Root-knot nematode (*Meloidogyne incognita*) infection alters vegetative growth and nitrogen uptake and distribution in grapevine

Feng Pan Wang, Lei Zhang, Yuan Peng Du and Heng Zhai

College of Horticulture Science and Engineering, Shandong Agricultural University, Key Laboratory of Crop Biology of Shandong Province, Tai'an, Shandong, China

Summary

Root-knot nematodes (RKN, *Meloidogyne* spp.) manipulate host cell developmental processes to build specialized feeding structure from which the larvae enlist nutrients. Nitrogen (N) is one of the most important components of plant metabolites, and isotopic tracers make it possible for us to study the transportation of the nitrogen metabolites in the whole plant. In order to figure out vegetative and physiological responses caused by RKN infection in vine, pot experiment was performed in this paper. The results showed that RKN infection weakened vine vigor with decreased biomass and increased root-shoot ratio. Whereas, before bursting the galls exhibited a higher metabolic activity, in comparison with control root, the root dehydrogenase activities improved 85 % and 71 % in the galls and adjacent roots respectively. In addition, RKN infection didn't significantly alter nitrogen content and distribution in various tissues, which might be due to feeding pressure or duration. ^{15}N Root labeling results indicated that RKN infection enhanced Nitrogen derived from fertilizer (Ndff) and nitrogen utilization efficiency of the annual root. It suggested that RKN temporarily turned grape root into nitrogen sinks to meet their demand. Finally, the infected plant retained relatively few storage nutrients in the root and shoot after defoliation.

K e y   w o r d s : root-knot nematode; vegetative growth; nitrogen trace; nutrient concentration.

Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.), plant endoparasitic nematodes, are obligate root pathogens for grape and other species. Different RKN species are widely distributed in vineyards causing direct and indirect damage on grape root system since they were identified worldwide in the last century (Boubals 1954, Lider 1956, Gonzales 1970, Stirling 1976, Smith 1977). The most common species are *M. incognita*, *M. hapla*, *M. arenaria* and *M. javanica* which threaten sustainable development of world viticulture.

It is well-known for the damage mechanism on the host root system caused by RKN (Jones and Payne 1978). The formation of specialized giant cells (GCs) induced by second stage RKN juvenile (J2) is essential for nematode growth and development until the female lay eggs (Abad *et al.* 2003). Compared with the normal root cells, GCs need to absorb more metabolites acting as the sole source of nutrients for the feeding nematode. It has been confirmed that nutrients were transported from phloem to GCs induced by *M. incognita* (Dorhout *et al.* 1993). Glutathione and homo-glutathione are required for *M. incognita* development and reproduction (Baldacci-Cresp *et al.* 2012). Like RKN, phylloxera infestation on the grape root induces nodosities which causes changes in the uptake and transportation of water, minerals and assimilates (Porten and Huber 2003). This suggests that the nodosity enriched in GCs become an abnormal nutrition sink, enlisting nutrients from leaves, stems and other roots. Thus, the loss of nutrients from these other organs causes weakening of plant vigor.

Previous studies have demonstrated that cyst nematode and phylloxera infestation result in accumulation of sugars, free amino acids and amides in developmental syncytia and nodosity (Ryan *et al.* 2000, Kellow *et al.* 2004, Hofmann *et al.* 2010). *Meloidogyne spp.* affects the concentration of non-reducing sugars and macro- and microelements distributions in galls (Melakeberhan *et al.* 1990; Hurchanik *et al.* 2004). Indeed, comprehensive genome-wide expression analysis between inoculated and non-inoculated plant indicates that genes encoding proteins with possible roles in sugar or carbohydrate, metal ion, and amino acid transport that are differentially regulated within developing syncytia and GC (Ithal *et al.* 2007, Jammes *et al.* 2005). It suggests that RKN infection may alter metabolite distribution among different organs, especially for essential substances for RKN. Nitrogen (N) is one of the most important components of plant metabolites; isotopic tracers make it possible for us to study the transfer of the nitrogen metabolites. Herein we are reporting the vegetative and physiological effects caused by RKN infection in grapevine. Simultaneously, ^{15}N-urea labeling is used to study local and systemic changes of the nitrogen uptake and distribution in RKN infected grapevine.

Material and Methods

C o - c u l t i v a t i o n   o f   R K N   w i t h   p o t t e d   g r a p e v i n e : One-year old grapevines on own roots 'Cabernet Sauvignon' (*Vitis vinifera* L.) were grown in pots (24 cm diameter × 19.5 cm higher) filled up with loamy sand with no RKN (60 % sand, 30 % loam and 10 % com-

Correspondence to: Prof. H. Zhai, Science and Engineering, Shandong Agricultural University, State Key Laboratory of Crop Biology, Tai'an, Shandong 271018, China. E-mail: hengz@sdau.edu.cn

post), which avoided new roots being hurt when the plants were removed from pots. Before planting, fresh weight of each plantlet was measured. All the pots were placed in a shallow gully with a depth of 14 cm and the spaces among these pots were filled with sand. The plants were managed under natural sunlight at a temperature of 28/23 °C in the greenhouse. Each plant was inoculated with 5,000 eggs of RKN collected from the tomato roots infected by *M. incognita* when plants had seven expanded leaves. Specifically, the egg suspension was evenly poured into five to eight little round holes around each plant, and the holes were sealed with sand when the water seeped into the soil. The control plants were treated with clean water in the same way. Each treatment included more than twenty plants.

