Eur J Plant Pathol (2009) 123:27–36
DOI 10.1007/s10658-008-9335-9

Effects of plants containing pyrrolizidine alkaloids on the northern root-knot nematode *Meloidogyne hapla*

Tim C. Thoden · Johannes Hallmann · Michael Boppré

Received: 6 February 2008 / Accepted: 8 May 2008 / Published online: 19 June 2008

Abstract 1,2-Dehydropyrrolizidine alkaloids (PAs), known to be nematotoxic *in vitro*, represent a class of secondary plant metabolites from hundreds of plant species worldwide. Pot experiments with the commercially available PA-containing plants *Ageratum houstonianum*, *Borago officinalis*, *Senecio bicolor*, and *Symphytum officinalis* demonstrate that *Meloidogyne hapla* is not *per se* repelled by these plants as all species were infested with nematodes. However, the development of *M. hapla* juveniles was completely suppressed on *A. houstonianum* and *S. bicolor*. Soil in which *A. houstonianum* and *S. bicolor* were cultivated and incorporated contained 200–400 times less nematodes than soil treated with *Lycopersicon esculentum*. Depending on their qualitative composition of PAs at least some of these plants thus appear to be valuable tools for integrated root-knot nematode management.

T. C. Thoden (✉) · M. Boppré
Forstzoologisches Institut, Albert-Ludwigs-Universität,
79085 Freiburg i.Br., Germany
e-mail: tim.thoden@fzi.uni-freiburg.de

J. Hallmann
Julius Kühn-Institut, Bundesforschungsinstitut
für Kulturpflanzen, Institut für Epidemiologie
und Pathogendiagnostik,
Toppheideweg 88,
48161 Münster, Germany

Keywords Biological control · Integrated pest management (IPM) · Secondary plant metabolites · Soil amendment · Green manure · Botanicals · *Crotalaria* spp.

Abbreviation
PAs pyrrolizidine alkaloids = 1,2-dehydropyrrolizidines

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most hazardous soilborne plant parasites and are responsible for large economic losses in a wide variety of crops worldwide. In Northern Europe *Meloidogyne hapla* is currently causing problems on several economic crops in conventional as well as organic farming (Hallmann et al. 2007). For several decades the management of plant-parasitic nematodes has been dominated by the use of synthetic nematicides. Their application is problematic because of negative environmental impacts and, consequently, many nematicides have been withdrawn from the market. Therefore, there is a strong demand to develop more sustainable and environmentally-friendly methods for nematode control.

One such alternative is seen in the use of plants containing nematicidal secondary plant metabolites (Halbrendt 1996; Chitwood 2002). Two well known and already applied examples are marigolds (*Tagetes* spp.) and cruciferous plants (*Brassica* spp.) (Ploeg

2002; Zasada and Ferris 2004). In the case of marigolds the nematicidal effect is due to α-terthienyl, a thiophene, produced in the roots (Topp et al. 1998). For cruciferous plants it has been shown that some hydrolysis products from glucosinolates—so called isothiocyanates—, set free during degradation of the plant, are nematotoxic (Lazzeri et al. 2004).

We consider 1,2-dehydropyrrolizidine alkaloids (PAs), a class of secondary plant metabolites found in many Asteraceae, Boraginaceae, Fabaceae and Apocynaceae (Rizk 1991) to have potential for nematode management. PAs are well-known as feeding deterrents against herbivores and are toxic for a wide range of non-adapted animals (Narberhaus et al. 2005 and references therein). Previous studies with the PA-containing *Chromolaena odorata* revealed a nematicidal *in vitro* effect of purified PAs as well as a beneficial, i.e. nematode-reducing, effect of an application of PA-containing plant material as mulch or aqueous plant extract (Thoden et al. 2007). We expect, therefore, that a wide range of PA-containing plants might be promising candidates for nematode management.

Of the more than 560 plant species known to contain PAs (Rizk 1991), we chose four commercially available and partly also commercially cultivated PA-plants: borage (*Borago officinalis*), comfrey (*Symphytum officinalis*), silver ragwort = dusty miller (*Senecio bicolor*) and floss flower (*Ageratum houstonianum*). Their PAs have been identified (Larson et al. 1984; Kim et al. 2001; Wiedenfeld and Andrade-Cetto 2001; Wiedenfeld et al. 2006) and these species have also been part of nematode host studies and/or studies concerning nematicidal effects of plant extracts made from them (Moreno et al. 1992; McSorley and Frederick 1994; Walker et al. 1994; Brinkmann et al. 1996; Lordello and Lordello 1996; Bernard and Jennings 1997; Dias et al. 2000; Costa et al. 2001).

