of increasing population densities of *H. schachtii* on white sugar yield was demonstrated by calculating expected values for white sugar yield at Pi_{SB} levels of 500, 1500, 2500 and 3500 E + J 100 g^{-1} soil (Fig. 5). With increasing Pi, expected white sugar yield decreased in both sugar beet varieties, but much more rapidly in the susceptible than in the resistant one.

Discussion

Methodology

The aim of the present study was to develop a new concept for variety trials with nematode resistant or tolerant sugar beet varieties to solve the problems of inhomogeneous distribution of *H. schachtii* in the field and/or of population densities too low to result in yield response of sugar beet. Presumably, populations more homogenous but with on-site variation were achieved by growing oilseed radish with different levels of nematode resistance/susceptibility as preceding intercrop. A similar setup was successfully established by Schlang and Müller (1996) at a single environment. In our study, changes in nematode population density showed the expected trend according to the preceding oil radish treatments, but significantly

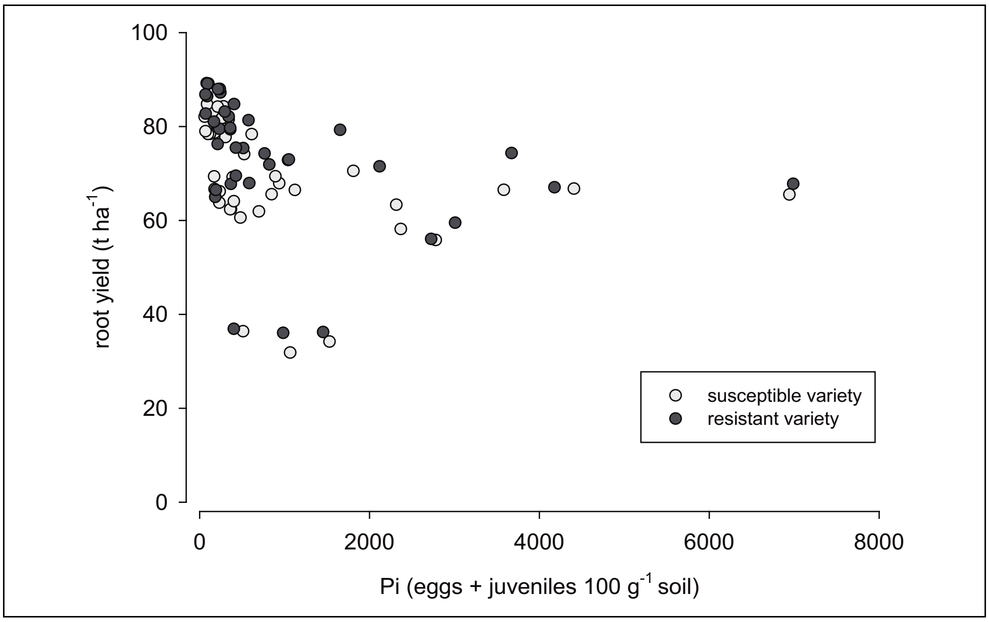

Fig. 4. Initial population density (Pi) of *Heterodera schachtii* and root yield of a susceptible and a resistant sugar beet variety. Thirteen environments, Germany 2002–2005; n = 5–8.

Tab. 4. White sugar yield of sugar beet susceptible or resistant to *Heterodera schachtii* following susceptible (S) or resistant (R) oilseed radish or a 50/50 mix of both as cover crop, Germany 2002–2005. Treatments with the same letter within a row were not significantly different (Tukey, $p \leq 0.05$), n = 5–8

