

Effect of Inoculum Density, Planting Depth, and Soil Temperature on *Urocystis colchici* Infection of Onion

W. C. Stienstra and M. L. Lacy

Former Graduate Assistant and Associate Professor, respectively, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48823.

Present address of first author: Department of Plant Pathology, University of Minnesota, St. Paul 55101.

Journal Series Article No. 5468, Michigan Agricultural Experiment Station.

Accepted for publication 23 September 1971.

ABSTRACT

Curves drawn using the multiple infection, log-probit, or log-log transformations tended to flatten at upper inoculum concentrations, but percentage of disease was linearly related to the distance between spores (D) over a wide inoculum range. The linearity using D and the nonlinearity using other transformations were explained by the nonlinear relationship between D and number of spores per gram of soil.

Soil was artificially infested in layers at 0-5, 5-10, and 10-15 mm deep, and in all possible combinations. Onion seeds were planted 5, 10, or 15 mm deep. Deeper planting resulted in more disease at a given inoculum density due

Additional key words: mathematical models.

to (i) increased amount of susceptible tissue exposed; and (ii) longer exposure of susceptible tissue to inoculum. Greatest amount of disease resulted when seeds were planted at 15 mm with all layers of soil infested. Least amount of disease resulted when inoculum was below the seed. At inoculum densities of 1,000 (D = 2.0 mm) or 2,000 (D = 1.7 mm) spores/g soil, amounts of disease were similar at each of four temperatures (12, 20, 24, and 28 C), but significantly more disease occurred at the higher inoculum density (16 C). Highest infection at both inoculum densities occurred at 20 C.

Phytopathology 62:282-286.

The density of pathogen propagules in soil is known to affect disease incidence and severity (3, 6), but the relationship of amounts of soil-borne inoculum to amount of disease has been discussed extensively with considerable controversy. There is general agreement that disease does not increase in a simple linear way with increased inoculum density unless inoculum concentration is low (6). Experts differ on the best way to transform data to obtain a linear relation between amount of inoculum and amount of disease (3, 5, 6, 8, 9, 15). Gregory (9) proposed a multiple infection transformation based on the probability of plants being infected at more than one site when exposed to different amounts of inoculum, and devised tables to transform per cent disease to number of infections. Van der Plank (15) devised a similar transformation based on the natural logarithm of the proportion of healthy plants in the population. Horsfall (10) and others (18) advocated the use of a logarithmic-probability plot based on dosage-response curves of inhibition of spore germination by fungicides. Baker (2) and Baker et al. (3) proposed logarithmic transformations for both amount of disease and amount of inoculum, and stated that inferences could be made about the nature of the relationship between root and inoculum from the slopes of resulting curves. Few data are available to confirm this hypothesis.

Our objectives were to determine the relationship of inoculum density to disease incidence, and the effects of soil temperature and depth of planting on that relationship. *Urocystis colchici* (Schlecht.) Rabenh. and *Allium cepa* L. 'Downing Yellow Globe' were used as the host-parasite combination in this study because (i) the fungus produces small, resistant teliospores which can be incorporated into soil in known amounts; (ii) the onion is susceptible to infection for a very short time, and no further

infection occurs after seedling emergence; and (iii) infection can be visually determined during the young seedling stage by the appearance of smut sori inside the lacunae of cotyledons.

MATERIALS AND METHODS.—Naturally infested field-grown onions were collected and dried at about 24 C. Dried leaves were fragmented, and the material passed through a 200-mesh sieve. This material contained ca. 2×10^8 teliospores/g, and was stored in a stoppered vial at 2 C for use as inoculum. Greenhouse soil (a 1:1:1 mixture of Conover loam, sand, and peat) was infested at varying inoculum densities by mixing a known number of teliospores into a specific weight of oven-dry soil for 20-30 min in a concrete mixer. Later, estimates of soil bulk density were made for calculations of the mean distance between spores (2, 3).

