FACTORS AFFECTING UROCYSTIS COLCHICI INFECTION OF ONION

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ABSTRACT

FACTORS AFFECTING *UROCYSTIS COLCHICI* INFECTION OF ONION

By

Ward C. Stienstra

The relation of *Urocystis colchici* teliospore density and disease response in *Allium cepa* 'Downing Yellow Globe' was studied. Data on disease severity as a function of the number of spores/g of soil have been treated by the multiple-infection transformation, the log-probability transformation, and the log-log transformation. These transformations produce linear results for only specific sections of the disease response curve. Disease was shown to be linearly related to the distance between propagules over the entire inoculum range tested.

The effects of planting depth, inoculum placement, and soil temperature were studied. The amount of disease increased 13-27% for each 5 mm increase in planting depth, depending on inoculum density. Eighteen percent disease occurred from inoculum (1,000 spores/g) present in the top 5 mm of soil when seeds were planted 5, 10, or 15 mm below

the surface. However, when a 5 mm zone of inoculum (1,000 spores/g) was placed immediately above the seed, 18, 25, and 67 percent disease occurred. Maximum infection occurred at 20°C. The time required for emergence was shortened at 24 and 28°C, and increased at 12 and 16°C, as compared to 20°C.

Exudation of ninhydrin- and anthrone-positive materials by onion seedlings was studied. Exudation after one day of incubation was 5.0 µg anthrone positive (glucose equivalent) material/seedling and 2.0 µg ninhydrin positive (glycine equivalent) material/seedling. Amounts decreased during the next two days to less than 1 µg/seedling for ninhydrin- and anthrone-positive materials on the fourth day.

Nutrients present in dormant onion seeds or in exudate from seeds incubated 48 hours were shown to support teliospore germination. Sucrose and asparagine did not support complete spore germination.

Maximum disease response occurred from inoculum present in the 5 mm zone immediately above the seeds where early exudation of nutrients would stimulate spore germination and the length of exposure to inoculum was the longest.

FACTORS AFFECTING *UROCYSTIS COLCHICI* INFECTION OF ONION

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Ward Curtis Stienstra

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I am especially grateful to my wife, Myrna, who encouraged and supported a student-husband and to our parents: Mr. and Mrs. Henry Stienstra and Mr. and Mrs. Arie Schuitman for their moral and financial support.

to my wife and son,

Myrna and Curt

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INTRODUCTION

Smut fungi played an important role in the early history of plant pathology. Tilletia caries, incitant of stinking smut or bunt of wheat, was used by Tillet (43) in 1755 to establish the principle that plant diseases were contagious. Prevost (31) in 1807 observed germination of wheat bunt spores and conceived the idea that this fungus penetrated the wheat plant and caused disease. The revolutionary discovery that fungal spores germinated as "seeds" of microscopic plants and produced new individuals was not accepted until 1847, when published studies on the wheat bunt fungus confirmed Prevost's observations of spore germination and fungal growth in the host plant (45). These early studies on smut diseases served as models for later scientific work on the nature and causes of plant disease.

The onion smut fungus (Urocystis colchici (Shlecht.) Rabenh.) is a soil-borne plant pathogen (1, 6, 42, 45). The overwintering stage is the teliospore, often described as a chlamydospore. The thick-walled teliospore is surrounded by 15-20 small sterile cells and is reported to contain a single diploid nucleus (1, 6, 13, 42, 45). Tachibana and Duran suggested that U. colchici teliospores

exhibited bipolar sexuality and produced unisexual mycelia (41). The nucleus is believed to undergo meiosis prior to teliospore germination (6), but Tachibana and Duran (41) were unable to infect onion seedlings with mycelium from single spores. The germinating teliospore produces a branching monokaryotic promycelium from which hyphae develop which directly penetrate the epidermal cells of the onion cotyledon (13). The mycelium progresses intercellularly, with the host cells being pushed apart as the lesion grows. Systemic infection is associated with the penetration of the mycelium to the onion meristem. The mycelium becomes dikaryotic just prior to teliospore formation (6), which can occur in the cotyledon, bulb scales, or leaves.

Smut was one of the first groups of diseases to be controlled by chemicals (24). Seed treatments collectively known as le Chaulage, involving lye, ammonia, or copper sulfate were used for the control of stinking smut by European farmers in the late 1700's. Chemical control methods for onion smut were developed in the early 19th century (42). The earliest control was a solution of formaldehyde dripped in the furrow near the seed (35). Formaldehyde inhibited the fungus until the onion plant developed beyond the three-week susceptible period. Smut control now consists of either seed treatment or in-furrow soil fumigation. This disease cannot be economically eradicated from soil because of longevity of the fungal

spores, and therefore remains an important disease in the northern onion growing areas despite effective control measures (45). Environmental conditions affect the period of susceptibility and the amount of infection (46). Infection is limited in climates with mean soil temperatures exceeding 25°C at the time of planting, apparently because of inhibition of the pathogen (12, 23, 47). In addition the onion seedling develops rapidly under these conditions, shortening the period of susceptibility (46).

The purpose of my research was to study the factors affecting Urocystis colchici infection of onion. These factors included inoculum density and placement, planting depth, temperature, and host exudates. Inoculum density has a profound influence on disease severity; therefore, most of the research reported herein was directed toward evaluating the relation of inoculum density and disease.

LITERATURE REVIEW

The challenge of dealing in a quantitative way with soil-borne inoculum was summarized by Dimond and Horsfall (11). They state that air-borne pathogen concentrations can be estimated by spore trapping, but in soil estimation is very difficult. Air-borne inoculum can be applied directly to the host surface and measurements made of the relationship between inoculum density and resultant disease production, but in soil direct observations are more difficult because of the opacity of soil. Data from diseases with air-borne inoculum have given reasonably satisfactory theories for predicting distribution of inoculum and disease severity, but in soil this has proven less applicable because of the relative immobility of inoculum and difficulty of direct observation of infection of host plants.

The various definitions of the term "inoculum potential" in soil have been reviewed recently by Dimond and Horsfall (10), Garrett (15), and Baker (3). Inoculum potential has been defined as the energy available for infection at the surface of the host to be infected (14). This is a resultant of the inoculum density and the environmental or capacity factors affecting the inoculum.

Disease potential has been defined by Grainger as the ability of the host to contract disease at different stages in its development (16). I will use the formula developed at the discussion following the symposium on Ecology of Soil-borne Plant Pathogens held at Berkeley in which:

Disease Severity = Inoculum Potential x Disease Potential.

Data of Rowell and Olien (32) indicated a simple, linear relation between the amount of inoculum and number of infections when the amount of inoculum was small. However, as inoculum became more abundant the resultant number of infections was no longer simply related to the number of diseased plants because a plant could be infected a second or third time and still be counted as one infection. Disease measured as percent diseased plants thus ignores the increase in the number of infections that occur on diseased plants (17). This shortcoming of assessing disease by percentage diseased plants led to computation of the number of infections corresponding to different percentages of diseased plants by Gregory (17). This multiple-infection transformation was called the semilogarithmic transformation by Dimond and Horsfall (11). A similar transformation proposed by van der Plank (30) was $\log_e 1/1 - X$, where X = the percentage of diseased plants and 1 = unity, or all susceptible plants in the population. His argument was that new infections occurred only in tissue not previously

infected. Gregory and van der Plank thus arrived at the same result from two different approaches. Gregory corrected for multiple infections by computing the number of infections corresponding to different percentages of diseased plants, while van der Plank assumed that multiple infections did occur and dealt with the number of healthy plants remaining.

Another hypothesis which deals with the relation between inoculum and disease states that the logarithm of inoculum density is linearly related to the probability of a host becoming infected (19, 49). Since living organisms are variable, it can be assumed that in a population of inoculum, virulence is not uniform, and in a population of infection sites, susceptibility is not uniform (11). The probability of highly virulent spores infecting less susceptible infection sites and the probability of less virulent spores infecting highly susceptible infection sites is smaller than the probability of average sites being infected by average spores. That is, virulence in the pathogen and susceptibility in the host follow a normal distribution. Therefore the response scale needs to be stretched at both ends to provide equal information for equal distance on the scale (19). The probability scale was employed for this purpose. The application of the log-probit grid was shown to hold for inoculum levels, using the data of Heald (18) on spore load of bunt in relation to

the percentage of smutted wheat plants, as well as for response of fungal spores to fungicide dosage (9). However, log-probit dosage-response curves become flat at high inoculum densities because multiple infections are ignored (11). Furthermore, this analysis can only straighten a symmetrical S-shaped curve.

The approaches described above have been used to straighten dosage-response curves so that the relation between disease and inoculum was linear. Pathologists interested in soil-borne inoculum have long attempted to define the events affecting the process of infection in soil. Since plants merely influence pathogens in soil by growing into proximity of pathogen propagules, infection is a matter of probability of contact of host and pathogen and is directly related to the number of spores that are in a position to be influenced. Therefore the geometric orientation of spores in soil should be examined.

Baker (2, 3) proposed that orientation of spores in soil could be as a lattice of trahedra, since the tetrahedron was the simplest possible three-dimensional figure conceivable. This hypothesis requires an ideal 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. In cultivated soils the actual situation may approach the ideal (3). The

tetrahedral surface consists of four equilateral triangles, and each apex is equidistant from the other three. If each apex represents a spore, the distance between spores, or length of an edge of the tetrahedron, is directly proportional to the volume enclosed within the tetraderon. The volume of a tetrahedron (V_t) is described by the formula:

$$V_t = 0.11785 \quad D^3 \qquad \text{(I)}$$

where (D) is the distance between apices, or spores. While tetrahedra do not completely "fill space," other geometric figures are less suitable (3).

In soil the influence of roots on propagules is one of two basic types: a rhizosphere or rhizoplane effect (2, 3). In the rhizosphere type, a cylinder of soil adjacent to, but not touching the root is affected; that is, the host root, hypocotyl, epicotyl, or cotyledon is surrounded by a cylindrical volume of soil under the host's influence. If pathogen propagules germinate and grow in the rhizosphere, Baker (3) stated that any additional inoculum in the soil volume influenced by the host should produce a directly proportional increase in disease. From equation (I) the number of points in a unit volume is inversely proportional to the cube of the distance between them:

$$I = \frac{k}{D^3} \qquad \text{(II)}$$

where (I) is inoculum density (spores/g), and (k) is a constant. The number of infections (S) as a result of rhizosphere influence is also inversely proportional to the cube of the distance between spores:

$$S = \frac{k}{D^3} \qquad \text{(III)}$$

The number of infections (S) can be expressed in terms of the number of spores/g as follows:

$$S = I \qquad \text{(IV)}$$

Thus the number of infections is directly proportional to the number of spores/g of soil, if the zone of influence is a rhizosphere (2, 3).

