

## A Stem Blight of Rose Caused by *Phytophthora megasperma*

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### ABSTRACT

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A stem blight of rose was first observed in Chiba Prefecture of Japan in May, 1968. Pathogenicity of *Phytophthora* sp. isolated from the diseased plants was proved on rose plants originating from the cuttings and grown in artificially infested soil, and on excised stems, shoots, or leaves

inoculated in vitro through artificial wounds. The fungus was identified as *P. megasperma* on the basis of morphology. This is the first report of the natural infection of rose by *P. megasperma*.

A new disease of rose characterized by wilting, yellowing, and defoliation first was observed in hydroponic culture of rose seedlings for breeding experiments at Yachiyo City, Chiba Prefecture in May, 1968. This same disease has occurred in serious proportions in rose plants originating from cuttings and used in commercial plantings in greenhouses in different areas of Chiba since June, 1973.

A part of the stem near the soil surface at first became water-soaked, dark green, and then changed to dark brown (Fig. 3-B). New shoots or immature branches wilted and the plants eventually died. The lower leaves of older plants were yellow and slightly wilted, and finally plants became defoliated (Fig. 3-A).

The disease occurred first in the poorly drained areas, and then was apparent in other areas. Several cultivars such as Carina, Golden Rapture, and Mary De Vor were severely diseased. Plants that originated from cuttings were more susceptible to this disease than ones grafted onto wild roses (*Rosa multiflora* Thunb.). A species of *Phytophthora* has been consistently isolated from the diseased plants.

This disease has never been reported in Japan, nor probably from other countries. Therefore, a study was undertaken to determine the cause of this disease and to identify the causal organism. A preliminary report has been published (8).

### MATERIALS AND METHODS

The causal fungus was isolated on water agar or potato-dextrose agar (PDA) plates containing 100-200 µg/ml streptomycin sulfate from the newly invaded water-soaked periphery of actively developing lesions on the diseased stems or branches.

Two isolates of *Phytophthora* sp., which were isolated from rose and designated as P-A and P-B, were used

throughout the study. A 6-mm diameter mycelial plug from the edge of a colony was transferred to PDA plates to determine rates of mycelial growth in relation to temperature. The average diameter of the colony minus the inoculum plug was determined after a 3-day incubation. The experiment was repeated twice with four replicate plates for each temperature.

Washed mycelial mats from cornmeal broth or V-8 juice cultures were placed in pond water or water filtered through soil, to induce sporangium production and zoospore discharge under various conditions.

Inoculation experiments were conducted by (i) burying washed oatmeal broth-cultured mycelial mats in the soil near healthy rose cuttings grown in pots, (ii) planting rooted-rose cuttings in autoclaved soil after inoculation by dipping the roots for 1 min into an inoculum suspension composed of one flask (300 ml) of cultured minced mycelial mats and 300 ml of sterilized water, and (iii) placing a 6-mm mycelium-agar plug onto excised 10- to 15-cm long immature branch or shoot segments, or leaves of rose. Twelve different rose cultivars were tested. All pathogenicity tests were conducted under greenhouse conditions at 24(18-30) C and soil around the inoculated plants was maintained near saturation. Excised branch or shoot segments and leaves and other plant parts that were inoculated were kept moist in 18-cm diameter petri dishes and incubated at 25 C for 2-5 days. All experiments were repeated at least twice.

### RESULTS

**Morphology.**—The colony characteristics of all of the many isolates of *Phytophthora* isolated from diseased rose plants during this study were identical. Two typical isolates, P-A and P-B, formed hyphal swellings and knotted hyphal tangles (Fig. 1-A). The sporangia were terminal, nonpapillate, ovate, or elliptical, and often proliferated internally or externally, 23-70 µm × 18-42 µm (average 46.8 × 29.5 µm) [Fig. 3-(B to D)]. Subsporangial elongation of hyphae often was observed. Sporangioophores often were inflated at the base, 150-350

$\mu\text{m}$  long and 2.5-10.0  $\mu\text{m}$  wide, and occasionally subsporangial septation was observed.

Numerous sporangia were formed on washed mycelial mats from cornmeal broth or V-8 juice cultures that had been placed in pond water or water filtered through soil. Zoospores were differentiated inside sporangia (Fig. 1-E), and liberated through the open apical part. Encysted spherical zoospores were 8.7-12.5  $\mu\text{m}$  (average 10.5  $\mu\text{m}$ ) in diameter.

