Vitis **53** (4), 227–231 (2014)

Phylloxera infestation and the uptake and distribution of ^{13}C and ^{15}N tracers in grape vines

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Summary

In order to study the reason phylloxera (*Daktulosphaira vitifolia* Fitch) feeding on roots leads to decreased plant productivity, the uptake and distribution of ^{13}C photosynthates and ^{15}N in the grape vine 'Wuhe 8612' in response to phylloxera infestation were investigated. Phylloxera and grapevines cocultivated in pots were treated with $^{13}CO_2$ and ^{15}N-urea. The plant weight, nitrogen concentration and accumulation, ^{15}N utilization efficiency, Nitrogen derived from fertilizer (Ndff%), and carbon isotope ratio ($\delta^{13}C$) of different organs were measured. Phylloxera infestation significantly reduced grape weight, shoot length, and N concentration and accumulation in different organs, whereas it increased the ratio between N content of the of roots and above-ground organs. Phylloxera infestation reduced leaf and root nitrogen ^{15}N utilization efficiency, by 24 % and 14 %, respectively compared to controls. Labeled leaves of infested plants took up rather more ^{13}C and ^{15}N and exported a substantial amount of these nutrients to roots. Labeled roots took up rather more ^{15}N and exported a small amount of these nutrients to upper leaves. This study found that phylloxera infestation reduced ^{13}C and ^{15}N uptake in leaves and roots, but increased N and photosynthates, which were mostly distributed to the roots, but also to the upper leaves. These factors together led to weak grape vine growth.

Key words: phylloxera; uptake; distribution; $^{13}CO_2$; $CO(^{15}NH_2)_2$.

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) (Homoptera: Phylloxeridae) is an obligate parasite and quarantined pest of grape vines in China. It was found in Jiading (Shanghai province) in 2005 and has since spread to Huaihua (Hunan province), Xi'an (Shaanxi province) and Xingcheng (Liaoning province). Damage to vineyards spreads with the insect's predominance. Understanding the nature of the damage is an important step towards ameliorating it without completely replanting.

Grape phylloxera cause substantial damage to susceptible *Vitis* spp. by inducing growth of root-galls known as nodosities and tuberosities. The insects induce a feeding site within the meristematic zone, where they stay attached to the root (HOFMANN 1957) and cause changes in the uptake and transportation of water, minerals and assimilates (PORTEN and HUBER 2003), which decrease plant vigor and cause leaves to yellow and fall. Nodosities accumulate starch, sugar, amino acids (RYAN *et al*. 2000, KELLOW *et al*. 2004), auxin, and cytokinin (DU *et al.* 2011). This suggests that the nodosities became a nutrition sink, drawing in nutrients from leaves, stems and other roots. Thus, loss of nutrients from the plant organs might cause weakening of shoot vigor. Isotopic tracers would allow us to study nutrient transfer. Here we report the results of $^{13}CO_2$ and ^{15}N-urea labeling to study the carbon and N uptake and distribution under phylloxera infestation.

Material and Methods

Co-cultivation of phylloxera with potted grapevine roots: Potted grapevines were cultivated and inoculated as described in FORNECK (2001). One-year old grapevines 'Wuhe 8612' ['Zhengzhou Zaohong' (*V. vinifera*) × 'Kyoho' ('Campbell Early' × 'Centennial')] obtained from Juxian, Shandong province (China) were grown in 30 cm diameter pots filled up with coarse sand (0.2 to 2.0 mm size grains) in order to avoid delicate new roots being hurt when the plants were removed from pots. When plants had seven expanded leaves, each plant was infested by 300 five to six day-old phylloxera eggs, by placing the eggs on moistened filter paper, and wrapping the filter paper around the mature root (FORNECK, 2001). The same handling of the plants was simulated without eggs on the filter paper for the control group. A total of 96 plants (60 infested plants, 18 uninfested control plants) were randomly placed in a greenhouse with shade screen to keep the potting mix temperature at approximately 25 °C. Each plant was watered with 300 mL of Hogland nutrient solution every week and watered with 100 mL deionized water daily to maintain soil moisture. After the phylloxera egg exposure, infestation was assessed and fifteen infested plants were selected to be used in Experiments 1, 2 and 3 (see below).

The remaining plants were removed from the sand 25 d after egg exposure, three replicates of each treatment were selected for sampling. The shoot length of each plant was measured, and the roots were divided into annual roots,

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perennial roots, stem, 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). Fresh and dried weights of all sampled plant tissues were measured, and the N concentration were estimated using the micro-Kjeldahl method.