However, in the rhizoplane type, only propagules touching the host surface infect, and theoretically no volume of soil is influenced (3). The host root, hypocotyl, epicotyl, or cotyledon surface area is the infection court, and only pathogen propagules in direct contact could germinate and infect. Thus, when inoculum is added to soil, it is essential to determine what proportion would be in contact with the host surface. The number of points of a tetrahedral lattice touching a unit surface area is two dimensional and inversely proportional to the square of the distance between the points:

$$S = \frac{k}{D^2} \qquad \text{(V)}$$

The number of infections (S) can then be expressed in terms of (I). The 2/3 power of equation (II) is:

$$I^{2/3} = \frac{k^{2/3}}{D^2} \qquad \text{(VI)}$$

which can be rewirtten as:

$$D^2 = \frac{k^{2/3}}{I^{2/3}} \qquad \text{(VII)}$$

Equation (VII) can be substituted into (V) as follows:

$$S = \frac{k}{k^{2/3} \div I^{2/3}} = kI^{2/3} \qquad \text{(VIII)}$$

The slope of the curve resulting when Log S is plotted against Log I can be determined from equation (VIII) when it is written in log form:

$$\log S = 2/3 \log I + \log k \qquad \text{(IX)}$$

The slope of log S plotted against log I is 2/3.

Thus, Baker (3) hypothesized that the slope of the curve resulting when log number of infections is plotted against log inoculum density should be 2/3 for a rhizoplane influence and one for a rhizosphere influence. Very little data are available to test the hypothesis.

Exudation of soluble organic substances is a phenomenon common to all higher plants (38). The importance of exudates in influencing soil microorganisms, both pathogens

and saprophytes, has been demonstrated for many plants. Major sources of exudates are: the hilum-micropylar area of intact germinating seeds (29, 36), the root, the region of developing root hairs, and sites where breaks appear in the eipdermis (29, 32, 39). The types of substances found in exudates are materials involved in cellular metabolism. Soil-borne plant pathogenic fungi typically invade only living tissue and do not grow through nonsterile soil. They generally exist in soil apart from the host as resting spores or sclerotia. Infection is promoted by the growth of the host into the micro-environment of the dormant spore. Thus as the plant invades the fungus habitat, it alters the micro-site nutritionally to favor spore germination and growth. There is good evidence that many propagules in soil require a carbon and nitrogen source for germination (7), and this stimulus is provided by the exudates from host plants (5, 8, 20, 28, 34). Spore germination in the rhizosphere or rhizoplane is not selective for pathogens (37, 44), but it appears to be a general response of many microorganisms to nutrients exuded from plant parts (40, 25). If, however, exudates from a suitable host induce spore germination of a pathogen, the possibility of a successful infection exists. Kinds and amounts of exudates profoundly influenced amount of infection resulting from inoculum by influencing germination of pathogen propagules (20).

MATERIALS AND METHODS

Source of inoculum

Onion smut teliospores were collected in June of each year from naturally infected field-grown onion plants with at least one visible sorus per plant. The infected areas were removed and allowed to dry at room temperature. The dry leaf sections were fragmented in a Waring blender and sieved to remove most of the plant material. Material passed through a 200 mesh sieve contained approximately 2 x 10^8 teliospores/g. Microscopic examination indicated a high percentage of smut spores with only an occasional spore of Helminthosporium or Alternaria. This material was stored in a stoppered vial at 2°C, and used as inoculum for soil infestation.

Field-collected spores were used as inoculum because Urocystis does not sporulate in culture. It was impossible to assay the viability of inoculum directly because contaminating microorganisms quickly overrun the slowly germinating smut spores (1). The viability of inoculum was assayed indirectly by determining the amount of inoculum required to give 50±5% infection under optimum conditions with freshly collected inoculum. If inoculum later fell below

this level of disease incidence more than 10%, the experiment was repeated with a newer inoculum collection.

Soil types

Since virtually all Michigan onions are grown on organic soils, it was first thought that this type of soil would be used for soil infestation experiments. However organic soils are difficult to handle because of their physical properties. A comparison of disease response to several inoculum levels was made using organic soil, a mixture of organic soil and fine sand (3:1 v/v), and the Michigan State University greenhouse mixture of Conover loam, sand, and peat (1:1:1 v/v). The results indicated that no difference in disease incidence existed between soils, and the pasteurized greenhouse mixture was used in all further studies.

Infestation of soil

Soil was infested at varying inoculum densities by mixing a known number of teliospores into a known weight of soil corrected to an oven dry basis. Soil and spores were mixed for 20 to 30 minutes in a concrete mixer. Infested soil was diluted by mixing with uninfested soil to obtain lower inoculum densities. Later, estimates of soil bulk density were made, which allowed calculation of the mean distance between spores (2, 3).

Growing plants and assessing disease

Surface-disinfested Allium cepa L. 'Downing Yellow Globe' seeds were planted either in eight inch plastic pots or metal flats (64 x 39 x 10 cm). To insure uniform planting depth in pots, seeds were pushed into soil with a small rod to a selected depth. Seeds planted in metal flats were placed on the smooth surface of a compact soil. They were then carefully covered with more soil, which was smoothed and compacted until the seeds were at the desired planting depth. The pots or flats were either placed in water bath controlled temperature tanks, growth chambers or on greenhouse benches, depending on the experiment, and fertilized weekly. The greenhouse was cooled by an evaporative cooler, and experiments have not been reported in which summer temperatures (above 25°C) may have interfered with the infection process. Natural light was supplemented with 16 hours of artificial light from four 40 watt fluorescent bulbs (1/2 warm and 1/2 cool white) suspended 18 inches above the bench for each 16 square feet of space. A minimum of 100 seedlings in each of four replicate pots were observed for smut infection. The criterion used for determining infection was visual perception of sori. All experiments were repeated at least twice with similar results. Variability in any given inoculum density experiment did not exceed ±5% diseased plants.

Planting depth and inoculum placement

Initial planting depth studies were carried out in eight inch plastic pots with an internal diameter of 19 cm. The pot was filled with a four inch gravel base and infested soil was added, leveled, and compacted. Seeds were placed on the smooth soil surface and covered with a measured volume of infested soil calculated to position the seeds 10 mm below the soil surface. Seeds could be planted at selected depths more rapidly and uniformly using the volume method than trying to prepare furrows or push seeds into soil individually. Later, to study the effect of inoculum placed in zones, another method of planting became necessary. The rectangular metal flats were used and a straight edge which could be adjusted to selected depths in the flat was used to level the soil. Soil was built up and compacted to a desired depth. Seeds were placed on the smooth, level soil surface and carefully covered with more soil. The straight edge was adjusted 5 mm above the seed placement depth and the newly added soil was leveled, compacted, and built up to the selected height. This process was repeated using infested soil or uninfested soil. This method allowed for precise control of planting depth and soil could be infested in layers by increments of 5 mm.

Time of exposure to inoculum

Length of exposure to inoculum may be important in determining disease response. Longer exposure of susceptible host tissue to inoculum should result in increased amounts of disease (3). The effect of planting depth and temperature on the length of time plants were exposed to inoculum was measured. Counts were made daily on cotyledon emergence and appearance of the first true leaf.

Exudate collection

Onion seeds were surface disinfested by immersion in a 10% Clorox solution for 10 minutes. The seeds were rinsed with sterile distilled water and treated in one of two ways.

(i) Four g of seeds were placed in a Buchner funnel and covered with a glass fiber filter paper. Distilled water slowly dripping onto the filter paper was collected either by suction through a 0.45 μ Millipore filter, or in a sufficient volume of ethanol to insure a final concentration of not less than 50% ethanol after 24 hours. Ethanol or filtration was used to prevent utilization of exudates by contaminating organisms.

(ii) Seeds were placed on a medium containing 5 g/liter each of yeast extract-malt extract-peptone (YEMEP medium) and assayed for sterility. Twenty five to 35 seeds and seedlings that remained sterile after four days were placed onto petri dishes 7 cm deep containing a base layer

of acid-washed, sterile fine sand and covered with a 1/2 inch layer of acid washed, sterile coarse sand. The dishes were specially modified with an inlet in the cover and an outlet in the side near the base (22). At 24 hour intervals, 100 ml of sterile distilled water was applied to the surface of the sand and collected in a sterile flask. All solutions containing exudates were tested for sterility on YEMEP medium. The exudate-containing solutions were reduced from 100 to 10 ml under vacuum in a rotary evaporator at 40°C and frozen until analyzed.

Exudate analysis

Conductivity of the exudate solutions was determined with a conductivity bridge. Results are expressed in μ mhos/seed and are corrected for background conductance.

Total carbohydrates were determined using a modification of Dreywood's anthrone reagent (27). The anthrone reagent was prepared by dissolving 0.2 g anthrone in 100 ml of 95% H_2 SO_4 (one liter concentrated sulfuric acid and 50 ml of water). One ml of the solution to be assayed was mixed with 9 ml anthrone reagent and placed in a boiling water bath for ten minutes. The solutions were cooled and the optical density at 600 mμ was determined with a Bausch and Lomb Spectronic 20 spectrophotometer. A standard curve was made using glucose (20-100 μg/ml).

Total amino acids were determined by the method of Moore and Stein (25). Ninhydrin reagent was made by dissolving 0.2 g ninhydrin and 0.03 g hydrindantin in 7.5 ml ethylene glycol monoethyl ether and 2.5 ml 4 N sodium acetate: acetic acid buffer (pH 5.5). The buffer was made by adding 54.4 g sodium acetate · $3H_20$ to 40.0 ml H_20, mixing in 10 ml glacial acetic acid and diluting to 100 ml with water. One ml of the sample to be assayed was mixed with one ml ninhydrin reagent in a boiling water bath for 15 minutes. The solution was diluted with 8 ml 50% ethyl alcohol, and optical density determined at 570 mμ in a spectrophotometer. A standard curve was made using glycine (4-32 μg/ml).

Spore germination

Urocystis colchici teliospores for germination studies were collected from 6-12 week-old onion seedlings grown in greenhouse soil infested with field-collected teliospores (23). Plants with sori in the leaves were immersed for ten minutes in 10% Clorox (containing one drop of Tween 20 surfactant/liter) to disinfest the leaf surface. The plants were rinsed three times in sterile distilled water and allowed to dry in a transfer hood for 10-15 minutes. Intact leaves containing sori were slit with a sterile spatula, the spores scraped from the lacunae of the leaves and suspended in sterile distilled water at a

concentration of 10^5 - 10^6 spores/ml. Single-spore units for germination studies were obtained by breaking spore clumps with high speed agitation in a sterile Sorval Omni-mixer. The spore suspensions were stored at 20-22°C for 24 hours and diluted to 2 x 10^3 spores/ml. One quarter ml of the suspension was spread evenly over the surface of each of four replicate agar plates with a sterile glass rod. Spores plated at this concentration were sufficiently separated so that colonies developing from germinating spores did not overgrow and conceal ungerminated spores.

