Rhizobial Nodulation of Alfalfa in Soils Conducive and Suppressive to *Fusarium* Disease

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In general, effectiveness of root-nodule bacteria symbiotic to legumes is attributed to the growth of host plants and activity of the bacteria in nodulation process, and also to ability of nitrogen fixation of the bacteria after nodule formation. Although the nitrogen-fixing ability depends on genetic character of the bacteria, growth of the host plants and activity of the bacteria in nodulation process are closely related with physical, chemical, and microbiological environments of soil. There are less information, however, about possible interaction between rhizobial nodulation and other microorganisms rather than about physical and chemical properties of soil.

On the other hand, it has been recognized that establishment and persistency of legume pastures are difficult, in particular, in southern districts of Japan with warm and humid climatic conditions. As for the difficulty, so-called summer depression, it seems likely that incidence of soil-borne diseases is suggested to be one of the most important causes.[5] It is also brought to the author's notice that pathogenic *Fusarium oxysporum* could be isolated from infected roots of alfalfa wilted in the first summer season in a field of our institute. The fungus *F. oxysporum* is known widely distributed in cropland and grassland soils in Japan, and to be one of the most important soil-borne pathogens throughout the country[3,11]. Furthermore, a forma specialis of the fungus that is pathogenic to clover and alfalfa has recently been reported as a causal agent of legume depression in Japan[1].

From these evidences, it may be of great importance for persistency of the legumes, and also for effectiveness of symbiotic rhizobia in grasslands, to notice an interaction between root-infecting *Fusarium* disease and the growth or nodulation of the host legume. The present paper deals with the rhizobial nodulation of alfalfa affected by *Fusarium* disease. Experimental data were compiled mainly from the author's original paper.[6]

Occurrence of *Fusarium* disease and nodule formation

Fusarium species can commonly be isolated from either root surface of various species of herbage grasses or plant residues in manured pasture soils, but not or rarely from forest and natural grassland soils. From this point, comparison of growth and nodulation of alfalfa seedlings was made with two soils taken from a sown pasture and a native grassland to examine whether occurrence of the root-infecting fungi affects the nodulation or not.

The sown pasture soil was taken from a 6-year grazing pasture in our institute where dominant vegetations were orchardgrass (*Dactylis*) and red top (*Agrostis*), and the native grassland soil from a *Miscanthus* and *Arundinella* grassland near the sown pasture. In this experiment, in order to eliminate a possible nutritional difference of the soils, seedlings were grown in each soil for 3 days before transplanting, together with closely adhering rhizosphere soil, onto Jensen's seedling-agar slant[12] in a test-tube and inoculated with rhizobia. Data obtained are shown in Table 1.

With the sown pasture soil that was naturally infested by *F. oxysporum*, brownish dis-

Table 1. Growth and nodulation of alfalfa seedlings affected by different rhizosphere soils in an early stage of the growth*

Rhizosphere soil	Fresh wt. shoot (mg per plant)	No. of nodules (per plant)	Discoloration of root	Root-infect. fungi
Sown pasture				
Not-sterilized	32	1	+	*F. oxysporum*
Sterlized**	48	2.7	—	
Native grassland				
Not-sterilized	56	2.5	±	—
Sterilized**	50	3.3	—	

* Seedlings were grown on the different soils for 3 days before transplanting to seedling agar, on which they were grown at 26–19°C (15–9 hr/light-dark) for 4 weeks, inoculated with 10^2 rhizobia per plant. Figures are means of 5 replications.

** By steam, 100°C, 1 hr.

coloration of roots and poor nodulation were remarkably observed. The *Fusarium* species isolated here apparently caused root-infection of alfalfa seedlings in an inoculation test showing brown necrotic symptom. When soils were sterilized, healthy growth of roots and an increase in number of nodules were also exhibited. These results emphasize that occurrence of root-infecting fungus, *F. oxysporum*, eventually affects the nodule formation of susceptible varieties of the host legumes.

Nodule formation in disease-suppressive soil

It has been known that there are some soils suppressive to disease which are restrictive to progress of the disease, even when the pathogen has been introduced[4]. Such suppressive nature of the soils apparently results from complex microbiological interactions that provide biological control of the disease, though the causes are not well known today.

As to *Fusarium* disease, an example that is designated as legume-persistent grassland soil has recently been reported in Hokkaido by Araki et al.[1,2].

In this respect, an experiment for nodule formation was conducted by comparing the soil suppressive (legume-persistent, Date soil) with the soil conducive (non-persistent, Hayakita soil) to *Fusarium* disease. Characteristics of the soils are given in Table 2. Disease-suppressive Date soil shows a finer texture and more bacterial plate counts than conducive Hayakita soil. In addition, according to soil-inoculation test with *F. oxysporum*, hyphal growth of the fungus that was observed on contact-slide glass, was consistently less in the suppressive soil as compared with the conducive soil. The soils placed in 400-m*l* beakers were moistened to approximately field capacity and limed prior to planting alfalfa.

From the results (Table 3), it was noted that almost no visible nodule was produced with the conducive soil showing discoloration of roots. Growth of the seedlings was also

Table 2. Analyses of experimental soils conducive(C) and suppressive(S) to *Fusarium* disease

Soil	pH	C/N	Mechanical analysis			Microbial analysis		
			Sand (%)	Silt(%)	Clay(%)	Fungi(10^4)	Acti.(10^6)	Bact.(10^6)
Hayakita(C)	5.5	13.9	86.3	9.1	4.7	31	1	7
Date(S)	6.3	11.8	70.1	21.3	8.6	5	1.3	31

pH, C/N, and mechanical analysis were compiled from data of Araki (1978).

