Plant Pathology Bulletin 11:75-84, 2002

Impact of Crop Rotation on Soilborne Diseases and Yield of Kidney Bean: A case study in northern Japan

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Accepted for publication : May 30, 2002

ABSTRACT

Huang, H. C. [1,5], Kodama, F. [2], Akashi, K. [3], and Konno, K. [4] 2002. Impact of Crop Rotation on Soilborne Diseases and Yield of Kidney Bean: A case study in northern Japan. Plant Pathol. Bull. 11:75-84.

The effects of crop rotation on diseases and yield of kidney bean were investigated using six of the plots in the long-term crop rotation experiment established since 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan. Data collected in 1994 showed that compared to the 6-year rotation, kidney bean cultivation in repeated monoculture resulted in a significant (P<0.05) reduction in plant height, aboveground plant biomass, pod number, seed yield, and seed size. During the last 12-year period (1989-2000), the average seed yield of kidney bean was 970 kg/ha in monoculture, representing a 59% loss as compared to the average yield of 2380 kg/ha in the 6-year rotation. Greenhouse tests of soil samples collected in 1994 revealed that the incidence of Pythium damping-off of kidney bean and sugar beet was highest (>66.1%) in the soil from bean monoculture, modest in the soil from bare fallow, and lowest (<38.9%) in the soil from bean in the 6-year rotation. Fusarium yellow of kidney bean was detected at high frequency (87%) only in the soil of bean monoculture. These results from testing of soil samples and examination of field plants suggest that the stunting and yellowing of kidney bean plants observed in monoculture plots were due to a root rot complex caused by *Pythium* spp. and *Fusarium* spp. Several isolates of *Pseudomonas* sp. from healthy bean rootlets of the rotation plots showed suppressive effects to the damping-off pathogens in this field. This study suggests that the soil testing method is useful for studying activities of soilborne pathogens and beneficial microorganisms in microplots where removal of plants is restricted.

Key words: Crop rotation, Pythium damping-off, Fusarium yellow, biocontrol, kidney bean, sugar beet

INTRODUCTION

Crop rotation is a cultural practice which has long been regarded as an effective method for control of plant diseases [(20)]. This is based on the notion that repeated cropping of the same annual crop can enhance the build-up of plant pathogens. For example, repeated bean monocultre resulted in severe outbreaks of Fusarium root rot caused by *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. in USA [(3,18)] and Japan [(19)]. Burke and Kraft [(3)] observed that the incidence of Fusarium root rot and Pythium damping-off was high in a field where beans were planted continuously for 15 years, whereas these two diseases were not detected in another field where bean had never been grown previously.

In northern Japan, a long-term crop rotation experiment to study effects of crop sequence on crop production was established in 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido. Previous investigations of this field revealed that continuous monocropping of kidney bean [(13)] and soybean [(14)] resulted in severe root rots on these crops. *Pythium myriotylum* Drechsler and an unidentified *Pythium* sp. were the causal agents for the growth retardation and root necrosis of the bean [(10,13)] and the soybean [(14)] in monoculture. Based on the field study conducted in 1979, Kageyama *et al*. [(13)] considered that *F solani* f. sp. *phaseoli* was not associated with the root rot of bean in the monoculture plots of the field at the Kitami

Agricultural Experiment Station. Ozaki and Kodama [16] reported that Fusarium yellow of bean caused by *F. oxysporum* Schlechtend was prevalent in Hokkaido.

Field inspection of the long-term crop rotation experiment in July, 1994 revealed that the plants of kidney bean, cultivar Taishokintoki, in the monoculture plots were short and most of the lower leaves turned yellow, whereas the plants in the 6-year rotation plots were tall and the leaves remained green. The symptoms of plant stunting and leaf yellowing suggested that these plants might have suffered from root diseases. However, removal of large number of plants from the microplots for direct examinations of root tissues was prohibited due to requirements of annual data on seed yield, plant height and biomass for this long-term experiment. The objective of this study was to determine the impact of crop rotation on growth, yield and root diseases of kidney bean and to assess the feasibility of using soil testing methods for studying these diseases without removal of large number of diseased plants from the plots.

MATERIALS AND METHODS

Source of plant and soil samples

The long-term crop rotation experiment established in 1959 at the Kitami Agricultural Experiment Station, Hokkaido, Japan, contained seven treatments with two replicates each. The treatments were: (1) bare fallow (no crops or weeds); (2) monoculture (potato, sugar beet, oat, kidney bean, or winter wheat); (3) two-year rotation (oat-sugar beet); (4) three-year rotation (soybean-sugar beet-oat); (5) four-year rotation (potato-sugar beet—oat-soybean); (6) five-year rotation (sugar beet—oat-winter wheat-red clover-potato); and 7) six-year rotation (potato-sugar beet—oat-kidney bean-winter wheat-red clover). The plots layout and the crops planted in 1994 were shown in Figure 1. The size of each plot was 6.5 m x 5 m (length x width).

