Formae Speciales Differentiation of *Phytophthora megasperma* Isolates From Soybean and Alfalfa

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Portion of a Ph.D. dissertation by the senior author, University of California, Riverside.
Accepted for publication 10 October 1979.

ABSTRACT

KUAN, T.-L., and D. C. ERWIN. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.

Colony morphology of thirty isolates of *Phytophthora megasperma* from various sources varied with isolates and growth media. There were no distinct differences in oospore morphology and the diameters of oogonia ranged from 30 μm to 58 μm and appeared to be of a single continuously varying population. Isolates from alfalfa and soybean were pathogenic only to the hosts from which they were isolated. Special forms were proposed to subdivide the alfalfa and soybean isolates of *P. megasperma* into *Phytophthora megasperma* f. sp. *medicaginis* for isolates that attack alfalfa and *Phytophthora megasperma* f. sp. *glycinea* for isolates that infect soybean.

The species *Phytophthora megasperma* Drechs. includes several morphologically and pathogenically different forms. To recognize these differences, subgroups of *P. megasperma* have been established (5,16,27). *P. megasperma,* as originally described by Drechsler (2), possesses nonpapillate sporangia and large oospores (avg 41.4 μm in diameter). Tompkins et al (23) later amended Tucker's key (24) to include within *P. megasperma* isolates from cauliflower with oospores larger than 30 μm in diameter. Since then, several other morphological and pathogenic forms of *P. megasperma* have been reported (3,5,10,13,14,25).. Both pathogenicity (5) and size of oogonia (14,25) have been used as criteria for subgrouping. Hildebrand (5) designated an isolate which caused root rot of soybean as *P. megasperma* var. *sojae* on the basis of its specific pathogenicity to soybean. Waterhouse (27) later separated *P. megasperma* into two morphological varieties based on sizes of oogonia. The isolates with smaller oogonia (< 45 μm) were designated as *P. megasperma* var. *sojae,* and those with larger oogonia (> 45 μm) were designated as *P. megasperma* var. *megasperma*. However, if all *P. megasperma* isolates with small oogonia are classified as *P. megasperma* var. *sojae,* regardless of host, there is confusion because isolates from alfalfa (3), soybean (5,10), and sugarcane (25) differ pathogenically, even though they all have small oogonia. Furthermore, according to the International Code of Botanical Nomenclature (22), specific pathogenicity is not a valid criterion for varietal classification. To determine whether size of oogonia and specific pathogenicity were valid criteria to establish intraspecific populations of *P. megasperma,* a study was made of the variation in pathogenicity, morphology, and size of sexual reproductive structures of 30 isolates, including an original small-oospore isolate from Hildebrand and a large oospore isolate from Waterhouse (5,27).

MATERIALS AND METHODS

Thirty isolates of *P. megasperma* were obtained from various sources (Table 1) and a single-zoospore isolate was chosen to be representative of each one. After isolates were grown on clear V-8 juice agar (V8C), potato dextrose agar (PDA), cornmeal agar (CM), and Ribeiro's synthetic medium (SM) (17) in the dark (24 C) for 6 days, colony morphology was recorded photographically.

The morphology of oospores was examined microscopically and measurements were made after each isolate had been grown on V-8 juice agar (V8A) for 1 mo. The oospores were observed by light and Normarski differential phase-contrast microscopy (Zeiss photomicroscope-III) at a magnification of × 1,000. Thirty measurements of the diameters of oogonia and oospores and the thickness of the oospore walls were taken for each isolate. Significant differences among means of each structure were analyzed by Duncan's multiple range test ($P = 0.01$).

