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EVALUATING THE HEALTH STATUS OF *ROBINIA PSEUDOACACIA* L. BY THE ROOT EXUDATES

WEI TIANXING*, SHI XIN[1], ZHU JINZHAO, ZHU QINGKE AND YUKUI RUI[2,3]

School of Soil and Water Conservation, Beijing Forestry University, Beijing, 100083, P.R. China

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Abstract

The components of root exudates released from healthy and unhealthy black locust (*Robinia pseudoacacia* L.) root were determined by GC-MS. The results showed that the kinds and contents of root exudates released from Black Locust are different for healthy and unhealthy plants, so it may be evaluated whether the Black Locust plants are healthy or unhealthy by the root exudates. Healthy plants can release more kinds of exudates than unhealthy plants, especially the kinds of acids, alcohol and ester. There are 46 kinds of compounds in the healthy Black Locust, but only 24 kinds in the unhealthy Black Locust. Many compounds are released from healthy plant but not from unhealthy, while many kinds of compounds are released from unhealthy plant but not from healthy one.

Introduction

Root exudates plays important role for plant, such as nodulation of legume plant (Peter and Astrid 1997). And the change of environment can also change the composition of root exudates (Jia *et al*. 2014), and the exudate will change with the change of season (Oe *et al*. 2011) so the compounds and their concentration could be an important indexes to prove whether the plant is healthy or not.

Black Locust (*Robinia pseudoacacia* L.) has been widely planted in north China. It showed strong vitality and adaptability in the growth potential. It can withstand drought and poor soil (Zhang *et al*. 2008). It was considered a promising tree for reforestation due to its fast growth and ability to fix atmospheric nitrogen and has become the pioneer tree in the Loess Plateau, though the trees are not native to the region (Zheng 1985). As an exotic tree species, Black Locust has some influence on ecosystem. Invasive species threaten the ecological integrity of natural areas by influencing community structure and function and by altering ecosystem processes (Wang *et al*. 2012, Dantonio and Vitousek 1992, Ehrenfeld *et al*. 2001, Evans *et al*. 2001, Mack and Dantonio 1998). The results confirmed that Black Locusts have long-term benefits on the improvement of soil properties (Mack *et al.* 2000). Black Locust supplements soil nitrogen pools, increases nitrogen return in litter fall, and enhances soil nitrogen mineralization rates when it invades nutrient poor, pine-oak ecosystems (Rice *et al.* 2004). Ecological benefits of Black Locust have decreased mainly due to lack of water and low tending management. In order to study evaluate the healthy status, the compounds of root exudates and their relative percentage were determined at different growth seasons.

Materials and Methods

The roots and rhizosphere soil of Black Locust were collected from the plantation in Pingquan County Hebei Province on March 2012.

*Author for correspondence: <weitx@bjfu.edu.cn>. [1]Zhejiang Guangchuan Engineering Consulting Co.Ltd., Hangzhou, 310020, P.R. China. [2]College of resources and environmental science, China Agricultural University, Beijing, 100193, P.R. China. [3]Stockbridge school of agriculture, University of Massachusetts, 01003 MA, USA.

The roots and the rhizosphere soil were washed with 50 ml mol/l NaOH after air-dried. The residual liquid was collected and filtered 2 - 3 times. Take 20 ml filtrate was taken as subject. The filtrate was adjusted pH to 2.5 - 3, dripped with saturated NaCl and then extracted with ether three times. The extraction was dried at 40ºC on a rotary evaporator and was dissolved by 1 ml methanol, then moved to diameter of 1.8 - 2.0 mm capillaries. The capillaries were placed in a vacuum desiccator and pumped to dry and then added in 8 µl BSTFA to derive 1 hr at 100ºC for further GC-MS analysis.

The detection method was referred to Dong's methods (Dong *et al*. 2013): Root exudates of black locusts were analyzed by Gas Chromatography-Mass Spectrum (GC-MS). Chromatographic column was a silica capillary column (30 m × 0.32 mm × 0.25 um) made in Perkin Elmer. Column temperature starts from 50ºC (kept for 3 min) with a 8ºC /min rate up to 180ºC (kept for 1 min), then with 10ºC /min rate up to 280ºC (kept for 5 min) with helium as the carrier gas at constant pressure. The interface temperature is set at 260ºC controlled by constant electronic flow. Split less injection with injection volume of 0.6 µl. EI-mass spectrum was taken at 70 eV, interface temperature was 25ºC, mass ranges from 29 to 500, voltage of the detector was set at 400 V and full-scan is finished within 0.2 s.

