土壤淋溶液对大豆胞囊线虫卵孵化的影响

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摘 要:大豆胞囊线虫病是一种世界性的大豆病害,利用寄主植物的抗性和作物轮作已长期用来控制该病害。但是为了进一步解释中国大豆主产区利用抗性品种和轮作对大豆胞囊线虫病控制作用机制,将大豆胞囊线虫病抗性品种-抗线4号和感病品种-合丰25移栽到6种不同种植顺序土壤中,测定各土壤淋溶液对大豆胞囊线虫(SCN)3号生理小种卵孵化的影响。结果表明:大豆胞囊线虫卵孵化明显被不同轮作系统中种植抗感品种后土壤淋溶液影响。在麦豆麦、豆麦豆、米豆米和大豆连作12年的茬口中种植合丰25后土壤淋溶液比休闲区淋溶液和蒸馏水对照刺激更多的卵孵化,并且孵化速度也快。但是抗线4号在豆麦豆种植后土壤淋溶液较蒸馏水对照和不栽苗的土壤淋溶液明显抑制了卵孵化。在豆麦米和休闲区种植合丰25和抗线4号后土壤淋溶液对SCN卵孵化影响不大。所以大豆胞囊线虫病抗性品种-抗线4号可以被种植在豆麦豆轮作系统,感病品种-合丰25能被种植在豆麦米种植顺序中。

关键词:大豆胞囊线虫;卵孵化;土壤淋溶液;抗感品种;轮作系统

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Effect of Soil Leachates on Egg Hatch of *Heterodera Glycines*

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Abstract: Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe), is a devastating pest. Although host plant resistance and crop rotation have been used as methods to reduce the damage of SCN for a long time, in order to further explain SCN management mechanism by resistant cultivars and crop rotation in Chinese producing area of soybean, SCN-susceptible soybean cultivar Hefeng 25 and SCN-resistant cultivar Kangxian 4 were transplanted into pots with the soil from 6 kinds of cropping sequences, then the effects of soybean genotypes and rotation systems on egg hatch of SCN in soil leachates were assessed in this study. The results showed that SCN egg hatch in the soil leachates was significantly affected by soybean genotypes and rotation systems. The soil leachates from Hefeng 25 planted in the cropping sequences of wheat-soybean-wheat, soybean-wheat-soybean, corn-soybean-corn and soybean continuous cropping for 12 years stimulated more and quicker egg hatch than those from Fallow (bare land) system and distilled water control, but that of Kangxian 4 in soybean-wheat-soybean inhibited markedly the egg hatch of SCN. In soybean-wheat-corn and fallow, there was no significant difference for egg hatch in the soil leachate between Hefeng 25 and Kangxian 4. Therefore, SCN-resistant soybean Kangxian 4 could be sown in soybean-wheat-soybean rotation system. Susceptible cultivar Hefeng 25 could be sown in soybean-wheat-corn cropping system.

Keywords: *Heterodera glycilnes*; Egg hatch; Soil leachate; Soybean genotypes; Rotation systems

Introduction

Soybean [*Glycine max* (L.) Merr.] is a very important crop globally because it is grown in vast geographical areas worldwide[1]. The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pathogen of soybean, which causes significant yield losses in soybean production[2]. Northeast China is the largest producing area of soybean in China. SCN was first found in Northeast China in 1899 and first reported in 1915 by Japanese[3]. Although the control strategies to SCN are improved, the pest is an increasing production problem for soybean producers in Northeast China. As a soil-borne pest, hatching, movement, feeding, development and reproduction of SCN are all influenced by many factors, including environmental conditions and growing stage of the host, such as soil type, soil pH, agricultural chemical, cultivation systems and so on[4-5]. For SCN management, decreasing *H. glycines* populations is very important. Hatching is the beginning of the development and reproduction of SCN, and therefore inhibition of hatching could play a key role in SCN management.

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Plant resistance and crop rotation have been used as methods to reduce the damage of SCN for a long time[6]. Host plant resistance is the most economic, environmentally safe and effective strategy to control SCN, but the availability of resistance sources is very limited. Crop rotation with non-host plants to control SCN is another widely accepted practice, because the density of SCN population was less in rotation system than continuous cropping area[7]. In Northern China, the number of SCN, including the number of cysts and all juveniles of different stages in soil and roots of soybeans was notably decreased in rotation field compared to continuous cropping soybean[8].

