Seed Transmission of Downy Mildews of Spinach and Soybean

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Downy mildew of crops is one of the most devastating diseases throughout the world. In conjunction with the completion of the life cycle of the downy mildew fungus, the seed transmission seems to play an important epidemiological role, especially as a source of primary infection. Seed transmission of the downy mildew is known in some species within Peronosporaceae, i.e., *Peronospora schachtii* (downy mildew of beets), *P. manshurica* (downy mildew of soybean), and *P. tabacina* (blue mould of tobacco).[16]

The present paper deals with the outline of the results obtained in Japan on the seed transmission of downy mildews of spinach and soybean.

Seed transmission of spinach downy mildew[8]

Downy mildew of spinach (*Spinacia oleracea* L.), caused by *Peronospora effusa* (Grev. ex Desm.) Ces. (syn. *P. spinaciae* Laub. and *P. farinosa* Fr.), occurs in many countries. It has not been determined previously whether downy mildew of spinach is transmitted by seeds. Leach and Borthwick[12] observed hyphae of the downy mildew fungus in the calyx tube, funiculus, integument, and nucellus of spinach seeds. These infected seeds were planted in a cool greenhouse for seed-transmission trials, but no infected seedlings were observed. Cook[1] reported that oospores of *P. effusa* were found mixed with commercial spinach seeds and that the crop grown from heavily infested seeds was severely damaged by downy mildew in the field. No conclusive evidence of seed transmission of spinach downy mildew has yet been presented, however. The objective of this study, which was carried out under controlled conditions, was to determine whether seed transmission of spinach downy mildew could occur.

1) Detection of oospores from commercial seeds

Commercial seeds from 11 cultivars were employed (Table 1). No macroscopic symptoms were observed on the commercial seeds and it was difficult to identify the seeds infested with either oospores or mycelia with a dissecting microscope. Therefore, oospores were collected from seeds by the seed-washing method as follows: 30 m*l* of seeds of each cultivar (813–1,461 seeds) were soaked in 50 m*l* of distilled water and stirred for 5 min, then the seeds were removed through one layer of cheesecloth. Water suspensions were then centrifuged at 3,000 rpm for 5 min and the precipitate was resuspended in 5 m*l* of distilled water. Oospores were counted in 10 drops of 10 μl each from the suspension of the precipitate with a microscope. Oospores were detected in washings of seed from six of 11 cultivars. The number of oosopores was high in seeds of cultivars Akagi, Kurobi, and Maruryu Münster and low in those of cultivars Kuroba Münster, Parade, and Three Carnel (Table 1). Oospores were round and had a smooth surface without protuberances. The diameters of oospores varied from 20 to 38.8 μm, with a mean of 30 μm. The size and shape of oospores found on seeds coincided with those of the oospores formed in the leaves infected with the mixture of conidia of two mating types, P1 and P2, of spinach downy mildew fungus.[10]

2) Seed transmission test

Commercial seeds from the same lots as those employed for detection of oospores on seed were

Table 1. Relationship between occurrence of oospores of *Peronospora effusa* in commercial spinach seed and seed transmission

Cultivar	Detection of oospores from commercial seed		Seed transmission		
	Number of seeds used[a)]	Number of oospores[b)]	Number of seedlings examined	Number of infected seedlings[c)]	Percentage of infected seedlings
Akagi	813	650	1,763	28	1.6
Fudo	1,328	0	1,037	0	0
Hokkai Ichiban	1,332	0	586	0	0
Kuroba Münster	1,461	50	2,059	6	0.3
Kurobi	1,097	1,100	1,134	33	2.9
Maruryu Münster	1,111	1,750	2,738	41	1.5
Maruryu Münsterland	1,365	0	1,112	0	0
Parade	1,371	50	1,841	0	0
Popeye	922	0	1,618	0	0
Three Carnel	938	50	1,272	8	0.6
Yoshu Münsterland	1,170	0	2,011	0	0

a) Number of seeds in 30 m*l* of seeds.
b) The number of oospores was determined based on those contained in 10 drops of 10 μl each from the suspension of the precipitate prepared from 30 m*l* of seeds by the seed-washing method.
c) On the 21st day after sowing (at the cotyledon stage), the potted seedlings were placed in a moist chamber. The number of infected seedlings was determined by observing, with the unaided eye, the conidium-producing cotyledons.

