Seed Transmission of Spinach Downy Mildew

T. INABA, K. TAKAHASHI, and T. MORINAKA, Plant Pathologists, Division of Plant Pathology, National Institute of Agricultural Sciences, Yatabe, Tsukuba, Ibaraki 305, Japan

ABSTRACT

Inaba, T., Takahashi, K., and Morinaka, T. 1983. Seed transmission of spinach downy mildew. Plant Disease 67:1139-1141.

Oospores were collected by the seed-washing method in commercial seed lots from six of 11 spinach cultivars. These seeds were planted in sterilized soil in a growth chamber set at 15 C under natural light. The percentage of infected seedlings was positively correlated with the degree of oospore infestation of seed. We concluded that *Peronospora effusa* could be transmitted by the seed.

Downy mildew of spinach (*Spinacia oleracea* L.), caused by *Peronospora effusa* (Grev. ex Desm.) Ces. (syn. *P. spinaciae* Laub. and *P. farinosa* Fr.) (5,6), is one of the most devastating diseases of spinach in many countries. It has not been determined previously whether downy mildew of spinach is transmitted by seeds. Leach and Borthwick (3) observed hyphae of the downy mildew fungus in the calyx tube, funiculus, integument, and nucellus of spinach seed.

Accepted for publication 2 May 1983.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

These infected seeds were planted in a cool greenhouse in soil steam-sterilized for seed-transmission trials, but no infected seedlings were observed. Cook (1) reported that oospores of *P. effusa* were found mixed with commercial spinach seed and that the crop grown from heavily infested seed 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.

MATERIALS AND METHODS

Collection of oospores from commercial seed. Commercial seeds from 11 cultivars were employed (Table 1). Oospores were collected from seeds by the seed-washing method as follows: 30 ml of seeds of each cultivar (813–1,461 seeds) were soaked in 50 ml of distilled water and stirred for 5 min, then seeds were removed through one layer of cheesecloth. Water suspensions were then centrifuged at 3,000 rpm for 5 min and the precipitate resuspended in 5 ml of distilled water. Oospores were counted in 10 drops of 10 μl each from the suspension of the precipitate with a microscope.

Seed-transmission test. Commercial seeds from the same lots as those employed for detection of oospores on seed were used. Unglazed pots (18 cm in diameter) were filled with a mixture of garden soil and manure (2:1, v/v), which was steam-sterilized at 1.2-kg pressure for 3 hr. 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 mixture, 100 seeds per pot, and the pots were placed for 21 days in a growth chamber set at 15 C under natural light. On the 21st day after sowing (at the cotyledon stage) the potted seedlings were placed in a

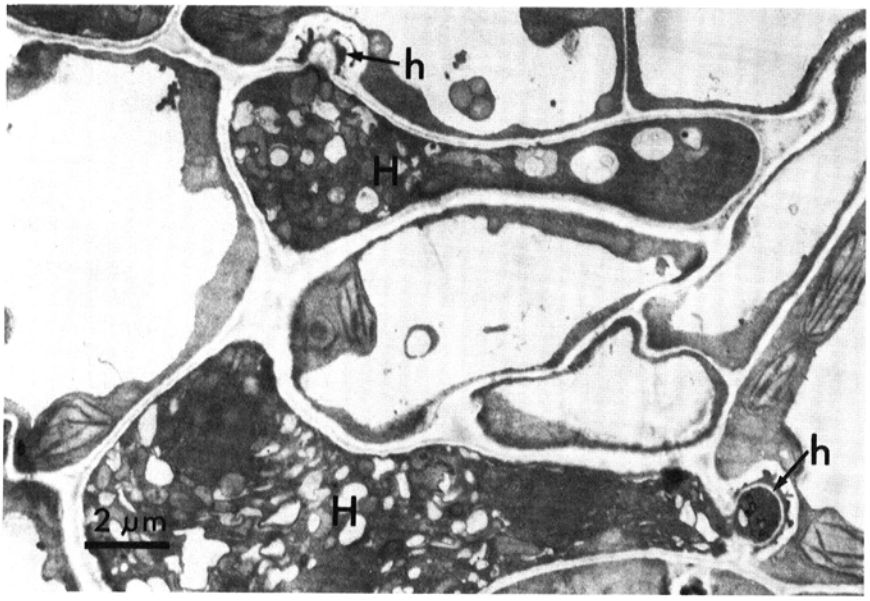

Fig. 1. Electron micrograph of spinach shoot tip of seedling infected with *Peronospora effusa* by seed transmission. Micrograph shows intercellular hypha (H) and haustorium (h) in the leaf primordium.