Many mechanisms studies of resistant soybean cultivar to SCN were reported. Xu et al. [9] found that inhibition of SCN development rather than invasion might be the resistance mechanism of SCN-resistant soybean cultivar Kangxian 4 to *H. glycines* race 3. During *in vitro* test, young root of SCN-susceptible cultivar Liaodou 10 had great attraction to J2 of SCN, while the effect in resistant cultivars was just contrary[10]. The difference of soybean cultivars in SCN egg hatching is significant. For example, the SCN egg hatching stimulated activity by root exudates of susceptible soybean cultivar Hefeng 25, is higher than those of resistant cultivar Kangxian 4[11]. The hatching rate of eggs in root diffusates of susceptible cultivars (*in vitro*) was significantly higher than that of resistant varieties and in deionized water[12]. Root diffusate from sugar beet in wheat-soybean-wheat, soybean-wheat-soybean and soybean continuous cropping for 12 years caused more egg hatch of SCN than other crops (wheat, corn, soybean and flax) on the same rotation system[13]. Before nematodes hatch, chemical components of the hosts' root exudates are bound to interact with the parasitic nematodes[3]. Masamuni et al. [14] obtained glycinoeclepin A, which stimulated SCN eggs' hatch, from root exudates of kidney bean and other legume crops.

Generally, the exudates from the roots of host plants or susceptible cultivars may have stronger effects on the hatching of SCN eggs according to *in vitro* experiments, compared with those from many non-hosts or resistant cultivars[11,13]. The root exudates from 8-week-old Williams 82 and A3127 (*H. glycines*-susceptible soybean cultivars) also stimulated more hatch and emergence of *H. glycines* from cysts of both race 3 and race 4 than Fayette (resistant to *H. glycines*) [15]. But the reports on effects of soil leachates on hatching of SCN eggs were far fewer than those of root diffusates of crops on hatching. In this study, soil leachates were collected from the field soil with growing soybean, not hydroponics and sand culture, and eggs of SCN were assayed, not cysts. The effect of soil leachates of soybean genotypes and rotation systems on egg hatch of H. glycines was studied. The study provides valuable information for SCN control mechanisms by resistant cultivars and crop rotation in the typical black soil agroecosystem of Northeast China.

Materials and methods

Rotation systems

Soil was taken from topsoil 0-20 cm of the long-term rotation system field in National Observation Station of Hailun (N47°26′, E126°38′) Agroecosystem, Chinese Academy of Sciences, in Northeast China. Soil samples were collected randomly at 6 different points as "X" shape in each plot for the first time experiment. The same sampling method was repeated for the second time experiment. The properties of these soil samples which were the typical mollisols (black soil) were tested by Academy of Agricultural Sciences of Heilongjiang Province listed in Table 1. The rotation systems with different crops in the experiment are explained in Table 1, and six cropping sequencing treatments were wheat-soybean-wheat (WSW), soybean-wheat-soybean (SWS), corn-soybean-corn (CSC), soybean-wheat-corn (SWC), soybean continuous cropping for 12 years named monoculture (S…SS), bare land (Fallow) without any plants with the tillage regime the same as other rotation systems. The rotation treatments were arranged in a randomized complete block design with three replicates. Individual plots were 3.3 m × 10 m (5 rows). Each crop plot received the same amount of fertilizer, for corn (Haiyu 6), 150 $kg \cdot ha^{-1}$ diammonium phosphate (18% N, 46% P_2O_5) and 110 $kg \cdot ha^{-1}$ carbamide (46% N) when planting, and the same dosage of carbamide again at corn V6 stage. For soybean (Heinong 35 with medial resistance to SCN), 150 $kg \cdot ha^{-1}$ diammonium phosphate (18% N, 46% P_2O_5) were used as the starter fertilizer. For wheat (Longmai 29), 84 $kg \cdot ha^{-1}$ diammonium phosphate (18% N, 46% P_2O_5) and 168 $kg \cdot ha^{-1}$ carbamide (46% N) were applied before sowing. Three kinds of crops were sown by ridge planting. One moldboard ploughing (about 20 cm deep) was conducted at October after soybean and corn harvest and August after wheat harvest.

Table 1 Characteristics of the soil (0-20 cm depth) before the initiation of the experiment

Treatment	Rotation systems	Soil organic Matter/%	Total N /%	Available N /mg · kg^{-1}	Available P /mg · kg^{-1}	Available K /mg · kg^{-1}	pH
WSW	S-W-S-W-S-W-S-W-S-W-S-W	2. 84	0. 27	205. 3	107. 4	141. 9	5. 5
CSC	S-C-S-C-S-C-S-C-S-C-S-C	3. 01	0. 20	230. 1	142. 8	114. 9	5. 4
SWC	S-W-C-S-W-C-S-W-C-S-W-C	2. 78	0. 29	192. 3	131. 5	118. 0	5. 5
SWS	W-S-W-S-W-S-W-S-W-S-W-S	2. 95	0. 20	275. 0	133. 1	107. 2	5. 0
S…SS	S-S-S-S-S-S-S-S-S-S-S-S	2. 89	0. 32	204. 9	141. 0	126. 6	5. 7
Fallow	Bare land	2. 82	0. 19	151. 0	59. 5	172. 3	5. 3

S = soybean; W = wheat; C = corn.