Surface-disinfested onion seeds were placed on a smooth surface of soil in metal flats (64 X 39 X 10 cm) or 20-cm plastic pots. Seeds were then covered with enough soil (infested or noninfested) to give a specified thickness above the seeds after compaction. Soil was placed over seeds in increment 5-mm layers. The pots or flats were placed in controlled temperature tanks or on greenhouse benches, watered daily, and fertilized weekly with a soluble 20-10-10 fertilizer. Natural light was supplemented 16 hr/day with artificial light from four 40-w fluorescent lamps (45 cm above the bench) per 1.5 square meter. A minimum of 100 seedlings in each of four replicate pots was visually observed for smut infection. All experiments were repeated at least twice with similar results. Variability in amount of disease between replicates in any given inoculum density did not exceed 5%.

RESULTS.—*Effect of inoculum density on disease incidence.*—Increases in inoculum densities from 125 to 8,000 spores/g soil caused percentage of diseased

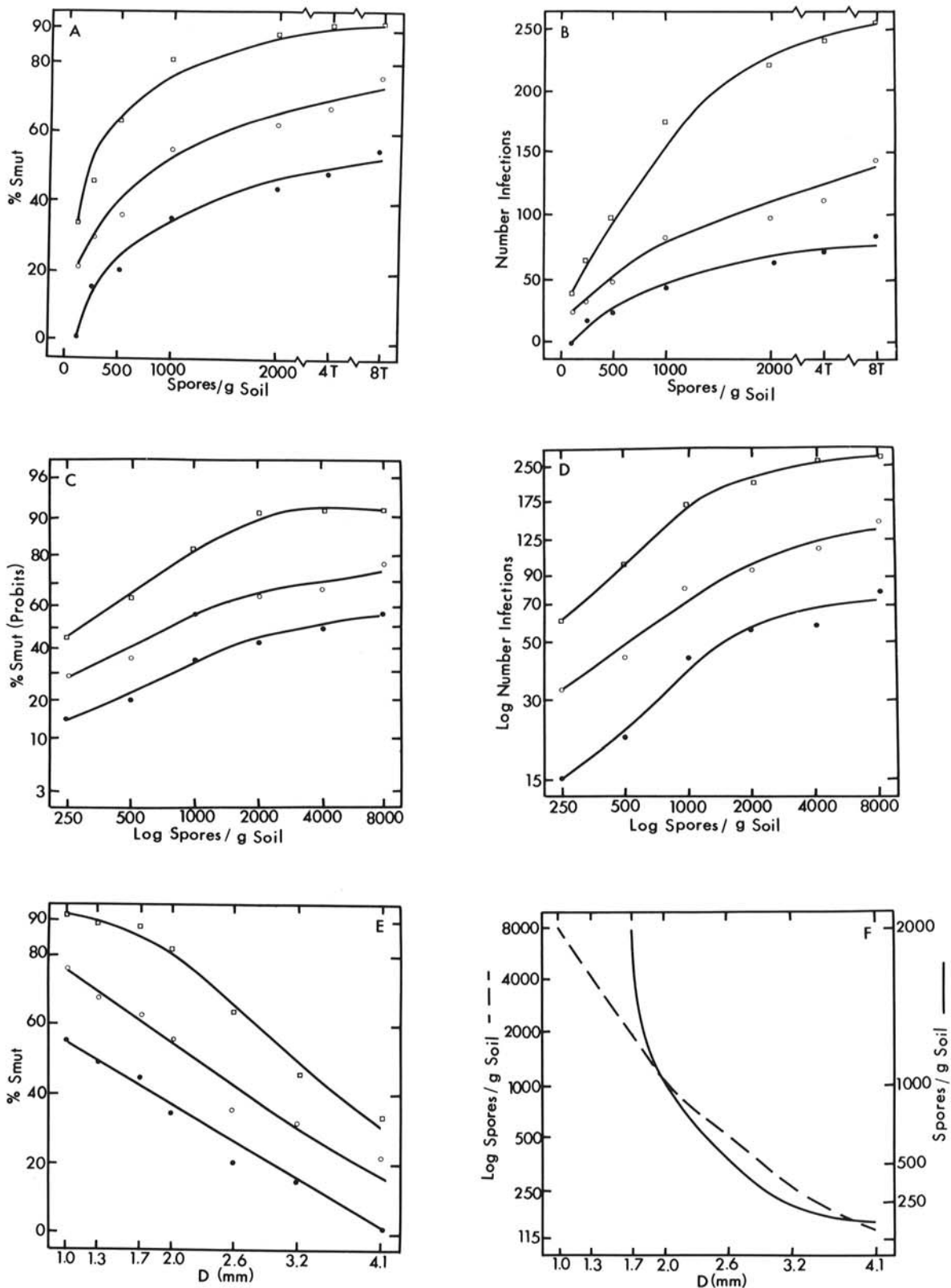


Fig. 1. The relationship of inoculum density and disease incidence when seeds were planted at the three depths below the soil surface: 5 mm (solid circles); 10 mm (open circles); and 15 mm (open squares). A) Inoculum density (arithmetic) and percentage disease (arithmetic). B) Inoculum density (arithmetic) and number of infections (arithmetic). C) Inoculum density (logarithmic) and percentage disease (probability scale). D) Inoculum density (logarithmic) and number of infections (logarithmic). E) Inoculum density (distance between propagules) and percentage of disease (arithmetic). F) The relationship between distance between propagules (D) and spores per gram of soil or log spores per gram of soil.

plants to increase very rapidly at lower inoculum densities, but the rate of increase was less at higher densities (Fig. 1-A). An arithmetic plot of the data gave curves difficult to analyze, since most of the increase in disease occurred over a small range of inoculum, and additional inoculum appeared less effective in producing a unit disease response. Thus, the relationship between inoculum density and percentage diseased plants was not simple.

Gregory (9) pointed out that, as inoculum density increases, the probability of a plant having more than one infection site also increases. Percentage disease does not measure multiple infections on individual plants. The multiple infection transformation (9) corrects for the faster rate of increase in numbers of infections than does percentage disease as inoculum density increases (Fig. 1-B). These curves, while more nearly linear than the curves in Fig. 1-A, were difficult to analyze because the response to increasing inoculum concentration was not linear.