Experimental lay-out

Experiment 1: Physiological and vegetative effects caused by RKN: In order to survey the activity of the non-lignified root after infection by *M. incognita*, both the infected and non-infected plants were removed from the pots. Immediately, the non-lignified roots were cleaned with water and dried with blotting paper before being broken into small pieces and mixed, and then the root pieces were used to measure the root dehydrogenase activity using TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) staining method (KURZBAUM *et al.* 2010). The sampling time points were 1, 3, 7, 10 and 15 weeks post inoculation (wpi). Five independent plants of each treatment were replicates in all sampling time point experiments.

The growth rate and root-shoot ratio were measured until ten weeks after inoculation. The fresh weights of the whole plants infected and non-infected were weighted in five independent replicates respectively. Almost immediately the fresh biomasses of these plants were respectively measured over-ground and under-ground. Moreover, five infected vines were reserved until defoliation to survey the storage nutrient (starch, soluble sugars, free amino acids and soluble proteins) in stem and root, so did the control ones.

Experiment 2: Root labeling with ^{15}N-urea: Two months later after inoculation, five control plants and five *M. incognita* infested plants were treated with 1g ^{15}N-urea (^{15}N abundance is 10.15 % made in Shanghai chemical Academy), respectively. The marked urea was dissolved in 300 mL water applied into the pots in two consecutive days irrigating with 100 mL deionized water daily to maintain soil moisture. One week later, the labeled plants were removed from the sand, washed with water, alconox detergent, water, 1 % hydrochloric acid and again three times with water to clear residual urea from roots. All organs were divided into different parts, leaves contained upper leaves (a third of the expanded leaves from the upper first fully expanded leaf), middle leaves and lower leaves (a third of the expanded leaves from the lowest leaf), so did as the stem while the roots were classified into annual roots, perennial roots and the roots with galls. These samples were ground into powder after drying to measure the contents of total nitrogen and the ^{15}N as well as some mineral elements.

Experimental measurements: Dried samples were used to measure a series of experimental indexes. The fresh plant tissues were broken into small pieces (less than 0.5 cm) and placed into an oven at 105 °C for 30 min, and then dried at 85 °C until no change of the weight. The concentrations of starch, soluble sugars, free amino acids and soluble proteins were determined using phenol-sulfuric acid, ninhydrin and coomassie brilliant blue G250 staining methods respectively. To measure nitrogen and mineral elements concentrations the samples were ground into powder and the powders were crushed through a 2 mm sieve. We measured phosphorus and other mineral elements (potassium, calcium, copper and zinc) using Mo-Sb colorimetry and atom absorption spectrophotometer methods respectively. N concentration and ^{15}N abundance was determined by a mass spectrometer (ZHT-03). Atom percent ^{15}N values were converted to N derived from the fertilizer (Ndff) using the following formula adapted from HAUCK and BREMNER (1976).

$$Ndff = \frac{(^{15}N_{\text{ natural abundance}}) - (atom\%^{15}N)_{\text{ tissue}}}{(^{15}N_{\text{ natural abundance}}) - (atom\%^{15}N)_{\text{ urea}}}$$

Statistical analysis: All analyses were performed using SPSS 7.5 (SPSS, Chicago, IL, USA) for Windows statistical package. Independent samples group t test and a LSD multiple range test analysis of variance (MCKONE and LIVELY 1993) was used to analyze data. Sigma plot (version 10.0) was employed to draw figures.

Results

Impact of RKN infestation on grape root system: Root dehydrogenase activities were measured at different periods after inoculation of *M. incognita*. No significant difference was detected in the first three weeks between the infected and the control (Fig. 1). One month later, the root dehydrogenase activity of the treated plant, including both the root with galls and the adjacent root material without visible swelling (uni-Root), increased markedly compared with the control root (c-Root). Especially at the 10 wpi, the infected plant displayed a significant increase of root dehydrogenase activity both in galls and uni-Root suggesting a systemic effect in response to RKN infection. In comparison with the control plant their root dehydrogenase activities improved by 85 % and 71 %, respectively. However, no change was detected at the last sampling time.