For pathogenicity tests on alfalfa, the various isolates were grown on V-8 juice agar medium for 6 days. Three-week-old alfalfa

TABLE 1. Sources of isolates of *Phytophthora megasperma* from different hosts

Isolate number	Host	Location	Source
P844	alfalfa	California	Hancock
P127	alfalfa	Australia	Purss
P348	alfalfa	Mississippi	Morgan
P1057	alfalfa	California	Erwin
P410	alfalfa	Minnesota	Frosheiser
P240	alfalfa (H.T.)[a]	California	Erwin
K15	soybean (Race 1)	Illinois	Paxton
K16	soybean (Race 2)	Illinois	Paxton
K17	soybean (Race 3)	Illinois	Paxton
K18	soybean (Race 4)	Illinois	Paxton
K19	soybean (Race 5)	Illinois	Paxton
K20	soybean (Race 6)	Illinois	Paxton
P174	soybean (Race 1)	Canada	Hildebrand
P405	soybean (Race 1)	Mississippi	Morgan
I2	*Brassica* sp.	U.K.	IMI56348[b]
P147	sugarcane	Louisiana	Van der Zwet
K1	cherry	California	Mircetich
K2	almond	California	Mircetich
K3	almond	California	Mircetich
K4	apple	California	Mircetich
K5	lilac	California	Mircetich
K6	raspberry	California	Mircetich
K7	raspberry	California	Mircetich
K8	pear	California	Mircetich
K9	juniper	California	Mircetich
K10	grape (from soil)	California	Mircetich
K11	grape (from soil)	California	Mircetich
K12	walnut (from soil)	California	Mircetich
K13	walnut (from soil)	California	Mircetich
K14	prune	California	Mircetich

[a] H. T. = high-temperature isolate (max > 39 C).
[b] IMI = Imperial Mycological Institute, Kew, Surrey, England.

0031-949X/80/04033306/$03.00/0
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plants (*Medicago sativa* [L.] 'Moapa 69') were inoculated with minced mycelia of each of the 30 isolates by the soil drench method (4). Two rows of alfalfa seeds were planted in each 15-cm diameter pot and thinned to 10 seedlings per pot. Two plates of 6-day-old cultures on V8A were blended at low speed for 15 sec in 1 L of sterile distilled water. Before inoculation, trenches were made at the side and between the two rows in which 50 ml of the suspension of minced mycelia was poured. Plants were irrigated by flooding for 3 days of the week (the pots were placed in water-filled saucers) and by watering when necessary for the remaining 4 days. Three weeks after inoculation, the roots were scored for disease according to the following disease index (4): 0 = no disease; 1 = small lesions, involving not more than 10% of the circumference of the tap root; 2 = slightly larger lesions, encompassing 10–20% of the circumference of the roots; 3 = lesions covering 20–50% of the circumference; 4 = lesions covering 50–100% of the root and with the plant still living; and 5 = the entire root rotted and the plant dead.

Pathogenicity of *Phytophthora* isolates to soybean was tested on 1-wk-old seedlings (*Glycine max* [L.] Merr. 'Harosoy') by puncturing the hypocotyl, inserting mycelium (11), and incubating the plants in a mist chamber for 3 days. Pathogenicity was determined by the appearance of soft necrotic lesions on the hypocotyl and the collapse of the seedling.

Pathogenicity of *Phytophthora* isolates from various sources was tested on cherry seedlings (*Prunus mahaleb* [L.] 'Mahaleb'). In a petri dish (9-cm diameter), five seedlings were inoculated by wounding the hypocotyl and inserting mycelium. The presence of soft rot on the hypocotyl was the indication of pathogenicity. Pathogenicity tests on different plants were repeated at least three times.

TABLE 2. Pathogenicity of isolates of *Phytophthora megasperma* on alfalfa (cultivar Moapa 69), soybean (cultivar Harosoy), and seedlings of cherry (cultivar Mahaleb)

		Pathogenicity		
Isolate	Host	Alfalfa[a]	Soybean[b]	Cherry[b]
P844	alfalfa	4.7	–	–
P127	alfalfa	3.9	–	–
P348	alfalfa	4.6	–	–
P1057	alfalfa	4.9	–	–
P410	alfalfa	2.9	–	···
P240	alfalfa	2.9	–	···
K15	soybean	0	+	–
K16	soybean	0	+	–
K17	soybean	0	+	–
K18	soybean	0	+	–
K19	soybean	0	+	–
K20	soybean	0	+	–
P174	soybean	0	+	–
P405	soybean	0	+	···
I2	*Brassica* sp.	0	–	–
P147	sugarcane	0	–	–
K1	cherry	0	–	+
K2	almond	0	–	···
K3	almond	0	–	···
K4	apple	0	–	···
K5	lilac	0	–	···
K6	raspberry	0	–	···
K7	raspberry	0	–	···
K8	pear	0	–	···
K9	juniper	0	–	···
K10	grape (from soil)	0	–	···
K11	grape (from soil)	0	–	···
K12	walnut (from soil)	0	–	···
K13	walnut (from soil)	0	–	···
K14	prune	0	–	···