Plants and inoculum

SCN-susceptible cultivar Hefeng 25 and SCN-resistant cultivar Kangxian 4 were used in this study. SCN race 3 was identified by using Niblack ' s classification scheme[16].

Soil leachate preparation

Five healthy 3-day seedlings (SCN-susceptible cultivar Hefeng 25 and SCN-resistant cultivar Kangxian 4) in the autoclaved vermiculite were transplanted to 9-cm-diameter and 12-cm-depth plastic pots filled with 350 g dry soil from previous mentioned six rotation systems. There were three pots with the soil from each rotation systems. The plants were grown in the greenhouse at approximately 20 to 28℃ with adding artificial light to provide a minimum of 14 hours of daylight[15]. The soil leachate was collected on 15 days after seedling transplanting. One day before collection, there was no watering for plants. Each pot was immersed with 50 mL distilled water for one hour, and then more distilled water (150 mL) was added to the pot until a total of 50 mL of soil leachate was collected from each pot, and then filtered with filter paper (Φ12. 5, Ruizekang Technology Ltd. in Beijing, China). For controls, the soil leachate was collected from the pots without seedlings in different rotation systems. The experiment was conducted twice completely.

SCN egg hatch assays

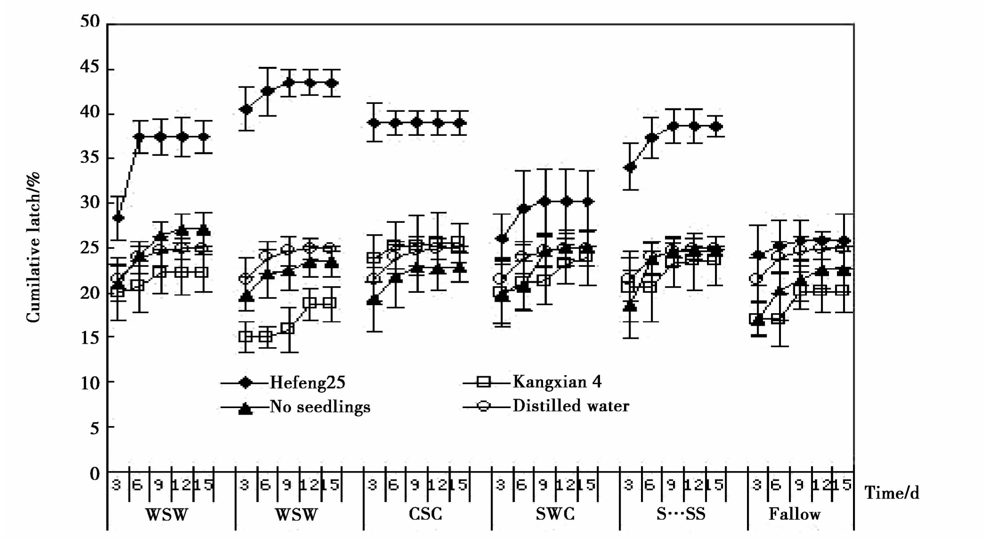
H. glycines (race 3) were cultured on the soybean *cv.* Hefeng 25 in sand-filled beakers using tap water in a constant moisture system[17], at 28 ± 0. 1℃ with 16 h of artificial light and 8 h of dark. Five weeks later, cysts were collected by gently stripping the washed roots. Cysts were rinsed with water and selected individually for uniformity under a dissecting microscope with 20 × magnification (Motic [Xiamen] Electric Group Co. , Ltd. China) using a needle. Uniform batches of yellow lemon-shaped cysts were prepared in each batch experiment [12]. SCN eggs were obtained by gently crushing the cysts in distilled water using rubber plug of flask. The released eggs were rinsed 3 × with distilled water to remove the cyst wall and contents. Eggs were then suspended in sterile deionized water at a concentration of 400 eggs. 0. 5 mL of the egg suspension containing approximately 200 eggs was transferred to a plastic petri dish (3. 5 cm diameter), and then 1. 5 mL of the soil leachates was added to the petri dish. Three petri dishes were used for each treatment. Eggs in the petri dishes were incubated at 25℃. All eggs and J2 in every Petri dish were counted with a stereoscopic microscope (Olympas Corporation, Japan) at 35 × magnification per 3 days from day 0 to day 15. 0. 2 mL of sterile deionized water was added the same petri dish after count per 3 days.