RKN infestation affects grape over-ground and under-ground growth: It was well-known that RKN parasitism resulted in decrease of grape biomass in the growing season (MELAKEBERHAN *et al.* 1988, 1989). RKN infection decreased grape fresh weight while the root-shoot ratio dramatically increased (Fig. 2a). When we dissected the biomass of over-ground and under-ground respectively, it was found that the fresh weigh of infected plant shoots decreased while that of the roots enhanced by 21.5 % (Fig. 2b). We measured storage me-
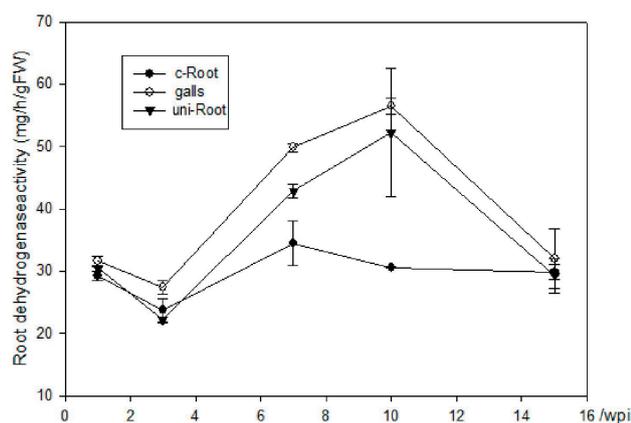
Fig. 1: Root dehydrogenase activities of control and infected plants. The error bars are standard deviations (n = 3), the same is as below. The legend c-Root, galls and uni-Root respectively represent the control roots, the roots with galls and the parts of adjacent roots without visible swelling. On X-axis wpi means weeks post inoculation.
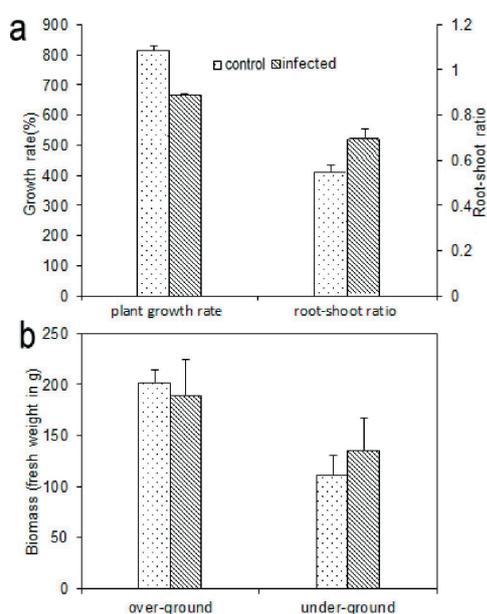

Fig. 2: RKN infection affects grape growth. **a**, grape growth rate and root-shoot ratio. The grape growth rate is analyzed using the equation, growth rate = added biomass (the added fresh weight since planted in pot) / the initial biomass (the fresh weight before planting). **b**, grape fresh weight of over-ground and under-ground. The two figures share the same legend.

tabolites remaining in the stem and root after defoliation to evaluate the plant overwintering ability. All detected metabolites in infected plants were less than those in control plants indicating weakened overwintering ability (Tab. 1). The contents of soluble sugars, free amino acids and soluble proteins in roots significantly decreased while the starch content showed no difference, and all the four measured metabolites were reduced in the stem. In particular, the soluble sugars in roots exhibited the most pronounced decline by 34.3 % while the greatest reduction in stem was starch with 27.4 %. The results indicated that the energy flowing to nodosities were competitively consumed by RKN instead of the root itself.

Impact of RKN infestation on total N content in different organs: One week later after root labeling with ^{15}N-urea, we measured total N contents and the distribution rates in different organs. Surprisingly, the total N content of the whole plant enhanced a little in the infected plant (Fig. 3a). When we measured the N contents in different organs, it was found that the root displayed an increase while the leaf and shoot didn't change (Fig. 3b). However, Upper leaves' N content elevated markedly while that of lower and middle leaves decreased. RKN infection enhanced the N contents of the annual root while that of the perennial root didn't vary (Tab. 2). In particular, the annual root with galls presented the highest N content compared with all other roots. RKN infection resulted in a significant accumulation of N in annual roots. What didn't change is that the leaves possessed the highest N content followed by the root and the shoot.

As a whole, the N distribution rate of infected plants showed a slight decrease in the leaf and shoot and an increase in the root (Fig. 3c). However, RKN reduced the N distribution rate of the perennial root and increased that of the annual root if adding the annual roots and galls together because the galls also belonged to annual roots (Tab. 2). Interestingly, the total N distribution rate of the root didn't show significant variation with a slight increase in comparison with the control.

RKN infection alters the ^{15}N Ndff% in different organs: The Ndff is an important test index to measure plant N uptake ability from the fertilizer. Just as the results shown in Tab. 3, the ^{15}N Ndff in leaves and shoots decreased on different levels. Conversely, RKN dramatically improved the ^{15}N uptake ability of the infect-

Table 1

Storage nutrients remaining in the stem and root after defoliation. Data are mean ± standard deviation (*n* = 3). The data within a line followed by different letters are significantly different using independent samples group *t* test. The different letters show significant difference at 5 % level. DW means dry weight as well as below

	Stem		Rate of change (%)	Root		Rate of change (%)
	Control	Infected		Control	Infected	
Soluble sugars (mg·g⁻¹ DW)	5.0 ± 0.06a	4.0 ± 0.19c	-20.0	6.4 ± 0.15a	4.2 ± 0.08c	-34.3
Free amino acids (mg·g⁻¹ DW)	50.0 ± 1.61c	47.0 ± 0.37d	-6.0	99.1 ± 1.36a	88.9 ± 1.84b	-10.3
Starch (mg·g⁻¹ DW)	17.5 ± 0.69b	12.7 ± 0.22c	-27.4	36.3 ± 0.43a	35.3 ± 0.20a	-2.7
Soluble proteins (mg·g⁻¹ DW)	6.0 ± 0.16c	4.8 ± 0.60d	-20.0	42.6 ± 0.24a	32.6 ± 0.84b	-23.5