[a] Tap root was scored on 0–5 basis 3 wk after inoculation: 0 = no root rot and 5 = completely rotted.
[b] + = soft rot occurred on hypocotyl and plants were killed; – = mechanical wounding at the site of inoculation, but plants were healthy; and ··· = not tested.

RESULTS

Colony morphology. The colony morphology varied among the isolates grown on the same medium, for each isolate when compared on the same medium, and also for each isolate when compared on the four media, V8C, PDA, CM, and SM (Fig. 1).

Sexual reproductive structure morphology. The morphology of oogonia + oospores of 29 isolates are shown in Fig. 2. Both amphigynous and paragynous antheridia were observed in all isolates; however, there was no distinctive differences in the morphology of oogonia and oospores among the isolates of *P. megasperma* observed.

Size of sexual organs. The diameter of oogonia and oospores and the thickness of oospore walls varied with isolates of *P. megasperma* and each of them formed a continuous distribution (Figs. 3–5). Therefore, there were no clear groups of isolates based on these characteristics. Most importantly, the size of oogonia ranged from 30 μm to 58 μm with no definite break (Fig. 3) between the "large" and "small" oogonia groups (27). There was a high correlation ($r = 0.98$) between the diameters of oospores and of oogonia among isolates of *P. megasperma*.

Pathogenicity of the isolates of *P. megasperma*. All the isolates from alfalfa caused a typical reddish brown rot on the roots of alfalfa. The shoots became wilted, necrotic, and the plant died. Isolates from other hosts did not infect alfalfa roots (Table 2). On soybean, only those isolates from soybean caused a soft rot on the hypocotyl and death of the inoculated plants. Isolates from other hosts did not cause any rot on the hypocotyl except for slight spotting due to mechanical wounding by the needle; the inoculated plants remained healthy and normal (Table 2). Neither the alfalfa isolates, the soybean isolates, nor those from other plants caused soft rot on hypocotyls of cherry seedlings. Brown lesions were caused by the isolate from cherry (Table 2).

DISCUSSION

Since *P. megasperma* includes isolates which differ either in size of oogonia and oospores or in pathogenicity, it is natural that subgrouping has been considered. Subgrouping of *P. megasperma* var. *sojae* (5) based on specific pathogenicity was logical. However, the variety status that Hildebrand proposed should be based on morphology (22). Because of the rules of the International Code of Botanical Nomenclature (22), Waterhouse (27) did not use specific pathogenicity as a taxonomic criterion, but separated *P. megasperma* into two populations based on a group with large oogonia (>45 μm), designated var. *megasperma,* and a group with small oogonia (<45 μm), designated var. *sojae.* While these groups can be distinguished, our data indicate a continuous range in size of oogonia varying from 30 μm to 58 μm (Fig. 3) with no definite break in size between the groups with "large" and "small" oogonia. Also, isolates K7, K9, K12, and I2 produced oogonia that ranged in size from 44.3 to 47.7 μm between the separate points designated for "small" and "large" oogonia groups. Our results suggest that the designation of varieties within *P. megasperma* based on oogonial size is questionable. This question is further supported by the report that the oogonia of an alfalfa isolate from Australia ranged from 27 to 58 μm (6). At least for the soybean and alfalfa isolates reported on here, we would agree with the amendation of Tucker's Key (24) by Tompkins et al (24) that "The average diameter of oospores of *P. megasperma* exceeding 30 microns" characterized *P. megasperma*.