Table 3. **Growth and nodulation of alfalfa seedlings in a disease-conducive and a suppressive soil**

Soil	pH	Shoot (mg per plant)	Root (mg per plant)	Disease index	No. of nodules (per plant)
Conducive soil					
Not-inoculated	7.4	31	33	+	—
Inoculated	7.4	33	38	+	0
Suppressive soil					
Not-inoculated	7.5	53	38	—	—
Inoculated	7.5	50	40	—	1.5

Soils were limed, and inoculated with or without 10^4 rhizobia per plant, grown at 26–19°C (15–9 hr/light-dark) for 3 weeks.

less than with the suppressive soil. Examination of infecting fungi on the roots showed that pathogenic *F. oxysporum* existed with both the conducive and suppressive soils. It seemed probably, therefore, that the root-disease caused by the fungus but not the fungus itself restricted the nodule formation in the conducive soil as previously shown in Table 1 for the pasture soil. On the other hand, it was noticeably indicated that suppressiveness of the disease and good nodulation could be seen with the suppressive soil though the pathogenic fungus existed.

According to the microscopic examination of well-washed root segments placed on water-agar plate, there appeared to be proportionately fewer *Fusarium* and more bacteria with the suppressive soil than with the conducive soil (Table 4, Plate 1). This evidence suggests that the bacteria multiplied on the root surface may, at least in part, possibly exhibit antagonism to *Fusarium*. Smith & Snyder[7] also observed that rate of bacterial multiplication was great in a disease-suppressive soil rather than in a conducive soil.

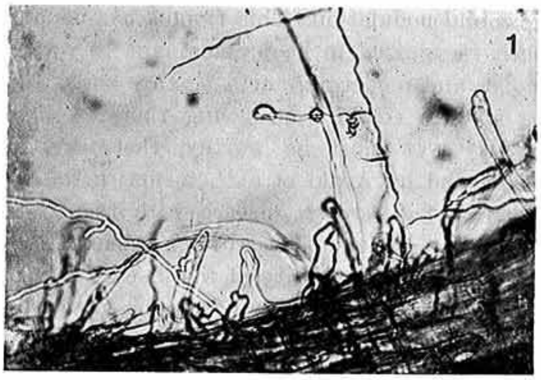
In addition, there has recently been indi-

Table 4. **Occurrence of *Fusarium* and bacteria on root surface of alfalfa grown in a disease-conducive and a suppressive soil***

Soil	*Fusarium* (%)**	Bacteria (%)**
Conducive soil		
Not-inoculated	31	53
Inoculated	38	31
Suppressive soil		
Not-inoculated	22	75
Inoculated	6	78

*: The data correspond to Table 3

**: Percent frequency of occurrence on root segments



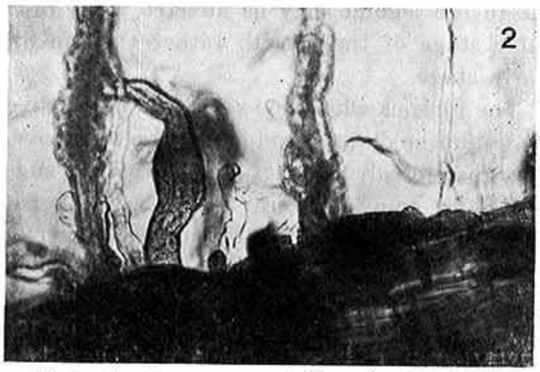


Plate 1. Occurrence of *Fusarium* (1), and bacteria (2), on the root segments of alfalfa grown in a disease-conducive, and a suppressive soil, respectively

cated a further interesting problem that a definite antagonistic interaction between root-nodule bacteria and pathogenic fungi may be present. Tu[9,10] reported that rhizobial nodulation reduced the severity of *Fusarium* root-rot in soybeans, and that, *in vitro*, rhizobial parasitism against root-rot fungi would be expected. The present author also observed that hyphal growth of *F. oxysporum* was suppressed by multiplying of rhizobia, *R. meliloti*, in a mixed culture. Further study is needed, however, on the pathogen-antagonist-soil combination with special reference to bacterial multiplication in the rhizosphere of host plants.

Concluding remarks

In the present work, it was found that naturally-infested *Fusarium oxysporum* pathogenic to alfalfa might eventually affect the rhizobial nodulation. This fungus has usually been recognized to be predominant in warm, moist, and moderately acid pasture soils, and be able to colonize on young roots of host plants[8]. It must be worthy, therefore, as mentioned by Araki et al.[1], to notice the incidence of *Fusarium* disease with regard to depression of legume pasture in Japan. Furthermore, since severity of the disease caused by *F. oxysporum* is revealed to be gradually progressive, it is possible to consider that nodulation and effectiveness of rhizobia symbiotic to the legume may be affected more in a later stage of the growth rather than in an early stage.

The reasons why some soils are suppressive to soil-borne disease, and others are conducive, are undoubtedly complex and varied as suggested by many workers[4]. However, it must be considered important to support bacterial multiplication, inclusive of inoculated rhizobia, in a given soil.

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