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[c]		
	Number of seeds used[a]	Number of oospores[b]	Number of seedlings examined	Number of infected seedlings[d]	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 ml 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 ml of seeds by the seed-washing method. The experiment was repeated twice. Each number represents the mean of two experiments.
[c] Three or five trials of seed transmission for each cultivar were performed. The number of seedlings examined and infected seedlings represent the total number from three or five trials.
[d] 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.

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. Three or five trials of seed transmission were performed for each cultivar.

Light microscopy. For observation of mycelia and haustoria in the infected seedlings, on the 21st day after sowing, the leaf pieces of cotyledons and of the developing small first true leaves were stained with aniline blue according to the method of Shipton and Brown (4) and observed with a microscope.

Electron microscopy. Shoot tips were collected from 15 infected seedlings of cultivar Maruryu Münster 21 days after sowing and cut into small pieces, fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3–4 hr at 4 C, and postfixed in 1% veronal-acetate-buffered osmium tetroxide (pH 7.2) for 3 hr at 4 C. After fixation, the materials were dehydrated through an acetone series and embedded in epoxy resin. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and observed with a Hitachi H-600 electron microscope.

RESULTS AND DISCUSSION

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. Oospores were detected in washings of seed from six of 11 cultivars. The number of oospores 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 200 oospores found in seeds of Maruryu Münster varied from 20 to 38.8 μm, with a mean of 30 μm. No differences in the size of oospores were observed in cultivars Akagi, Kurobi, and Maruryu Münster. 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 (T. Inaba, *unpublished*).

Infected seedlings were produced from the seed of five of 11 cultivars. 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 (2) characteristics of *P. effusa* were observed. On the developing small first true leaves of infected seedlings 21 days after sowing, neither symptoms nor sporulation were observed, although in stained tissues, intercellular, nonseptate mycelia and haustoria were sometimes detected. Hyphae and haustoria were observed in the leaf primordium of one shoot tip of the 15 examined with an electron microscope (Fig. 1).

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.

Leach and Borthwick (3) observed hyphae of *P. effusa* in the spinach seed; it can be considered that the hyphae may also be present in the seeds infested with oospores. In this study, however, it was not possible to determine whether mycelia were actually present in the seeds

infested with oospores or whether the inoculum carried with the seed existed as both mycelia and oospores. Further studies are required.

Because 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. Cook (1) reported that in the field, crops grown from heavily infested seed bearing oospores were severely damaged by downy mildew. 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.

ACKNOWLEDGMENT

We thank Dr. T. Kajiwara, Head of the Division of Plant Pathology, National Institute of Agricultural Sciences, for critical reading of the manuscript.

LITERATURE CITED

1. Cook, H. T. 1935. Occurrence of oospores of *Peronospora effusa* with commercial spinach seed. (Abstr.) Phytopathology 25:11-12.
2. Ikata, S., and Yamauti, K. 1941. Notes on the haustoria of some species of *Peronospora*. Ann. Phytopathol. Soc. Jpn. 10:326-328.
3. Leach, L. D., and Borthwick, H. A. 1934. Distribution of downy mildew mycelium in spinach fruits. Phytopathology 24:1021-1025.
4. Shipton, W. A., and Brown, J. F. 1962. A whole-leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology 52:1313.
5. U.S. Department of Agriculture. 1960. Index of Plant Diseases in the United States. U.S. Dep. Agric. Handb. 165. 62 pp.
6. Yerkes, W. D., and Shaw, C. G. 1959. Taxonomy of the *Peronospora* species on Cruciferae and Chenopodiaceae. Phytopathology 49:499-507.