used. Seeds were soaked in distilled water for 1 day at 15°C, then drained and incubated for 3 days at 15°C to promote germination. Germinating seeds were then sown in the sterilized soil, and the pots were placed for 21 days in a growth chamber set at 15°C and located outdoors to receive natural light through the glass sides and roof. On the 21st day after sowing (at the cotyledon stage) the potted seedlings were placed in a moist chamber for 20 hr at 20°C to induce sporulation and the number of infected seedlings was determined by observing, with the unaided eye, the conidium-producing cotyledons. Infected seedlings were produced from the seed of five of 11 cultivars (Table 1). The percentage of infected seedlings was 1.5–2.9 for cultivars Akagi, Kurobi, and Maruryu Münster, among seeds of which the number of oospores detected was high. The two cultivars, Kuroba Münster and Three Carnel, in which the number of oospores detected on seed was low, produced seedlings with 0.3–0.6% infected plants. In contrast, no infected seedlings were observed in five cultivars in which oospores could not be detected on the seeds. Oospores were detected in the cultivar Parade but were not transmissible in this study.

No macroscopic symptoms were observed on cotyledons 21 days after sowing, although after incubation under humid conditions, a heavy coating of conidiophores and conidia appeared on the lower surface of the infected cotyledons. In stained tissues of conidium-producing cotyledons, intercellular, nonseptate mycelia and branched, fingerlike haustoria characteristic of *P. effusa* were observed. Hyphae and haustoria were observed in the leaf primordium of shoot tip with an electron microscope.

For observation of oospore germination, oospores were collected by the seed-washing method of germinated seeds of Maruryu Münster, which were soaked in distilled water for 1 day at 15°C, then drained and incubated for 3 days at 15°C. A few oospores germinated via a germ tube.

Since infected seedlings were observed 21 days after sowing (at the cotyledon stage), it is assumed that the infection observed in the seedlings was the primary one. Throughout our trials, seed from certain cultivars repeatedly produced infected seedlings, whereas others always produced seedlings free of infection. The percentage of infected seedlings was positively correlated with the degree of oospores infestation of seed under controlled conditions. From these results, we concluded that *P. effusa* could be transmitted

by seed.

Seed transmission of soybean downy mildew[7,9]

Downy mildew of soybean, *Glycine max* Merril, caused by *Peronospora manshurica* (Naoum.) Sydow ex Gäumann is wide-spread and commonly found in Japan. Downy mildew of soybean has been proved to be seed-transmitted in the United States.[6,11] Some of the seedlings which originated from oospore-encrusted seeds showed systemic infection.[6,11] Although the percentage of oospore-encrusted seeds collected in natural fields has been extensively surveyed in the United States,[2,11,14] the degree of contamination of oospore-encrusted seeds in field and commercial samples has not been surveyed in Japan. On the other hand, with respect to the way in which oospore-encrusted seeds are produced, it is only known that oospore-encrusted seeds can be

Table 2. Percentage of oospore-encrusted seeds in soybean samples produced in Japan