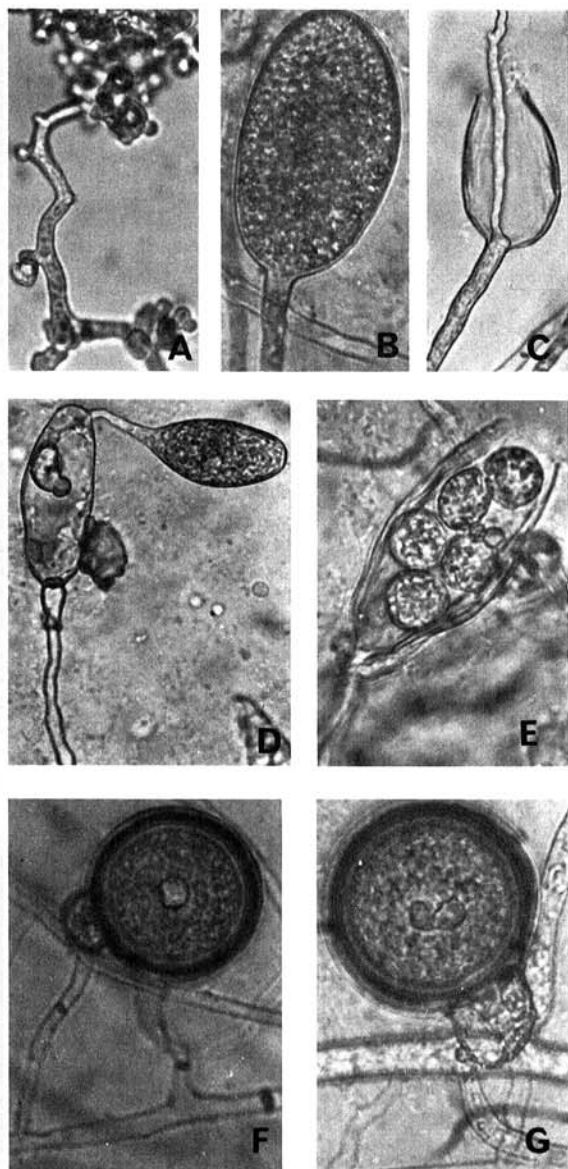


Fig. 1-(A to G). Morphology of *Phytophthora megasperma* causing a stem blight of rose. A) Hyppha with knotted hyphal tangles ( $\times 400$ ). B) Nonpapillate sporangium ( $\times 750$ ). C) Internally-proliferated sporangium ( $\times 500$ ). D) Externally-proliferated sporangium ( $\times 550$ ). E) Zoospores differentiated inside sporangium ( $\times 750$ ). F) Oogonium and oospore with paragynous antheridium ( $\times 600$ ). G) Oogonium and oospore with amphigynous antheridium ( $\times 700$ ).

Sexual organs were formed abundantly on oatmeal agar, cornmeal agar, and V-8 juice agar, but rarely on PDA. Oogonia were smooth, globose, elliptical, and usually 35-55  $\mu\text{m}$  (average 42.5  $\mu\text{m}$ ) in diameter. The oogonium stalks occasionally were observed to be funnel-shaped, and were 7.5-120  $\mu\text{m} \times 10$ -25  $\mu\text{m}$ . Oospores (Fig. 1-F, G) were nearly plerotic, 27.5-49.0  $\mu\text{m}$  (average 39.0  $\mu\text{m}$ ) in diameter, and often contained an oil globule, 13.7-15.0  $\mu\text{m}$  in diameter.

Antheridia were predominantly (approximately 88%) paragynous (Fig. 1-F) or occasionally amphigynous (Fig. 1-G), subglobose, 7.5 to 25.0  $\mu\text{m}$  in length by 10.0 to 20.0  $\mu\text{m}$  in diameter. They were terminal or intercalary on the antheridial stalks.

**Growth-temperature relations.**—The two *Phytophthora* sp. isolates, P-A and P-B, from rose showed similar temperature responses when tested at 11 different temperatures (Fig. 2). The optimum temperature for mycelial growth on PDA was 25 C. The fungus did not grow at 5 C or 38 C, even after incubation for 20 days, but it grew within the range 10 to 35 C.

**Pathogenicity.**—Three-mo-old rooted cuttings of rose inoculated by burying the inoculum in the soil near healthy rose cuttings grown in pots or by dipping the roots in the inoculum suspension were seriously damaged, especially when artificially wounded prior to inoculation by cutting a part of the roots. The new shoots and lower leaves of the inoculated plants initially wilted (Fig. 3-C) and the plants finally died.