All agar media used in spore germination studies contained (g/liter): KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4$, 0.5; $FeSO_4$, 0.01; and bacteriological agar, 15.0. Onion seeds, broken with high speed agitation in a Sorval Omni-mixer, were used to amend agar, and intact seeds, autoclaved or surface disinfested with 10% Clorox for 10 minutes, were placed under solidified water agar to determine the effect on spore germination. All media were adjusted to pH 6.2 and amount of germination was determined after eight days at 22°C. One hundred spores were observed for germination on each of four replicate plates. Spores were considered germinated if germ tubes were as long as spore diameter. Spore germination level and rate were compared to the level and rate obtained on malt extract (10 g/liter)-phytone (5 g/liter) medium reported to be optimum for teliospore germination (23). All experiments were repeated at least twice.

RESULTS

Effect of inoculum density

The effects of inoculum densities (125 to 8,000 spores/g of soil) on disease incidence when measured by percent diseased plants (X), and on disease severity by the calculated number of infections (S), are presented (Table 1). Also presented are the calculated mean distances between spores as inoculum density is increased, calculated assuming that spores in soil are distributed uniformly in a three dimensional medium (2, 3). The number of spores required to establish a selected distance between spores in a given volume of soil, assuming spores represent the apices of tetrahedra, is calculated by the formula:

$$N = \frac{V_s}{(0.11785\ D^3)} \qquad (X)$$

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 desired.

Percent diseased plants and calculated number of infections increased with increasing inoculum density and with decreasing distance between spores. Amount of disease

Table 1.--The effects of inoculum density on percent disease and number of infections at three planting depths

Number spores/g	D(mm)	Planting depth					
		5 mm		10 mm		15 mm	
		X	S	X	S	X	S
125	4.1	1	1	22	25	34	42
250	3.2	15	16	29	34	46	62
500	2.6	20	22	36	45	63	99
1,000	2.0	35	43	56	82	82	172
2,000	1.7	49	67	62	97	89	221
4,000	1.3	44	58	67	111	91	241
8,000	1.0	56	82	77	148	92	253

D = distance between spores in mm

X = percent infection

S = number of infections per plant

at any inoculum density also increased with deeper planting. These data were analyzed several ways in Figures 1-5.

Percent diseased plants typically increased very rapidly at first and then the curve flattened as inoculum density was further increased (Figure 1). An arithmetic plot of this type produces a curve that is very difficult to analyze, since most of the increase in disease response occurs in a small inoculum range, and additional inoculum appears less effective in producing a unit disease response. There is not a simple relation between the number of infections and the percent diseased plants because a plant can become infected a second or third time and still be counted as a single diseased plant. The data were therefore plotted as the calculated number of infections versus the number of spores/g of soil (Figure 2). In contrast with Figure 1 where multiple infections are ignored, Figure 2 corrects for the increase in number of infections as inoculum density increases. These curves, while more nearly linear than the curves in Figure 1, were difficult to analyze because the response to increasing inoculum was not constant.

A logarithmic-probability plot (Figure 3) usually gives a linear relation between the percentage of disease units on a probability scale and the amount of inoculum on a logarithmic scale (11, 19). This hypothesis assumes that susceptible plants are normally distributed in accordance with the logarithm of the inoculum level. Therefore the

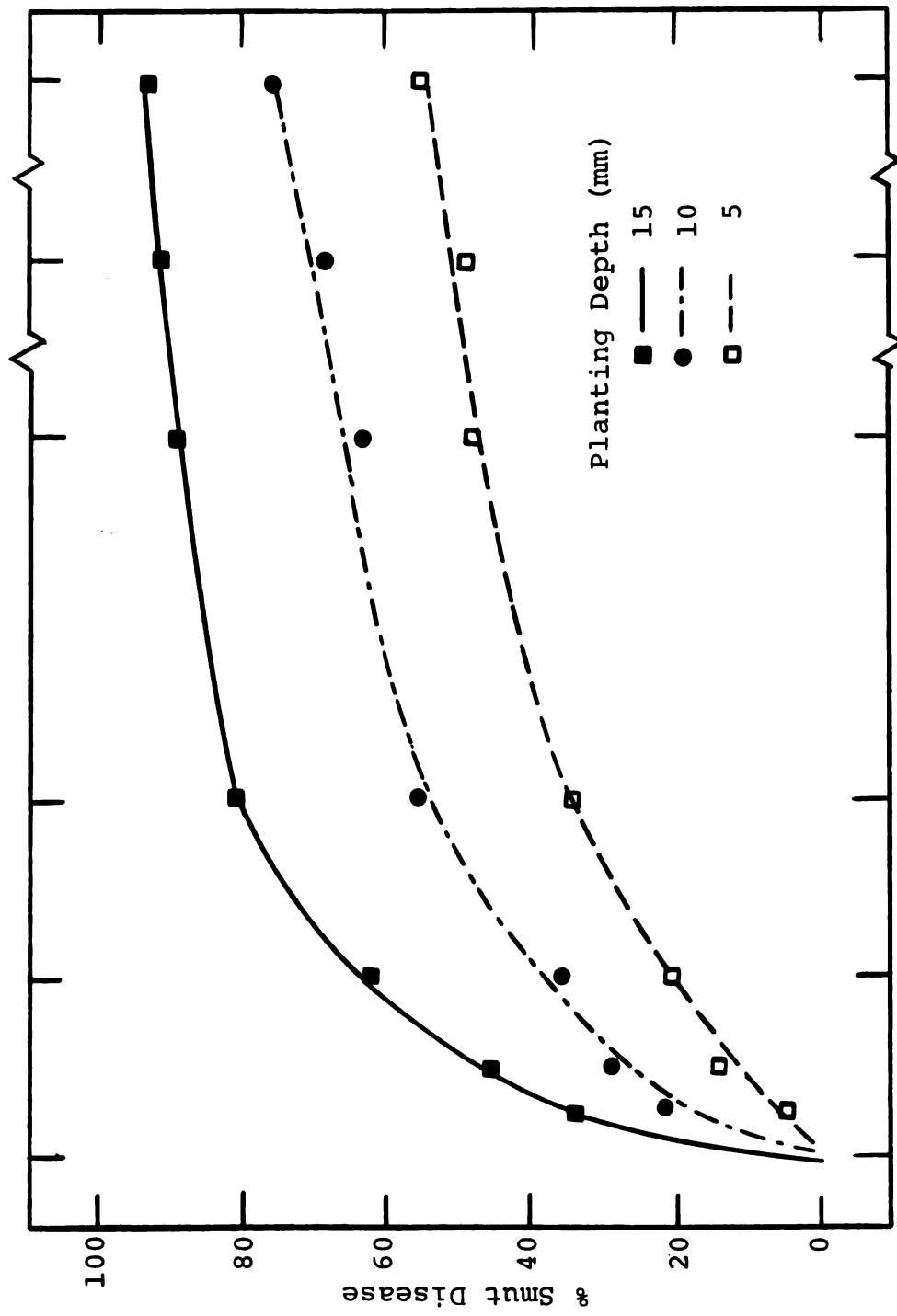

Figure 1. Effect of inoculum density on percent smut disease.

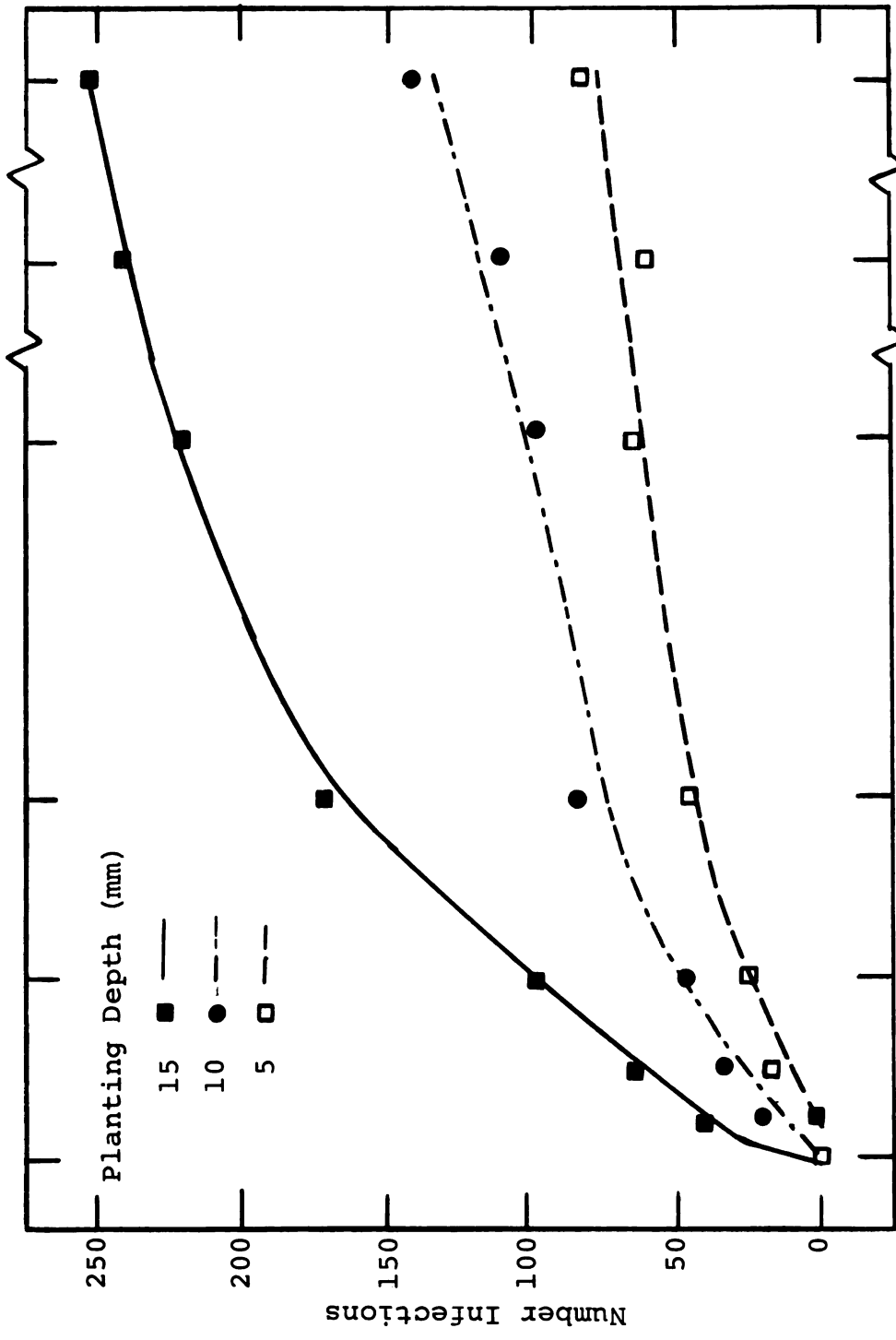

Figure 2. Effect of inoculum density on the number of infections.