Sample No.	Cultivar	Number of seeds examined	Percentage of oospore-encursted seeds (%)	Locality
Seeds collected from fields				
1	Akisengoku	1,617	0	Kumamoto
2	Akiyoshi	1,349	0.4	Kumamoto
3	Asomusume	1,282	0	Kumamoto
4	Gogaku	1,427	2.3	Kumamoto
5	Higomusume	1,506	2.8	Kumamoto
6	Himeyutaka	867	0	Hokkaido
7	Hyuga	1,225	0.2	Kumamoto
8	Kitakomachi	1,024	0	Hokkaido
9	Kitamishiro	1,327	0.8	Hokkaido
10	Kitamusume	1,142	0.9	Hokkaido
11	Koganedaizu	1,281	0.6	Kumamoto
12	Okuharawase	797	0.6	Chiba
13	Orihime	1,392	10.5	Kumamoto
14	Sayohime	1,441	2.6	Kumamoto
15	Shirosaya	1,129	3.4	Kumamoto
16	Tamanishiki	5,737	1.1	Shizuoka
17	Toyosuzu	1,011	0.1	Hokkaido
Commercial seeds				
18	Denko-okuharawase	1,229	23.4	Hokkaido
19	Guntsuru	860	1.0	Gunma
20	Guntsuru	1,069	0.2	Gunma
21	Hakucho	1,241	3.9	Hokkaido
22	Hakucho	1,287	4.4	Hokkaido
23	Hakucho	1,032	1.2	Hokkaido
24	Kaori	876	1.4	Iwate
25	Kegon	1,482	4.3	Saitama
26	Mikawashima	1,287	2.0	Gunma
27	Mikawashima	1,421	1.2	Gunma
28	Okuharawase	1,286	16.1	Hokkaido
29	Okuharawase	1,293	23.1	Hokkaido
30	Okuharawase	1,214	9.6	Hokkaido
31	Raicho	1,240	1.5	Hokkaido
32	Raicho	1,335	2.3	Gunma
33	Ryokuko	871	1.0	Hokkaido
34	Wasemidori	1,167	3.3	Hokkaido
35	Wasemidori	1,324	0.8	Hokkaido
36	Waseosodefuri	1,314	1.9	Hokkaido
37	Waseosodefuri	1,238	0.8	Hokkaido
38	Waseosodefuri	1,110	0.7	Hokkaido

produced on the systemically-infected plants originating from oospore-encrusted seeds.[6]

The purpose of this study reported here was to examine the percentage of oospore-encrusted seeds in soybean samples collected from various parts of Japan, the production of oospore-encrusted seeds by means of inoculation of conidia to flower and pod, and the mechanism of systemic infection.

1) Percentage of oospore-encrusted seeds in soybean samples collected from various parts of Japan

Encrustation of oospores of the fungus on seed coat which is dull white, and formed a thin layer covering 1/10–1/4 of the surface of the seed coat in most cases, was easily detected with the unaided eye. Seventeen seed samples from the fields and twenty-one commercial seed samples were collected from various parts of Japan. The oospore-encrusted seeds were identified in seed samples mostly with unaided eye. Percentages of oospore-encrusted seeds surveyed in 38 samples from 27 cultivars are shown in Table 2. No oospore-encrusted seed was observed in 4 cultivars (4 samples); Akisengoku (sample No. 1), Asomusume (No. 3), Himeyutaka (No. 6) and Kitakomachi (No. 8). Most of the samples showed a percentage lower than 5%. However, in 3 samples from 2 cultivars, Okuharawase (sample No. 28 and 30) and Orihime (No. 13), the percentages ranged between 9.6 and 16.1%. Denko-okuharawase (sample No. 18) and Okuharawase (No. 29) showed the remarkably high percentages of 23.4% and 23.1%, respectively. Oospore-encrusted seeds were produced on various cultivars throughout Japan, from the north (Hokkaido) to the south (Kumamoto) of the country.

The degree of downy mildew occurrence, however, could not be determined in the fields where the samples were collected. The relationship between the percentage of oospore-encrusted seeds and the fluctuations in the disease occurrence has not been elucidated yet and should be investigated in the future.