Two cultivars of rose, Super Star and Yellow Belinda, and wild rose differed in susceptibility (Table 1). Wild

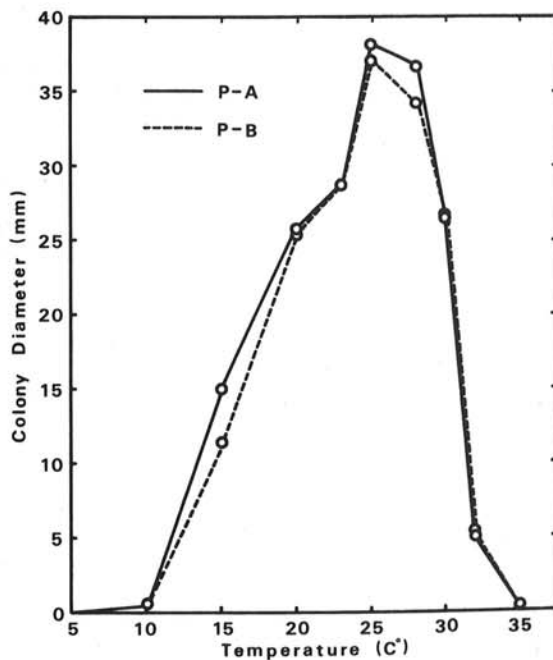


Fig. 2. Average colony diameters of two isolates, P-A and P-B, of *Phytophthora megasperma* on PDA after incubation for 3 days at 5-35 C.

rose cuttings were not susceptible; no symptoms followed wound inoculation.

The excised branch segments of 12 cultivars and wild rose became diseased when artificially wounded by pricking with a needle and then inoculated, but were healthy in noninoculated control segments which received sterile PDA plugs in wounded areas. Among cultivars tested, Blue Moon, Fure-Daiko, Mister Lincoln, and Super Star were most susceptible; these developed more than 40-mm long water-soaked lesions after 4 days of incubation. Cultivars Anabell, Bridal Pink, Belinda, Yellow Belinda, and Harrison's Yellow were moderately susceptible, showing 30- to 40-mm-long lesions. Two cultivars, Winna Sharme and Yuki-San, and wild rose were resistant, showing lesions less than 30 mm long. Mature branches were somewhat more resistant to the pathogen than young shoots.

Other plants that were infected following wound inoculations were as follows: unripe fruits of eggplant (*Solanum melongena* L., 'Senryo'), tomato (*Lycopersicon esculentum* Mill., 'Hikari'), bell pepper (*Capsicum annuum* L. var. *domestica* Mill., 'Ace'), cucumber (*Cucumis sativus* L., 'Tokiwa'), and apple (*Pyrus malus* L., 'Delicious'). The fruit of watermelon (*Citrullus vulgaris* Schrad., 'Kyokuto'), branches of eggplant, tomato, bell pepper, and tobacco (*Nicotiana tabacum* L., 'Bright Yellow'), and leaves and petioles of strawberry (*Fragaria chiloensis* Duch. var. *anannassa* Bailey, 'Hokhowase'), hollyhock (*Althaea rosea* Cav.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium repens* L.), and white clover (*Trifolium pratense* L.) were either slightly damaged or not damaged by inoculations into wounds.

When hollyhock seedlings were inoculated by burying washed mycelial mats from oatmeal-broth culture near the test plants grown in the autoclaved soil, hollyhock seedlings wilted within 14 days after inoculation, but alfalfa, red clover, white clover, soybean *Glycine max* (L.) Merr., 'Shiroseennari', strawberry, and rice (*Oryza*

*sativa* L., 'Honenwase') seedlings were free from the disease after inoculation of artificial wounds made by cutting a part of the roots.

The rose *Phytophthora* was consistently reisolated from the diseased plants, but not from noninoculated healthy plants.

## DISCUSSION

No reports of disease of rose caused by *Phytophthora* sp. has been reported from Japan or elsewhere. The disease first occurred on seedlings grown in hydroponic culture in 1968, and plants originated from cuttings also have been diseased in poorly-drained low-land areas previously used as rice-paddy fields. In addition, the plants that originated from cuttings were more susceptible to this disease than the ones grafted onto wild rose which was shown experimentally to be resistant to the rose *Phytophthora*.

Evidence that rose was susceptible to this fungus by wound inoculations, but rather resistant without wounds may indicate that the disease is not likely to occur naturally except on physiologically-damaged plants grown in poorly-drained soils.