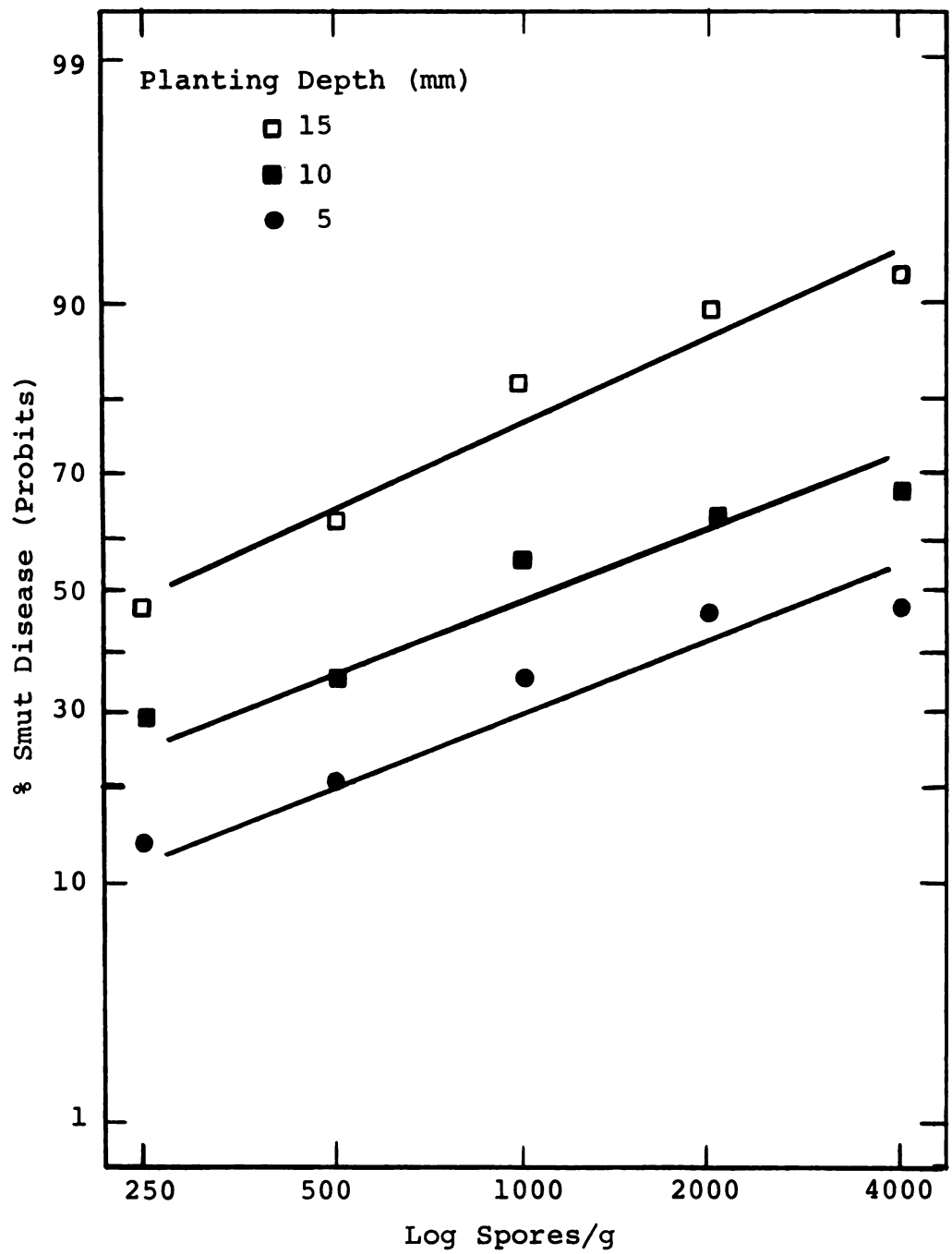

Figure 3. The relation between percent smut disease (probability scale) and the number of spores/g of soil (logarithmic scale).

ordinate is symmetrical about the ED_{50}, and either percentage healthy or percentage diseased plants gives a linear relation. In these experiments the slope was approximately 0.5 for the three curves which represent the three planting depths. However at the higher inoculum densities the slope of the curve flattened.

The data from Table 1 are plotted as log number of infections versus log number of spores/g of soil (Figure 4). The relation between these two variables on a log-log scale was only linear over the inoculum range of 250-2,000 spores/g of soil, where the slope ranged from 0.64 to 0.72, and the correlation coefficient ranged between 0.90 and 0.95. However, above 2,000 spores/g of soil the slope flattened significantly. Thus the actual relation between the log number of spores/g of soil and log number of infections was very close to the predicted linear relation between inoculum density and disease severity up to 2,000 spores/g of soil (2, 3).

Disease incidence in response to increasing inoculum densities, when measured as the distance between spores, was linear (Figure 5). The correlation coefficient ranged between 0.90 and 0.95 for the entire inoculum range tested. A decrease in the distance between spores, which is another way of expressing an increase in the number of spores/g of soil, resulted in a linear increase in disease incidence. That is, a unit change in the distance between propagules

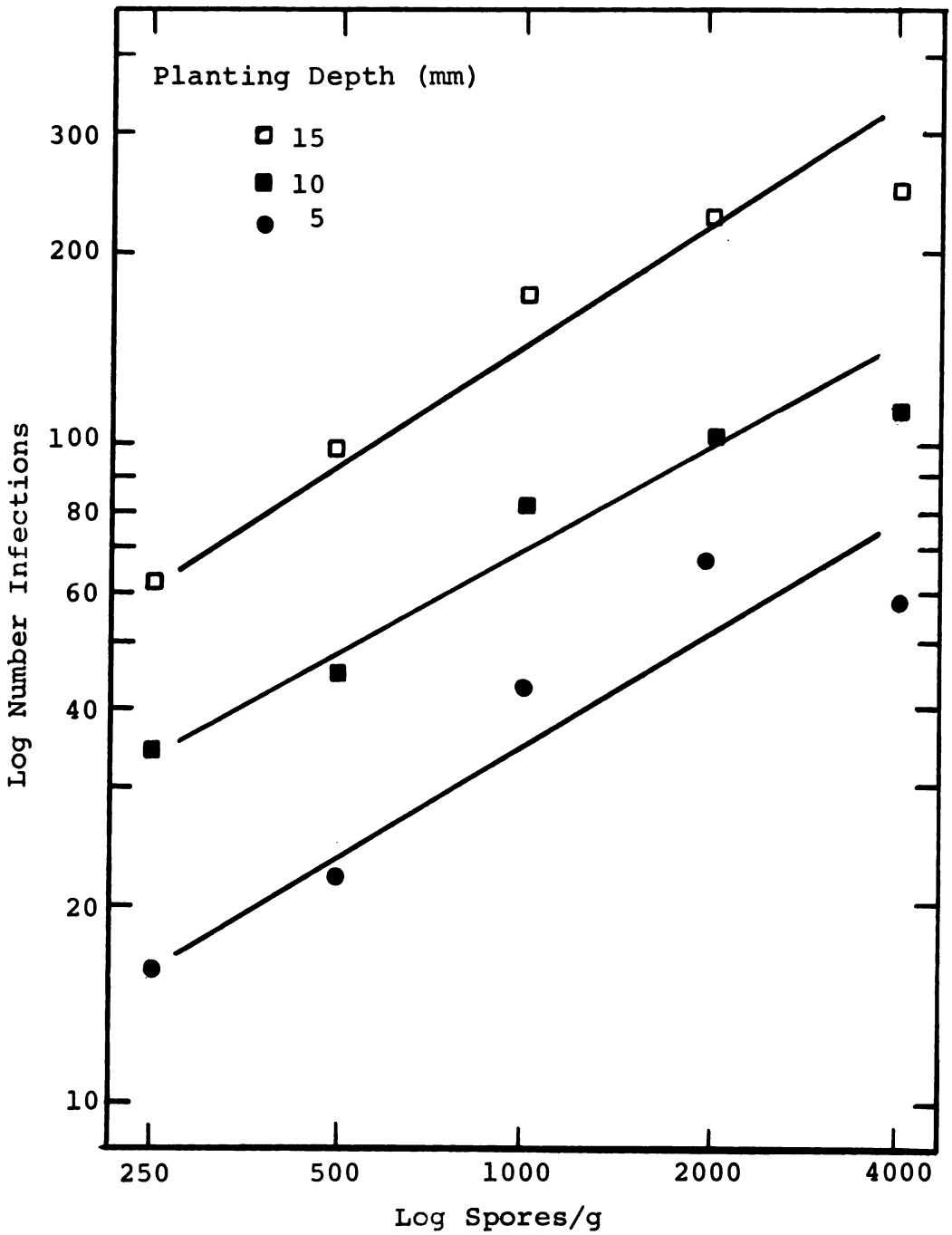

Figure 4. The relation between the log number of infections and the log number of spores/g of soil.