Results and Discussion

The root exudates released from healthy black locust better-grown are mainly of acids accounting for 31.84%, ester 29.73% , alcohols 15.28%, alkanes 12.69%, aldehydes 7.22%, phenol 2.82 and 0.42%, respectively and contain 46 active substances, respectively (Table 1). Tables 1 and 2 show black locust of different growth states have different types of root exudates.

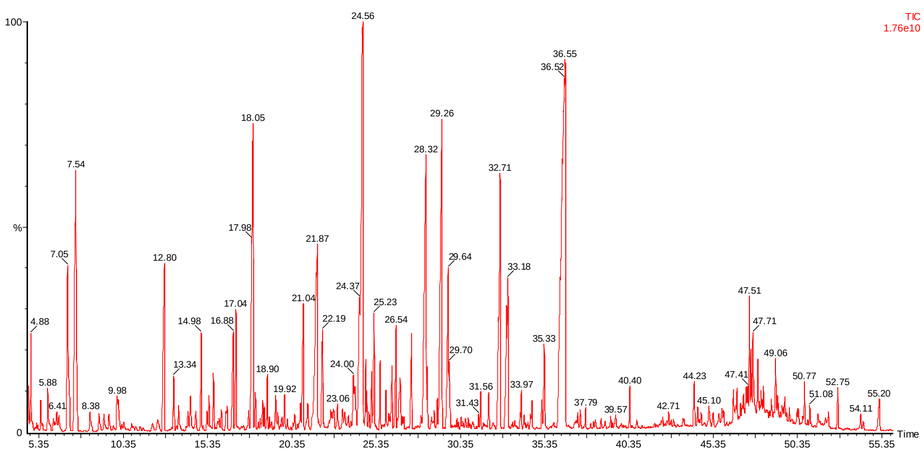

Fig. 1. GC/MS of root exudates from healthy locust.

The root exudates released from unhealthy black locust are mainly of acid 48.39%, ester 27.66%, alcohol 15.49%, alkane 5.21%, arene 2.51%, and phenol 0.74%, respectively (Table 2). It was detected only 24 active substances in the unhealthy black locust.

The chemical composition of the forest soil near the plantation have been analyzed in the same time (Table 3). N-hexadecanoic acid was detected in the healthy, unhealthy Black Locust and forest soil. It is known that the n-hexadecanoic acid is existed in the soil. The other 3 substances are from root exudates, such as, oleic acid, 9-octadecenoic acid (Z)-, methyl ester, hexadecanol.

Table 1. Main components of root exudates released from healthy black locust.

