Germination of Fusarium solani f. sp. pisi Chlamydospores in the Spermosphere of Pea

G. E. Short and M. L. Lacy

Graduate Assistant and Associate Professor, respectively, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

Journal Series Article No. 6443, Michigan Agricultural Experiment Station.
Accepted for publication 5 November 1973.

ABSTRACT

A method was developed for directly determining amount of spore germination in pea spermospheres in 1-mm increments of distance from the seed coat. The greatest distance at which *Fusarium solani* f. sp. *pisi* chlamydospores germinated was established within 24 h at 22 or 30 C, and never exceeded 7 mm under any conditions tested. More spores germinated, and the spermosphere was larger at 50% than at 20% soil moisture. More germination occurred near the emerging radicle than in other areas of the spermosphere. Greatest distances from seeds at which spores germinated decreased with increasing temp at 50% soil moisture. At 20% soil moisture, a higher percentage of spores germinated at 22 than at 10 or 30 C. The wrinkle-seeded pea cultivar 'Miragreen' supported more spore germination in the spermosphere than did the smooth-seeded cultivar 'Alaska'. If Miragreen seeds were soaked in aerated water for 48 h prior to planting, spores germinated only in the millimeter of soil nearest the seed and percentage germination was one-sixth that of spores in the same zone near unsoaked seeds.

Phytopathology 64:558-562.

Additional key words: agar-embedded soil columns.

Spores of most plant pathogenic fungi do not germinate in soil unless provided with some external stimulus (10). Germinating seeds exude nutrients capable of stimulating microbial activity, including spore germination (17). The zone around the seed into which exudates diffuse and microbial activity increases, has been designated the spermosphere (24) or spermatosphere (22). *Fusarium solani* f. sp. *phaseoli* chlamydospores were reported to germinate as far as 10-12 mm from germinating bean seeds (23). Attempts to ascertain sporangial germination (23) and populations (21) of *Pythium ultimum* at varying distances from seed surfaces, were less successful.

Inadequate techniques of observing activities of microorganisms in soil has been a major obstacle in determining quantitative dimensions of the spermosphere. The objective of this investigation was to determine the amount of chlamydospore germination of *F. solani* (Mart.) App. & Wr. em. Snyd. & Hans. f. sp. *pisi* occurring in different regions, and at various distances from germinating seeds of *Pisum sativum* L. as influenced by: (i) cultivar; (ii) soil temp; (iii) soil moisture; and (iv) soaking of seeds prior to planting. A preliminary report has been published (19).

MATERIALS AND METHODS.—*Selection and preparation of seeds.*—Wrinkle-seeded ('Miragreen') and smooth-seeded ('Alaska') pea cultivars obtained from Ferry-Morse Seed Co., Mountain View, Calif. were used. Individual seeds were selected on the basis of uniformity in size and color (yellow in the case of Miragreen; green for Alaska) and freedom from spots or cracks on the seed coat. Prior to planting, seeds were surface disinfested for 10 min in 0.5% sodium hypochlorite containing 1 ml of Tween 20 (polyoxyethylene sorbitan monolaurate) per liter, followed by 5 min of rinsing in sterile distilled water. In one experiment, seeds were soaked for 48 h in aerated water prior to planting.

Source, preparation, and infestation of soil.—Conover loam soil from the Michigan State University farm, collected from an area free from recent pesticide application, was used in all experiments. Soil was stored at 15-20% moisture at 22-25 C in closed plastic containers. Prior to use, soil was air-dried and passed through a 30-mesh sieve. Water-holding capacity of this soil was 61%, organic matter content was 3.4%, and pH was 6.6. The soil contained 18% clay, 15% silt, and 67% sand.

Chlamydospores of *F. solani* f. sp. *pisi* were produced in shaken liquid culture as follows: Macroconidia were removed from potato-dextrose agar (PDA) plates in 25 ml of sterile distilled water, combined with 40 ml of potato-dextrose broth, and agitated on a reciprocal shaker for 48 h. The germinated conidia were washed and suspended in 40 ml of sterile soil extract [prepared by mixing 1 liter of water with 1 kg of Conover loam, allowing the mixture to stand for 48 h, and filtering the supernatant through a 2.2-μ Gelman membrane filter (1)]. Germinated conidia agitated in soil extract produced an abundance of chlamydospores free from mycelium within 7 days. Chlamydospores were washed, resuspended in distilled water, and agitated at low speeds for 1-2 h in a Sorvall Omni-Mixer to break up aggregates of chlamydospores. The chlamydospore suspension was adjusted to 2.5×10^7 chlamydospores/ml using a hemacytometer. Air-dried, sieved soil was placed in a mixing apparatus, and the spore suspension was applied with an atomizer until a final concn of 1.6×10^6 chlamydospores/g dry wt of soil and a 20% soil moisture were simultaneously attained.