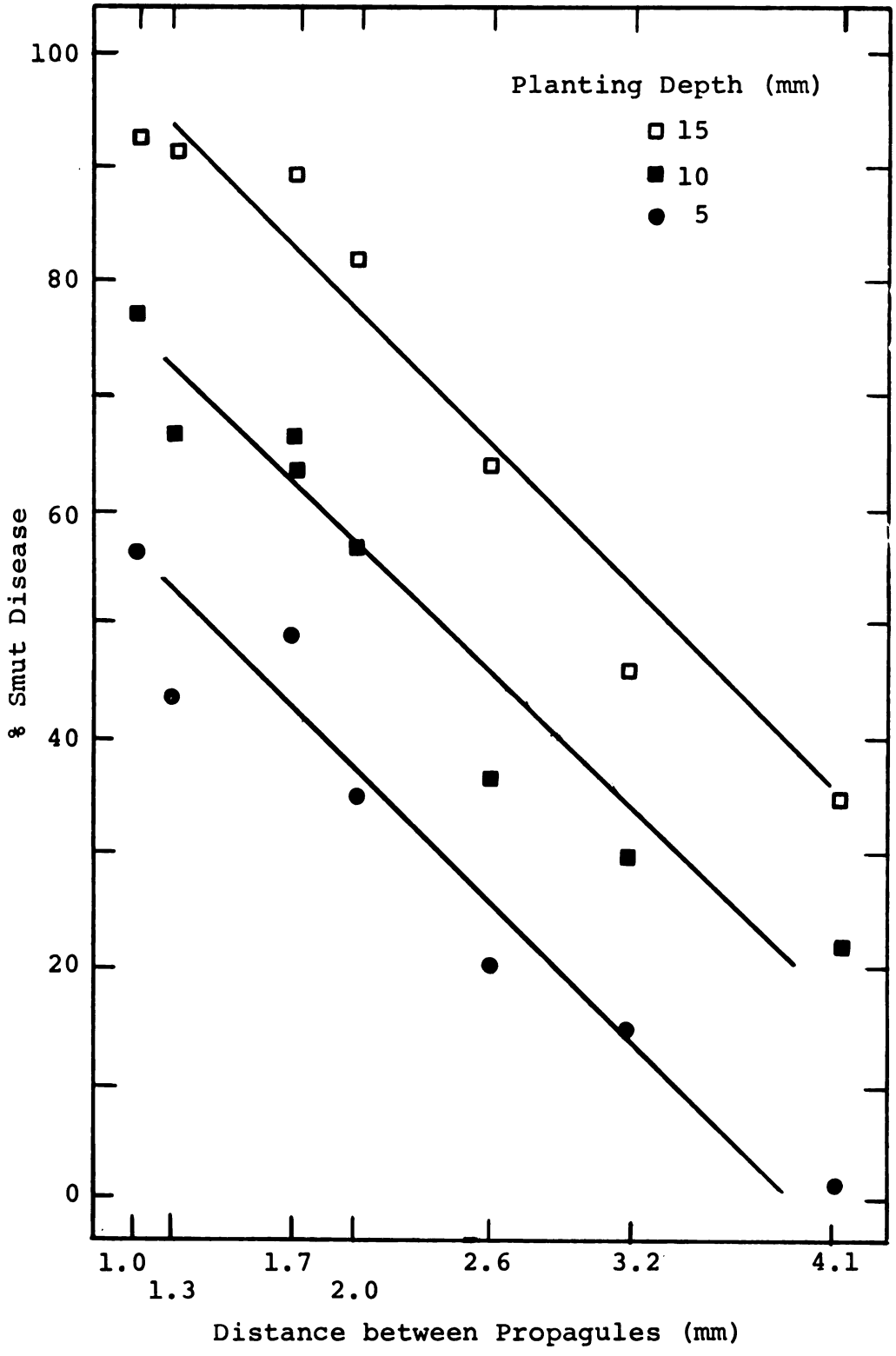

Figure 5. The relation between percent smut disease and the calculated distance between spores.

produced a unit change in percent diseased plants over all inoculum densities tested. Inoculum density measurements have been normally given as the number of propagules/g of soil, or conidia/ml (2, 11); however, a more meaningful way of expressing this measurement in soil may be the distance between propagules.

Effect of temperature

Eight inch plastic pots in water bath controlled temperature tanks at 12, 16, 20, 24, and 28°C in an evaporatively cooled greenhouse were used to measure the effect of temperature on the relation of inoculum density to disease incidence. Disease incidence rose with increasing temperature, reaching a maximum at 20°C, and decreased at 24 and 28°C (Figure 6). At 12°C the amount of infection after five weeks was near zero, however when plants were removed from 12°C and incubated for two weeks at 20°C, the disease incidence rose approximately 10%.

The effect of temperature on percent diseased plants was analogous to the effect of temperature on spore germination *in vitro* (23), although the optimum temperature for spore germination *in vitro* was somewhat higher.

There was a significant difference in the disease response at 16 and 20°C as inoculum was increased from 1,000 to 2,000 spores/g of soil. Two thousand spores/g of soil gave 49% infection at 16°C and 53% at 20°C, while

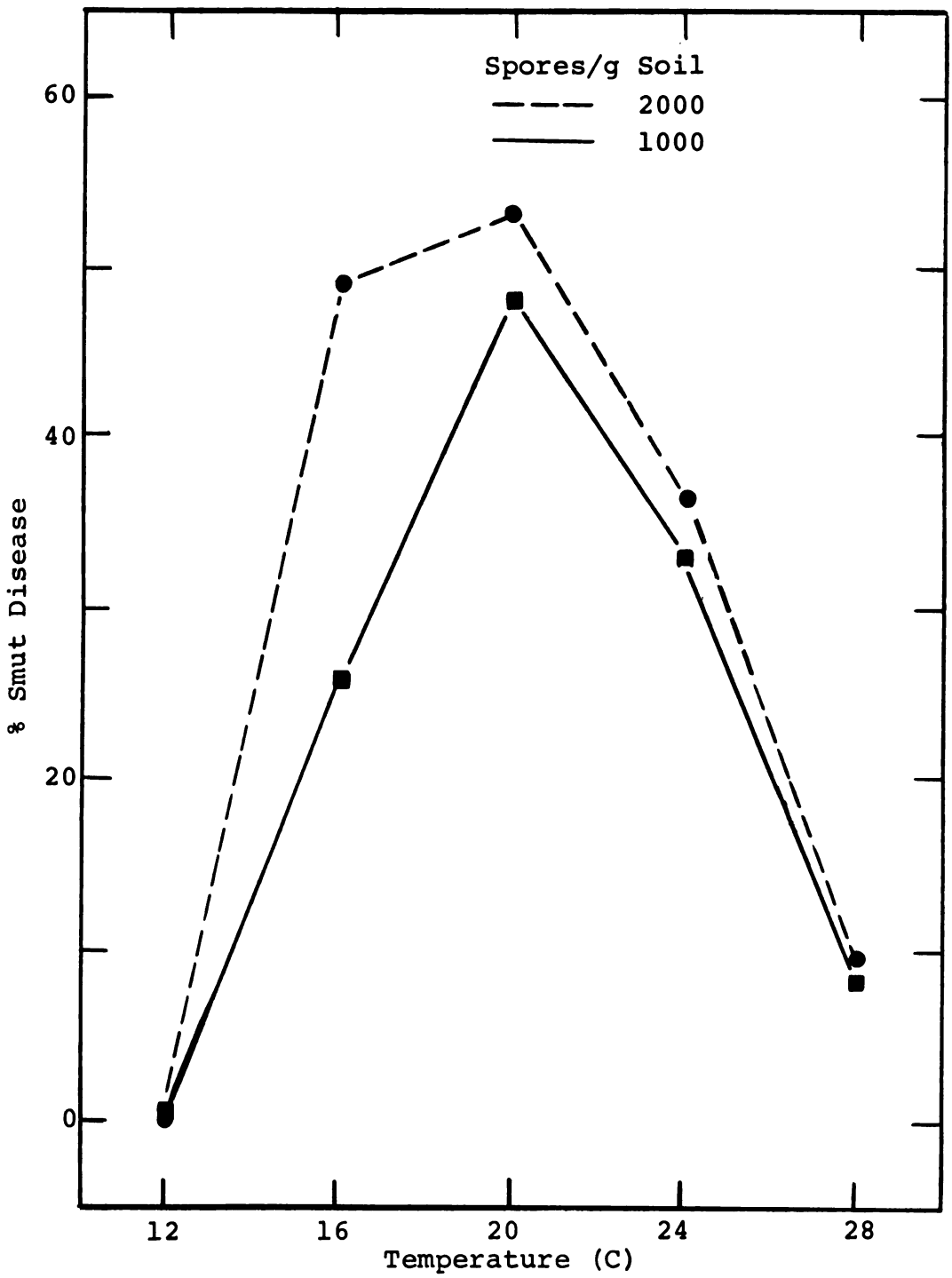

Figure 6. The effect of temperature on disease incidence at two inoculum levels.

1,000 spores/g of soil gave 27% infection at 16°C and 48% at 20°C. There was a much greater decrease in percent diseased plants at 16°C at the lower inoculum density than at the higher inoculum density.

Effect of planting depth

Onion seed germination is characterized by cotyledon elongation, most of which occurs underground. Most, if not all, smut infection occurs in the cotyledon (1). It was assumed that increased planting depth directly increased the amount of cotyledon elongation in soil, and thereby increased the amount of susceptible host tissue exposed to inoculum. Deeper planting increased time of exposure to inoculum slightly (Table 3), but the effect was small and could not be separated from increased exposure of host tissue. The amount of host tissue surface area exposed at the 5 mm planting depth was taken as one unit, the amount exposed at the 10 mm planting depth as two units, and the amount exposed at 15 mm planting depth as three units. Percentage diseased plants at four inoculum densities and three planting depths was determined (Table 1), and plotted as the relative amount of susceptible host surface area exposed to inoculum (Figure 7). Disease incidence was directly proportional to the amount of susceptible host tissue exposed to inoculum at all inoculum levels tested. Percentage diseased plants, for example, at 250 spores/g

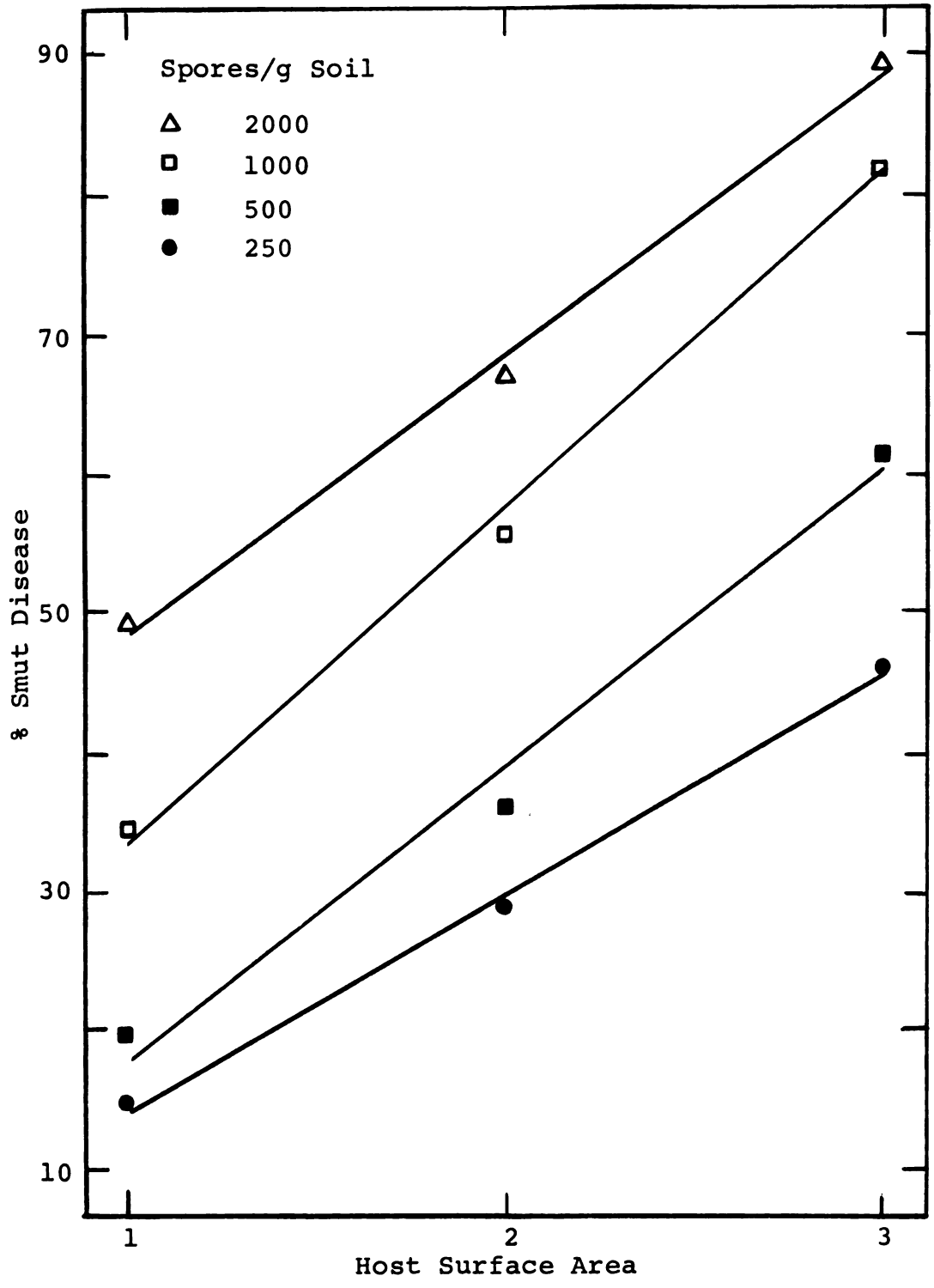

Figure 7. The effect of relative host surface area exposed to four inoculum densities on percent disease.