The objectives of our study were: (i) to evaluate the host status of these plants for *M. hapla* and (ii) to investigate their nematicidal potential when being cultivated and subsequently used for soil amendment.

Materials and methods

Plant material nematodes

Seeds of the PA-plant species *A. houstonianum* (cv. Capri; Asteraceae), *B. officinalis* (Boraginaceae), *S. officinalis* (Boraginaceae) and *S. bicolor* (formerly *Senecio cineraria*, *S. maritimus*, *Cineraria maritima*) (cv. Silberzwerg; Asteraceae) were purchased from Kiepenkerl (Kiepenkerl Pflanzenzüchtung, Everswinkel, Germany). Tomato (*Lycopersicon esculentum* cv. 82 Davis), barley (*Hordeum vulgare* cv. Adonis) and bare fallow were used as controls.

M. hapla Chitwood 1949, originally collected from a tomato field near Münster, Germany, were cultivated on tomato (cv. 82 Davis) under greenhouse conditions (20± 3°C). Second stage juveniles (J2) of *M. hapla* were extracted from heavily galled tomato roots by the mistifier extraction technique described by Hooper et al. (2005).

Host study

For two separate experiments considering: (i) the infestation of PA-plant species with *M. hapla* and (ii) the reproduction of *M. hapla*, seeds of the different plant species were germinated in sterile sand and seedlings were 5–10 days later transplanted into small (∅=8 cm) or large (∅=12 cm) plastic pots, filled with either 200 or 700 ml of steam sterilised field soil/sand mixture (1:2, *v*/*v*), respectively. Three days later the small pots were inoculated with 400 J2 and the large pots with 5,600 J2 of *M. hapla*. Each treatment was repeated 12 times. Pots were kept in a greenhouse at 20±3°C, arranged as randomised complete blocks, fertilised once a week with Wuxal® (0.3%, *v*/*v*, N/P_2O_5/K_2O—8:8:6; Wuxal®, Bayer Crop Science, Germany) and watered regularly.

Infestation of PA-plants by M. hapla Seven days after nematode inoculation, plants from the small pots were removed to measure juvenile penetration into the roots. The roots were carefully washed free of soil debris in a waterbath, bleached for 5–10 min in an aqueous solution of commercial bleach (20%, *v*/*v*; DanKlorix®, Colgate, Germany) and afterwards stained with acid fuchsin (Byrd et al. 1983). The stained roots were pressed between glass plates and the number of juveniles inside the roots counted under a stereomicroscope (×20–50). In addition, we also differentiated between second and third stage juveniles (J3).

Reproduction of M. hapla *on PA-plants* To measure reproduction of *M. hapla*, the remaining plants (large pots) were cultivated for another 9 weeks (in total 70 days). Then the plants were removed from the pots as described above and evaluated for: (i) the degree of

root galling using the index described by Zeck (1971), (ii) the number of eggs per root system and (iii) the number of J2 per 100 ml soil. Eggs were obtained by chopping the entire root system into 2–3 cm segments and shaking them for 3 min in 100 ml aqueous NaOCl solution (0.53%, *w*/*v*; Hussey and Barker 1973). The number of eggs was determined in 100 µl aliquots. J2 of *M. hapla* were extracted from 100 ml soil using the Baermann technique (extraction for 48 h).

Soil amendment study

The impact of cultivating and subsequently incorporating PA-plants on the population density of *M. hapla* was determined by using plastic pots (∅=21 cm) filled with 3.6 l of a steam sterilised field soil/sand mixture inoculated with approx. 18,000 J2 of *M. hapla* per pot. Seven to 15 seeds of either *B. officinalis*, *S. officinalis*, *A. houstonianum*, *S. bicolor* or the non-PA-plants *L. esculentum* and *H. vulgare* were sown into each pot and soon after germination thinned to five (*B. officinalis*, *S. officinalis*, *L. esculentum*), seven (*H. vulgare*) or ten (*A. houstonianum*, *S. bicolor*) plants per pot. In addition we included two further treatments: (i) a bare fallow representing the natural decline of nematodes in the absence of a plant host and (ii) cultivating and incorporating *B. officinalis* in *M. hapla*-free soil as an indicator for potential phytotoxic effects. Each treatment was repeated 12 times and plants were kept in a greenhouse at 20±3°C arranged in randomised blocks, watered regularly and fertilised weekly with Wuxal® (0.3%, *v*/*v*, $N/P_2O_5/K_2O$—8:8:6; Wuxal®, Bayer Crop Science, Germany).