Because the size of oogonia of both alfalfa and soybean isolates are in the small end of the distribution, these isolates cannot be separated based on the size of oogonia. Pathogenicity, on the other hand, always differentiated the alfalfa isolates from the soybean isolates. In addition to our pathogenicity data, many authors have previously reported this. Kaufmann and Gerdemann (10) reported that the soybean isolates were distinct, and because of their specific pathogenicity to soybean, elevated them to a new species, *P. sojae.* Hildebrand proved that his variety *sojae* was specifically pathogenic to soybean and not to many other hosts (5). Jones and Johnson (9) also found that the soybean isolate was neither pathogenic to nine nonlegume plants nor to alfalfa and 15 other

leguminous plants. However, lupine was susceptible. Erwin (3) reported that alfalfa isolates were limited in pathogenicity to the genus *Medicago*. There are reports of pathogenicity of alfalfa isolates to the leguminous plants *Cicer arietinum* L. and *Sesbania* sp. (1,3), but the natural occurrence of a Phytophthora root disease of these crops has not been noted in the field. Recently Matsumoto and Araki (13) reported that isolates of *P. megasperma* from alfalfa in Japan were pathogenic only to alfalfa. Irwin et al (7) also found that *P. megasperma* isolated from soybean was not pathogenic to alfalfa.

The idea of using specific pathogenicity as a basis to set apart populations of a species has been accepted by many authors. Recommendation 4 of the International Code of Botanical Nomenclature (22) states: "In classifying parasites, especially parasitic fungi...authors...should distinguish within the special forms (formae speciales) characterized by their adaptation to different hosts." Johnson (8), Luttrell (12), Munk (15), Savile (18), and Walker (26) all considered data on parasitism as important as morphological data in the assessment of taxa. Snyder and Hansen (19–21) recognized the host specificity of numerous parasitic forms within *Fusarium oxysporum* and *F. solani* and employed "formae speciales" to designate host specificity. In his paper on reclassifying the causal agent of root rot of alfalfa from *P. cryptogea* to *P. megasperma* (3), Erwin suggested the possibility of using "formae speciales" to separate the alfalfa and soybean isolates of *P. megasperma*.

Therefore, we suggest the subgroups: *P. megasperma* f. sp. *medicaginis* for isolates that attack alfalfa (*Medicago sativa* L.) and