increased from 15 at one unit of host surface area to 29% at two units, and to 46% at three units. Percent diseased plants at 2,000 spores/g increased from 49 at one unit, to 67% at two units, and to 89% at three units of host surface area. At all inoculum densities, the average increase in diseased plants with increased exposure of host tissue due to deeper planting was approximately 20%.

The calculated number of infections resulting from seeds planted 10 mm deep was approximately twice that of seeds planted at 5 mm (Table 1). However, increasing the planting depth from 10 to 15 mm again doubled the number of infections, which was twice the increase expected from extrapolation of the difference between 5 and 10 mm planting depth. For example, with 500 spores/g, 48 infections occurred at 10 mm planting depth and 94 infections at the 15 mm planting depth, a doubling of the number of infections. At 2,000 spores/g, 100 infections occurred at 10 mm compared to 210 at the 15 mm planting depth. The number of infections resulting from seeds planted 15 mm deep was thus approximately twice that of seeds planted 10 mm deep. The doubling in disease severity at the 10 mm planting depth over the 5 mm depth was expected because twice as much host tissue was exposed to inoculum. However, the increase in infections at the 15 mm depth cannot be explained by increased surface area alone, since surface area was not doubled.

Effect of inoculum placement

The effect of planting depth on disease incidence raised the question of whether all parts of the cotyledon are equally susceptible, and whether the response of the cotyledon is the same or different when exposed to a given amount of inoculum at different planting depths. That is, does the additional expenditure of energy required for emergence from deeper planting result in a higher degree of susceptibility and more infection from a constant amount of inoculum? It was known from preliminary experiments that inoculum placed below the seed was not effective in producing disease, even if the seed was placed just above the inoculum.

Seeds were planted 5, 10, and 15 mm below the soil surface in these experiments (Table 2). Infested soil (1,000 spores/g) was placed in the following zones: (a) the top 5 mm, (b) 5 to 10 mm below the surface, (c) 10 to 15 mm below the surface, (d) the upper 10 mm, (e) from 5 to 15 mm below the soil surface, and (f) the entire 15 mm. More disease occurred from inoculum in the 5 mm zone immediately above the seed than occurred from a 5 mm inoculum zone separated from the seed by 5 mm of uninfested soil. Sixty-seven percent diseased plants occurred when spores were confined to the first 5 mm above seeds planted 15 mm below the soil surface. Eighty percent diseased plants occurred when spores were present in all three 5 mm zones

Table 2.--The effects of planting depth and infestation of soil by zones on percent disease

Seed Placement depth mm	Percent Smut Disease (Location of infested soil[a] zone mm)					
	0 - 5	5 - 10	10 - 15	0 - 10	5 - 15	0 - 15
5	9.0	3.1	0.5	19.3	4.9	20.1
10	16.9	25.2	3.4	47.8	22.3	49.6
15	18.2	47.5	67.0	61.4	70.9	79.5

[a]Soil infested at 1,000 spores/g

of soil covering the seeds. Also, when seeds were planted 10 mm deep and infested soil was used in all three 5 mm zones, 50% disease occurred, compared with 25% when inoculum was confined to the 5 mm immediately above the seeds.

The amount of disease from inoculum present in the zone 5 to 10 mm below the soil surface was greater when seeds were planted 15 mm deep than when seeds were planted 10 mm deep. Also, more infection occurred from inoculum present in the top two 5 mm zones of soil when seeds were planted 15 mm deep than when seeds were planted 10 mm deep. It appeared that more disease occurred from inoculum in soil in close proximity to the seed than occurred from inoculum in soil above this region.

Time of exposure to inoculum

In the temperature and planting depth studies the time of exposure of susceptible host tissue to inoculum also varied because lower temperatures and deeper planting depths delayed seedling emergence. The length of exposure is an important variable in the disease response (3). Since it was not possible to separate length of exposure to inoculum from direct effects of temperature or depth, measurements of time required for emergence of the cotyledon and appearance of the first true leaf are reported (Table 3). Cotyledons emerged from soil in seven days when planted 5 mm below the soil surface. Cotyledon emergence was delayed

Table 3.--Number of days after planting required for 50% emergence of cotyledons and 50% appearance of true leaves

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

by one day at the 10 mm planting depth and by two days at 15 mm planting depth. In addition deeper planting delayed the appearance of the first true leaves by one day.

The effect of temperature on time of cotyledon and leaf emergence was investigated (Table 3). At 20°C eight days were required for cotyledon emergence and 19 days for appearance of the first true leaves. Cotyledon emergence was delayed by two days and true leaf appearance by four days, at 12°C. Cotyledon emergence was not delayed at 16°C but true leaf appearance required two more days than at 20°C. At 28 and 24°C, cotyledon emergence occurred four and two days earlier and the first true leaves appeared four and three days earlier than at 20°C.

Onion seedling exudation

Since spore germination in soil is thought to occur in response to nutrients (25, 38), experiments were designed to determine the nutrient exudation pattern of developing onion seeds. Four g onion seeds in a Buchner funnel covered by a glass fiber filter paper were washed with 100 ml distilled water daily. The conductivity of the solution containing the exudate was determined with an electrode and a conductivity bridge. Conductivity was used as an indication of the amount of exudation occurring. Most of the electrolytes were lost in two days (Figure 8). After the second day a very small amount of electrolytes were present in the solution containing exudate.

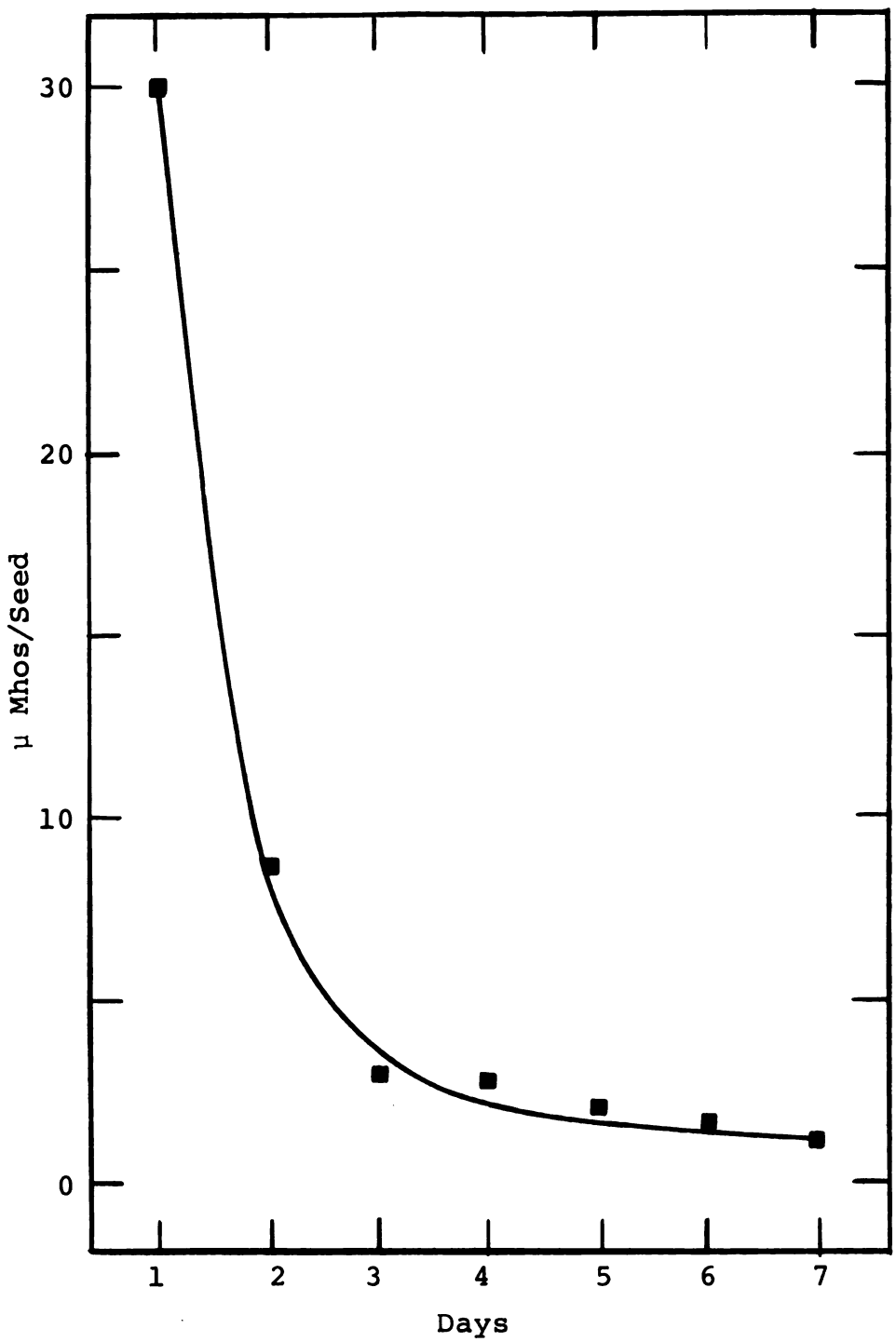

Figure 8. Conductivity of exudates of onion seeds germinating under moist glass fiber filter paper and leached daily.