Nine weeks (63 days in total) later the plants—except *H. vulgare*—were removed from each pot. For each treatment, seven randomly chosen root samples were evaluated for the number of root galls and eggs using the same procedures as described for the host study. The soil from each pot was mixed in a plastic bowl and 100 ml used for nematode extraction as described above (Baermann technique). The corresponding plants of each pot were then weighed, cut into small pieces (2–3 cm) and incorporated into the soil which was then transferred back into the pots. The bare fallow treatment was treated in the same way. In the treatment with *H. vulgare*, plants were left growing and 100 ml soil aliquots sampled directly in the pots by using a soil sampler.

Five weeks after incorporating the plant material (98 days in total), the soil from each pot was mixed in a plastic bowl again and another 100 ml used for nematode extraction as described above. In the treatment with *H. vulgare* plants were uprooted and discarded. A single 14 day-old tomato seedling—as a bioindicator for *M. hapla* infestation—was then planted in each pot and cultivated for 10 weeks as described above. At harvest (168 days in total), plant fresh weight (roots plus leaves) and root galling were recorded (Zeck 1971), and nematodes were extracted from 100 ml soil as described before.

PA-analyses

Quantitative and qualitative composition of PAs For the quantitative and qualitative determination of PAs we analysed: (i) two samples of each PA-plant species from the soil amendment study (63 days old; separated into roots and leaves) and (ii) seedlings of each PA-plant species from the host study (10 days old; only roots). Samples were oven-dried for 24 h at 30°C, weighed, ground with pestle and mortar and total PAs, i.e. PA free bases and their *N*-oxides, extracted as described in Thoden et al. (2007). It is important to note that this extraction method is accompanied by a reduction of the PA *N*-oxides into their free bases.

To check the presence and qualitative composition of PAs, the purified plant extracts were subjected to TLC-analyses (Molyneux and Roitman 1980). These were conducted on Alugram® silica gel TLC-plates (0.20 mm Silica Gel 60, Macherey-Nagel, Düren, Germany). Ten to 40 µg of the purified extracts dissolved in methanol were spotted on the plates. The TLC-plates were developed in a mixture of methanol–dichloromethane–ammonia (15:82:3, *v*/*v*) in saturated glass chambers. The air-dried plates were sprayed with *o*-chloranil (1 g in 70 ml chloroform), heated for 3 min at 75°C and finally resprayed with Ehrlich-Reagent (10 g 4-dimethylaminobenzaldehyde dissolved in 90 ml hydrochloric acid).

Determination of PA N*-oxides and PA free bases* To get an initial estimation of the proportion of PA *N*-oxides and free bases present in the plant material, we carried out some TLC-analyses with the crude plant extracts before the reduction step. Three-hundred to 400 µg of the dried crude extracts dissolved in methanol were spotted on the plates. For detecting PA-free bases plates were developed as described above. For detecting PA *N*-oxides plates were developed as described by Thoden et al. (2007).

Data analyses

All data were analysed using SPSS® 14.0. Before we separated treatment means by ANOVA, the data were first tested for equality of variances (Levene tests). If this was not the case the data were either transformed to $\log_{10}(x+1)$ or—if percent values—to arcsin values and again tested for equality of variances. Treatment means were then separated by ANOVA using either the Duncan test if equality of variances was given, or the Games–Howell test if not.

Results

Host study

Infestation of PA-plants by M. hapla Ten days after inoculating 400 J2 per plant, all PA- and non-PA-plant species exhibited clear symptoms of nematode infestation. The degree of galling varied significantly, *S. officinalis* being the most and *A. houstonianum* the least affected one (Table 1). Significantly more J2 had penetrated the roots of *L. esculentum* and *S. officinalis* than the roots of *S. bicolor* and *A. houstonianum* (Table 1). The proportion of juveniles that had already reached the J3-stage was three to ten times higher for roots of *B. officinalis*, *S. officinalis* and *L. esculentum* than for roots of *A. houstonianum* and *S. bicolor* (Table 1).