A logarithmic-probability plot (Fig. 1-C) gave a more nearly linear relationship between the percentage of diseased units and the density of inoculum. The assumption implicit in this plot is that susceptible plants are normally distributed in accordance with the logarithm of the inoculum concentration. Therefore, the ordinate is symmetrical about the ED_{50} , and percentage of either healthy or diseased plants should be linearly related to log inoculum density. The slopes of the three curves which represented the three planting depths were estimated at 0.5; however, the slopes of the curves flattened at the higher inoculum densities, and the relationships were no longer linear.

The relationship between log number of infections and log number of spores/g of soil (Fig. 1-D) was linear over the inoculum range of 250-2,000 spores/g of soil. The slope for this range was from 0.64 to 0.72 (linear regression analysis); correlation coefficients were 0.90 to 0.95. However, the slope flattened for concentrations higher than 2,000 spores/g of soil.

Roots of plants have an influence on pathogens in soil. Infection is a matter of probability of contact of growing host root and pathogen propagule. Baker (2) and Baker et al. (3) proposed that orientation of spores in soil could occur ideally as a lattice of tetrahedra, as the tetrahedron was the simplest three-dimensional figure possible. This hypothesis requires a situation in which any addition of inoculum to a soil system redistributes all inoculum, so that the propagules initially present and the added propagules always reorient themselves to become equidistant from each other. The tetrahedral surface consists of four equilateral triangles, and each apex, which represents the position of a spore, is equidistant from the other three. The distance between spores, or length of an edge of the tetrahedron, is thus directly proportional to the volume enclosed within the tetrahedron, and can be calculated. The volume of a tetrahedron (V_t) is described by the following formula:

$$V_t = 0.11785 D^3 \quad (1)$$

where (D) is the distance between apices of the

tetrahedron or spores. The number of spores required to establish a selected distance between spores in a given volume of soil is calculated by the following formula:

$$N = \frac{V_s}{(0.11785) D^3} \quad (II)$$

where N is the number of spores per volume of soil, V_s is the volume of soil to be infested, and D is the distance between spores. If the desired distance between spores in mm is substituted for D, and the volume of soil to be infested is substituted for V_s , the equation can be solved for the number of spores per volume of soil (N) needed to give the selected distance between spores (D).