Ta b l e 2

Total nitrogen contents and distribution rates of different organs. Data are mean ± standard deviation ($n = 3$). The data belonging to the same organ (leaf, shoot and root) are significantly different using independent samples group t test. Lowercase letters show significant difference at 5 % level. The same is below

|		Leaf		
		Upper	Middle	Lower
Distribution rate (%)	Control	14.8 ± 6.62ab	12.3 ± 2.97abc	8.3 ± 1.56c
	Infected	18.3 ± 3.56a	11.2 ± 1.16bc	7.8 ± 1.50c
Content (mg·g⁻¹ DW)	Control	27.6 ± 6.85ab	28.2 ± 1.47ab	21.9 ± 0.64b
	Infected	31.7 ± 3.20a	27.0 ± 4.22ab	22.7 ± 4.94b
		Shoot		
		Upper	Middle	Lower
Distribution rate (%)	Control	8.1 ± 0.71bc	10.3 ± 1.76ab	12.4 ± 0.83a
	Infected	6.9 ± 1.50c	9.4 ± 0.28b	12.3 ± 1.34a
Content (mg·g⁻¹ DW)	Control	9.7 ± 1.56a	7.8 ± 0.87ab	7.9 ± 0.59ab
	Infected	9.4 ± 1.72a	8.6 ± 1.41ab	7.3 ± 0.19b
		Root		
		Perennial	Annual	Galls
Distribution rate (%)	Control	16.5 ± 3.76a	14.1 ± 1.48a	
	Infected	13.9 ± 2.10a	9.8 ± 0.49b	8.9 ± 1.50b
Content (mg·g⁻¹ DW)	Control	10.4 ± 0.69c	15.0 ± 0.52a	
	Infected	11.0 ± 1.13c	18.1 ± 2.31ab	19.5 ± 2.79a
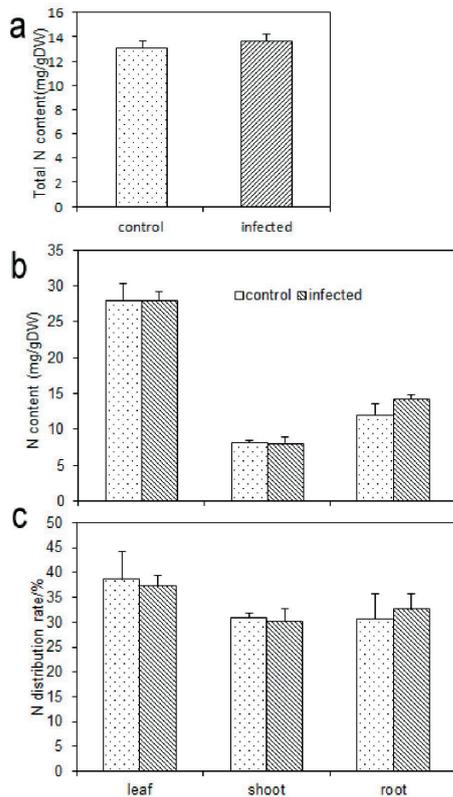
Fig. 3: RKN infection affects nitrogen distribution in grape plant. **a**, total N contents in control and infected plant. **b**, N contents in different organs. **c**, N distribution rate in different organs. a and b share the same legend and abscissa.

Ta b l e 3

The ¹⁵N Ndff (%) in different organs. Data are mean ± standard deviation ($n = 3$). The data belonging to the same organ (leaf, shoot and root) are significantly different using independent samples group t test. Lowercase letters show significant difference at 5 % level

	Leaf		
	Upper	Middle	Lower
Control	3.7 ± 0.02a	3.6 ± 0.79a	3.5 ± 0.89ab
Infected	3.3 ± 0.43ab	3.0 ± 0.04ab	2.6 ± 0.30b
	Shoot		
	Upper	Middle	Lower
Control	3.8 ± 0.35a	3.2 ± 0.04a	3.5 ± 0.20a
Infected	3.0 ± 0.71a	3.6 ± 0.27a	3.1 ± 0.36a
	Root		
	Perennial	Annual	Galls
Control	6.9 ± 0.70c	11.7 ± 0.54b	
Infected	7.5 ± 1.15c	16.1 ± 0.66ab	16.2 ± 2.85a

Ndff of the whole root system while decreased that of the over-ground organs.

RKN infection affects the absorption and distribution of ¹⁵N: ¹⁵N tracing experiment demonstrated a different result for the content of the total N (Tab. 4). The ¹⁵N content of all organs was reduced while the annual roots decreased the most in infected plants, the annual roots and the galls diminished by 14.11 and 9.93 %, respectively. Further analysis found that the root decreased the most followed by the leaf and the shoot. However, the ¹⁵N utilization efficiency increased in infected roots while it decreased in leaves and shoots compared with the control (Fig. 4). Similarly, the ¹⁵N distribution rates of leaves and shoots decreased in infected plants, except for the upper leaf and the lower shoot (Tab. 4). When it came to the root

ed grape roots, both for annual and perennial roots. Compared with the control plant roots, the annual roots with galls showed the highest ¹⁵N Ndff which was higher than that of the perennial roots as well as the annual roots on the same infected plant. RKN infection enhanced the ¹⁵N