Adjustment of soil moisture.—Infested soil was placed in 2.5-cm-diam Pyrex glass tubes with a fine screen fastened to the base. Uniform compaction in the upper 2.5 cm of soil was attained by dropping the tubes from a height of 15 cm until further

compaction ceased. One pea seed, with the hilum oriented downward, was centered in the upper 2.5 cm of each soil column prior to the addition and compaction of the uppermost 1.25 cm of soil. The lower ends of the columns were immersed to a 1-mm depth in a water bath, permitting the upward movement of water by capillary action. Moisture levels of 20% and 50% were established in the upper 2.5 cm of soil by using 33 and 8.1-cm soil columns, respectively. This system maintained a constant moisture level in the upper 2.5 cm of soil during all stages of seed germination.

Determination of spore germination.—Spores were incubated at 10, 22, or 30 C in soil columns for 24-96 h following seed placement. Columns were then dried with a stream of air by applying a vacuum to the lower ends, infiltrated with 2% molten water agar, cooled, and the agar hardened by immersing in ethanol for 12 h. Agar-embedded soil columns were extruded from the Pyrex tubes and a 2-mm-thick cross-section in the vicinity of the seed was removed with a razor blade. A small sharpened spatula was used to serially remove blocks of soil at millimeter increments from the seed surface in the area of radicle emergence and opposite the radicle area (Fig. 1-F). Soil cores above and below the seed were removed with a 4-mm-diam cork borer, and serially sectioned in millimeter increments with a razor blade.

Each block of soil was placed on a microscope slide, and a drop of 5 N HCl added to dissolve the agar. Soil smears were made with 0.1% aniline blue in lactic acid, similar to the technique of Nash et al. (13). The smears were examined for chlamydospore germination at a magnification of X430. Fifty chlamydospores were counted per slide. Treatments were replicated five times and experiments were repeated once with similar results. Statistical differences between treatments were determined using a two-way analysis of variance following angular transformation of data.

RESULTS.—Preliminary experiments were carried out to determine the optimum time after planting to sample chlamydospore germination in the spermosphere. Germination at 22 C was determined after 24, 42, 48, or 72 h, using both pea cultivars and moisture levels of 20% and 50% (Table 1). The greatest distance from the seed at which spores germinated in any treatment was established within 24 h. Percentage germination was greatest 42 h after planting at 50% soil moisture and 48 h after planting at 20% soil moisture. Extensive mycelial growth after 42 h at 50% moisture obscured additional germination; however, this did not occur at 20% soil moisture. During preparation of soil smears from the 50% moisture samples, many ungerminated spores appeared to become dislodged from entangled hyphae of germinated spores, possibly resulting in an underestimate of spore germination. Germ tube lysis further reduced apparent spore germination 72 h after planting at 22 C (Table 1). No lysis was observed up to 96 h after planting at 10 C, but at 30 C lysis was already evident 30 h after planting. In later experiments (Fig. 1), soil columns were incubated for 24 h at 30 C; for 42 and 48 h at 50% and 20% soil moisture, respectively, at 22 C; and for 96 h at 10 C.

Effect of different regions of the spermosphere.—Spore germination was always greater near the emerging radicle than in other regions of the spermosphere (Fig. 1-A, B, E, F). However, no differences in germination in the other areas (hilum, opposite hilum, and opposite radicle) were found; hence, only data from the radicle and opposite radicle regions were plotted. At 50% soil moisture and 22 C, spores germinated with greater frequency at a given distance from the seed near the radicle than in other regions of the spermosphere. At 20% soil moisture, spore germination near the emerging radicle of either cultivar was consistently greater than in other areas only in the millimeter of soil directly adjacent to the seed. Maximum spore germination observed was 70% in the millimeter of soil (50% moisture) adjacent to the radicle area of the cultivar Miragreen. Spore germination declined with increasing distance from the seed, and was never detected further than 7 mm from the seed (Fig. 1-C).