			White sugar yield (t ha⁻¹)					
			Susceptible sugar beet			Resistant sugar beet		
			Oilseed radish (cover crop)					
Year		Site	S	50:50	R	S	50:50	R
2002	1	Ottbergen	9.11 a	9.34 a	9.54 a	9.59 a	9.37 a	9.28 a
	2	Niedernjesa	12.91 a	13.03 a	13.17 a	12.35 a	12.67 a	13.05 a
	3	Koslar	10.39 a	11.21 ab	12.24 d	10.99 bc	11.83 cd	12.32 d
	4	Geroldshausen	9.76 ac	10.21 ab	11.91 bc	10.87 bd	11.48 cd	11.53 bc
2003	1	Ottbergen	10.08 ac	9.83 bc	10.02 ab	11.75 bdf	11.23 ade	11.84 cef
	2	Niedernjesa	11.43 ab	11.70 ab	12.15 b	11.17 ab	11.08 ab	11.00 a
	3	Koslar	8.84 ab	9.33 ab	10.69 c	8.37 a	8.81 ab	9.67 bc
	4	Geroldshausen	5.91 a	5.66 a	6.28 a	5.57 a	5.69 a	5.74 a
2004	3	Niederembt	9.64 a	10.30 ab	11.40 d	10.52 bc	10.79 bd	11.20 cd
2005	1	Borsum	12.68 a	12.93 a	12.78 a	13.07 a	13.39 a	12.89 a
	2	Obernjesa	12.66 a	13.29 a	13.28 a	12.78 a	13.10 a	12.97 a
	3	Kelz	10.77 a	11.07 a	11.34 a	11.60 a	11.85 a	11.36 a
	4	Geroldshausen	13.39 a	13.11 a	13.55 a	13.13 a	13.21 a	13.17 a
		Mean	10.53 a	10.82 ab	11.42 b	10.88 ab	11.10 ab	11.24 ab

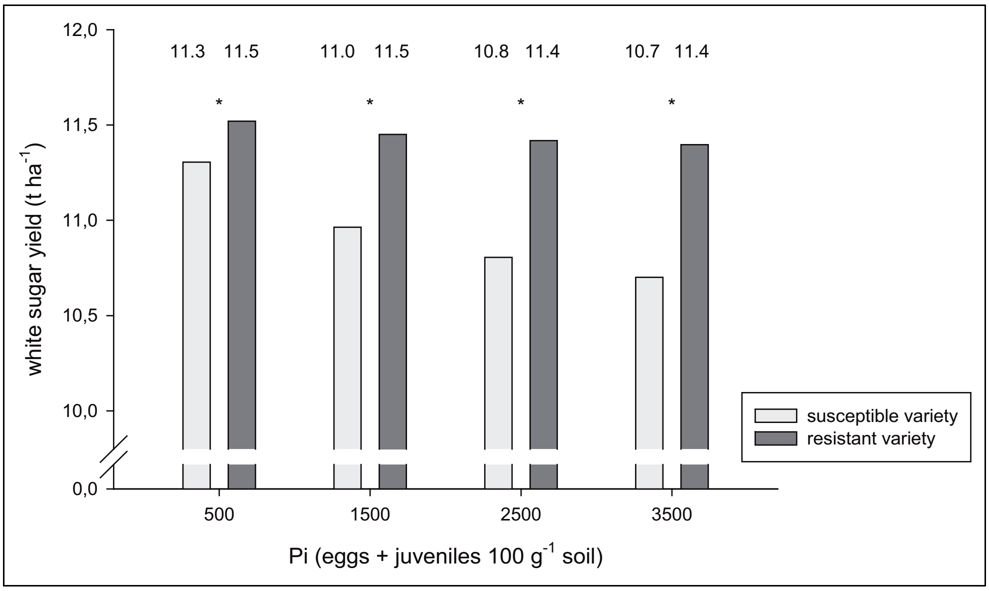

Fig. 5. Influence of population density (Pi) of *Heterodera schachtii* on white sugar yield of a susceptible and a resistant sugar beet variety (expected values). Twelve environments, Germany 2002–2005. Asterisks indicate significant differences between the varieties at a given Pi level (Tukey, $p \leq 0.05$).

different population densities were achieved only at six out of 13 environments. Significant varietal differences in r_{SB} and white sugar yield mainly occurred at these six environments. It is thus arguable whether trials without successful on-site variation of Pi_{SB} should have been pursued at all, but at two of these environments, significant differences in white sugar yield were measured.