Kidney beans, cultivar Taishokintoki, from the treatments of monoculture and 6-year rotation were compared in this study. The crop was planted on May 20, 1994, with 50 cm between rows and 25 cm between planting spots within rows. There were two plants per planting spot. On August 25, 1994, 40 plants (or 20 planting spots) from each plot were measured for height. On September 5, 1994, 96 plants (or 48 planting spots) from each plot were harvested, air-dried, and threshed to measure seed yield and the total biomass of the aboveground tissues which included seeds and chaff. Data on number of pods per planting spot (two plants) and single seed weight were also collected. In addition to the results of 1994, seed yields of kidney bean, cultivar Taishokintoki, collected from monoculture and 6-year rotation during 1989-2000 were also compared.

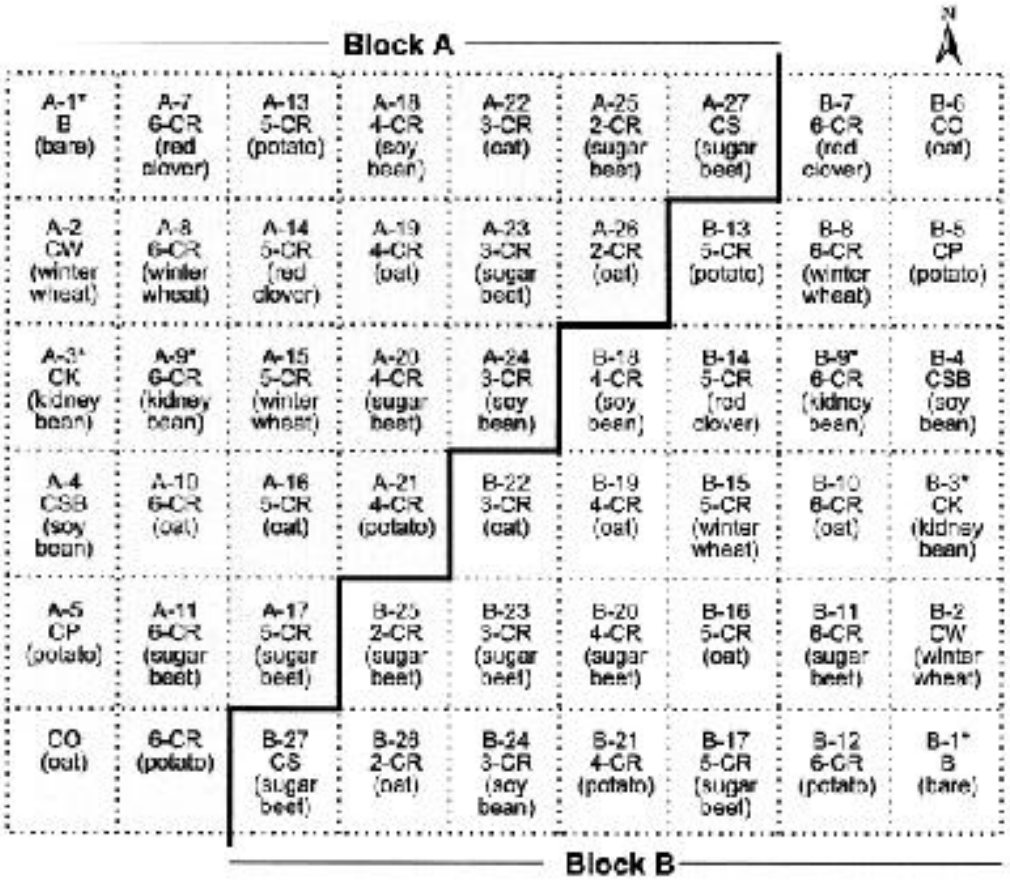
Soil samples were collected on July 14, 1994, from the plots of bare fallow, kidney bean monoculture, and kidney bean in 6-year rotation. Soil samples were collected up to a depth of 10 cm from random spots of the fallow plots and from the row spacing of the bean plots, 20 samples/plot. The samples from each plot were mixed thoroughly, sieved through a screen (2 mm mesh), and air-dried. All soil samples were stored in wooden flats at room temperature until used for the experiments.