No.	Classification	%	Main Root Exudates	Formula
1	Acid	3.67	3-Methyl- butanoic acid	$C_5H_{10}O_2$
2		3.27	Oleic acid	$C_{18}H_{34}O_2$
3		4.55	Octanoic acid	$C_8H_{16}O_2$
4		4.21	Cinnamic acid	$C_9H_8O_2$
5		6.72	n-hexadecanoic acid	$C_{16}H_{32}O_2$
6		2.68	Benzoic scid	$C_7H_6O_2$
7		1.02	Acetic acid	$C_2H_4O_2$
8		1.25	Hexanoic acid	$C_6H_{12}O_2$
9		1.97	2-methyl-butanoic acid	$C_5H_{10}O_2$
10		1.33	Carbolic acid	C_6H_6O
11		0.53	Acetic acid	$C_2H_4O_2$
12		0.64	Octadecanoic acid	$C_{18}H_{36}O_2$
		31.84		
13	Ester	3.87	1,2-Benzenedicarboxylic acid, Bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
14		2.59	Undecanedioic acid dimethyl ester	$C_{13}H_{24}O_4$
15		4.21	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
16		1.32	Dimethyl phthalate	$C_{10}H_{10}O_4$
17		0.87	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
18		1.74	N-Butyl myristate	$C_{18}H_{36}O_2$
19		7.38	Dibutyl phthalate	$C_{16}H_{22}O_4$
20		6.54	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
21		0.55	Hexanedioic acid, diisooctyl ester	$C_{22}H_{42}O_4$
22		0.66	Acetyl-butanedioic acid dimethyl ester	$C_8H_{12}O_5$
		29.73		
23	Alcohol	2.16	Isotridecanol-	$C_{13}H_{28}O$
24		1.25	2,5-dimethylcyclohexanol	$C_8H_{16}O$
25		2.26	1-decanol, 2-hexyl-	$C_{16}H_{34}O$
26		1.64	2,3-butanediol	$C_4H_{10}O_2$
27		2.44	1-octanol, 2-butyl-	$C_{12}H_{26}O$
28		1.52	2-furanmethanol, tetrahydro-	$C_5H_{10}O_2$
29		1.87	1-butanol	$C_4H_{10}O$
30		1.02	1-hexacosanol	$C_{26}H_{54}O$
31		0.87	1-tridecanol	$C_{13}H_{28}O$
32		0.25	Hexadecanol	$C_{16}H_{34}O$
		15.28		
33	Alkanes	3.64	Hexatriacontane	$C_{36}H_{74}$
34		3.45	Cyclohexane	C_6H_{12}
35		2.87	Undecane	$C_{11}H_{24}$
36		1.13	Tridecane	$C_{13}H_{28}$
37		0.37	Nonadecane	$C_{19}H_{40}$
38		0.84	Eicosane	$C_{20}H_{42}$
39		0.39	Tetratriacontane	$C_{34}H_{70}$
		12.69		
40	Aldehyde	5.57	2,4-decadienal, (E,E)-	$C_{10}H_{16}O$
41		1.26	Benzaldehyde, 2-hydroxy-	$C_7H_6O_2$
42		0.17	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$
43		0.22	p-isobutylbenzaldehyde	$C_{11}H_{14}O$
		7.22		
44	Phenol	2.68	Phenol, 3,4-dimethoxy-	$C_8H_{10}O_3$
45		0.14	Phenol, 3-ethyl-	$C_8H_{10}O$
		2.82		
46	Biphenyl	0.42	1,1'-biphenyl, 2-methyl-	$C_{13}H_{12}$

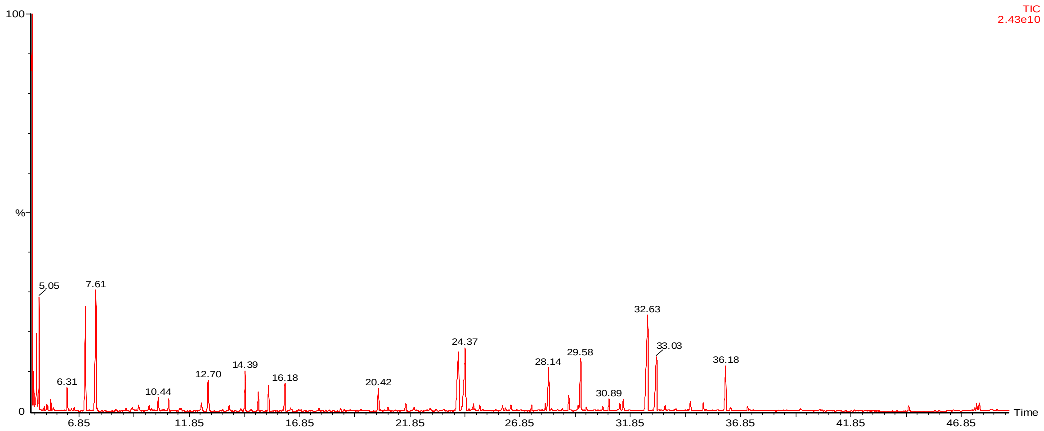

Fig. 2. GC/MS of root exudates from unhealthy locust.

Table 2. Main components of root exudates released from unhealthy black locust.