Experimental layout

Experiment 1: Leaf labeling with ^{15}N-urea: The middle leaves of six control plants and six selected phylloxera infested plants were treated with 0.5 % $CO(^{15}NH_2)_2$ solution, made in Shanghai chemical academy with a 10.15 % concentration of ^{15}N, by applying 5 mL at 8:30 on a clear day and another 5 mL on the following day. To ensure leaves were treated with identical volumes of the ^{15}N solution, the technique of BONDADA *et al.* (2001) was adopted which involved brushing the ^{15}N-urea solution onto both surfaces of the leaves using a small camel hair brush. One and three weeks after labelling, the infested and control plants were removed from the sand and treated to a series of washes including water, alconox detergent, water, 1 % HCL, and finally three times with water to remove residual urea from the external surfaces. Plants were divided into annual roots, perennial roots, stem, upper leaves, labeled leaves, and lower leaves.

Experiment 2: Root labeling with ^{15}N-urea: Six control plants and six selected phylloxera infested plants were irrigated with 300 mL 0.1 % ^{15}N-urea solution, made in Shanghai chemical academy with a 10.15 % concentration of ^{15}N, and watered with 100 mL deionized water daily to maintain soil moisture. Preliminary experiments indicated that 300 mL ^{15}N-urea solution and the daily 100 mL deionized water per plant remain in the soil, and didn't leave the pot through the bottom. Sampling time and washing procedure were similar to those in experiment 1. The plants were divided into roots (all roots combined), stems, upper leaves, labeled leaves, and lower leaves.

Experiment 3: Plant labeling with $^{13}CO_2$: Three control plants and three selected plants were labeled with $^{13}CO_2$ at 8:30 on a clear day. All plants' middle leaves were enclosed within PVC plastic containers, which had been specially made for labeling experiments. To expel the gas in the PVC container, a small bottle filled with 0.15 g $Ba^{13}CO_3$ was put in each container. Then, the entrance hole of the PVC container was filled with RTV silicone rubber (Dow Corning). A solution of 1 mol·l^{-1} HCl was injected into each small bottle by syringe until the $Ba^{13}CO_3$ ran out completely, which insured that the $^{13}CO_2$ concentration was above 350 ppm. The labeling process continued for 4 h, the remaining $^{13}CO_2$ was trapped by 5 mL of 2 mol·L^{-1} NaOH solution, and then the PVC container was removed. The plants remained in the pots for 48 h and then were pulled out of the soil, plants were divided into annual roots, perennial roots, stem, upper leaves, labeled leaves, and lower leaves.

Experimental measurements: The plant tissues were placed into an oven at 105 °C for 30 min, and then 85 °C until dried, and the powder was crushed through 2 mm sieve. N concentration and ^{15}N abundance was determined using 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):

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

The δ^{13}C values in plant tissues were also determined using a mass spectrometer (MAT 253, Finnigan, Germany) coupled with an elemental analyzer (FlashEA 1112, Finnigan, Italy).

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

Results

Impact of phylloxera infestation on vine weight and root-shoot ratio: After 25 d of the phylloxera infestation, the shoot length, root weight, and shoot weight of grape plants infested by phylloxera were significantly decreased by 56 %, 24 % and 35 %, respectively as compared to the control. Furthermore, the root-shoot ratio of grape plants infested by phylloxera was significantly greater than control (Tab. 1).

Impact of phylloxera infestation on N content in different organs: After 25 d of the phylloxera infestation, N concentration was significantly decreased (Tab. 2), N concentration of the upper leaves, middle leaves, lower leaves, and annual roots decreased by 17 %, 13 %, 10 %, 7 %, respectively. The reduction was greatest for upper leaves, and least for the annual roots. There was no reduction for the perennial roots.

Table 1

Impact of phylloxera infestation on grape vine weight and root-shoot ratio

	Shoot length (cm)	Root weight (g)	Shoot weight (g)	Root-shoot ratio
Infested plant	32.2 ± 8.8b	23.0 ± 1.8b	28.4 ± 2.4b	0.8 ± 0.03a
Control	73.8 ± 23.2a	30.4 ± 1.1a	44 ± 4.2a	0.7 ± 0.02b

Data are mean ± standard deviation (n = 3); for data within a column, different letters denote significant differences using independent samples group t test; small letters show significant difference at the 5 % level. The same below.