Percentage of disease when plotted against the calculated distance between spores (D) increased linearly over the entire inoculum range (Fig. 1-E). That is, a unit change in the distance between propagules produced a unit change in percentage of diseased plants over all inoculum densities tested. The correlation coefficients were 0.90 to 0.95 for the entire inoculum range. Slopes of curves resulting from seeds being planted at depths of 5, 10, or 15 mm were similar, but ordinate intercepts were increased with planting depth. Thus, position of the curve, but not slope, was altered by planting depth.

Effect of inoculum placement on disease incidence.—Initially, disease occurrence was low when inoculum was placed below the seed, even when the seed was placed directly on soil containing inoculum. However, planting depth did affect disease incidence (12, 13), raising the question of whether the cotyledon was more susceptible when exposed to inoculum (1,000 spores/g soil) deep in the soil. That is, did the additional expenditure of energy required for emergence from deeper planting result in a higher degree of susceptibility and more infection from the same amount of inoculum?

Seeds were planted either 5, 10, or 15 mm deep, with infested soil (1,000 spores/g) being placed in the following layers: (i) the top 5 mm; (ii) the center 5 mm; (iii) the lowest 5 mm below the surface; (iv) the top and center 5 mm; (v) the center and lowest 5 mm; and (vi) the top, center, and lowest 5-mm layers.

Sixty-seven per cent of the plants were infected when soil containing 1,000 spores/g was confined to a single 5-mm zone immediately above seeds planted at 15 mm (Table 1). When all three 5-mm zones above the seeds contained inoculum, the percentage of disease plants increased only 13%. A similar response occurred when seeds were planted 10 mm deep. Disease incidence from inoculum in the center 5-mm layer only was greater when seeds were planted at the 15-mm than at the 5- or 10-mm depth. Also, more infected plants occurred from inoculum in the top two 5-mm layers of soil when seeds were planted 15 mm deep than when seeds were planted 10 mm deep. However, this increased disease response from deeper planting did not occur when inoculum was only in the top 5-mm zone. Inoculum in the soil layer immediately above seeds produced more diseased plants than similar amounts of inoculum in layers 5 or 10 mm above seeds.

TABLE 1. The effects of planting depth and infestation of soil by zones on *Urocytis colchici* infection of onion

Location of infested soil layers (mm below surface) ^a	Seed planting depth (mm below surface)		
	5	10	15
	% Disease		
0-5	19.0	16.9	18.2
5-10	3.1	25.2	47.5
10-15	0.5	3.4	67.0
0-10	19.3	47.8	61.4
5-15	4.9	22.3	70.9
0-15	20.1	49.6	79.5

^a Soil infested at 1,000 spores/g.

Time of exposure to inoculum.—Length of exposure of onion tissue to inoculum is an important variable in disease response (1, 2, 14, 16). Deeper planting may delay seedling emergence, allowing longer exposure of seedlings to the pathogen during the susceptible phase. Average times required for cotyledon emergence and appearance of the first true leaf were observed for different planting depths (Table 2). Cotyledons emerged from soil in 7 days when seeds were planted 5 mm below the soil surface. Cotyledon emergence from seeds planted 10 or 15 mm deep was delayed by 1 and 2 days, respectively. The appearance of the first true leaves was delayed by 1 day.

The effect of temperature on time required for seedling emergence, and thus time of exposure to inoculum, was investigated (Table 3). At 20 C, cotyledons emerged in 8 days, and first true leaves, in 19 days. At 12 C, cotyledon emergence was delayed 2 days, and true leaves by 4 days. Cotyledon emergence was not delayed at 16 C, but true leaf appearance required 2 more days than at 20 C. At 28 and 24 C, cotyledon emergence occurred 4 and 2 days earlier, and the first true leaves appeared 4 and 3 days earlier, respectively, than at 20 C.

Disease incidence rose with increases in soil temperature, reaching a high at 20 C; temperatures above 20 C resulted in decreased disease. At 12 C, the amount of infection after 5 weeks was near zero; however, when plants maintained at 12 C were incubated for an additional 2 weeks at 20 C, disease incidence was approximately 10%. The disease response pattern for both inoculum densities (1,000 and 2,000 spores/g soil) as temperature increased from 12 to 28 C was similar, except at 16 C. Optimum temperature for disease incidence agreed closely with optimum temperature for spore germination in vitro (11). Differences in amount of disease at different temperatures appears to be due partly to rapidity of seedling emergence and amount of spore germination.