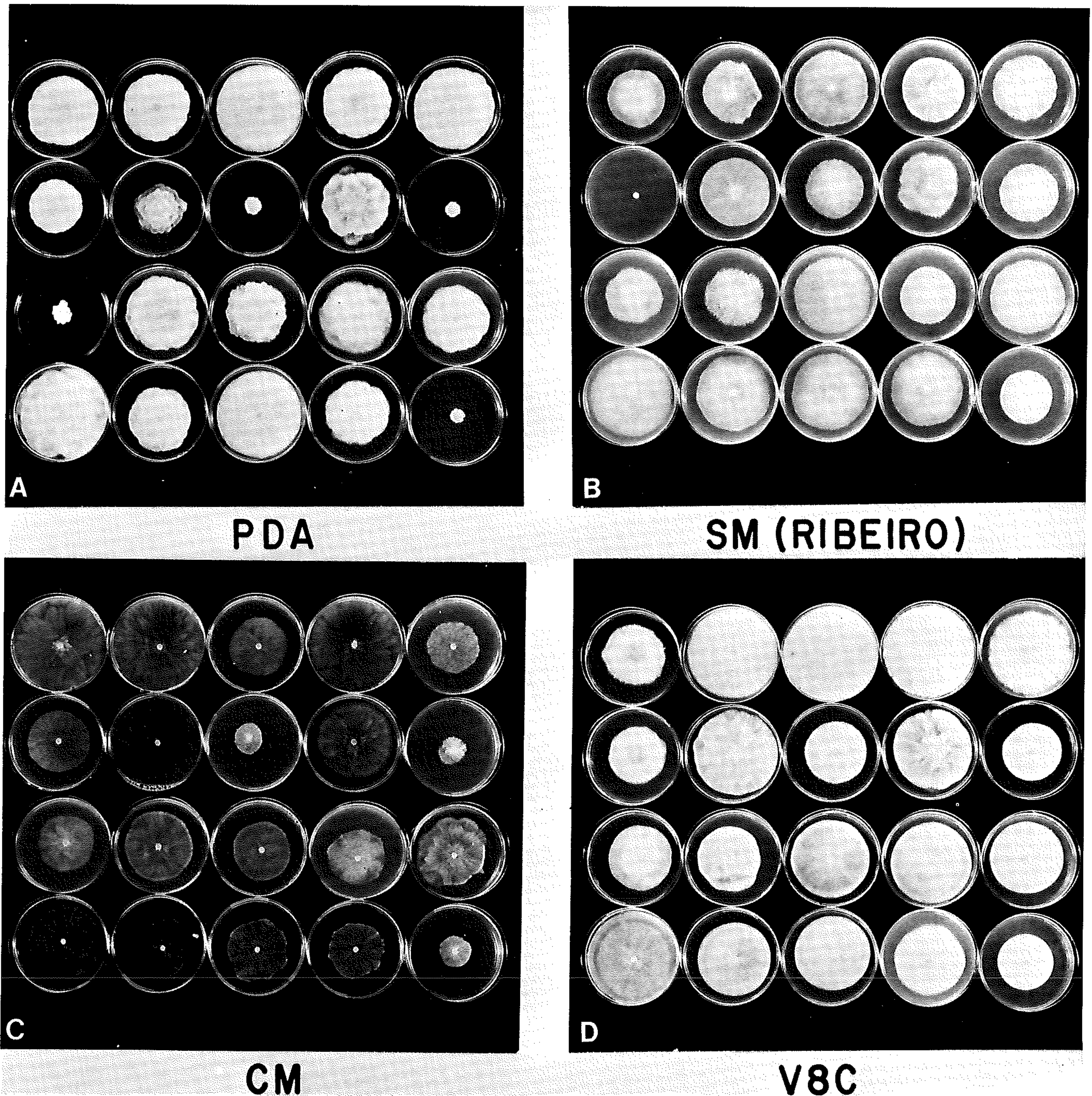

Fig. 1. Colony morphology of 20 isolates of *Phytophthora megasperma* grown on four different media at 24 C for 6 days. PDA = potato dextrose agar; SM = Ribeiro's synthetic medium (15); CM = corn meal agar; and V8C = clear V-8 juice agar. *P. megasperma* cultures pictured within each substrate group are (left to right): *Row 1*–P844, P410, P1057, P240, P348; *Row 2*–P127, K4, P405, P147, P174; *Row 3*–K19, I2, K1, K2, K5; and *Row 4*–K8, K11, K13, K14, K17.