Isolation of soilborne pathogens

Methods including direct root examination and indirect soil testing were used to study the cause of stunting and leaf yellowing of the kidney bean plants in monoculture. For direct plant inspection, six bean plants showing symptoms of stunting and yellowing were removed from the monoculture plots and washed to remove soil particles on the roots. Lesions, dark brown and reddish brown, on taproots and lateral roots were excised, surface sterilized in 70% ethanol for 2 min., placed on potato dextrose agar (PDA) and incubated at room temperature (20-26℃) for 2-5 days. Fungi derived from the tissues were isolated and purified by single hyphal tip and/or single spore isolations. Pure cultures on PDA were examined for morphological characteristics with a compound microscope. Stock cultures were stored at 20℃.

For the indirect testing, sugar beet, cultivar Monohomare, and kidney bean, cultivar Taishokintoki, were used as baits to detect the pathogens in the soils of bare fallow, bean monoculture and bean in 6-year rotation. Seeds of sugar beet were sown 2 cm deep in the soil in pots (9 cm diam.), 14 seeds/pot. After adjusting the soil moisture to field capacity (about 35%), the pots were kept in a clear plastic chamber in a greenhouse (18-26℃) for 6 days and examined for the number of emerged seedlings and incidence of damping-off. The treatments were arranged in a randomized complete block design with four replicates per treatment. Ten sugar beet seedlings showing damping-off symptoms were collected from each treatment to isolate the causal organism. Isolation and purification of fungi derived from the diseased tissues were made using the same procedure for the bean tissues described above. Six isolates of *Pythium* sp. from damping-off of sugar beet were tested for pathogenicity in a greenhouse. The experiment was set up by planting sugar beet seeds, 14 seeds/pot, in a commercial growth substrate made of pasteurized soil and fertilizers (Hokkaisankyo Co. Ltd., Hokkaido), inoculating the seeds with *Pythium* sp., using the methods described by Huang *et al*. [9], and checking for damping-off after incubating in a greenhouse for 10 days. The experiment was repeated in another greenhouse (air conditioned with a temperature range of 20-26℃) using the same procedure except for 16 seeds per pot. Data on seedling emergence and incidence of damping-off were collected 10 days after seeding.

Two experiments were carried out to test the soils for root pathogens of kidney bean by the same baiting method used for sugar beet. In the first experiment, there were 4



a The experiment was established in 1959. The symbols are: B = bare fallow; C = monoculture; 2-CR = 2-year rotation (oat- sugar beet); 3-CR = 3-year rotation (soybean- sugar beet- oat); 4-CR = 4-year rotation (potato- sugar beet—oat- soybean); 5-CR = 5-year rotation (sugar beet—oat- winter wheat- red clover- potato); and 6-CR = 6-year rotation (potato- sugar beet—oat- kidney bean- winter wheat- red clover).

b * : Soil samples were collected from the plots of bare fallow (A-1 and B-1); bean monoculture (A-3 and B-3); and bean in 6-year rotation (A-9 and B-9) on July 14, 1994.

Fig. 1. A diagram showing the plot design of the long-term crop rotation experiment at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan (1994).

replicates per treatment with 7 seeds per replicate (pot). The pots were kept in a clear plastic chamber under the greenhouse bench (18-26°C) for 8 days and examined for number of emerged seedlings and incidence of damping-off. For the second experiment, there were five replicates (pots) for each treatment and 5 seeds in each replicate. The pots were kept in the greenhouse (26-40°C) for 10 days, with the first 4 days in clear plastic chambers, and examined for seedling emergence and root rot. Isolations and pathogenicity tests of the fungi from bean seedlings were done by the same aseptic procedure described above for the sugar beet experiments.

Isolation of rhizosphere bacteria

Rootlets of kidney bean plants collected from plots of bean monoculture and bean in 6-year rotation in July, 1994 were washed, cut into small pieces, and divided into 1-g samples. To make a bacterial suspension, each 1-g rootlet sample was soaked in 100 ml deionized water and shaken vigorously for three times at one min each to make a bacterial suspension. After filtering through a double-layered cheesecloth, the filtrate was diluted 100 fold and flooded on PDA containing 5 g of chitin/L in 5 Petri dishes, 5 ml/dish. After incubation at room temperature (20-26°C) for 2 to 3 days, single bacterial colonies were isolated and the stock cultures were maintained on PDA slants at 20°C. The rhizosphere bacterial cultures were identified by the method described by Nishiyama [15].

Biocontrol of damping-off by rhizosphere bacteria

The bacterial isolates from bean rootlets were assessed for antagonism against an isolate of *Pythium* sp. isolated from

a diseased seedling of sugar beet from a previous experiment. Dual cultures were set up by streaking each bacterial isolate on PDA near the edge of the Petri dish, incubating at 20℃ for 4 days and then inoculated with the pathogen by placing an agar block containing mycelial mats (5 mm diam) of *Pythium* sp. in the center of each dish. The dual cultures were incubated at 20℃ for 6 days and the inhibition zone measured by the categories of no inhibition (-, 0 mm), slight inhibition (+, <4 mm), moderate inhibition (++, >4 mm and <8mm) or strong inhibition (+++, >8mm).