Reproduction of M. hapla *on PA-plants* Seventy days after inoculating 5,600 J2 per plant, root galling was most severe on *S. officinalis*, *L. esculentum* and *B. officinalis*, but root galls were also present on *S. bicolor* and *A. houstonianum* (Table 2). Despite the presence of galls, we did not find any eggs of *M. hapla* on roots from *S. bicolor* and *A. houstonianum*, indicating that these two PA-plants do not allow completion of the life cycle (i.e. are resistant) (Table 2). In contrast, we found eggs on both of the other PA-plants and on *L. esculentum* (Table 2). However, there were approximately ten times as many eggs on *L. esculentum* as on *B. officinalis* and *S. officinalis*. The number of J2 per 100 ml soil also differed drastically and reflected the egg density on the roots (Table 2). So, we found 1,000 times more J2 in soil from *L. esculentum* and *S. officinalis* than in soil from *A. houstonianum* or *S. bicolor*.

Soil amendment study

Sixty-three days after inoculating 18,000 J2 per pot, all PA-plant species again showed symptoms of nematode infestation (Table 3). As in the host study the reproduction of *M. hapla* was only completed on *L. esculentum*, *S. officinalis* and *B. officinalis* (Table 3). The number of eggs was again significantly higher for *L. esculentum* than for *S. officinalis* and *B. officinalis*.

The effect of first cultivating and then incorporating PA-plants on the population density of *M. hapla* is shown in Table 4. After growing the different plant species for 63 days the amount of produced and incorporated biomass differed significantly (Table 3). At this time most J2 were found in soil from *L. esculentum* and the fallow, and fewest in soil from *H. vulgare*, *S. officinalis* and *S. bicolor* (Table 4). The overall small numbers of extracted J2 indicated that the life-cycle of *M. hapla* was not yet fully completed.

Five weeks after the incorporation of the plant material (98 days in total) these numbers had changed (Table 4). There was an increase in the number of juveniles from soil treated with *B. officinalis*, *S.*

Table 1 Infestation of plants containing pyrrolizidine alkaloids 10 days after inoculating 400 J2 of *Meloidogyne hapla* per plant

Plant species	No. of root galls per plant, D	No. of J2 per root system, D	% J3 of *M. hapla*, D
Ageratum houstonianum	4.17 d	33.00 c	0.05 c
Borago officinalis	9.00 ab	52.33 bc	0.27 b
Senecio bicolor	6.09 c	32.55 c	0.09 c
Symphytum officinalis	10.75 a	84.50 a	0.38 ab
Lycopersicon esculentum	7.75 bc	72.58 ab	0.51 a

Values are means of 12 repetitions. Means in the same column followed by the same letter are not significantly different following Duncan or Games Howel test ($P \leq 0.05$)

D Duncan

Table 2 Reproduction of *Meloidogyne hapla* on plants containing pyrrolizidine alkaloids 70 days after inoculating 5,600 J2 per plant

	Gall index, D	No. of eggs g^{-1} fresh root, GH	No. of J2 100 ml^{-1}, GH
Ageratum houstonianum	3.08 c	0	6 d
Borago officinalis	6.50 a	13,618 b	220 c
Senecio bicolor	5.00 b	0	16 d
Symphytum officinalis	7.50 a	17,659 b	1,085 b
Lycopersicon esculentum	7.25 a	150,583 a	5,716 a

Values are means of 12 repetitions. Means in the same column followed by the same letter are not significantly different following Duncan or Games Howel test ($P \leq 0.05$)

D Duncan, GH Games Howel

officinalis and *L. esculentum* and a decrease for the numbers of J2 found in soil treated with *A. houstonianum*, *S. bicolor*, *H. vulgare* and the fallow (Table 4). Overall, there were approx. 200–400 times as many J2 in soil from *L. esculentum* and *S. officinalis* than in soil from *S. bicolor* or *A. houstonianum*. However, there was no clear relation between the amount of incorporated biomass and the observed nematode reduction. Therefore, it seems that the observed reduction in nematodes is more likely to be due to the observed resistance than to the incorporation of the plant material.