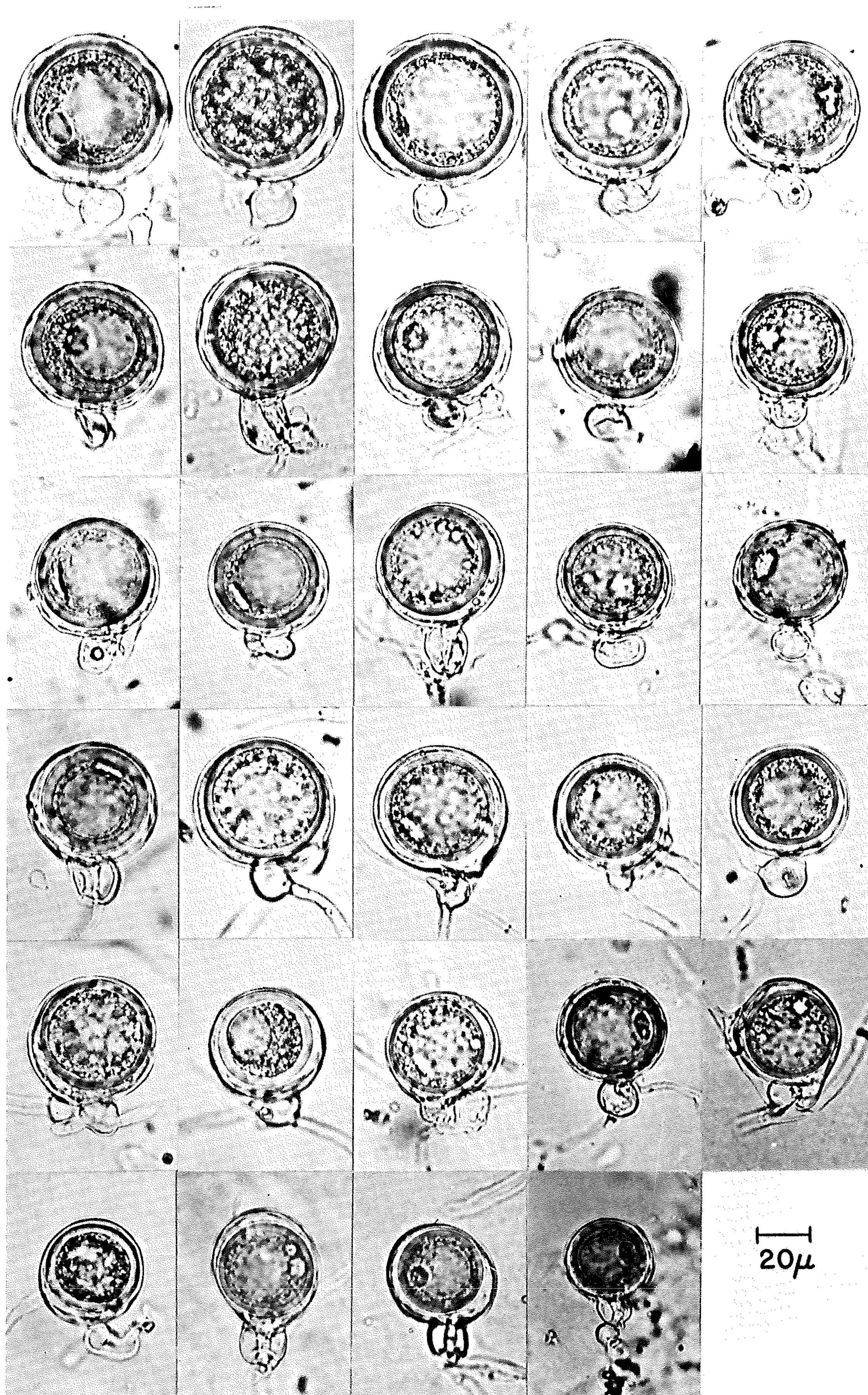

Fig. 2. Oospore morphology of 29 isolates of *Phytophthora megasperma* grown on V-8 juice agar at 24 C for 1 mo. Left to right: *Row 1*–K4, K8, K10, K2, K1; *Row 2*–K9, I2, K12, K7, K11; *Row 3*–K13, K6, K18, K5, K14; *Row 4*–K3, K19, P410, P405, K20; *Row 5*–K16, K15, K17, P1057, P174; and *Row 6*–P147, P348, P127, P844.

P. megasperma f. sp. *glycinea* for isolates that infect soybean (*G. max*). The use of *glycinea* to replace *sojae* was suggested by D. P. Maxwell to be consistent with the use of the name of the genus of the host attacked and avoid the confusion which might result from the use of *sojae*.

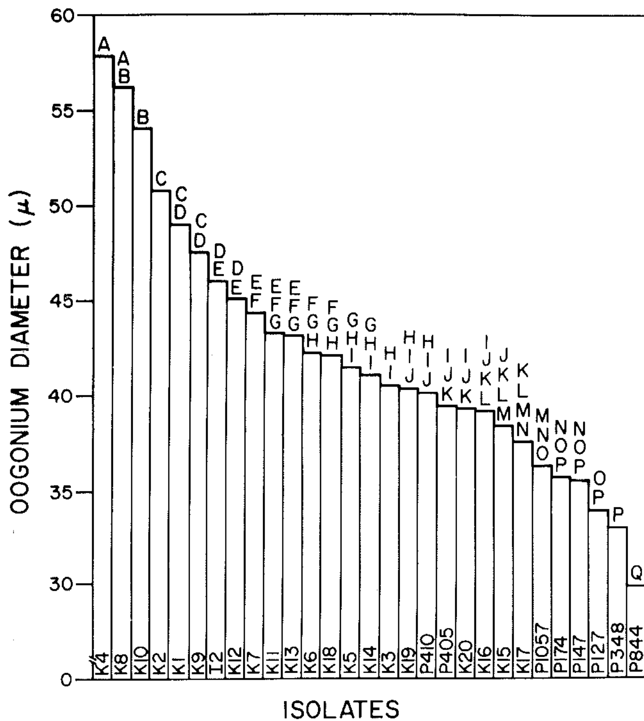

Fig. 3. Oogonia diameters of 29 isolates of *Phytophthora megasperma.* Each isolate was grown on V-8 juice agar at 24 C for 1 mo. Value represents mean of 30 measurements of each isolate. Mean values followed by same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