Four isolates of *Pseudomonas* sp., isolates 3a-1, 3b-1, 3b-9 and 3b-14, from the bean plots in 6-year rotation, and one unidentified isolate 2b-5 (orange colored colony on PDA) were tested for control of damping-off of sugar beet. Soil samples collected from fallow, bean monoculture and bean in 6-year rotation were mixed at 1:1:1 (v/v/v) ratio. Inoculum of each bacterial isolate was prepared with and without primer. For the inoculum without primer, the bacterial suspension was made by flooding the 1-day-old PDA culture with sterile water at 1.5 ml/dish and for the inoculum with primer, the bacterial suspension was made by flooding the 1-day-old PDA culture with a sterile solution containing a 0.6% nutrient broth and 2% gelatin at 1.5 ml/dish. For the treatments without primer, seeds of sugar beet, cultivar Monohomare, were surface sterilized in 70% ethanol for 2 min., air-dried on paper towel, soaked in the bacterial suspensions for 10 min. and planted in the soil in pots, 16 seeds/pot. The same planting method was used for the treatments with primer, with the exception of soaking sugar beet seeds in the bacterial suspensions for 24 hrs before planting. Seeds soaked in sterile water or in the nutrient solution were also planted in the soil as controls. After watering, the pots were kept in a greenhouse (air conditioned, 20-26℃) for 10 days (in a plastic chamber for the first 6 days) and the incidence of damping-off was recorded. There were 12 treatments in this experiment with 4 replicates per treatment.

Statistical methods

Statistical analyses were conducted for the data in this study using SAS/STAT[7] software [(17)]. Student=s *t*-test was used to determine significant differences in seed yield of kidney bean between monoculture and 6-year rotation, for each of the years 1989-2000. Student=s *t*-test was also used to analyze the data on plant height and total biomass (seed and other above ground tissues), pod number and single seed weight of kidney bean, collected from monoculture and 6-year rotation in 1994. Disease data of kidney bean and sugar beet collected from soil testing in 1994 were analysed by analyses of variance. Treatment means were compared using Duncan=s Multiple Range Test. Analyses of variance and Duncan=s Multiple Range Test were also used for the data collected from the control of damping-off of sugar beet by bacterial seed treatment study in 1994.

RESULTS

Effect of crop rotation on yield of kidney bean

Data collected from the plots during 1989-2000 showed that seed yield of kidney bean in monoculture was significantly (P<0.05) lower than the kidney bean under 6-year rotation for each of the years except 1989 and 1997 (Table 1). The annual yield reduction in monoculture varied from 44.9% in 1997 to 72.9% in 1996, averaging 59% of annual loss during the 12-year period. In addition to the reduction in seed yield, the data in 1994 also showed a significant (P<0.05) reduction in plant height and total biomass (seeds and aboveground tissues), pod number and seed size for the kidney bean in monoculture (Table 2). Compared to the plants under 6-year rotation, plant height, total biomass, pod number and seed size of kidney bean in monoculture were reduced by 26%, 56%, 52% and 8%, respectively. While the bean plants in the plots of the 6-year rotation remained green and healthy in mid-July, the plants in the monoculture plots showed symptoms of severe stunting and leaf yellowing (Fig. 2). By late July, most of the yellow leaves in monoculture had fallen, resulting in the appearance of a thin canopy, whereas the plants in the plots of the 6-year-rotation continued to grow and form a thick, close canopy.

Root rot diseases of kidney bean and sugar beet

The six bean plants collected from the monoculture plots showed symptoms of plant stunting and leaf yellowing and the taproots and lateral roots were covered with numerous dark brown to reddish brown lesions. *Pythium* spp. and *Fusarium* spp. were isolated from all of these plants and

Table 1. Seed yield of kidney bean, cultivar Taishokintoki, under 6-year rotation and monoculture (1989-2000).[1]

Year	Yield (kg/ha)		Loss in monoculture (%)
	6-year rotation [2]	Monoculture	
1989	2105	845	59.8
1990	2830	1400	50.5 [3]
1991	3065	1115	63.6 [3]
1992	2675	910	65.9 [3]
1993	2125	770	63.7 [3]
1994	2700	945	65.0 [3]
1995	2800	1350	51.7 [3]
1996	1830	495	72.9 [3]
1997	1625	895	44.9
1998	2385	950	60.1 [3]
1999	2090	970	53.5 [3]
2000	2330	1025	56.0 c

1. The experiment was established in 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan.
2. The crop sequence for the 6-year rotation: potato- sugar beet – oat- kidney bean- winter wheat- red clover.
3. Yield loss in monoculture is significant (P<0.05, Student's *t*-test).