Table 4

The ^{15}N contents and distribution rates of grape organs in control and infected plants. Data are mean ± standard deviation (n = 3). The data belonging to the same organ (leaf, shoot and root) are significantly different using independent samples group t test. Lowercase letters show significant difference at 5 % level

|		Leaf		
		Upper	Middle	Lower
Distribution rate (%)	Control	6.4 ± 0.11ab	5.2 ± 0.97bc	5.0 ± 1.25c
	infected	7.3 ± 0.78a	5.1 ± 0.07bc	4.0 ± 0.52c
Content (mg·g^{-1} DW)	Control	7.3 ± 0a	7.2 ± 0.81a	6.6 ± 0.28ab
	infected	6.8 ± 0.45ab	6.6 ± 0.06ab	6.2 ± 0.31b
		Shoot		
		Upper	Middle	Lower
Distribution rate (%)	Control	11.2 ± 1.85c	16.1 ± 1.25b	18.6 ± 1.81ab
	infected	9.6 ± 0.78c	16.8 ± 2.84b	21.2 ± 2.54a
Content (mg·g^{-1} DW)	Control	7.5 ± 0.35a	6.8 ± 0.07b	7.1 ± 0.20ab
	infected	7.6 ± 0.21a	7.2 ± 0.23ab	6.7 ± 0.36b
		Root		
		Perennial	Annual	Galls
Distribution rate (%)	Control	21.5 ± 3.14a	16.7 ± 0.55b	
	infected	17.9 ± 2.65ab	10.3 ± 0.64c	8.9 ± 0.77c
Content (mg·g^{-1} DW)	Control	8.3 ± 0.55cd	10.8 ± 0.44a	
	infected	7.6 ± 0.66d	9.7 ± 0.14b	9.3 ± 0.70bc
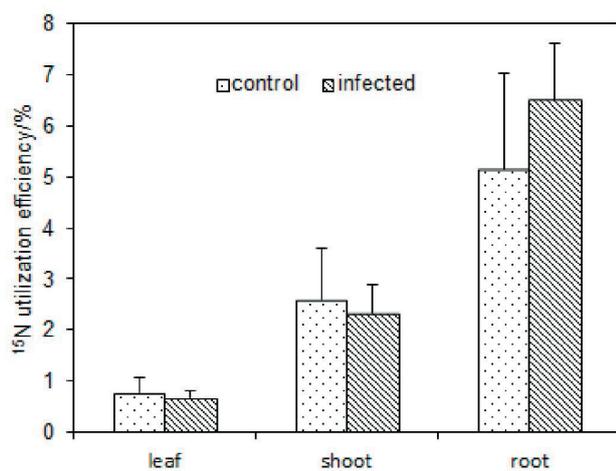
Fig. 4: ^{15}N utilization efficiency in different organs. The values are calculated using the following formula, utilization efficiency (%) = [Ndff × total nitrogen in organ (g)] /fertilized nitrogen amount (g) × 100.

system, RKN reduced the ^{15}N distribution rate of perennial roots and increased that of annual roots (add the annual roots and the galls together) in accordance with the variation of the total N distribution.

R K N   i n f e c t i o n   a f f e c t s   t h e   a c c u m u l a -
t i o n s   o f   m i n e r a l   e l e m e n t s   i n   r o o t : Root-knot nematode destroyed host root system especially for the rootlets, which finally affected the ability of the root system. In this paper, it was found that RKN infection didn't lead to significant changes for the mineral elements contents of the leaves and shoots (Tab. 5). However, the contents of the microelements copper and zinc notably increased in perennial roots as well as the galls. In addition, the contents of potassium and phosphorus improved in the galls compared with both the adjacent annual roots and

control roots. Above all, the content changes of the mineral elements caused by RKN occurred mainly in the root system, and the infected plant absorbed much more minerals from the soil and retained them in the roots.

Discussion

Fungal plant pathogens are generally classified as biotrophic, necrotrophic or hemibiotrophic pathogens based on their parasitic strategies (OKMEN and DOEHLEMANN 2014). Biotrophic pathogens caused limited damaged within host tolerance instead of killing it, as opposed to hemi-biotrophic or necrotrophic parasites, which ultimately kill the host in a short time (RAMAN et al. 2009, NEUHAUSER et al. 2010). Compared with the obligate pathogens, the pests with stylet adopted the similar feeding strategy to some degree, especially for phylloxera and nematodes. Root-knot nematodes induce swelling feeding structures termed root gall visible for the naked eye, which is fundamental to finish its lifestyle (WYSS et al. 1992). The galls as the feeding source provide all what the nematode needs to grow and reproduce. The specialized cells in the gall perform really different developmental and metabolic processes compared with the normal cells (MACHADO et al. 2012). Metabolic profiling analysis reveals a highly active metabolism in the syncytial feeding structure induced by *Heterodera schachtii* at the early stage (HOFMANN et al. 2010). Indeed molecular researches have demonstrated that the highest number of differentially expressed genes is involved in metabolism in the gall (JAMMES et al. 2010). The nutrient consumption derived by the nematodes and morphological rearrangements of the gall cells suggest enhanced biosynthetic and metabolic activity to meet the high energy demand. It seems to be that RKN infection unexpectedly benefits the host root

Table 5

The contents of mineral elements in different organs. Data are mean ± standard deviation ($n = 3$). The data belong to the same organ (leaf, shoot and root) are significantly different using independent samples group *t* test. Lowercase letters show significant difference at 5 % level