Two *Pythiomorpha* spp., *P. miyabeana* Ito & Nagai, and *P. oryzae* Ito & Nagai, now considered to be a synonym of *Phytophthora megasperma* Drechsler (13), and *Phytophthora oryzae* (Ito & Nagai) Waterhouse (14), respectively, have been isolated from rice seeds and seedlings in rice-paddy fields in Japan (5).

According to Ito and Nagai (5), the former species differs from the latter in the size, mode, and later formation of sporangia as well as in the absence of oogonia. The sporangia of these two species are 36-53  $\mu\text{m}$   $\times$  17-36  $\mu\text{m}$  (mostly 46-48  $\mu\text{m}$   $\times$  24-29  $\mu\text{m}$ ), and 41-84  $\mu\text{m}$   $\times$  26-48  $\mu\text{m}$  (mostly 60-84  $\mu\text{m}$   $\times$  29-43  $\mu\text{m}$ ), respectively. Later-formed sporangia (probably secondary sporangia) were produced on the hyphae and proliferated inwardly through the empty preceding ones in both former and



Fig. 3-(A to C). Symptoms of a stem blight of rose caused by *Phytophthora megasperma*. A) Defoliation of lower leaves of diseased rose plants originated from cuttings. B) Discoloration of stems of the diseased rose plants near the soil surface. C) Wilt of a young shoot and leaves after artificial inoculation under greenhouse conditions.

latter species, but in the latter species, they also were produced on the lateral subsporangial branch from the base. The sexual organs were formed in the former, but not in the latter. *Pythiomorpha miyabeana* barely grew on the apricot agar medium; *P. oryzae* did not grow. The optimum temperatures for mycelial growth of these two species are 26-28 C, but the mycelial growth (the average colony diameter) at about 10 C was 19 mm in the former, but only a trace in the latter after incubation for 4 days on the rice-grain agar.

All of the characteristics used to determine speciation of these two species are judged not to be significant criteria in view of the reasons discussed below:

Sporangia of the fungus isolates identified as *Phytophthora megasperma* Drechsler ranged in size from 15.0 to 67.0  $\mu\text{m}$  in length by 6.0 to 52.5  $\mu\text{m}$  in width, but were consistently identical in being proliferous and nonpapillate (1, 2, 3, 7, 9, 10, 11, 12). Therefore, *P. oryzae* (Ito & Nagai) Waterhouse may be slightly larger than *P. megasperma* in the size of some sporangia, but both fungi are almost identical in size and shape.

Sporangium-bearing hyphae of *P. megasperma* were illustrated in three different ways, namely (i) by internal proliferation, (ii) by subsporangial elongation, and (iii) by both of (i) and (ii) methods (1, 3). Presence of the second type of sporangium-bearing hyphae was considered to be one of the criteria to separate *P. oryzae* from *P. megasperma*, but this morphology commonly was found in both fungi.

*Phytophthora oryzae* did not form oospores, and this is one of the basic characteristics by which *P. oryzae* is separated from *P. megasperma* (5). But oospore production of *P. megasperma* may differ for different isolates, although most isolates were reported to form sexual reproductive organs readily and abundantly. For example, one of the three groups among isolates of *P. megasperma* from Wisconsin alfalfa fields formed few oospores, and the colony morphology on V-8 juice agar was different from that of the other two groups that formed oospores abundantly (9).

In the original description of *Phytophthora megasperma* Drechsler (1), knotted hyphal tangles were present, but large globose chlamydozoospores were not formed. However, most of the fungi identified as *P. megasperma* by different investigators were characterized by presence of hyphal swellings and sometimes chlamydozoospores except for the cherry isolates (7).

Hyphae of the soybean *Phytophthora*, identified as *P. megasperma* var. *sojae* by Hildebrand (3), were irregularly knobby or tuberculate with more complicated skeins. Hyphae of *P. megasperma* and *P. oryzae* isolated from rice plants in Japan (5) also had knoblike appearances at irregular intervals and had chlamydozoospores. In addition, hyphal clumps were present on the hyphae of *P. oryzae* (5), the illustration of which is similar to the hyphal morphology of the rose *Phytophthora* shown in Fig. 1-A.

*Phytophthora oryzae* and *P. megasperma* also are quite similar in other morphological and physiological characteristics. Therefore, *P. oryzae* may be a variant of *P. megasperma* on the basis of the characteristics aforementioned.