Table 2. Effect of 6-year rotation and monoculture on plant height, biomass, pod formation and seed size of kidney bean, cultivar Taishokintoki.(1994). [1]

Treatment	Plant height [2] (cm)	Biomass [3] (kg/10a)	No. pods (2 plants) [4]	Seed weight (g/seed)
Bean, 6-year rotation	45.0 a [5]	471.5 a	17.8 a	0.585a
Bean, monoculture	33.3 b	207.0 b	8.5 b	0.538b
P =	0.0457		0.0138	0.030

1. The experiment was established since 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan. The crop sequence for the 6-year rotation: potato- sugar beet—oat- kidney bean- winter wheat- red clover.
2. Plant height was measured on August 25, 1994 based on average of 40 plants (or 20 planting spots) per plot.
3. Biomass was measured at harvest (September 5, 1994) based on the combined results of seed weight and chaff of 96 plants (or 48 planting spots) per plot.
4. Two plants per planting spot.
5. Means in each column with the same letter are not significantly different at 0.05 level, using Student's *t*-test.

frequently, both organisms were isolated from the same piece of diseased root, especially from the dark brown lesions. *Fusarium* spp. were isolated more readily from reddish brown lesions than the dark brown ones.

Results of greenhouse tests on soil samples collected in 1994 from the plots of fallow, bean monoculture and bean in 6-year rotation showed that there was a significant ($P<0.01$) reduction in seedling emergence of bean and sugar beet grown in the soil from bean monoculture plots (Table 4) and that the poor emergence was due to pre-and post-emergence damping-off on these crops (Figs. 3, 5). Isolations from diseased seedlings showed that *Pythium* spp. was the causal agent for the damping-off of these two crops. Among the three soil samples examined, incidence of Pythium damping-off of bean and sugar beet was the highest in the soil from the bean monoculture (66.1% in bean and 74.8% in sugar beet), modest in the soil from bare fallow, and the lowest in the soil from bean in the 6-year rotation (16.1% in bean and 38.9% in sugar beet) (Table 3).

Fusarium yellow of kidney bean was detected in another greenhouse test but the disease was found only in the soil of bean monoculture and was absent in the soils from bare fallow or bean in 6-year rotation (Table 5, Fig. 4). Although sudden wilt symptoms (Fig. 4) occurred in all seedlings in the treatment of monoculture soil, results of isolations from diseased tissues of these plants showed that 57% of taproots and 87% of hypocotyls yielded *Fusarium oxysporum*. The diseased tissues that failed to yield *F. oxysporum* were heavily contaminated with bacteria.

Biocontrol of damping-off by bacterial seed treatment

Thirty-two isolates of bean rhizosphere bacteria including 11 from the plots of bean monoculture and 21 from the plots of bean in 6-year rotation were tested against *Pythium* sp. isolate beet-1 in dual culture. Results showed that one isolate (3a-1) was highly inhibitory to *Pythium* sp. with an inhibition zone of 13 mm and four isolates (3a-2, 3b-1, 3b-5 and 3b-8) were slightly inhibitory to the pathogen with an inhibition zone of less than 2 mm. All these antagonistic isolates were isolated from healthy rootlets of kidney bean in the 6-year rotation and they were identified as *Pseudomonas* sp.

Treatment of sugar beet seeds with rhizosphere bacteria with or without primer significantly ($P<0.0001$) reduced the incidence of damping-off and increased seedling emergence (Table 5). The treatments of *Pseudomonas* sp. 3a-1 with primer, *Pseudomonas* sp. 3b-1 with primer, *Pseudomonas* sp. 3a-1 without primer, *Pseudomonas* sp. 3b-9 with primer, *Pseudomonas* sp. 3b-14 with primer, unidentified isolate 2b-5 with primer, and control II (primer only), resulted in significantly higher emergence than the control I (water only). No interaction between antagonistic isolates and primer was found, and both the primer ($P<0.0001$) and isolate ($P<0.001$) effects were significant. Meanwhile, seed treatment with primer improved the vigor of sugar beet seedlings (Fig. 6) for all the treatments including the control II (primer only) (Table 5).