Data analyses

Statistical analyses were done using SAS8. 0 (SAS Institute, Cary, NC, USA). Mean cumulative percentage of hatch of SCN eggs in each rotation system was compared using three-way ANOVA with Duncan's Multiple Range Test ($P < 0.05$), with 6 × 4 × 5 factorial with rotation systems, soybean cultivars (including no seedling and distilled water) and observation time were treated as factors. Cumulative percent hatch means of SCN eggs was calculated as ([{J_2 on day_x-J_2 on day_0}/eggs on day_0] × 100). J_2 on day_x and J_2 on day_0 indicate the number of J_2 on day_x and day_0; eggs on day_0 indicate the number of egg on day_0.

Resultsand analysis

Egg hatch of SCN in the soil leachates was impacted obviously by soybean genotypes and rotation systems. Average cumulative percentages of egg hatch in the soil leachates from SCN-susceptible cultivar Hefeng 25 planted in WSW, SWS, CSC and S…SS cropping sequences were significantly greater than those from SCN-resistant Kangxian 4, no seedling and distilled water on the day 3 until 15 days after hatching, and those of Hefeng 25 were higher 1.6-2.3 times than Kangxian 4 on 15th day in the four cropping sequences (Fig. 1). Especially in SWS cropping sequence, cumulative hatch rate from Hefeng 25 was 40.5%-43.5%, but that of Kangxian 4 was 15.1%-18.7% and significantly lower than that of distilled water and no seedlings. In SWC cropping sequence and Fallow, cumulative hatch rate were not significantly different among the four treatments.



Each value is the mean of accumulative hatch rate of SCN in three replicates ± SD. Hefeng 25 indicates the soil leachate of Hefeng 25 planted in all the rotation systems. Kangxian 4 indicates the soil leachate of Kangxian 4 planted in all the rotation system. No seedlings indicates the soil leachate of no seedlings from all the rotation system. Distilled water serves as control.

Fig. 1 Effect of cropping sequencing and soybean genotype on egg hatch of SCN

The multivariate analysis of variance (Table 2) showed that cumulative percent hatch mean of SCN eggs in the soil leachates was not markedly affected by observation time during hatching but was significantly impacted by soybean genotypes, rotation systems and rotation systems x soybean genotypes ($P<0.0001$).

Table 2 Analysis of variance of the factors affecting average accumulative percentage of egg hatch of SCN in the soil leachates.

Source	DF	Sum of squares	Mean square	*F* value	*P*
Observation time	4	555.98	138.99^{ns}	2.33	0.0569
Rotation systems	5	1100.88	220.18*	3.69	0.0031
Soybean genotypes	3	8196.49	2732.16**	45.78	<.0001
Observation time × Rotation systems	20	32.82	1.64^{ns}	0.03	1.0000
Observation time × Soybean genotype	12	76.22	6.35^{ns}	0.11	0.9999
Rotation systems × Soybean genotypes	15	3825.51	255.03**	4.27	<.0001
Observation time × Rotation systems × Soybean genotypes	60	119.59	1.99^{ns}	0.03	1.0000

* indicates significant at $P<0.05$; ** indicates significant at $P<0.01$; ns indicates no significant, respectively; DF stands for degree of freed.

Conclusions and disscussion

Effect of soybean genotypes on egg hatch of SCN

Source of genetic resistance and crop rotation were the most important factors to consider in maximizing seed yield and decreasing *H. glycines* populations[18-20]. DaRocha et al.[21] found that the number of females and eggs per female were significantly higher on susceptible soybean cultivar S20-20 than resistance cultivars Bell. The results of our study may provide useful information for how to choose soybean cultivars planted in different rotation systems to reduce the damage of SCN race 3. Soil leachates of resistant soybean cultivar Kangxian 4 inhibited egg hatch level of SCN in SWS rotation system (Fig. 1), so Kangxian 4 could be planted in SWS cropping system in Hailun region. Because percent hatches of SCN eggs in the soil leachates of Kangxian 4 in SWS, CSC and S…SS cropping systems, were significantly lower than those of Hefeng 25 during hatch, Kangxian 4 is considerable in these three systems sown when other crops are not available. Furthermore susceptible cultivar Hefeng 25 did not considerably increased egg hatch of SCN in SWC rotation systems, so Hefeng 25 could be cultivated in SWC rotation systems when resistance cultivar is not available, but Hefeng 25 could not be cultivated in SWS, CSC, WSW and S…SS cropping systems, because it stimulated more egg hatching, SCN disease would occur seriously to result in reducing soybean yield. The root exudates of susceptible and resistant cultivars in soil leachate might play a main role in egg hatch of SCN in SWS, CSC, WSW and S…SS cropping systems. Egg hatch rate of four treatments in Fallow were not significantly different, because there were not accumulative root secretions and exudates, degradation substance of plant debris and so on in the soil leachates from the bare areas without any plant for 12 years.