Table 2

Impact of phylloxera infestation on nitrogen concentration (mg·g^{-1}) and accumulation in different organs (mg)

		Upper leaves	Middle leaves	Lower leaves	Annual roots	Perennial roots	Above-ground organs	Roots	Roots/organs above-ground	Total/ plant
Nitrogen concentration (mg·g^{-1})	Infested plant	25.9 ± 2.7b	25.8 ± 0.3b	21.9 ± 1.6a	13.3 ± 0.5b	9.11 ± 0.8a				
	Control	31.2 ± 0.2a	29.5 ± 0.2a	24.2 ± 0.4a	14.3 ± 0.1a	9.03 ± 1.1a				
Nitrogen accumulation in organs (mg)	Infested plant	54.4 ± 4.7b	120.2 ± 8.2b	46.2 ± 4.0b	85.5 ± 1.8b	20.7 ± 7.8b	220.8 ± 9.9b	106.2 ± 4.0b	0.48a	327.0b
	Control	148.4 ± 1.2a	163.7 ± 7.5a	101.4 ± 4.9a	107.3 ± 5.0a	28.2 ± 1.3a	413.5 ± 5.7a	135.5 ± 4.0a	0.33b	548.95a

Table 3

Impact of phylloxera infestation on ^{15}N utilization efficiency (%)

	Labeled leaves	Labeled roots
Infested plant	57.2 ± 0.9b	2.4 ± 0.1b
Control	75.5 ± 1.4a	2.9 ± 0.02a

The same trends occurred for N accumulation, except that N accumulation was also reduced in the perennial roots. The total N accumulation per phylloxera infested plant decreased by 40 % compared to control plants, with above-ground organs decreasing by 47 %, roots decreasing by 22 %, and the greatest reduction of 63 % in the upper leaf, The N content ratio of roots to above ground organs was significantly greater in plants infested by phylloxera compared with control plants.

Impact of phylloxera infestation on leaf and root ^{15}N utilization efficiency: After leaves and roots were labeled with ^{15}N-urea for three weeks (Experiments 1 and 2), the ^{15}N utilization efficiency of infested plants significantly decreased. This decrease, compared to controls, was by 24 % for labeled leaves and 14 % for labeled roots, which indicates that phylloxera infestation reduced N absorption by leaves and roots.

Impact of phylloxera infestation on Ndff% in different organs determined using labelled ^{15}N absorbed through leaves: Nitrogen derived from fertilizer (Ndff%) is an assessment of the ratio of N absorbed from fertilizer relative to total N content. After leaves were labeled with ^{15}N-urea for one week (experiment 1), the rank of infested plant parts ^{15}N Ndff%, from highest to lowest was: labeled leaves > annual roots > lower leaves > upper leaves > perennial roots. For control plant parts, the ^{15}N Ndff% rank was: labeled leaves > upper leaves > annual roots > perennial roots > lower leaves (Tab. 4). The upper leaf Ndff% of controls was 1.88 times that of infested plant, while the Ndff% of infested annual roots was 2.67 times that of controls, which indicates that ^{15}N mostly distributed to new shoots in control plants and roots in phylloxera infested plants.

Impact of phylloxera infestation on Ndff% in different organs determined using labelled ^{15}N absorbed through roots: After one week of root ^{15}N-urea labelling (Experiment 2), the ^{15}N Ndff% rank of plant parts for infested plants and controls both followed the order: roots > upper leaves > middle leaves > lower leaves (Figure). ^{15}N Ndff% in each control plant organ was significantly higher than that of infested plants, except for the lower leaves. ^{15}N Ndff% in upper leaves and roots of control plants was 3.73 and 1.26 times that of infested plants, respectively. The ratio between the ^{15}N Ndff% of upper leaves and roots of control plants (0.86) was higher than that of infested plants (0.29), indicating that phylloxera infestation induced ^{15}N absorbed by the roots to be mostly retained in the roots, with little export to new shoots.

Table 4

Impact of phylloxera infestation on ^{15}N Ndff% in different organs through labeled leaves

	Upper leaves	Labeled leaves	Lower leaves	Annual roots	Perennial roots
Infested plant	1.84 ± 0.50b	19.95 ± 0.27a	2.23 ± 1.04a	4.19 ± 0.72a	1.55 ± 0.01a
Control	3.45 ± 0.04a	13.72 ± 0.11b	0.82 ± 0.34b	1.57 ± 0.04b	1.05 ± 0.12b

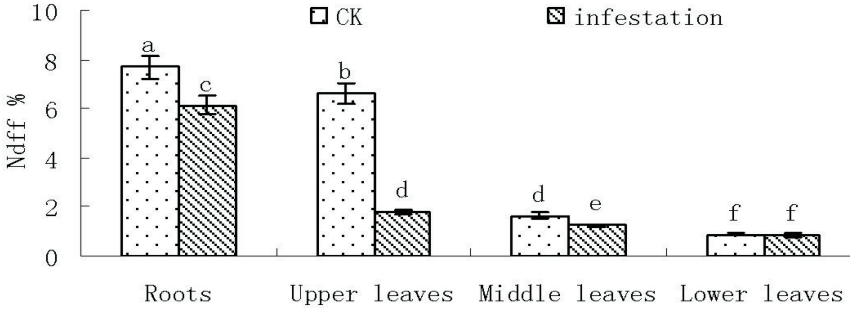

Figure: Impact of phylloxera infestation on ^{15}N Ndff% in different organs by labeled roots. Different letters are significantly different using a LSD multiple range test.