During the subsequent cultivation period of the tomatoes, nematode numbers increased for all treatments (Table 4); but at the end, there were still 30–45 times more J2 in pots treated with *L. esculentum* than in pots treated with *S. bicolor* and *A. houstonianum*.

Both the root galling of the tomato plants as well as their final fresh weights reflected the reported differences in the final numbers of juveniles (Table 5). The tomatoes were growing significantly better in pots treated with *S. bicolor*, *A. houstonianum* and the fallow than in pots treated with *B. officinalis*, *S. officinalis* and *L. esculentum* (see Table 5, Figs. 1, 2)

As already observed in earlier studies, there was a slight phytotoxic effect from the incorporation of a PA-containing plant like *B. officinalis* which was obvious from stunting in growth (see Fig. 1b).

PA-analyses

Quantitative and qualitative composition of PAs PAs were present in all samples extracted from the host study as well as from the soil amendment study (Fig. 3). Overall, the plants contained a bouquet of two to four PAs (Fig. 3). *B. officinalis* and *S. officinalis* contained a similar mixture of PAs—both belong to the Boraginaceae—which differed from the mixture found in *A. houstonianum* and *S. bicolor*. The PA concentrations, calculated on the basis of the obtained purified extracts, are given in Table 6.

Table 3 Infestation and reproduction of *Meloidogyne hapla* on plants containing pyrrolizidine alkaloids and the amount of biomass used for soil amendment 63 days after inoculating 18,000 J2 per pot

Plant species	No. of galls g^{-1} root, D	No. of eggs per pot, GH	Biomass incorporated g fresh weight, GH
Borago officinalis	13.75 c	3,225 b	138.92 a
Borago officinalis without *Meloidogyne hapla*	–	–	131.50 a
Symphytum officinalis	22.62 b	10,837 b	124.45 ab
Senecio bicolor	42.92 a	0	16.50 c
Ageratum houstonianum	5.85 d	0	89.33 b
Hordeum vulgare	–	–	–
Lycopersicon esculentum	38.74 ab	106,617 a	105.58 b
Fallow	–	–	–

Values are means of seven to 12 repetitions. Means in the same column followed by the same letter are not significantly different following Duncan or Games Howel test ($P \leq 0.05$)

D Duncan, GH Games Howel

Table 4 Number of *Meloidogyne hapla* J2 in 100 ml soil at the point of incorporating the plant material, 5 weeks later and after cultivating tomatoes for 10 weeks

Plant species	No. of J2 100 ml^{-1}		
	At 63 days = time of incorporation, D	At 98 days = 5 weeks after incorporation, GH	At 168 days = 15 weeks after incorporation, GH
Borago officinalis	29 c	547 a	12,976 a
Borago officinalis without *Meloidogyne hapla*	–	–	–
Symphytum officinalis	23 c	1,640 a	12,018 ab
Senecio bicolor	26 c	8 b	418 c
Ageratum houstonianum	30 c	4 b	268 c
Hordeum vulgare	13 c	10 b	3,158 b
Lycopersicon esculentum	90 a	1,481 a	12,466 ab
Fallow	59 b	34 b	706 c

Values are means of 12 repetitions. Means in the same column followed by the same letter are not significantly different following Duncan or Games Howel test ($P \leq 0.05$)

D Duncan, GH Games Howel

Determination of PA N-*oxides and free bases* In the non-reduced crude extract of *S. bicolor* PAs were solely present as *N*-oxides, only free bases could be detected in *A. houstonianum* and both chemical forms were present in the non-reduced crude extracts of *S. officinalis* and *B. officinalis* (Table 6).

Discussion

PAs are known as feeding deterrents against herbivores and *in vitro* studies have shown their nematicidal and nematostatic effects on plant-parasitic nematodes (Thoden et al. 2007 and unpublished). We therefore investigated the effect of commercially available PA-containing plants on *M. hapla*. Our results show that: (i) *M. hapla* is not principally repelled by those plants but some of them are resistant to *M. hapla* and others not, (ii) there is no clear relationship between the observed resistance and the presence of PAs and (iii) the observed reduction in nematodes is more likely to be due to this resistance than to the incorporation of the PA-plant material.