DISCUSSION

The high incidence of Pythium damping-off of kidney bean and sugar beet observed in the soil samples from bean monoculture in 1994 confirms the study in 1979 by Kageyama *et al*. [13] that *Pythium* spp. were the major cause for plant stunting and root necrosis of kidney bean observed in the monoculture plots of the crop rotation experiment at the Kitami Agricultural Experiment Station, Hokkaido. However, bioassay of soil samples and isolation of pathogens from diseased plants collected from this field indicate that the stunting and leaf yellowing of kidney bean observed in 1994 were due to root rot complex caused by *Pythium* spp. and *F. oxysporum*. Thus, the soil testing method is particularly useful for monitoring root diseases of crops in long-term crop rotation experiments where excessive removal of plants is restricted in a consideration of crop yield and other agronomic parameters. Furthermore, the present study on the control of Pythium damping-off by seed treatment with rhizosphere bacteria isolated from healthy bean plants suggests that, in addition to soilborne pathogens, the soil testing method is also valuable in studying population

Fig 2. The kidney bean, cultivar Taishokintoki, showing stunting plants with yellow leaves in monoculture (Fig. 2, left; plot A-3 in Fig. 1) and tall plants with healthy green leaves in 6-year rotation (Fig. 2, right; plot A-9 in Fig. 1). (July 17, 1994, Kitami Agricultural Experiment Station, Hokkaido).

Fig. 3. Incidence of Pythium damping-off of kidney bean, cultivar Taishokintoki, in soils collected in 1994 from the plots of bare fallow (left), bean monoculture (center) and bean in 6-year rotation (right). Note the disease was severe in the soil from bean monoculture.

Fig. 4. Incidence of Fusarium yellow of kidney bean, cultivar Taishokintoki, in soils collected in 1994 from the plots of bare fallow (left), bean in monoculture (center) and bean in 6-year rotation (right). Note the disease occurred only in the soil from bean monoculture.

Fig. 5. Incidence of Pythium damping-off of sugar beet, cultivar Monohomare, planted in soil samples collected in 1994 from the plots of bare fallow (left row), bean in monoculture (center row) and bean in 6-year rotation (right row). Note high disease incidence in the soil from bean monoculture (center row). Top row: soil samples from Block B; bottom row: soil samples from Block A (Fig. 1).

Fig. 6. Control of Pythium damping-off of sugar beet, cultivar Monohomare, by seed treatment with rhizosphere bacteria. The soil used in this test was collected from the plots of bare fallow, bean in monoculture and bean in 6-year rotation, which were mixed at 1:1:1 (v/v/v) ratio. Seeds were treated with water (control I) (bottom left), *Pseudomonas* sp. isolate 3b-9 in water (bottom center), and *Pseudomonas* sp. isolate 3a-1 in water (bottom right) or treated with the primer (0.6% nutrient broth) (control II) (top left), *Pseudomonas* sp. isolate 3b-9 in primer (top center), and Pseudomonas sp. isolate 3a-1 in primer (top right). Note the difference in incidence of Pythium damping-off and growth vigor of sugar beet between controls (left row) and bacterial treatments (center and right rows).

Table 3. Incidence of Pythium damping-off of kidney bean and sugar beet in the soils of bare fallow, bean monoculture and bean in 6-year rotation (1994). [1]

Source of soil (Treatment)	Kidney bean [2] Emergence (%)	Kidney bean [2] Damping-off (%)	Sugar beet [3] Emergence (%)	Sugar beet [3] Damping-off (%)
Bare fallow	57.1 b [4]	42.9	48.2 a [4]	51.8
Bean, monoculture	33.9 b	66.1	25.2 b	74.8
Bean, 6-year rotation	83.9 a	16.1	61.1 a	38.9
P =	0.0022		0.0027	

1. The experiment was established since 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan. The crop sequence for the 6-year rotation: potato- sugar beet—oat- kidney bean- winter wheat- red clover.
2. Kidney bean, cultivar Taishokintoki, was planted in the soils in pots, 7 seeds per pot and 4 pots for each treatment. Data were collected 8 days after seeding.
3. Sugar beet, cultivar Monohomare, was used for two experiments. Combined results of two experiments were presented based on 14 seeds/pot and 4 pots/treatment for the first experiment and 16 seeds/pot and 4 pots/treatment for the second experiment. Data were collected 6 and 10 days after seeding for experiments 1, and 2, respectively.
4. Means in each column with the same letter are not significantly different at 0.05 level, using Duncan's Multiple Range Test.

Table 4. Incidence of Fusarium yellow of kidney bean in the soils of bare fallow, bean monoculture and bean in 6-year rotation (1994). [1]

Source of soil	Kidney bean [2] Emergence (%)	Kidney bean [2] Fusarium yellow (%)
Bare fallow	100	0
Bean, monoculture	100	87 [3]
Bean, 6-year rotation	100	0

1. The experiment was established in 1959 at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan. The crop sequence for the 6-year rotation: potato- sugar beet—oat- kidney bean- winter wheat- red clover.
2. Kidney bean, cultivar Taishokintoki, was planted in the soils in pots, 5 seeds per pot and 5 pots for each treatment. Data were collected 10 days after seeding.
3. All seedlings wilted but only 87% of the diseased plants yielded *Fusarium oxysporum* by isolation of water-soaked lesions on hypocotyls.

dynamics of beneficial microorganisms, such as biocontrol agents, associated with crops grown under rotation or monoculture.