Effect of soil moisture.—Spores germinated 2-5 mm further from the seed when soil moisture was increased from 20% to 50%, regardless of cultivar or temp used (Fig. 1-A, B, C, D). Spore germination at a given distance from the seed was also considerably greater at 50% than at 20% soil moisture.

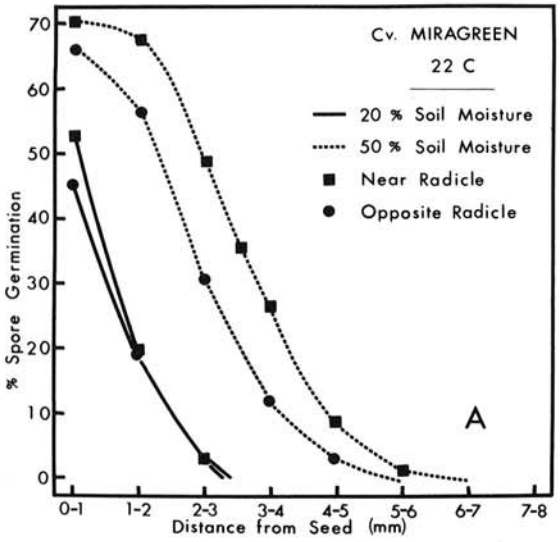
Effect of cultivar.—Spores germinated at greater distances and in higher frequencies at comparable distances from the Miragreen than the Alaska cultivar under all soil moisture and temp conditions (Fig. 1-A, B, C, D).

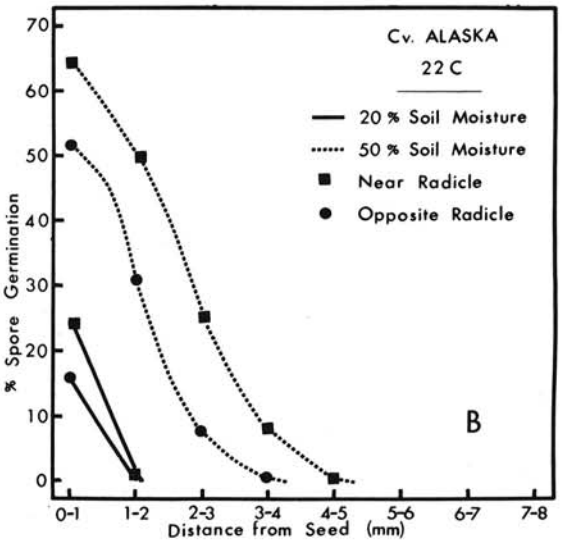
Effect of temperature.—The region of the spermosphere sampled was opposite the radicle (Fig. 1-F), since the amount of spore germination in this area was indicative of that in most other regions of the spermosphere. At 50% soil moisture, the maximum distance from the seed at which chlamydospores germinated decreased with increasing temp with both cultivars (Fig. 1-C, D). However, at 20% soil moisture, germination was greatest at 22 C and was least at 10 C.

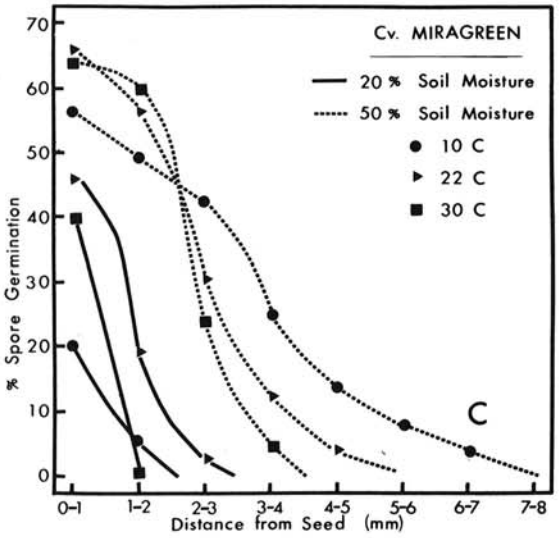
Effect of soaking seeds.—Spore germination was confined within 1 mm of the surface of Miragreen pea seeds soaked in aerated water for 48 h before planting (Fig. 1-E). Germination within 1 mm of soaked seeds was one-sixth that of spores in the same zone near unsoaked seeds.