As PAs are feeding deterrents we expected that *M. hapla* might be repelled by plants containing this group of secondary plant metabolites. This was not the case as all species in both experiments were infested with *M. hapla*. However, during the course of the experiments only the juveniles in roots of *B. officinalis* and *S. officinalis* matured whereas those in roots of *S.*

Table 5 Plant growth and infestation of tomatoes planted in pots inoculated with 18,000 J2 of *Meloidogyne hapla* after cultivation and incorporation of different PA-plants

Plant species	Gall index tomatoes after 70 days, GH	Shoot fresh wt tomatoes (g/pot), D	Total fresh wt tomatoes (g/pot), D
Borago officinalis	7.92 a	54.08 cd	103.08 cd
Borago officinalis without *Meloidogyne hapla*	–	64.08 bc	133.00 bc
Symphytum officinalis	8.09 a	46.91 d	85.09 d
Senecio bicolor	3.58 cd	82.08 a	190.25 a
Ageratum houstonianum	2.75 d	70.16 ab	152.25 b
Hordeum vulgare	7.17 b	51.08 cd	130.58 bc
Lycopersicon esculentum	8.50 a	47.08 d	82.66 d
Fallow	5.20 c	77.27 ab	177.27 ab

Values are means of 12 repetitions. Means in the same column followed by the same letter are not significantly different following Duncan or Games Howel test ($P \leq 0.05$)

D Duncan, GH Games Howel

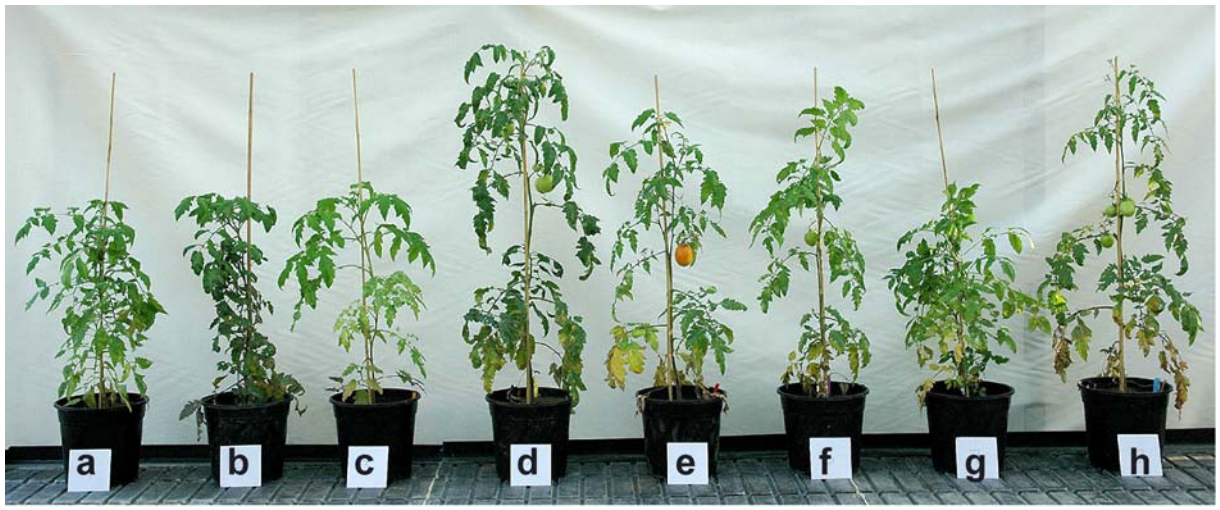

Fig. 1 Plant growth differences between tomatoes planted in pots inoculated with 18,000 J2 of *M. hapla* after cultivation and incorporation of *B. officinalis* (**a**), *S. officinalis* (**c**), *S. bicolor* (**d**), *A. houstonianum* (**e**), *H. vulgare* (**f**), *L. esculentum* (**g**), and fallow (**h**). For comparison cultivation and incorporation of *B. officinalis* without *M. hapla* (**b**)