The crop rotation experiment at the Hokkaido Prefectural Kitami Agricultural Experiment Station, Hokkaido, Japan, has lasted for 41 years (1959-2000). Kageyama *et al*. (13) reported a 30% reduction in seed yield for the kidney bean in monoculture in 1979, compared to the crop in 6-year rotation. The yield data for1989-2000 showed that compared to the bean in 6-year rotation, the averaged annual loss of seed yield of kidney bean in monoculture was 59% for the 12-year period (Table 2). These results indicate that since 1979 (13), a further decline of 29% of seed yield has occurred due to bean monoculture in this field. In addition to seed yield reduction, the study also reveals a reduction in seed quality as evidenced by the small seed size for the bean plants in monoculture.

Table 5. Biocontrol of Pythium damping-off of sugar beet by seed treatment with rhizosphere bacteria from bean plots (1994). [1]

Seed treatment [2]	Bacterial suspension	Seedling emergence (%) [4]	Damping-off (%)
Control BI	in water	32.8cd [5]	67.2
Unidentified, 2b-5	in water	17.1d	83.9
Pseudomonas sp. 3a-1	in water	75.0ab	25.0
Pseudomonas sp. 3b-1	in water	54.7bc	45.3
Pseudomonas sp. 3b-9	in water	56.3bc	43.7
Pseudomonas sp. 3b-14	in water	48.4bc	51.6
Control BII	with primer [3]	64.1ab	35.9
Unidentified, 2b-5	with primer	67.2ab	32.8
Pseudomonas sp. 3a-1	with primer	89.1a	10.9
Pseudomonas sp. 3b-1	with primer	84.4a	15.6
Pseudomonas sp. 3b-9	with primer	75.0ab	25.0
Pseudomonas sp. 3b-14	with primer	73.4ab	26.6
P =		0.0001	

1. Soil samples collected in July, 1994 from fallow, bean monoculture and bean in 6-year rotation were mixed at 1:1:1 ratio (v/v/v). The soil was naturally infested with damping-off pathogens.
2. Bacteria were isolated from rootlets of bean. *Pseudomonas* sp. 3a-1, 3b-1, 3b-9, and 3b-14 the plots of 6-year rotation and the unidentified isolate 2b-5 were from 3b-14 the plots of 6-year rotation and the unidentified isolate 2b-5 was from a monoculture plot.
3. Primer is 0.6% nutrient broth containing 2% gelatin.
4. Based on 16 seeds/replicate, 4 replicates/treatment. Data were collected 10 days after seeding.
5. Means in the column with the same letter are not significantly different at 0.05 level, using Duncan's Multiple Range Test.

From the disease perspectives, an effective crop rotation is based not only on the type of crops and the length of rotation, but also on the sequence of the crops. An early report by Coons and Kotila (6) showed that when sugar beet follows alfalfa, there is much more damping-off of sugar beet

seedlings than when sugar beet follows corn. Goss and Afanasiev [8] studied effects of rotations on potato diseases and recommended that when sugar beet and potato are in rotation with alfalfa, it is important that alfalfa should precede potato, not sugar beet. Previous studies of this crop rotation field at the Kitami Agricultural Experiment Station showed that P. myriotylum and an unidentified *Pythium* sp. were the causal agents for root rot of kidney bean [10,11,13] and soybean [14] in the monoculture plots of this field and that these organisms were also pathogenic to sugar beet and pea [12]. Since kidney bean, soybean and sugar beet are used in this crop rotation study (Fig. 1), using these crops in sequence may increase the diseases caused by the pathogens such as *P. myriotylum*. Thus, the design of soybean preceding sugar beet as shown in the 3-year rotation in this experimental field (Fig. 1) might increase the risk of Pythium damping-off of soybean.

Crop rotation has often been recommended for managing crop diseases without carrying out systematic research on the effect of previous crops on reducing or increasing disease problems of the following crops. There are very few examples of crop rotation experiments like the one at the Kitami Agricultural Experiment Station, which lasted for 41 years. The data on crop production, soil quality, and pests including diseases, insects and weeds collected from this field are precious. The beneficial or harmful effects involved in repeated monocroppimg and rotations on crops like bean [13] and soybean [14] were available after 20 years of studies in this field. Thus, it might be possible to minimize the period of a crop rotation experiment if annual data on crop productions, soil properties, and pests are collected and analyzed.