		Leaf		
		Upper	Middle	Lower
P (mg·g⁻¹ DW)	Control	3.4 ± 0.33a	3.1 ± 0.26ab	3.2 ± 0.33ab
	Infected	3.3 ± 0.19ab	3.0 ± 0.16ab	2.8 ± 0.19b
K (mg·g⁻¹ DW)	Control	13.9 ± 2.34b	15.8 ± 0.52a	13.8 ± 1.27b
	Infected	14.3 ± 2.69ab	14.2 ± 2.50ab	12.7 ± 0.41b
Ca (mg·g⁻¹ DW)	Control	47.3 ± 0.53c	53.2 ± 0.29b	62.3 ± 0.99a
	Infected	47.4 ± 2.05c	55.2 ± 1.16b	62.8 ± 2.39a
Cu (mg·g⁻¹ DW)	Control	26.4 ± 0.50a	26.3 ± 1.50a	24.1 ± 2.82a
	Infected	25.5 ± 0.50a	26.1 ± 1.19a	22.9 ± 8.45a
Zn (mg·g⁻¹ DW)	Control	42.7 ± 14.39d	181.3 ± 12.16a	160.6 ± 2.71ab
	Infected	83.7 ± 5.98c	171.4 ± 8.27ab	145.0 ± 4.20b
		Shoot		
		Upper	Middle	Lower
P (mg·g⁻¹ DW)	Control	2.7 ± 0.20a	2.4 ± 0.48ab	2.3 ± 0.56b
	Infected	2.6 ± 0.13ab	2.5 ± 0.43ab	2.3 ± 0.59b
K (mg·g⁻¹ DW)	Control	18.3 ± 1.19a	15.7 ± 1.90ab	11.9 ± 0.95cd
	Infected	17.7 ± 0.40ab	14.5 ± 0.74bc	10.3 ± 0.17d
Ca (mg·g⁻¹ DW)	Control	42.2 ± 0.29a	44.8 ± 2.51a	44.8 ± 2.78a
	Infected	43.8 ± 1.93ab	45.0 ± 1.37a	46.1 ± 1.27a
Cu (mg·g⁻¹ DW)	Control	20.7 ± 8.16b	22.4 ± 9.00ab	22.0 ± 3.72b
	Infected	24.9 ± 3.77ab	24.7 ± 5.43ab	31.8 ± 3.04a
Zn (mg·g⁻¹ DW)	Control	50.0 ± 2.83ab	46.5 ± 0.71ab	35.4 ± 16.70b
	Infected	61.1 ± 3.14a	40.1 ± 9.35b	37.2 ± 22.16b
		Root		
		Perennial	Annual	Galls
P (mg·g⁻¹ DW)	Control	3.8 ± 0.63ab	3.1 ± 0.90b	
	Infected	4.4 ± 0.81a	3.8 ± 0.44ab	4.2 ± 0.90a
K (mg·g⁻¹ DW)	Control	9.0 ± 0.46b	7.7 ± 0.25b	
	Infected	9.0 ± 1.52b	8.1 ± 0.87b	11.5 ± 0.63a
Ca (mg·g⁻¹ DW)	Control	48.33 ± 1.50a	45.3 ± 0.15b	
	Infected	48.8 ± 1.39a	44.7 ± 0.50b	45.7 ± 1.70b
Cu (mg·g⁻¹ DW)	Control	54.3 ± 2.25b	34.7 ± 7.25c	
	Infected	72.7 ± 9.82a	37.0 ± 2.83 c	59.2 ± 8.27b
Zn (mg·g⁻¹ DW)	Control	44.5 ± 8.50b	19.3 ± 0.64c	
	Infected	64.0 ± 6.90a	23.5 ± 0.44c	63.5 ± 9.55a

in biomass due to the formation of feeding structures at the early stage. However, RKN infection actually stimulates plant systemic responses to reduce the host plant above-ground growth in accordance with an increased root-shoot ratio. Earlier research has demonstrated that the nematodes consume appropriate 15 % of the total energy when entering the plant system (Melakeberhan *et al.* 1989). Finally, at the end of the growth season the infected vine retained relatively few nutrients compared to those of the control in the root and shoot after leaf fall, which indicated that the infected plant showed a weaker vigor.

Nematode growth and reproduction fully depends on the successful induction and maintenance of the gall cells. The nematode larvae cause limited damage at the feeding site before becoming the mature adults (Wyss *et al.* 1992). The soil-borne diseases didn't affect plant root system before the root gall bursting, which suggests that the rootlets keep their abilities to absorb and transport nutrients at the early stage. Conversely, the infected root absorbs more what it needs to meet the nutrient sink demand. Meanwhile, the gall as the sink retains metabolites reducing transport to the above-ground organs. In this research, the RKN infection slightly enhanced the total nitrogen content of the infected vine, especially for the infected root. Nitrogen assimilation initially occurs in plant roots by nitrate and nitrite reductase transforming nitrate and nitrite into ammonia. The microarray analysis indicates that the genes, encoding nitrate reductase, display increased expression pattern in the gall and syncytia (Jammes *et al.* 2010, Szakasits *et al.* 2009). It suggests a high level of nitrogen assimilation in the feeding structures. Furthermore, several numbers of amino acid transporter family genes (amino acid permease, AAP) are up-regulated in syncytia (Szakasits *et al.* 2009). Conversely, Das *et al.* (2010) and Jammes *et al.* (2010) point out that *AAP* genes are down-regulated in galls of Arabidopsis and *Vigna unguiculata* based on GeneChips analysis. Amino

acid transporter proteins may differentially function in a biological process, *OsAAT* genes exhibit diverse expression patterns under nitrogen starvation (Lu *et al.* 2012). No matter the variation of the amino acid transporter genes, it has been demonstrated that many free amino acids accumulate in these specialized feeding structures, gall (Machado *et al.* 2012), syncytia (Hofmann *et al.* 2010) and nodosity induced by phylloxera (Kellow *et al.* 2004). In particular, glutamine and glutamic acid notably increase in syncytia (Hofmann *et al.* 2010), both of the two metabolites are essential for biosynthesis of other amino acids in plant. We detected the enhanced ^{15}N Ndff and a higher ^{15}N utilization efficiency in RKN infected grape root system, which directly supported that RKN turned the specialized feeding structures into nitrogen sinks.