DISCUSSION.—A linear relationship at inoculum densities of 125-8,000 spores/g was obtained when percentage of infection was plotted against the distance in mm between spores (Fig. 1-E), whereas other types of plots were nonlinear, at least at high inoculum densities. Baker & McClintock (4) were the

TABLE 2. Number of days after planting required for 50% emergence of cotyledons and 50% appearance of true leaves

Planting depth	Temperature (C)	Days required for 50% of:	
		Cotyledons	First leaves
5 mm	20	7	18
10 mm	12	10	23
	16	8	21
	20	8	19
	24	6	16
15 mm	28	4	15
	20	9	19

first to suggest that the number of propagules per unit volume of soil and the distance between propagules are not linearly related. Since the probability of infection was linearly proportional to the distance between propagules, and not number of propagules per unit volume, it became clear why disease response curves flattened out at higher inoculum densities with the commonly used log-probit and log-log plots (Fig. 1-C, D). The fact that the magnitude of decrease in distance between propagules was very small as inoculum density increased above 1,000 spores/g of soil meant that the increased probability of additional infections was also very small. This factor has been generally omitted as a reason for the typical decrease in slope of disease response curves at high inoculum densities.

Plots of log number of propagules/g of soil against log inoculum densities between 250 and 1,000 propagules/g of soil were linear because the relationship between the log number of propagules/g of soil and distance between propagules was linear in this density range (Fig. 1-F). However, above 1,000 propagules/g of soil, the relationship was no longer linear and slope decreased. Thus, the hypothesis of Baker et al. (3) that a slope of two-thirds when log successful infections are plotted against log inoculum density reflects a rhizoplane effect was only true at relatively low inoculum densities (Fig. 1-D); at higher inoculum densities, slopes were less than two-thirds. This hypothesis also assumes that the relationship between propagule and infection count is two-dimensional. However, spores occupy space and

TABLE 3. The effect of soil temperature on % disease at two inoculum densities

Soil temperature (C)	Inoculum density (Spores/g soil)	
	1,000	2,000
	% Disease	
12	1	1
16	27	49
20	48	53
24	33	36
28	9	10

are three-dimensional. In addition, the amount of influence exerted in a rhizosphere may not be uniform, but decreases in intensity with increased distance from the root surface. Thus, it would seem nearly impossible to clearly separate rhizoplane from rhizosphere effects.

The effects of temperature on the relationship between inoculum density and disease incidence were best illustrated at 16 C (Table 3). The differences in amounts of disease at 16 C with 1,000 or 2,000 spores/g may be explained, in part, by the effect of temperature on spore germination. Spore germination data (11) indicated 48% germination at 16 C and 84% at 24 C under optimum conditions after 8 days. Thus, effective spore concentration would be reduced by half because of incomplete germination at the suboptimum temperature. The temperature effect on rate of emergence of seedlings (Table 3) and on growth of *U. colchici* at 16 and 20 C was reported to be small (7, 18), so this was probably not an important factor. The change in percentage disease in a population of plants due to a decrease in inoculum density from 2,000 (D = 1.7 mm) to 1,000 (D = 2.0 mm) spores/g was very small (Fig. 1-E); however, the change in disease response due to a decrease from 1,000 (D = 2.0 mm) to 500 (D = 2.6 mm) spores/g was much greater. Thus, in the past, the effect of suboptimal temperatures on disease incidence may have been masked by using high inoculum densities (16), as reductions in pathogen propagules at high densities have a relatively small effect on percentage of disease.