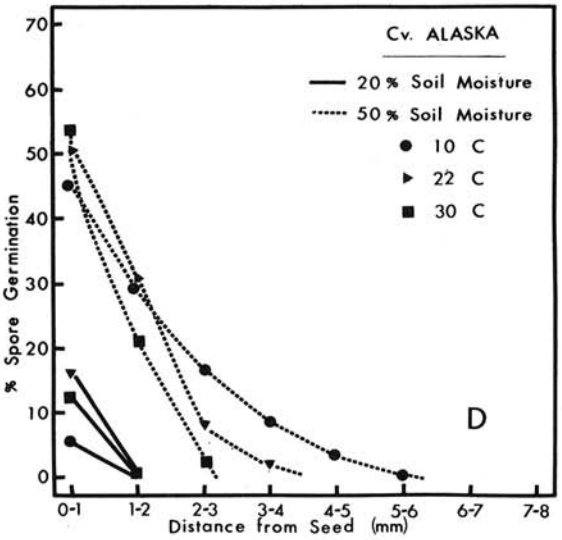
DISCUSSION.—The method of embedding and sectioning soil described enabled quantitative measurement of spore germination in the spermosphere with a precision heretofore not possible (14, 21, 22, 23). This technique could also be employed for determining spore germination in the rhizosphere. Undoubtedly, amount and rapidity of exudation are two very important considerations in selecting a host for study. If amount of exudation restricted spore germination to within 1 mm of the seed or root, this technique would not be as applicable. We have not been able to remove soil sections in increments of less than 0.5 mm.

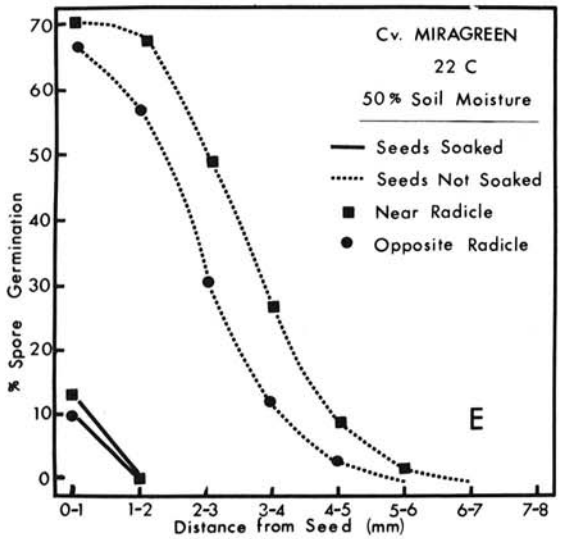
The ideal time for assessing spore germination in


Cv. MIRAGREEN
22 C
20 % Soil Moisture
50 % Soil Moisture
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
A


Cv. ALASKA
22 C
20 % Soil Moisture
50 % Soil Moisture
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
B


Cv. MIRAGREEN
20 % Soil Moisture
50 % Soil Moisture
10 C
22 C
30 C
% Spore Germination
Distance from Seed (mm)
C


Cv. ALASKA
20 % Soil Moisture
50 % Soil Moisture
10 C
22 C
30 C
% Spore Germination
Distance from Seed (mm)
D


Cv. MIRAGREEN
22 C
50% Soil Moisture
Seeds Soaked
Seeds Not Soaked
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
E

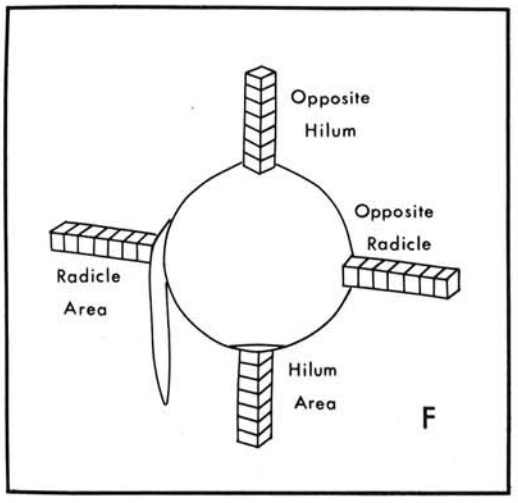

Opposite Hilum
Opposite Radicle
Radicle Area
Hilum Area
F

TABLE 1. Apparent germination of *Fusarium solani* f. sp. *pisi* chlamydospores at 22 C in the spermospheres of two pea cultivars at various times after planting in soil at 20% and 50% moisture