2) Production of oospore-encrusted seeds by inoculation of conidia to flower and pod

Soybean cultivars used for the flower- and pod-inoculations were Denko-okuharawase,

Table 3. Production of oospore-encrusted seeds of soybean by inoculation of conidia of *Peronospora manshurica* to flower

Cultivar	Percentage of oospore-encrusted seeds (%)					
	Exp. I		Exp. II		Exp. III	
	Inoculated	Control	Inoculated	Control	Inoculated	Control
Denko-okuharawase	24.8	0	56.3	0	—[a]	—
Hakucho	3.3	0	0	0	3.2	0
Okuharawase	29.0	0	48.1	0	39.4	0
Raicho	0	0	0	0	11.4	0
Wasemidori	1.9	0	0	0	21.7	0

a) —: Experiment was not performed.

Table 4. Production of oospore-encrusted seeds of soybean by inoculation of conidia of *Peronospora manshurica* to pod

Cultivar	Percentage of oospore-encrusted seeds (%)			
	Exp. I		Exp. II	
	Inoculated	Control	Inoculated	Control
Denko-okuharawase	29.0	0	13.2	0
Hakucho	3.4	0	0	0
Okuharawase	19.3	0	19.3	0
Raicho	0	0	8.5	0
Wasemidori	3.9	0	0	0

Hakucho, Okuharawase, Raicho and Wasemidori. The floral organs appearing 30–33 days after sowing and the pods (0.8–2.5 cm in length) appearing 40–43 days after sowing were inoculated with conidial suspension using a small painting brush, respectively. The inoculated plants were kept in a greenhouse. The whole plants except the roots were harvested 34–40 days after inoculation (67–70 days after sowing) in the case of flower-inoculation and 27 days after inoculation (67–70 days after sowing) in the case of pod-inoculation. After drying at room temperature for 10 days, all the seeds were removed from the pods and the percentages of oospore-encrusted seeds were examined. The results are shown in Table 3 and Table 4. Among the 5 cultivars used in the experiments on the infection of floral organ and young pod, Denko-okuharawase and Okuharawase produced abundant oospore-encrusted seeds, whereas Hakucho, Raicho and Wasemidori produced only a few oospore-encrusted seeds. Thirty-two races of soybean downy mildew fungus have been identified in the United States[2,3,4,5,13] and the existence of races has also been suggested in Japan.[15] It is thus conceivable that Hakucho, Raicho and Wasemidori cultivars might produce more oospore-encrusted seeds, if different races were used.

3) *Systemically infected plants from oospore-encrusted seeds*

Oospore-encrusted seeds of cultivar Okuharawase were planted in growth chambers each set at 15, 20, or 25°C and located outdoors to receive natural light through the glass sides and roof. The number of systemically infected plants grown at 15 and 20°C and that of the plants grown at 25°C was estimated 30 and 20 days after planting, respectively. The percentages of systemically infected plants were 16% at 15°C and 1% at 20°C. No systemically infected plants were produced at 25°C. To determine the presence of hyphae in stems of all the plants tested, i.e., systemically infected plants and plants without symptoms, a piece of the first internode of stem 1 cm long (stem between cotyledon and primary leaf) of all the plants was excised, sectioned by hand (1 mm thickness) and examined under microscope. Hyphae were found in the stem tissues of all the systemically infected plants, but not in those of the plants without symptoms. Thus it seems that encrusted-oospores on the seed coat germinate and infect host plant, resulting in systemically infected plants in every case.

The distribution of hyphae in systemically infected plant was observed. Paraffin sections of various tissues were prepared from systemically infected plants. The hyphae were found in petioles and laminae of developed and undeveloped leaves, stems, flower stalks, bracts, calyces, petals, and pods, except pistil and stamen. On the other hand, the hyphae in the stem apex were observed under electron microscopy. The intercellular hyphae and haustoria were found in the stem apex. From these results, it was demonstrated that hyphae distributed in every tissue of the host plant, penetrated into the stem apex, inducing systemic infection of the plant.

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