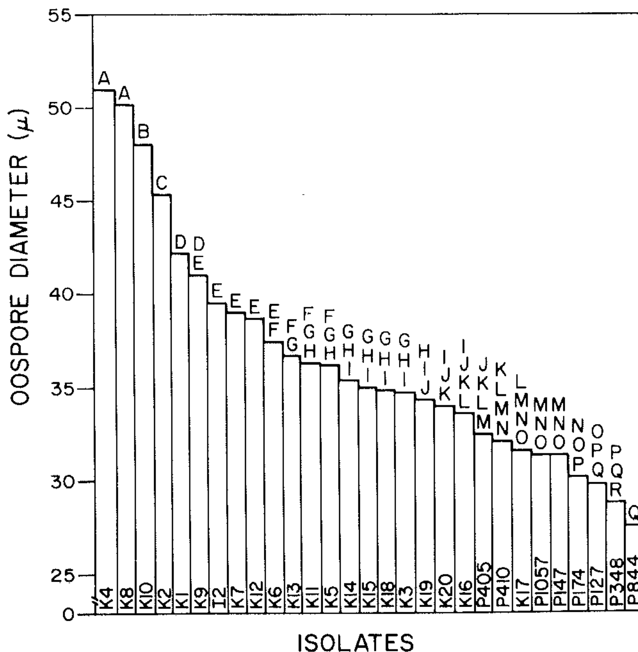

Fig. 4. Oospore diameters of 29 isolates of *Phytophthora megasperma.* Each isolate was grown on V-8 juice agar at 24 C for 1 mo. Value represents mean of 30 measurements of each isolate. Mean values followed by same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

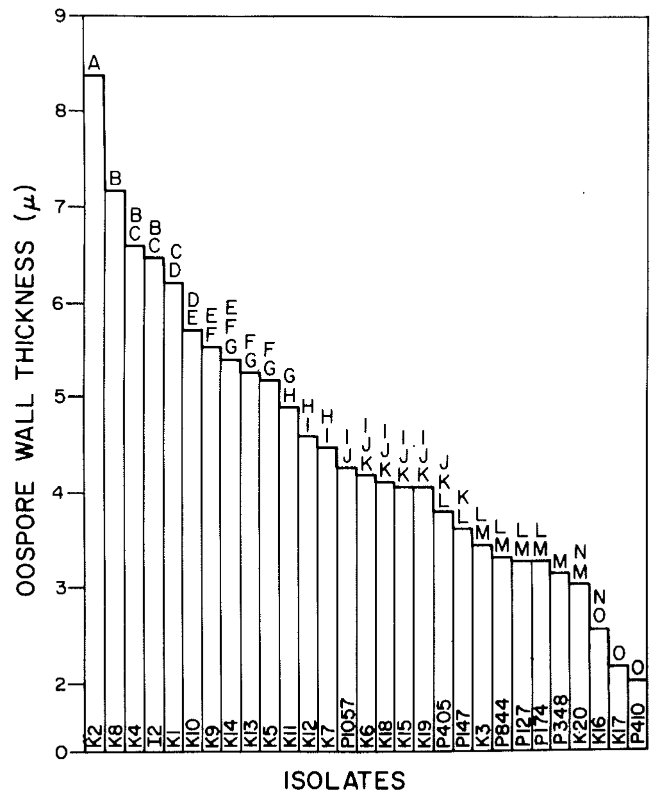

Fig. 5. Oospore wall thicknesses of 29 isolates of *Phytophthora megasperma.* Each isolate was grown on V-8 juice agar at 24 C for 1 mo. Value represents mean of 30 measurements of each isolate. Mean values followed by same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

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