No.	Classification	%	Main root exudates	Formula
1		4.68	1,2-benzenedicarboxylic acid	$C_8H_6O_4$
2		3.26	Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$
3		4.69	n-decanoic acid	$C_{10}H_{20}O_2$
4		5.47	Pentanoic acid	$C_5H_{10}O_2$
5	Acid	7.55	Oleic acid	$C_{18}H_{34}O_2$
6		4.25	Lactic acid	$C_3H_6O_3$
7		8.74	n-hexadecanoic acid	$C_{16}H_{32}O_2$
8		4.13	Stearic acid	$C_{18}H_{36}O_2$
9		5.62	Nonanoic acid	$C_9H_{18}O_2$
		48.39		
10		9.41	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-Hydroxy-1-methylethyl) propyl ester	$C_{12}H_{24}O_3$
11	Ester	5.28	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$
12		4.97	Pentyl phenylacetate	$C_{13}H_{18}O_2$
13		4.26	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$
14		3.74	Acetic acid, butyl ester	$C_6H_{12}O_2$
		27.66		
15		1.16	1-eicosanol	$C_{20}H_{42}O$
16		10.24	Hexadecanol	$C_{16}H_{34}O$
17	Alcohols	0.87	Benzeneethanol, 4-hydroxy-	C_7H_8O
18		1.97	3,4-Furandiol, tetrahydro-, cis-	$C_4H_8O_3$
19		1.25	1-hexanol, 2-ethyl-	$C_8H_{18}O$
		15.49		
20	Alkanes	4.87	Heptane, 2,4-dimethyl-	C_9H_{20}
21		0.34	Octane, 4-methyl-	C_9H_{20}
		5.21		
22	Arene	2.51	Benzene, 1,4-diethyl-	C_8H_{10}
		2.51		
23	Phenol	0.57	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$
24		0.17	Phenol, 4-(1-methylpropyl)-	$C_{10}H_{14}O$
		0.74		

Table 3. Chemical composition of the forest soil by GC/MS.

No.	Main root exudates	Formula
1	n-hexadecanoic Acid	$C_{16}H_{32}O_2$
2	Benzothiazole	C_7H_5NS
3	Heptane, 2,4-dimethyl-	C_9H_{20}
4	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$
5	Indole	C_8H_7N
6	Benzene, 1,4-diethyl-	C_8H_{10}
7	2-butanone, 3-hydroxy-	$C_4H_8O_2$

The kinds and contents of root exudates released from black locust are different for healthy and unhealthy plants, so we can evaluate whether the black locust plants are healthy or unhealthy by the root exudates.

(1) Healthy plants can release more kinds of exudates than unhealthy plants, especially the kinds of acids, esters and alcohols. There are 46 kinds of compounds in the healthy black locust, but only 24 kinds in the unhealthy black locust. The exudates from healthy root contained 13 kinds of acids, 10 kinds of esters, 10 kinds of alcohols, 7 kinds of alkanes, 4 kinds of aldehydes and 2 kinds of phenols and a biphenyl respectively; but they are only 9, 5, 5, 0, 2 and 3 in from unhealthy plants, respectively.

(2) The most three acids released from healthy plant but not from unhealthy one are octanoic acid (4.55%), cinnamic acid (4.21%) and 3-methyl-butanoic acid (3.67%); The most three esters released from healthy plant but not from unhealthy are dibutyl phthalate (7.38%), hexadecanoic acid, methyl ester (4.21%), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (3.87%); The most three alcohols released from healthy plant but not from unhealthy are 1-octanol, 2-butyl- (2.44%), 1-decanol, 2-hexyl- (2.26%) and isotridecanol- (2.16%).

Four kinds of aldhydes were released from healthy plants, they are 2,4-decadienal, (E,E)-, benzaldehyde, 2-hydroxy-, benzaldehyde, 4-hydroxy-3,5-dimethoxy- and p-isobutylbenzaldehyde, especially the contents of 2,4-decadienal, (E,E)- is 5.57%. If there are the above nine exudates, we can believe the Black locust plant are healthy.

(3) The major three acids released from unhealthy plant but not from healthy are nonanoic acid (5.62%), pentanoic acid (5.47%) and n-decanoic acid (4.69%). The most three esters released from unhealthy plant but not from healthy are propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester (9.41%), pentyl phenylacetate (4.94%) and octadecanoic acid, methyl ester (4.26%). The three alcohols released from healthy plant but not from unhealthy are 3,4-furandiol, tetrahydro-, cis- (1.97%), 1-hexanol, 2-ethyl- (1.25%) and 1-eicosanol (1.16%). If there are the above exudates, we can believe the Black Locust plants are unhealthy.

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