Earlier research has confirmed that these specialized feeding structures accumulate not only the nitrogen metabolites but also all needed nutrients for the pests (Hofmann *et al.* 2010). Some mineral elements, Mn, Cu and Zn, appear to accumulate in RKN infected coffee plants (Hurchanik *et al.* 2004). However, little is known about how the pests hijack the plant cells to meet their nutrient demand. It is speculated that the phytohormones play important roles in initial induction and subsequent maintenance of feeding structures (Beneventi *et al.* 2013). The nematodes, both cyst and root-knot nematodes, strongly activate an auxin-inducible promoter, which indicate an accumulation or an increased sensitivity of auxin in the feeding site (Karczmarek *et al.* 2004). Subsequent research has confirmed an increase in auxin accumulation in young syncytia (Absmanner *et al.* 2013). It is thought that nematodes are capable of using and manipulating the endogenous molecular and physiological pathways to facilitate their parasitism (Grunewald *et al.* 2009a). As a whole, the aggressive nematode alters host metabolites transportation network to benefit itself meeting nutrient demand in the compatible interaction (Caillaud *et al.* 2008). Du *et al.* (2011) determine the concentrations of endogenous plant hormones at different phylloxera developmental stages, indole acetic acid (IAA) and gibberelic acid (GA$_3$) present increased contents at the stage of nodosity formation. Furthermore, the rate of IAA/ABA (abscisic acid) dramatically increased until the larvae nearly change into adults, which likely keeps the nodosity in a state of continual hunger.

In this paper, we confirmed that RKN infection weakened potted vine vigor and brought about a higher metabolic activity in the whole root system before the galls bursting. Furthermore, RKN infection improved the accumulation ability of infected vine roots resulting in increasing concentrations of nitrogen and some mineral elements. However, decreased storage nutrients in root and shoot after defoliation revealed that nematode competitively consumed part of the plant carbon and nitrogen metabolites.

References

Abad, P.; Favery, B.; Rosso, M. N.; Castagnone-Sereno, P.; 2003: Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol. Plant Pathol. **4**, 217-224.

Absmanner, B.; Stadler, R.; Hammes, U. Z.; 2013: Phloem development in nematode-induced feeding sites: the implications of auxin and cytokinin. Front. Plant Sci. **4**, 1-14.

Baldacci-Cresp, F.; Chang, C.; Maucourt, M.; Deborde, C.; Hopkins, J.; Lecomte, P.; Bernillon, S.; Brouquisse, R.; Moing, A.; Abad, P.; He´rouart, D.; Puppo, A.; Favery, B.; Frendo, P.; 2012: (Homo) glutathione deficiency impairs root-knot nematode development in *Medicago truncatula*. PLoS Pathogens **8**, 1-12.

Beneventi, M. A.; Da Silva, O. B.; De Sá, M. E. L.; Firmino, A. A. P.; De Amorim, R. M. S.; Albuquerque, V. S.; Da Silva, M. C. M.; Da Silva, J. P.; De Araújo Campos, M.; Lopes, M. J. C.; 2013: Transcription profile of soybean-root-knot nematode interaction reveals a key role of phytohormones in the resistance reaction. BMC Genom. **14**, 1-17.

Boubals, D.; 1954: Les nematodes parasites de la vigne. Progr. Agr. Vitic. **141**, 204-207.

Das, S.; Ehlers, J. D.; Close, T. J.; Roberts, P. A.; 2010: Transcriptional profiling of root-knot nematode induced feeding sites in cowpea (*Vigna unguiculata* L. Walp.) using a soybean genome array. BMC Genom. **11**, 1-16.

Dorhout, R.; Gommers, F. J.; Kollöffel, C.; 1993: Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. Physiol. Mol. Plant Pathol. **43**, 1-10.

Du, Y. P.; Wang, Z. S.; Zhai, H.; 2011: Grape root cell features related to phylloxera resistance and changes of anatomy and endogenous hormones during nodosity and tuberosity formation. Aust. J. Grape Wine Res. **17**, 291-297.

Caillaud, M. C.; Dubreuil, G.; Quentin, M.; Perfus-Barbeoch, L.; Lecomte, P.; Engler, J. A.; Abad, P.; Rosso, M.; Favery, B.; 2008: Root-knot nematodes manipulate plant cell functions during a compatible interaction. J. Plant Physiol. **165**, 104-113.

Gonzalez, H.; 1970: Nuevas especies de nematodos que atacan la vid en Chile. Agr. Tec. **30**, 31-37.