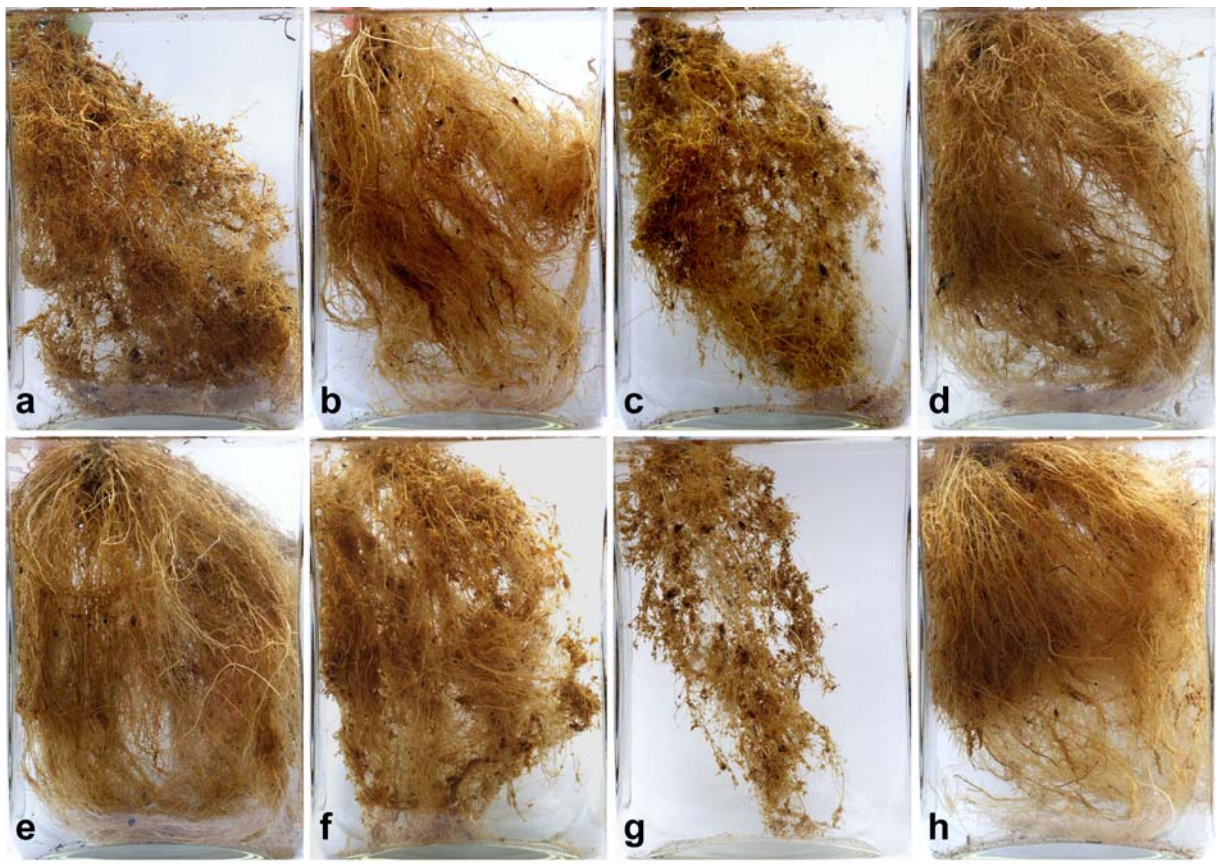

Fig. 2 Differences between roots of tomatoes planted in pots inoculated with 18,000 J2 of *M. hapla* after cultivation and incorporation of *B. officinalis* (**a**), *S. officinalis* (**c**), *S. bicolor* (**d**), *A. houstonianum* (**e**), *H. vulgare* (**f**), *L. esculentum* (**g**), and fallow (**h**). For comparison cultivation and incorporation of *B. officinalis* without *M. hapla* (**b**)

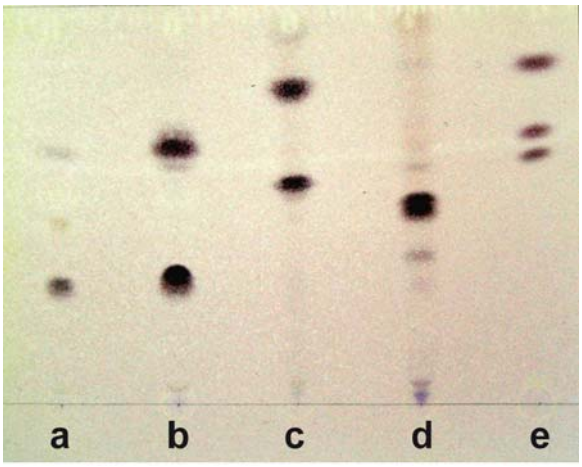

Fig. 3 TLC tracks of 10 day-old roots from the host study: *B. officinalis* (**a**), *S. officinalis* (**b**), *S. bicolor* (**c**), *A. houstonianum* (**d**). For comparison a mixture of the PA-free bases from senecionine, heliotrine and monocrotaline (**e**)