Effect of rotation systems on egg hatch of SCN

Generally, the population density of SCN was higher in continuous cropping soybean than rotation with non-host. In North China, the number of SCN including the number of cysts and all juveniles of different stages in soil and roots of soybeans was notably increased under continuous soybean cropping field compared with those under rotation[8]. Many non-host rotation crops have been shown to reduce SCN populations[22]. The population of SCN cysts was also reduced in long-term rotation cultivation of wheat-soybean-rape-corn-soybean, and reached homeostasis after rotation for a long term[23]. Rodríguez-Kábana et al.[24] suggested that tropical corn-soybean was useful to manage SCN problem because number of *H. glycines* juveniles in soil was higher in monoculture than in rotation systems. In our study, wheat-corn-soybean rotation system was an effective tactic for controlling SCN in typical mollisol agroecosystem of Northeast China, according to egg hatch of SCN in the soil leachates of susceptible cultivar Hefeng 25 in SWC rotation system being significantly lower than continuous soybean monoculture, in agreement with the above findings. Moreover, egg hatches of SCN from Hefeng 25 in WSW, SWS and CSC were also higher than SWC, which suggested that the susceptible soybean cultivar could be sown in the rotation system in which non-host plants were planted for at least last two years, although soybean had ever been planted two years ago, but it should not be planted in continuous and alternate cropping soybean fields. Therefore, soybean cultivars and rotation systems should be considered where serious SCN occurs.

In all the cropping systems, cumulative hatch rate from Hefeng 25 in continuous cropping soybean for 12 years was not the highest (but in SWS rotation system), in concert with the conclusion of previous researcher[23]. If one susceptible cultivar is cultivated continuously in one field for many years (at least 5 years), the former serious nematode disease will disappear suddenly or will greatly lessen. So far, this phenomenon has been found in at least 9 kinds of cyst nematode disease[23]. We speculated that there might be some inhibition substance produced, such as parasitic fungus or bacteria to maintain ecological balance, after continuous soybean cropping sequence for many years. The complex root exudates, degradation substance of debris and exfoliation of preceding soybean in previous one or two years in the soil leachate might play an important direct or indirect role in egg hatch in WSW, SWS, CSC and SSS cropping systems. Moreover, in SWC, variance of hatch percentage of SCN between Hefeng 25 and Kangxian 4 was not significant during the hatch period. This result suggested that SCN reproduction should not be affected significantly by susceptible and resistant soybean cultivars planted in soybean-wheat-corn rotation system, and the effect of root secretions and exudates, degradation substance of plant debris and exfoliation of preceding soybean on egg hatch was probably weakened after rotation with wheat and corn. Therefore, SWC rotation system would be also effective measure for management of SCN in typical mollisol agroecosystem in northeast China.

In Minnesota, the lowest densities of SCN eggs were typically found in cropping sequences that involved continuous corn[25]. Creech et al.[26] thought that inclusion of corn into a cropping sequence is a much more valuable SCN management tool. Mock et al.[27] also suggested that corn and soybean rotation system appeared to be an effective way for SCN management in the midwestern United States compared with continuous cropping of soybean. However, in our study, CSC rotation system did not show obvious reduction of egg hatch of SCN in the soil leachates of Hefeng 25 and Kangxian 4 compared with other cropping systems, which wasn't seemingly in agreement with previous studies. The reason may be due to different soybean and corn cultivars, agrotype and climatic conditions from Minnesota and the midwestern United States which resulted in unobvious efficacy of CSC rotation system for management of SCN.

Our work showed that egg hatch of SCN in the soil leachates was affected significantly by soybean genotypes and rotation systems. The soil leachates of susceptible cultivar Hefeng 25 in the different rotation systems except SWC and Fallow promoted egg hatch level and speed of SCN in comparison with distilled water control and no seelding, but that of resistant cultivar Kangxian 4 reduced egg hatch level and speed in SWS. But egg hatch of SCN in the soil leachate of Hefeng 25 was lower in SWC rotation system than SWS, WSW, CSC and S…SS monoculture on 15th day.

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