Grunewald, W.; Cannoot, B.; Friml, J.; Gheysen, G.; 2009: Parasitic nematodes modulate PIN-mediated auxin transport to facilitate infection. PLoS Pathog. **5**, 1-7.

Hauck, R.; Bremner, J.; 1976: Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. **28**, 219-266.

Hewezi, T.; Piya, S.; Richard, G.; Rice, J. H.; 2014: Spatial and temporal expression patterns of auxin response transcription factors in the syncytium induced by the beet cyst nematode *Heterodera schachtii* in Arabidopsis. Mol. Plant Pathol. **15**, 730-736.

Hofmann, J.; El Ashry, A. E. N.; Anwar, S.; Erban, A.; Kopka, J.; Grundler, F.; 2010: Metabolic profiling reveals local and systemic responses of host plants to nematode parasitism. Plant J. **62**, 1058-1071.

Hurchanik, D.; Schmitt, D. P.; Hue, N. V.; Sipes, B. S.; 2004: Plant nutrient partitioning in coffee infected with *Meloidogyne konaensis*. J. Nematol. **36**, 76-84.

Ithal, N.; Recknor, J.; Nettleton, D.; Maier, T.; Baum, T. J.; Mitchum, M. G.; 2007: Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Mol. Plant-Microbe Interact. **20**, 510-525.

Jammes, F.; Lecomte, P.; Almeida-Engler, J.; Bitton, F.; Martin-Magniette, M. L.; Renou, J. P.; Abad, P.; Favery, B.; 2005: Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. Plant J. **44**, 447-458.

Jones, M.; Payne, H.; 1978: Early stages of nematode-induced giant-cell formation in roots of *Impatiens balsamina*. J. Nematol. **10**, 70-84.

Karczmarek, A.; Overmars, H.; Helder, J.; Goverse, A.; 2004: Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. Mol. Plant Pathol. **5**, 343-346.

Kellow, A. V.; Sedgley, M.; Van Heeswijck, R.; 2004: Interaction between *Vitis vinifera* and grape phylloxera: Changes in root tissue during nodosity formation. Ann. Bot. **93**, 581-590.

Kurzbaum, E.; Kirzhner, F.; Armon, R.; 2010: A simple method for dehydrogenase activity visualization of intact plant roots grown in soilless culture using tetrazolium violet. Plant Root **4**, 12-16

Lider, L. A.; 1954: Inheritance of resistance to a root-knot nematode (*Meloidogyne incognita* var. *acrita* Chitwood) in *Vitis* spp. Proc. Helmintholog. Soc. Washington **21**, 53-60.

Machado, A. R. T.; Campos, V. A. C.; Da Silva, W. J. R.; Campos, V. P.; De Mattos Zeri, A. C.; Oliveira, D. F.; 2012: Metabolic profiling in the roots of coffee plants exposed to the coffee root-knot nematode, *Meloidogyne exigua*. Eur. J. Plant Pathol. **134**, 431-441.

McKone, M. J.; Lively, C. M.; 1993: Statistical analysis of experiments conducted at multiple sites. Oikos **67**, 184-186.

Melakeberhan, H.; Ferris, H.; 1988: Growth and energy demand of *Meloidogyne incognita* on susceptible and resistant *Vitis vinifera* cultivars. J. Nematol. **20**, 545-554.

Melakeberhan, H.; Ferris, H.; 1989: Impact of *Meloidogyne incognita* on physiological efficiency of *Vitis vinifera*. J. Nematol. **21**, 74-80.

Melakeberhan, H.; Ferris, H.; Dias, J, M.; 1990: Physiological response of resistant and susceptible *Vitis vinifera* cultivars to *Meloidogyne incognita*. J. Nematol. **22**, 224-230.

Neuhauser, S.; Bulman, S.; Kirchmair, M.; 2010: Plasmodiophorids: The challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. Molecular identification of fungi, 51-78. Springer.

Okmen, B.; Doehlemann, G.; 2014: Inside plant: biotrophic strategies to modulate host immunity and metabolism. Curr. Opin. Plant Biol. **20**, 19-25.

Porten, M.; Huber, L.; 2003: An assessment method for the quantification of *Daktulosphaira vitifoliae* (Fitch) (Hem., Phylloxeridae) populations in the field. J. Appl. Entomol. **127**, 157-162.

Raman, A.; Beiderbeck, R.; Herth, W.; 2009: Early subcellular responses of susceptible and resistant *Vitis* taxa to feeding by grape phylloxera *Daktulosphaira vitifoliae*. Bot. Helvet. **119**, 31-39.

Ryan, F.; Omer, A.; Aung, L.; Granett, J.; 2000: Effects of infestation by grape phylloxera on sugars, free amino acids, and starch of grapevine roots. Vitis **39**, 175-176.

Smith, P.; 1977: Distribution of plant parasitic nematodes in vineyards in the Western Cape Province. Phytophylactica **9**, 27-28.

Stirling, G.; 1976: Distribution of plant parasitic nematodes in South Australian vineyards. Animal Prod. Sci. **16**, 588-591.

Szakasits, D.; Heinen, P.; Wieczorek, K.; Hofmann, J.; Wagner, F.; Kreil, D. P.; Sykacek, P.; Grundler, F. M.; Bohlmann, H.; 2009: The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots. Plant J. 57, 771-784.

Wyss, U.; Grundler, F. M.; Munch, A.; 1992: The parasitic behaviour of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica **38**, 98-111.