Burke and Miller [4] reported that rotation of beans with crops such as small grains tends to reduce root rot of beans. Their observation supports the finding of Snyder *et al*. (18) that amendment of soil with mature barley straw which is rich in carbon and low in nitrogen gave good control of Fusarium root rot of bean. The healthy kidney bean crop observed in the plots of the 6-year rotation in 1994 (Fig. 2) may be related to the cereal crops in this rotation, oat as the preceding crop and winter wheat as following crop of bean (Fig. 1), as the soil testing revealed a high incidence of Fusarium yellow of bean in the soil from bean monoculture plots but not in the soil from fallow or bean in 6-year rotation. Meanwhile, the strong suppression of damping-off of sugar beets by isolates of *Pseudomonas* sp. obtained from healthy rootlets of kidney bean in the rotation plots suggests that antagonistic microorganisms might have been associated with the root health of bean plants observed in the rotation plots in 1994. Further studies on population dynamics of pathogens and beneficial microorganisms such as antagonists are essential for understanding mechanisms involved in the reduction of plant diseases and the improvement of crop production in a particular crop rotation sequence.

Studies in Canada showed that a rotation of legume crops with cereals is superior to the cereal monoculture because the legume-based rotation system improves yield of cereal crops [5,21], improves fertility and quality of soils [1,7] and increases soil microbial populations including fungi, bacteria and actinomycetes [2]. The present study of the rotation experiment in northern Japan revealed that a legume-based rotation system also minimizes yield loss of the legume crop through a reduction of soilborne diseases of this crop.

ACKNOWLEDGEMENTS

The senior author would like to express his sincere thanks to the Government of Hokkaido, Japan, for the Visiting Professorship for conducting this study at the Hokkaido Prefectural Kitami Agricultural Experiment Station in 1994. Also, we are grateful to Dr. H.-H. Muendel, bean breeder, and Dr. F.J. Larney, soil scientist, for reviewing this manuscript and to Mr. R. S. Erickson, for conducting statistical analyses. This is a Lethbridge Research Centre Contribution, No. 3870078.

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摘 要

黃鴻章[1,5]、Kodama, F. [2]、Akashi, K. [3]、Konno, K [4]. 2002. 用日本北部的一項試驗來探討輪作對菜豆產量及根部病害之影響. 植病會刊 11:75-84. ([1.] 加拿大農業及業食品部。Letasridze 研究中心；[2.] 日本北海道Kitami農業試驗所；[3.] 日本北海道中央農業試驗所；[4.] 日本北海道農業試驗所；[5.] 聯絡作者：電子郵件：huangh@emagr.ca；傳真：(403) 382-3156)

本文旨在用日本北海道 Kitami 農業試驗所的一項長期輪作栽培試驗 (1959-2000) 來比較輪作體系對菜豆產量及根部病害發生的影響。由 1994 年的資料顯示，和六年輪作處理 (即馬鈴薯、甜菜、燕麥、菜豆、冬小麥、紅三葉草) 相比，菜豆連作處理區之植物平均高度、地上部結夾數與產量、以及種子大小和產量均顯著較差。由 12年 (1989-2000) 平均資料顯示，菜豆種子的年平均產量在六年輪作區是 2380 kg/ha，而在連作區只有 970 kg/ha，平均年損失 59%。於 1994 年進一步採集菜豆連作區、六年輪作區及長期休耕 (bare fallow) 區作的土壤，進行微生物分離與比較，結果顯示土壤中均含有引起菜豆及甜菜發生猝倒病的病菌 (*Pythium* sp.)，以長期連作區的密度最高 (＞66.1% 的幼苗死亡率)，長期休耕區次之，而六年輪作區最低 (＜38.9% 的幼苗死亡率)。又造成菜豆萎凋病的病菌 (*Fusarium* sp.)，僅出現於長期菜豆連作區之土壤中 (87% 病株)。另外於長期連作區採集的菜豆萎黃病株，根部病原菌分離結果多屬 *Pythium* sp. 與 *Fusarium* sp. 病菌，而健康的菜豆植株根部則容易分離到 *Pseudomonas* spp. 等拮抗細菌。其中有些菌株做為種子處理劑時，能顯著降低幼苗猝倒病的發生。這些研究顯示，用土壤採樣分析法來探討 “ 輪作對根部病害的影響及對生物防治菌的增殖效果 ”，是一種不會破壞輪作試驗進行的好方法。

關鍵詞：輪作栽培，*Pythium* 幼苗猝倒病、*Fusarium* 萎黃病、生物防治、菜豆、甜菜