Most of the ninhydrin- and anthrone-positive materials were exuded in the first two days of incubation, similar to the conductivity measurements (Figure 9). Seeds were treated with Clorox in an attempt to reduce bacterial contamination; however, after one day the number of bacteria present in the exudate from Clorox-treated seeds was not significantly lower than that in untreated seeds. Ninety percent of the untreated seeds germinated in 48 hours, but Clorox-treated seeds required 72 hours for the same level of germination. After two days untreated seeds exuded 83% of the total ninhydrin- and anthrone-positive materials exuded during the first four days. After two days Clorox-treated seeds exuded 60% of the total ninhydrin- and anthrone-positive materials exuded during the first four days. In either treated or untreated seeds after three days 92-95% of the total ninhydrin- and anthrone-positive material exuded in the first four days was recorded. Apparently more ninhydrin- and anthrone-positive material was exuded from the seeds during the early germination stage. The amounts measured may be lower than the actual amounts exuded, because some material may have been metabolized by bacteria before collection.

Since germination and growth of the fungus (12, 23, 47) and host (46, 47) were affected by temperature, this study was done at 12, 16, 20, 24, and 28°C. Surface-disinfested onion seeds were aseptically removed from nutrient

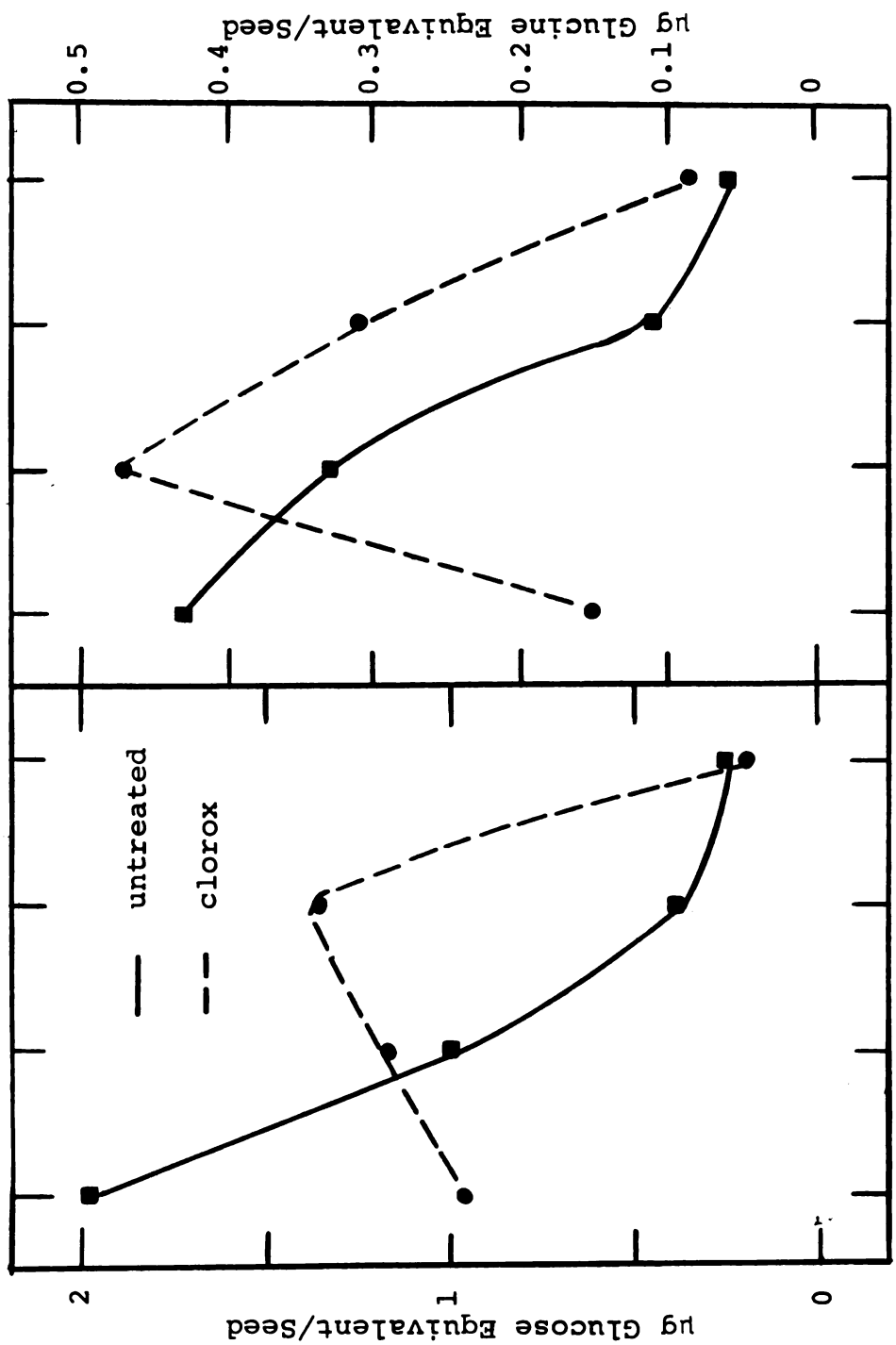

Figure 9. Carbohydrates and amino acids exuded by onion seeds germinating under moist glass fiber filter paper and washed daily.

agar after four days, placed in a sterile container, and covered with sterile sand. These seeds were allowed to continue germination, and ninhydrin and anthrone determinations were made daily from the sterile exudate. The process of surface disinfestation and assay for uncontaminated seeds required a minimum of four days. Thus all seeds selected for this study had begun germination.

The amounts of anthrone- and ninhydrin-positive materials in exudates from seedlings grown at temperatures of 16, 20, and 24°C after one day were approximately 4.8 and 2.2 µg/seed respectively at all three temperatures (Figures 10 and 11). These amounts dropped over the next two days to 1.0 and 0.9 µg/seed respectively on the fourth day. The amounts exuded from plants grown at 12°C were lower, and at 28°C a significantly larger amount was exuded for the first five days. The amounts exuded after the sixth day at any temperature were less than 1 µg/seed. Apparently exudation was affected slightly as temperature increased, but at 28°C a significantly larger amount of nutrient was exuded.

Spore germination

Spore germination in soil is thought to occur in response to nutrients lost by host plants (25, 38). Teliospores which floated on the surface of water containing 10-20 germinating onion seeds all germinated; however, the

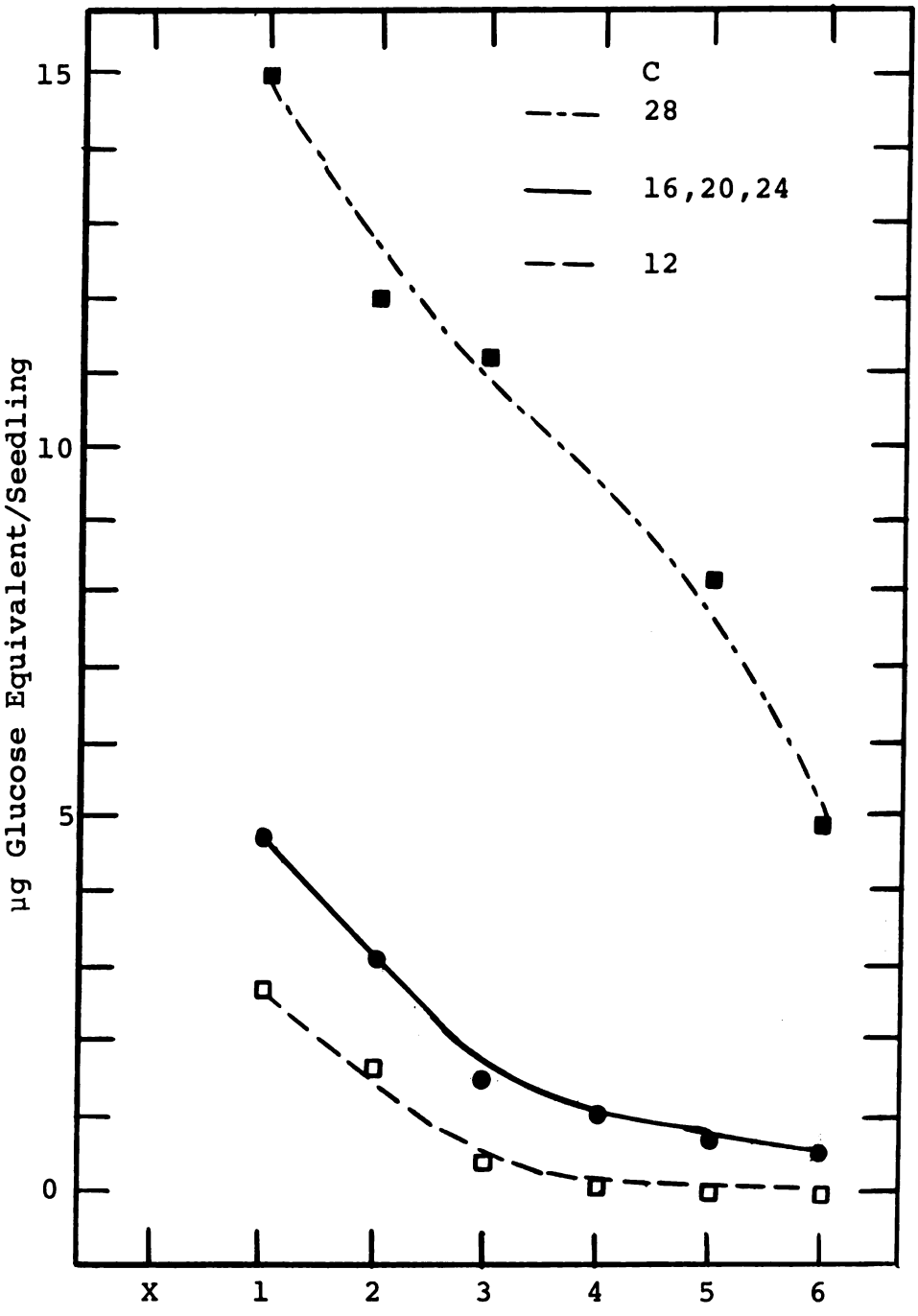

Figure 10. Carbohydrates exuded by uncontaminated onion seedlings after seeds were incubated four days (X) on agar and then placed in sterile chambers and washed daily.