bicolor and *A. houstonianum* died. These two plants can thus be classified as resistant against *M. hapla*, an observation which has been made in other studies on *Senecio* (Townshend and Davidson 1962; Belair and Benoit 1996; Ehwaeti et al. 1999) and—to a lesser extent—*Ageratum* species (McSorley and Frederick 1994; Walker et al. 1994). In addition, there are numerous similar reports on the resistance of *Crotalaria* spp., PA-plants already used for nematode control (Wang et al. 2002; Germani and Plenchette 2004), and other plant species containing PAs (Thoden et al. unpublished).

We further expected to find a clear relationship between the presence of PAs and plant resistance against nematodes. This was not the case as all plant species verifiably contained PAs; resistance against *M. hapla* however was only observed on *A. houstonianum* and *S. bicolor*. We do not believe that PAs have nothing to do with plant resistance against nematodes, as the resistance or non-resistance might be due to differences in: (i) the quantity of PAs, (ii) the qualitative composition of PAs or (iii) the proportion of PA *N*-oxides and PA free bases. To us, the observed differences in the quantities of PAs do not appear to be big enough to explain why *A. houstonianum* and *S. bicolor* were resistant while *S. officinalis* and *B. officinalis* were not. Interestingly the qualitative composition of PAs was quite similar for *B. officinalis* and *S. officinalis* which both were susceptible against *M. hapla*. In contrast, the resistant plants *S. bicolor* and *A. houstonianum* contained different types of PAs. *In vitro* experiments with *M. incognita* have shown that there are big differences in the nematicidal activity of PAs depending on their chemical structure (Thoden et al. unpublished). The same is true for the chemical form in which the PAs are present; either as free base or *N*-oxide. The latter are usually less toxic for vertebrates (Mattocks 1968) and or nematodes (Thoden et al. unpublished). Unfortunately, we found no clear results as in *A. houstonianum* only free and bases in *S. bicolor* only *N*-oxides could be detected.

Besides PAs, the tested plant species are known to contain additional secondary plant metabolites which might be responsible for nematicidal effects. Especially, *Ageratum* spp. contains a broad spectrum of different secondary plant metabolites including flavonoides, polyphenoles and tannins (Quijano et al. 1985). These are also known to exert nematotoxic effects (Wuyts et al. 2006) and might therefore be the responsible agents for the observed resistance. On the other hand, many of these substances have also been identified in *B. officinalis* and *S. officinalis* (Bandoniene and Murkovic 2002).

Despite these open questions our results indicate that from a practical point of view, at least, some PA-containing plants have the potential for nematode management, as the cultivation and incorporation of *S. bicolor* and *A. houstonianum* led to a stronger and longer-lasting reduction in *M. hapla* than the conventional methods such as fallow or rotation with

Table 6 Concentrations (percent dry weight) of pyrrolizidine alkaloids (PAs) extracted from plants of the host and soil amendment study

Plant species	PA concentration shoots (% dry wt)	PA concentration roots (% dry wt)	*N*-oxide vs free base
Ageratum houstonianum	0.26–0.31	0.41–0.48	Free bases
Borago officinalis	0.079	0.3–0.36	Both
Senecio bicolor	0.19–0.74	0.7–1.6	*N*-oxide
Symphytum officinalis	0.14–0.57	0.17–0.37	Both

Values give the lower and upper bound of PA-concentrations from three extracted samples

H. vulgare. Fortunately, both plant species and other PA-containing plants are already grown for economic reasons and therefore it should be possible to obtain sufficient quantities of seed and access to knowledge concerning their cultivation.

Further studies should identify which PAs might be responsible for a plant being resistant or not; we believe that it is the qualitative composition of PAs that determines this difference.

Acknowledgements We are most grateful to Tim Burzlaff and John Edgar for their valuable contributions, to R.I. Vane-Wright for critical revision of the English, to the staff of both the Forstzoologisches Institut and the Julius Kühn-Institut für Kulturpflanzen for technical assistance and to the Deutsche Bundesstiftung Umwelt for financial support. Further, we would like to thank S. Schlag for advice on statistical questions.

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