Differences in Pi_{SB} between oilseed radish treatments tended to be larger after sowing in July than after sowing in August and the four highest population densities were achieved after sowing in July. This is in accordance with previous studies demonstrating that changes in the population density of *H. schachtii* are highly depending on the sowing date of the cover crop (KOCH and GRAY, 1997;

Müller and Steudel, 1983). In the 2004/05 trials, population densities decreased or remained on a low level at all environments, even after susceptible oilseed radish sown already in May (Borsum and Obernjesa). So, this whole season-long crop did not seem to provide any benefit for nematode population density adjustments.

Population dynamics of Heterodera schachtii *in sugar beet*

The reproductive rate of *H. schachtii* in sugar beet (r_{SB}) differed between environments and was influenced by both sugar beet variety and Pi_{SB} as a result of the preceding oilseed radish treatment. As expected, the r_{SB} was lower in the resistant variety than in the susceptible one and higher at low than at high Pi_{SB} in both varieties as reported before (Heijbroek et al., 2002; Schlang and Müller, 1996). Varietal differences in r_{SB} were greater following resistant than following susceptible oilseed radish.

Reproductive rates > 1 were observed in both sugar beet varieties. Multiplication of the nematode in resistant sugar beet can be attributed to incomplete transmission of the resistance gene during the seed production process (Müller, 1999; Niere, 2009). For example, the transmission rate in Paulina was indicated at 92% (Niere, 2009), meaning that 8% of the plants will be fully susceptible to infection by *H. schachtii*. Müller et al. (1995) demonstrated that with a share of 7% of susceptible plants in a resistant variety, multiplication of *H. schachtii* can occur. While this biological phenomenon presumably impacted our study, current resistant sugar beet varieties have higher transmission rates than Paulina (Niere, personal communication), and suppress population densities more strongly (Krüssel and Warnecke, 2014).

The transmission rate substantially controls the host specific equilibrium density of a variety, at which r = 1 (Schlang and Müller, 1996). In the resistant variety, equilibrium density was approximately 300 E + J 100 g^{-1} soil or lower which is in accordance with values reported by Heinrichs (2000) for another nematode resistant sugar beet variety. However, great differences between environments became obvious as maximum equilibrium density was 1000 E + J 100 g^{-1} at Niederembt 2004. The reason for this variation remains unclear. As seeds from the same lot were used in all thirteen trials, different equilibrium densities at the environments cannot be due to differences in transmission rate. No environmental factor – soil or weather conditions – distinguishing trials with high and low equilibrium densities was identified on the basis of the available data. Environment-specific differences in equilibrium density were thus presumably due to an unknown variance of biological reproductive patterns (e.g. seedling penetration, larval emergence or fitness and virulence) between certain local nematode populations as they were described by Griffin (1981) and Lange et al. (1993).

In summary, although a general pattern was noted with overall reproductive rates being as expected, great differences between environments occurred and neither r_{OR} nor r_{SB} were predictable due to unknown environmental factors.

Sugar beet yield and quality

White sugar yield decreased with increasing Pi_{SB} in both varieties but more severely in Macarena than in Paulina, i.e. the resistant variety exhibited a certain degree of tolerance to *H. schachtii*. Heijbroek et al. (2002) also found that nematode resistant sugar beet suffered yield losses with increasing Pi, and explained this effect with the physiological cost of the hypersensitive response of the beet to invading juveniles. Independent of nematode population density, sucrose concentration was lower and concentrations of root impurities were higher in the resistant than in the susceptible variety. Poor root quality may be a consequence of the introduction of resistance genes (Biancardi et al., 2005). However, an effect of Pi_{SB} on root quality was not measured in either of the two varieties supporting finds for sucrose concentration by Cooke and Thomason (1978). By contrast to our results, Deumelandt et al. (2010) reported that concentrations of Na and amino-N in sugar beet decreased significantly with increasing nematode population density.