Although the effect of temperature on amount of disease at 16 C may be explained in part by a reduction of spore germination, other factors became important at 12, 24, and 28 C. At 12 C, the length of exposure of host tissue to inoculum was increased (Table 3), but germination and growth of the fungus were reported to be very low (7, 11, 17); therefore, amount of disease would be limited due to poor spore germination. Spore germination and vegetative growth of the fungus were reported to be optimum at 24 C (7, 11), but were nearly as good at 20 C, where optimum infection occurred. The mean length of exposure of the cotyledon to inoculum was 6 days at 24 C, and 8 days at 20 C. The first true leaf appearance was 3 days earlier at 24 than at 20 C. The appearance of the first true leaf was stated to be approximately coincident with the development of immunity in the onion cotyledon (1, 16); thus, at 24 C, immunity to infection may have been reached earlier. At 28 C, the length of exposure of host tissue to inoculum was shortest, and the immune condition occurred earliest as judged by the appearance of the first leaf. Also, the growth and germination of the fungus were reported to be greatly restricted at 28 C (7, 11). There was general agreement, with results reported earlier (17), that infection can occur at either high or low temperature, but at above 25 C, a decided reduction in disease occurs.

The amount of disease resulting from inoculum in the top 5 mm of soil was not significantly increased by deeper planting, but a significant increase in

disease occurred as seeds were planted deeper with the same amount of inoculum placed immediately above the seed. The length of exposure to inoculum in the top 5 mm of soil was not increased by deeper planting. However, exposure time was increased by deeper planting when the infested 5-mm layer was immediately above the seed. The cotyledon was uniformly susceptible, and increased disease was related to increased exposure to inoculum.

LITERATURE CITED

- ANDERSON, P. J. 1921. Development and pathogenesis of the onion smut fungus. *Mass. Agr. Exp. Sta. Bull.* 4:99-133.
- BAKER, R. 1965. The dynamics of inoculum, p. 395-403. *In* K. F. Baker & W. C. Snyder [ed.]. *Ecology of soil-borne plant pathogens*. Univ. California Press, Berkeley.
- BAKER, R., C. L. MAURER, & R. A. MAURER. 1967. Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. *Phytopathology* 57:662-666.
- BAKER, R., & D. L. MCCLINTOCK. 1965. Populations of pathogens in soil. *Phytopathology* 55:495.
- DIMOND, A. E., & J. G. HORSFALL. 1960. Inoculum and the diseased population, p. 1-22. *In* J. G. Horsfall & A. E. Dimond [ed.]. *Plant pathology: an advanced treatise*. III. Academic Press Inc., New York.
- DIMOND, A. E., & J. G. HORSFALL. 1965. The theory of inoculum, p. 404-419. *In* K. F. Baker & W. C. Snyder [ed.]. *Ecology of soil-borne plant pathogens*. Univ. California Press, Berkeley.
- DOW, R. L., & M. L. LACY. 1969. Factors affecting growth of *Urocystis colchici* in culture. *Phytopathology* 59:1219-1222.
- GARRETT, S. D. 1956. *Biology of root-infecting fungi*. Cambridge University Press, London & New York. 292 p.
- GREGORY, P. H. 1948. The multiple-infection transformation. *Ann. Appl. Biol.* 35:412-417.
- HORSFALL, J. G. 1956. Principles of fungicidal action. *Chronica Botanica*, Waltham, Mass. 280 p.
- LACY, M. L. 1968. Optimum conditions for germination of *Urocystis colchici* teliospores. *Phytopathology* 58:1460-1463.
- STIENSTRA, W. C., & M. L. LACY. 1969. Effect of inoculum density and planting depth on infection of onion by *Urocystis colchici*. *Phytopathology* 59:1052. (Abstr.).
- STIENSTRA, W. C., & M. L. LACY. 1970. Effect of planting depth and thiram formulations on stands and incidence of smut in field-seeded onions. *Plant Dis. Repr.* 54:290-293.
- THAXTER, R. 1890. The smut of onions (*Urocystis cepulae* Frost). *Ann. Rep. Conn. Exp. Sta.* 1889:129-153.
- VAN DER PLANK, J. E. 1963. *Plant diseases: epidemics and control*. Academic Press, New York & London. 349 p.
- WALKER, J. C., & L. R. JONES. 1921. Relation of soil temperature and other factors to onion smut infection. *J. Agr. Res.* 22:235-262.
- WALKER, J. C., & F. L. WELLMAN. 1926. Relation of temperature to spore germination and growth of *Urocystis cepulae*. *J. Agr. Res.* 32:133-146.
- WILCOXON, F., & S. E. A. MCCALLAN. 1939. Theoretical principles underlying laboratory toxicity tests of fungicides. *Contrib. Boyce Thompson Inst.* 10:329-338.