Impact of phylloxera infestation on $\delta^{13}C$ in different organs: After leaves were labeled with $^{13}CO_2$ for 48h (experiment 3), the control plant organ $\delta^{13}C$ ranked, from highest to lowest: labeled leaves > upper leaves > annual roots > perennial roots > lower leaves. Organs of infested plants ranked: labeled leaves > annual roots > perennial roots > upper leaves > lower leaves (Tab. 5). The $\delta^{13}C$ of infested plants' upper leaves was significantly lower than of the control plants, while the $\delta^{13}C$ of the roots of the infested plants was higher than that of the control plants, which indicates that phylloxera infestation induced allocation of photosynthates primarily to the infested roots, while the photosynthates of the control plants were mostly directed to the new shoots.

Discussion

Phylloxera infestations of grape vines cause roots to swell, primary roots to form nodosities and mature roots to form tuberosities. The swelling stops rootlet growth, and facilitates fungal infections causing roots to decay (BUCHANAN 1987). Our study confirmed that phylloxera infestation decreased plant shoot growth and decreased plant weight and N content of every vine organ.

Many researchers suggest that the vigor decline with phylloxera infestation is related to restraints on nutrient and water absorption (SCOTT 2002), and that root decay by secondary fungal infection accelerates the loss of ability to absorb nutrients (OMER *et al.* 1995, GRANETT *et al.* 1998). In our study, we used leaf and root ^{15}N labeling methods and found that phylloxera infestation affected leaf and root N utilization efficiency (Tab. 3). But this is not the only mechanism behind vigor decline, we also discovered reductions in plant weight as well as lower N content in roots than in above-ground organs. This was characterized by a higher root-shoot ratio and a higher ratio of N content between the roots and above-ground organs, which suggests that declines in plant vigor with phylloxera infestation may be caused by both nutrient absorption declines and distribution of nutrients to roots, The result of the ^{15}N and ^{13}C labeling experiments supported this finding. The ^{15}N-urea leaf labeling experiment showed that the N absorbed by the leaves was preferentially directed toward the roots, and the ^{15}N-urea root labeling experiment showed that the N absorbed by the roots is mostly retained in the roots with little distribution to new shoots (Figure). Furthermore, the results of leaf labeling with $^{13}CO_2$ showed that photosynthates were primarily distributed to roots (Tab. 5). Therefore, we hypothesize that the nodosities and tuberosities formed by phylloxera infestation act as nutrition sinks. Previous research has shown that photosynthates and N are transported from source to sink, and that the source and sink are relative, and will change with time and environmental conditions (LAWN and BRUN 1973, SCHNYDER 1993, ROITSCH 1999). New shoots act as nutrition sinks and induce the distribution of nutrition to themselves before bloom, while the fruits become nutrition sinks after fruiting begins (MENG *et al.* 2010). The tumors on stems of *Ricinus communis* L. var. *gibsonii* 'Carmencita' were shown to act as a strong metabolic sinks after *Agrobacterium tumefaciens* strain C58 infestation (MISTRIK *et al.* 2000). Preliminary studies

Table 5

Impact of phylloxera infestation on $\delta^{13}C$ in different organs

	Upper leaves	Labeled leaves	Lower leaves	Annual roots	Perennial roots
Infested plant	-20.12 ± 1.54b	358.12 ± 23.28a	-21.84 ± 2.06a	70.32 ± 8.64a	-1.43 ± 2.02a
Control	4.47 ± 3.01a	414.5 ± 30.02a	-24.4 ± 3.01a	-11.04 ± 3.01b	-21.16 ± 3.08b

also showed that the nodosities and tuberosities formed by phylloxera infestation accumulated much more starch and amino acids than the plant parts (Ryan *et al.* 2000). Based on these previous findings combined with our findings, we concluded that plant vigor decline phylloxera-infestation is not only due to decreased nutrient absorption ability, but also due to the infested-roots acting as nutrient sinks. These sinks alter the sink-source relationship, inducing the distribution of above-ground nutrients to roots, and thus accelerating plant vigor decline.

Conclusion

This study found that plants modulate biomass, photosynthate and N allocation in response to phylloxera infestation.

Acknowledgements

We sincerely thank Prof. em. J. Granett for editing the manuscript. We would also like to thank C. Verhille at the University of British Columbia for her assistance with English language and grammatical editing of the manuscript. This research was supported by the National Natural Sciences Foundation of China in 2012 (31201609), Promotive research fund for young and middle-aged scientisits of Shandong Province (BS2012NY007).

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Received December 11, 2013