Cultivar	Soil moisture (%)	Time after planting (h)	Chlamydospore germination (%) at incremental distances from radicle (mm)						
			0-1	1-2	2-3	3-4	4-5	5-6	6-7
Miragreen	50	24	61	53	38	23	13	2	0
		42	70	68	49	27	9	2	0
		48	50	34	23	8	3	0	
		72	...[a]	...	...	...	...	...	...
Alaska	50	24	45	33	21	12	0		
		42	65	50	25	8	1	0	
		48	61	38	13	2	0		
		72	45	13	1	0			
Miragreen	20	24	50	31	4	0			
		48	66	26	2	0			
		72	45	13	1	0			
Alaska	20	24	22	8	0				
		48	32	4	0				
		72	22	1	0				

[a]No data due to extensive germ tube lysis.

soil is when the last germ tube has appeared. Unfortunately, by that time some germ tubes have lysed, and at 22 C and 50% soil moisture extensive mycelial growth also had occurred and made counting of germinated spores difficult. Both chlamydospore germination and germ tube lysis were less at the lower soil moisture (Table 1), in agreement with Cook and Flentje (5). It was possible, however, to accurately ascertain the greatest distance from seeds at which spores germinated. Lysis, mycelial growth and delayed spore germination were most pronounced within 2 mm of the seed. Thus, the values in Fig. 1 probably underestimate the final extent of chlamydospore germination close to the seed, but become increasingly more accurate toward the periphery of the spermosphere.

Severity of pre-emergence damping-off in peas has been directly correlated with amount of carbohydrate exudation during seed germination (7, 12). Wrinkle-seeded cultivars exuded more sugars than the less-susceptible smooth-seeded cultivars (7). Pre-emergence damping-off and carbohydrate exudation were greater in wet than dry soils (6, 7, 8, 9). The data (Fig. 1-A, B) suggest that at constant temp, spermosphere size is directly related to carbohydrate exudation. The considerably larger spermosphere at 50% soil moisture is likely due to (i) an increase in exudation, and (ii) a facilitated diffusion of these sugars through soil water (23).

The greater spermosphere effect near the radicle than in other areas of the seed (Fig. 1-A, B) is also likely due to greater amounts of nutrient exudation. The micropyle is a major portal through which seeds imbibe water (11) and simultaneously exude sugars (18, 20). Exudation from soybean (4), bean (16), and broad bean (15) was greatest in the micropylar zone, where the radicle penetrated the seed coat.

Schroth et al. (18) reported a 50% increase in carbohydrate exudation from seeds of the pea cultivar Alaska as temp was increased from 15-30 C. Bacterial competition for these nutrients would also increase with temp in the range of 10-30 C (2). Thus, at 10 C, the slow rate of bacterial consumption of these exudates may have enabled their diffusion further from the seed than at warmer temp. Hence, spermosphere size appeared to be inversely related to temp at the 50% soil moisture level (Fig. 1-C, D). However, at 20% soil moisture, the spermosphere was larger at 22 C than at 10 C, conceivably due to an insufficient increase in microbial activity to consume the greater amount of exudates as quickly. But when temp was further increased to 30 C, the smaller spermosphere effect was likely due to increased microbial competition. Any direct effect of temp on spore germination seems unlikely, since chlamydospore germination on PDA was greater than 90% at 10, 22, or 30 C.

Size of the spermosphere in which pathogenic spores germinate may be directly related to disease severity. Pre-emergence rotting of peas is most severe in cool wet weather (3, 8), conditions which resulted in a large spermosphere (Fig. 1-C, D). The Miragreen

Fig. 1-(A to F). Germination of *Fusarium solani* f. sp. *pisi* chlamydospores in the spermospheres of pea cultivars 'Miragreen' and 'Alaska.' **A & B)** Effect of soil moisture in two regions of the spermosphere. **C & D)** Effect of temp and soil moisture. Region of sampling was opposite the radicle. **E)** Effect of soaking seeds for 48 h prior to planting. **F)** Regions of the spermosphere sampled.

cultivar had a larger spermosphere than the less susceptible Alaska cultivar (Fig. 1-C, D). Flentje and Saksena (7) obtained a 5-fold increase in emergence by soaking wrinkle-seeded peas for 20 h prior to planting in soil infested with *Pythium*.

Since little exudation occurred after imbibition of water was complete (approximately 8 h) (20), fully swollen seeds when planted should exude considerably less nutrients than unsoaked seeds. Soaking pea seeds for 48 h prior to planting drastically reduced the volume and intensity of the spermosphere effect (Fig. 1-E), and presumably inoculum potential. Knowledge of the effect of soil moisture, temp, type of cultivar, cultural practices, and other factors in altering spermosphere dimensions may be useful in controlling pre-emergence rotting of peas and other agricultural crops.