Resistance to pests or diseases in agricultural crops often comes along with a yield penalty in the absence of the pathogen (Brown, 2002). Schlang and Müller (1996) confirmed this effect for nematode resistant sugar beet hybrids. In the present study, the resistant variety did not necessarily yield lower at low Pi_{SB} than the susceptible one but site-specific differences in yield response occurred. A positive yield effect of the resistant compared to susceptible oilseed radish as cover crop became obvious at most environments, especially in the susceptible variety and where great variation in nematode population had been achieved (e.g. Koslar 2002, 2003). At Niedernjesa 2003, however, yield response was relatively light despite high variation in Pi_{SB}, presumably caused by severe drought stress that can limit the pest activity (Steudel et al., 1981). Due to these heterogenous and partly controversal findings, a general relation between Pi_{SB} and yield response valid for all environments could not be derived. An unpredictable relation of *H. schachtii* population density to sugar beet yield was reported before (Steudel and Thielemann, 1970, 1979) although close relations were found at single environments (Arndt, 2002; Deumelandt et al., 2010; Heijbroek et al., 2002).

In both varieties, other environmental factors beyond population density must have influenced yield response to *H. schachtii*. In some cases, the deep and heavy soils at the trial sites may have buffered the nematode's influence which is more severe on light soils (Santo and Bolander, 1979), and a certain annual effect became evident in 2003 with low precipitation rates. Furthermore, especially at sites with high population densities of *H. schachtii*, parasitic fungi can prevent a yield effect of the nematodes (Balke, 2001; Steudel et al., 1990). The relation between nematode population density and white sugar yield may have been closer if population density below the topsoil had also been measured. Steudel et al. (1989) found high densities of *H. schachtii* in 30–40 cm soil depth and Westphal (2013) demonstrated that deep occurring *H. schachtii* in

30–60 cm depth can decrease white sugar yield of sugar beet.

Finally, data interpretation was additionally complicated by laboratory specific differences in the measured nematode population densities. In further studies, all samples should thus be analysed by the same laboratory to get more homogeneous data allowing for a more precise evaluation.

Conclusions

Population dynamics of *H. schachtii* in both oilseed radish and sugar beet were highly variable across environments. White sugar yield decreased with increasing Pi_{SB} in both susceptible and resistant sugar beet, but this relation was not consistent in each trial and yield response was thus hardly predictable. Due to year interactions, the need for precise timing of cover crop sowing and the high space requirements when testing a large number of entries, the introduced methodology is not suitable to establish a new system for official variety testing of sugar beet. The results obtained are nevertheless valuable for agricultural extension since the high number of field trials reveals the complexity of both population dynamics of nematodes and yield response of sugar beet. Resistant sugar beet can be part of a management strategy in fields with high nematode population density as they can suppress nematode population density by the same factor as resistant oilseed radish.

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Introduction Sugar beet (Beta vulgaris L.) is an important arable crop that plays a vital role in the crop rotation systems of northern hemisphere agriculture. It produces about 27% of the total world sucrose production annually (http://www.faostat.fao.org).Â Some sugar beet accessions initially developed for resistance to H. schachtii by interspecific hybridisation with species of Procumbentes showed partial resistance (reviewed by Van Geyt et al., 1990). Partial resistance to H. trifolii was also found in advanced breeding line of B. vulgaris spp. maritima crossed with sugar beet (Mesken & Lekkerkerker, 1988).Â Resistant sugar beet lines showed a low rate of maggot survival and less yield loss than susceptible varieties. Production of haploid sugar beets (Beta vulgaris L.) by ovule culture. [In:] Genetic Manipulation in Plant Breeding, W. Horn, C.J. Jerser, W. Odenbach, O.Schieder, (eds). Walter de Gruyter Berlin, New York. 307â€“309. Doctrinal M., Sangwan R.S., Sangwan-Norreel B.S. 1989. In vitro gynogenesis in Beta vulgaris L.: Effects of plant growth regulators, temperature, genotypes and season. Plant Cell Tiss. Org.