The rose *Phytophthora* formed sexual reproductive organs within 14 days abundantly on V-8 juice, cornmeal, or oatmeal agar plates. Formation of the large oogonia (average 42.5  $\mu\text{m}$ ) and oospores (average 39.0  $\mu\text{m}$ ) also was characteristic of *P. megasperma* together with the predominantly paragynous antheridia (approximately 88%). All of these characteristics agree with those of the fungi identified as *P. megasperma* by previous workers (1, 2, 3, 10, 12, 13, 14).

According to Waterhouse (14), the average diameter of oogonia of *P. megasperma* var. *meiasperma* is over 45  $\mu\text{m}$ , but for *P. megasperma* var. *sojae* Hildebrand, the average diameter is under 40  $\mu\text{m}$ , and rarely over 45  $\mu\text{m}$ . In addition, Waterhouse (14) listed the maximum temperature for mycelial growth of the former variety as 30 C, and for the latter as 33-35 C. However, two out of seven isolates of *P. megasperma* tested by Savage et al. (10) could not be identified well to any variety, because two isolates, N352 and N353, were morphologically identified as var. *sojae*, but physiologically better fitted to var. *meiasperma*. Similar results were obtained with alfalfa isolates by Erwin (2) and Pratt and Mitchell (9), and with the rose *Phytophthora* tested in this study.

The rose *Phytophthora* was not pathogenic on rice, but two *Phytophthora* spp. from rice plants, originally identified as *Pythiomorpha* spp., were pathogenic (5).

According to Erwin (2), the alfalfa isolates appeared to have a narrow host range, and may in nature be limited to the genus *Medicago*. Isolates of *P. megasperma* from other hosts were not pathogenic to alfalfa. *Phytophthora megasperma* isolated from subterranean clover (*Trifolium subterranean* L.) was shown to be different in

TABLE 1. Pathogenicity of *Phytophthora megasperma* to the clones of three rose cultivars artificially inoculated<sup>a</sup> and species grown in autoclaved soil maintained at near saturation of water under the greenhouse conditions at 24 C

Species or cultivar	Pathogenicity <sup>b</sup>			
	Inoculated		Control	
	Nonwounded	Wounded <sup>c</sup>	Nonwounded	Wounded
Super Star	1/10	4/10	0/10	0/10
Yellow Belinda	5/10	10/10	0/10	1/10
<i>Rosa multiflora</i>	0/10	0/10	0/10	0/10

<sup>a</sup>Inoculated by dipping roots of healthy rose cuttings in the inoculum suspension of minced mycelium and sterilized water; noninoculated controls were dipped in sterilized water.

<sup>b</sup>Denominator is the number of cuttings tested; numerator is the number of cuttings killed.

<sup>c</sup>Artificially wounded by cutting a part of the roots.

pathogenicity to different host plants including soybean (6). The four soybean cultivars and two species of lupine were attacked by the clover isolate. In addition, seedlings of Alaska garden pea and Marglobe tomato were killed by stem inoculation tests. It also caused severe damping-off of seedlings of Delta alfalfa, Meechee arrowleaf clover, and two cultivars of crimson clover, when seeds of these legumes were planted in infested soil or sand. The clover isolate of *P. megasperma* thus possesses a wider range of pathogenicity than had been recognized previously for the var. *sojae*. Therefore, Johnson and Keeling (6) concurred with Erwin (2) and claimed that it should not be assigned the varietal designation. Almost similar opinions have been presented by many workers, including Pratt and Mitchell (9).

Our isolate of *Phytophthora* from rose did not show pathogenicity to soybean and other leguminous plants. Hilty and Schmitthenner (4) showed that there is a variation in the pathogenicity of 94 single-zoospore isolates of *P. megasperma* var. *sojae* obtained from nine mass-culture sources using two resistant and two susceptible varieties of soybeans. For example, one isolate was nonpathogenic on all test cultivars; another was slightly virulent on resistant cultivars. Other differences in virulence on susceptible cultivars also were noticed. All of these experimental results indicate that the pathogenicity of *P. megasperma* is variable in isolates, host plants tested, inoculation method used, or environmental conditions (2, 4, 6). We believe, therefore, that pathogenicity can not always be used as a criterion for speciation.

Therefore, we identified the rose *Phytophthora* isolates to be *Phytophthora megasperma* mainly on the basis of morphological characteristics such as nonpapillate proliferating sporangia, large oogonia and oospores, predominance of paragynous antheridia, and the ready formation of sexual reproductive organs, according to the broad concept of *P. megasperma* presented by previous workers (2, 9, 11).

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