LITERATURE CITED

1. ALEXANDER, J. V., J. A. BOURRET, A. H. GOLD, and W. C. SNYDER. 1966. Induction of chlamydospore formation by Fusarium solani in sterile soil extracts. Phytopathology 56:353-354.
2. ALEXANDER, M. 1961. Introduction to soil microbiology. John Wiley and Sons, New York 472 p.
3. BAYLIS, G. T. S. 1941. Fungi which cause pre-emergence injury to garden peas. Ann. Appl. Biol. 28:210-218.
4. BROWN, G. E., and B. W. KENNEDY. 1966. Effect of oxygen concentration on Pythium seed rot of soybean. Phytopathology 56:407-411.
5. COOK, R. J., and N. T. FLENTJE. 1967. Chlamydospore germination and germling survival of Fusarium solani f. pisi in soil as affected by soil water and pea seed exudation. Phytopathology 57:178-182.
6. FLENTJE, N. T. 1964. Pre-emergence rotting of peas in South Australia. II. Factors associated with the soil. Austr. J. Biol. Sci. 17:651-664.
7. FLENTJE, N. T., and H. K. SAKSENA. 1964. Pre-emergence rotting of peas in South Australia. III. Host-pathogen interaction. Austr. J. Biol. Sci. 17:665-675.
8. HULL, R. 1937. Effect of environmental conditions, and more particularly of soil moisture upon the emergence of peas. Ann. Appl. Biol. 24:681-689.
9. KERR, A. 1964. The influence of soil moisture on infection of peas by Pythium ultimum. Austr. J. Biol. Sci. 17:676-685.
10. LOCKWOOD, J. L. 1964. Soil fungistasis. Annu. Rev. Phytopathol. 2:341-362.
11. MANOHAR, M. S., and W. HEYDECKER. 1964. Effects of water potential on germination of pea seeds. Nature 202:22-24.
12. MATTHEWS, S., and W. T. BRADNOCK. 1968. Relationship between seed exudation and field emergence in peas and french beans. Hortic. Res. 8:89-93.
13. NASH, S. M., T. CHRISTOU, and W. C. SNYDER. 1961. Existence of Fusarium solani f. phaseoli as chlamydospores in soil. Phytopathology 51:308-312.
14. PAPAVIZAS, G. C., and C. B. DAVEY. 1961. Extent and nature of the rhizosphere of Lupinus. Plant Soil 14:215-236.
15. PEARSON, R., and D. PARKINSON. 1961. The sites of excretion of ninhydrin-positive substances by broad bean seedling. Plant Soil 13:391-396.
16. SCHROTH, M. N., and R. J. COOK. 1964. Seed exudation and its influence on pre-emergence damping-off of bean. Phytopathology 54:670-673.
17. SCHROTH, M. N., and W. C. SNYDER. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus, Fusarium solani f. phaseoli. Phytopathology 51:389-393.
18. SCHROTH, M. N., A. R. WEINHOLD, and D. S. HAYMAN. 1966. The effect of temperature on quantitative differences in exudates from germinating seeds of bean, pea, and cotton. Can. J. Bot. 44:1429-1432.
19. SHORT, G. E., and M. L. LACY. 1972. Direct observation of Fusarium solani f. pisi chlamydospore germination in the spermosphere of peas. Phytopathology 62:1111 (Abstr.).
20. SIMON, E. W., and R. M. RAJA HARUN. 1972. Leakage during seed imbibition. J. Exp. Bot. 23:1076-1085.
21. SINGH, R. S. 1965. Development of Pythium ultimum in soil in relation to presence and germination of seeds of different crops. Mycopathol. Mycol. Appl. 27:155-160.
22. SLYKHUIS, J. T. 1947. Studies on Fusarium culmorum blight of crested wheat and brome grass seedlings. Can. J. Res. 25:155-180.
23. STANGHELLINI, M. E., and J. G. HANCOCK. 1971. Radial extent of the bean spermosphere and its relation to the behavior of Pythium ultimum. Phytopathology 61:165-168.
24. VERONA, O. 1963. Interaction entre la graine en germination et les microorganismes telluriques. Ann. Inst. Pasteur (Paris) 105:75-98.