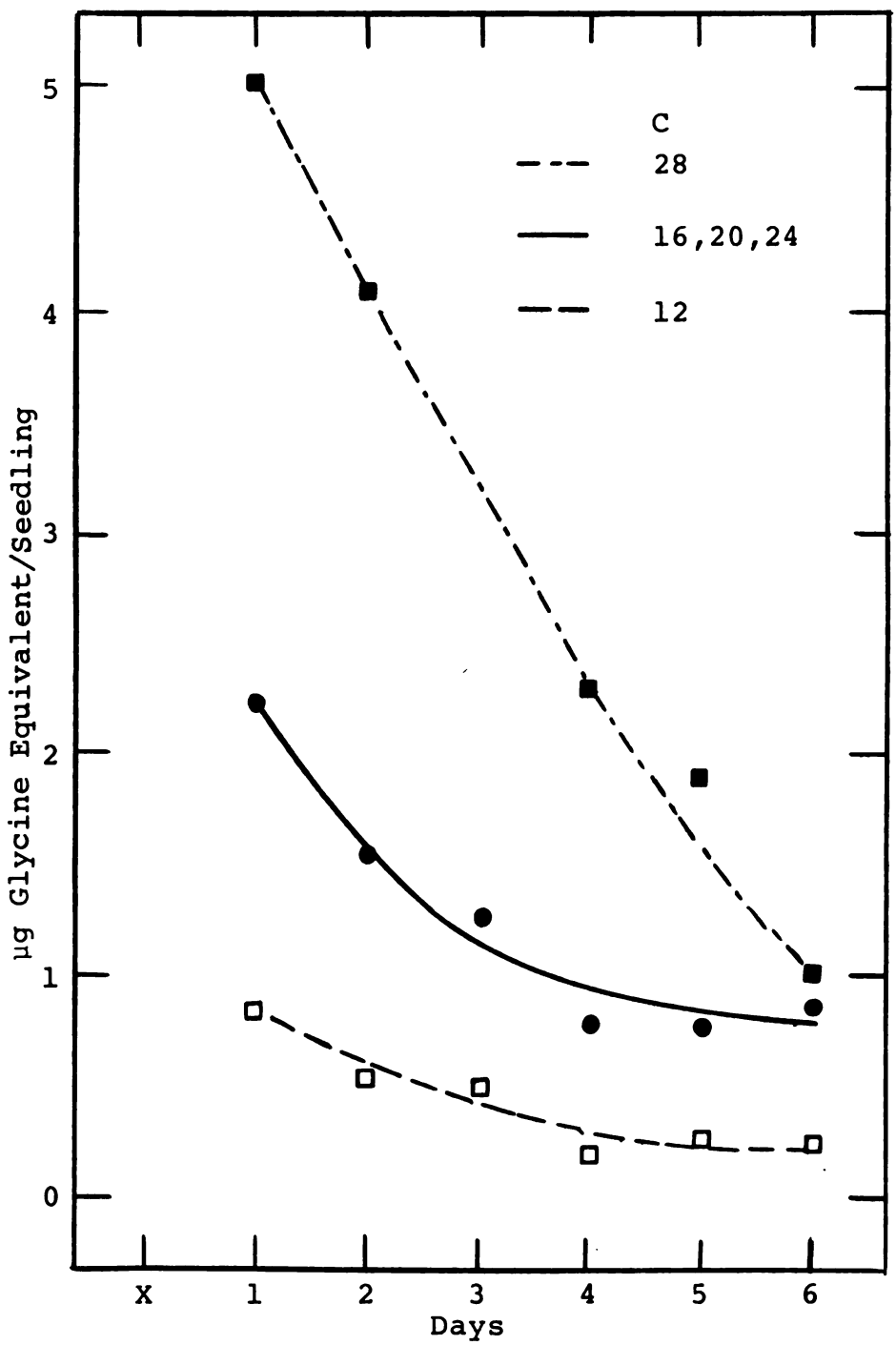

Figure 11. Amino acids exuded by uncontaminated onion seedlings after seeds were incubated four days (X) on agar and then placed in sterile chambers and washed daily.

majority of the spores, which sank below the surface, failed to germinate. Later studies indicated that spores failed to germinate when an agar surface contained a continuous water film. This confirmed a requirement for oxygen for spore germination, as reported by Anderson (1).

Since the seed is the only nutrient source in the early development of the onion plant, it may also be the major source of nutrients for teliospore germination. Dormant onion seeds were broken and mixed in agar containing basal salts. After eight days teliospore germination of 48, 56, and 65% occurred on agar containing 2.5, 5.0, and 8.0 g/liter of ground onion seed respectively (Table 4). Teliospore germination on malt extract-phytone agar (MEPA) control was 78%. Teliospores on water agar placed over intact, ungerminated seeds supported 58% germination. However, if seeds were allowed to germinate for 48 hours, rinsed, and then placed under water agar only 29% germination resulted. Teliospores germinated on water agar placed on top of MEPA as well as when placed directly on MEPA, presumably because nutrients diffused into the water agar (21).

Teliospore germination on agar containing exudate from onion seeds which were incubated for 48 hours was 45% when the exudate was filtered and mixed with hot (60°C) agar plus basal salts (Table 4). If the exudate was autoclaved before mixing with agar, spore germination was only

Table 4.--Germination of Urocystis colchici teliospores on agar media and on water agar placed over onion seeds or agar media

Nutrient Source	Concentration	Germination[a]
	g/liter	Percent
1. Ground onion seed	2.5	48
2. Ground onion seed	5.0	56
3. Ground onion seed	8.0	65
4. Malt extract-phytone (MEPA)	10 & 5	78
5. Intact, ungerminated onion seeds, autoclaved[b]		58
6. Intact, 24 hr. germinated onion seeds, autoclaved[b]		55
7. Intact, 48 hr. germinated onion seeds, autoclaved[b]		29
8. Intact, ungerminated, surface disinfested onion seeds[b]		59
9. Autoclaved exudate from seeds allowed to germinate 48 hr.	16	2
10. Filtered exudate from seeds allowed to germinate 48 hr.	16	45
11. MEPA under water agar	10 & 5	78
12. Water agar		1

[a]Average number of spores germinated/100 on each of four plates.

[b]Twenty seeds/plate.

2%. The pH of the exudate ranged from 6.8-7.4. The reason for the loss of ability to support germination is not known.

The standard teliospore germination procedure developed in this laboratory (23), required that spores be stored for 24 hours at high concentration before plating. Spores from a single collection were plated on MEPA concentrated or dilute storage, with and without leaching. Spore leaching was done in the model system described by Ko and Lockwood (23). Teliospores were placed on sterile Millipore filters on sterile sand in a petri dish. Sterile distilled water from a separatory funnel was dripped onto the sand at the rate of 10-30 ml/hr. The water moved through the sand and drained from the outlet. The spores remained sterile during the leaching process. The germination rate and level were lower after 48 hours leaching (Table 5). After 48 hours dilute storage, germination was 10% lower than after 24 hours dilute storage. The rate of germination was lower in treatments 1, 2, and 4, but the level after eight days was not lower than the control (treatment 3). Storage of spores on Millipore filters over natural soil for 24 or 48 hours and then placed on MEPA resulted in germination levels of 75% and 40% respectively. It thus appears that any treatment which removes nutrients from the teliospores tends to reduce germination.

Mycelial growth of U. colchici was reported as excellent on a liquid medium containing sucrose (30 g/l)

Table 5.--Germination of *Urocystis colchici* teliospores on malt extract-phytone agar at 22°C after different treatments

Treatment	Percent Germination[a] (Days of incubation)					
	3	4	5	6	7	8
1. Plated immediately	20	32	45	56	65	79
2. 24 hr., dilute storage[b]	19	33	48	56	68	77
3. 24 hr., conc. storage[c]	22	50	68	71	76	79
4. 24 hr., leached spores[d]	20	35	50	65	73	78
5. 48 hr., dilute storage	20	28	50	56	61	67
6. 48 hr., conc. storage	23	52	68	69	73	78
7. 48 hr., leached spores	18	25	30	34	43	45

[a]Average number of spores/100 germinated on each of four plates.

[b]Dilute storage (10^3 spores/ml).

[c]Concentrated storage (10^5 - 10^6 spores/ml).

[d]Leached spores were subjected to dripping water for the time indicated.

and asparagine (4.7 g/l) (12). To determine if nutrient requirements for growth were the same as for spore germination, 16 different combinations of sucrose and asparagine were tested for teliospore germination (Table 6). Germination ranged from 6-15%. The sucrose-asparagine medium failed to support complete germination, but no indication of the reason for this was obtained.

Table 6.--Germination of Urocystis colchici teliospores on sucrose and asparagine in agar media at 22°C

Nutrient	Concentration	Nutrient	Concentration	Germination[a]
	g/liter		g/liter	
1. Sucrose	5	Asparagine	0.5	10
2. Sucrose	5	Asparagine	1.0	9
3. Sucrose	5	Asparagine	3.0	13
4. Sucrose	5	Asparagine	5.0	9
5. Sucrose	10	Asparagine	0.5	11
6. Sucrose	10	Asparagine	1.0	13
7. Sucrose	10	Asparagine	3.0	13
8. Sucrose	10	Asparagine	5.0	12
9. Sucrose	20	Asparagine	0.5	14
10. Sucrose	20	Asparagine	1.0	7
11. Sucrose	20	Asparagine	3.0	10
12. Sucrose	20	Asparagine	5.0	6
13. Sucrose	30	Asparagine	0.5	15
14. Sucrose	30	Asparagine	1.0	6
15. Sucrose	30	Asparagine	3.0	9
16. Sucrose	30	Asparagine	5.0	10
17. Malt Extract	10	Phytone	5.0	75

[a]Average number of spores/100 germinated on each of four plates.

DISCUSSION

Initially only the effect of inoculum density was considered in relating inoculum potential and disease potential to disease severity. Theoretically the addition of more inoculum to soil should have resulted in proportional increases in disease severity; however, a point was reached at which increased amounts of inoculum did not increase percent disease proportionately (Figure 1). Since disease measured by percentage ignores any increase in disease severity that occurs on diseased plants, the multiple-infection transformation was used to calculate the number of infection per plant (17). The multiple-infection transformation (Figure 2) straightened the dosage-response curve somewhat, but response was not completely linear. The log-probit grid (Figure 3) produced a linear response, but only for inoculum densities below 2,000 spores/g of soil. The log-log plot of the number of infections against the number of spores/g of soil (Figure 4) also was linear up to 2,000 spores/g of soil; however, above this inoculum level the curve flattened. This decrease in the slope of the curve has been attributed to the lack of susceptible tissue in which infection can occur (29, 49).

In this study a linear relationship over the inoculum range of 125-8,000 spores/g was obtained when percent infection was plotted against the distance in mm between spores (Figure 5). In Baker's model (page 9) the number of propagules that fall on a surface area (rhizoplane) is directly related to the distance between propagules (3). Likewise the number of propagules that fall within a specific volume is also directly related to the distance between propagules (3). Since the number of propagules in a position to infect is directly related to the distance between the propagules, disease severity is linearly proportional to the distance between propagules.

Baker and McClintock (4) first suggested that the number of propagules/unit volume of soil and the distance between propagules are not linearly related (Figure 12). Since the probability of infection is directly related to the distance between propagules and not the number of propagules/unit volume, it becomes clear why disease response curves flatten out at higher inoculum densities (Figures 1 and 2). The fact that the magnitude of decrease in distance between propagules is very small as inoculum density increases above 1,000 spores/g means that the increased probability of further infection is also very small. This factor has been omitted as a reason for the typical decrease in slope of the disease response curve at high inoculum densities, although Baker and McClintock (4)

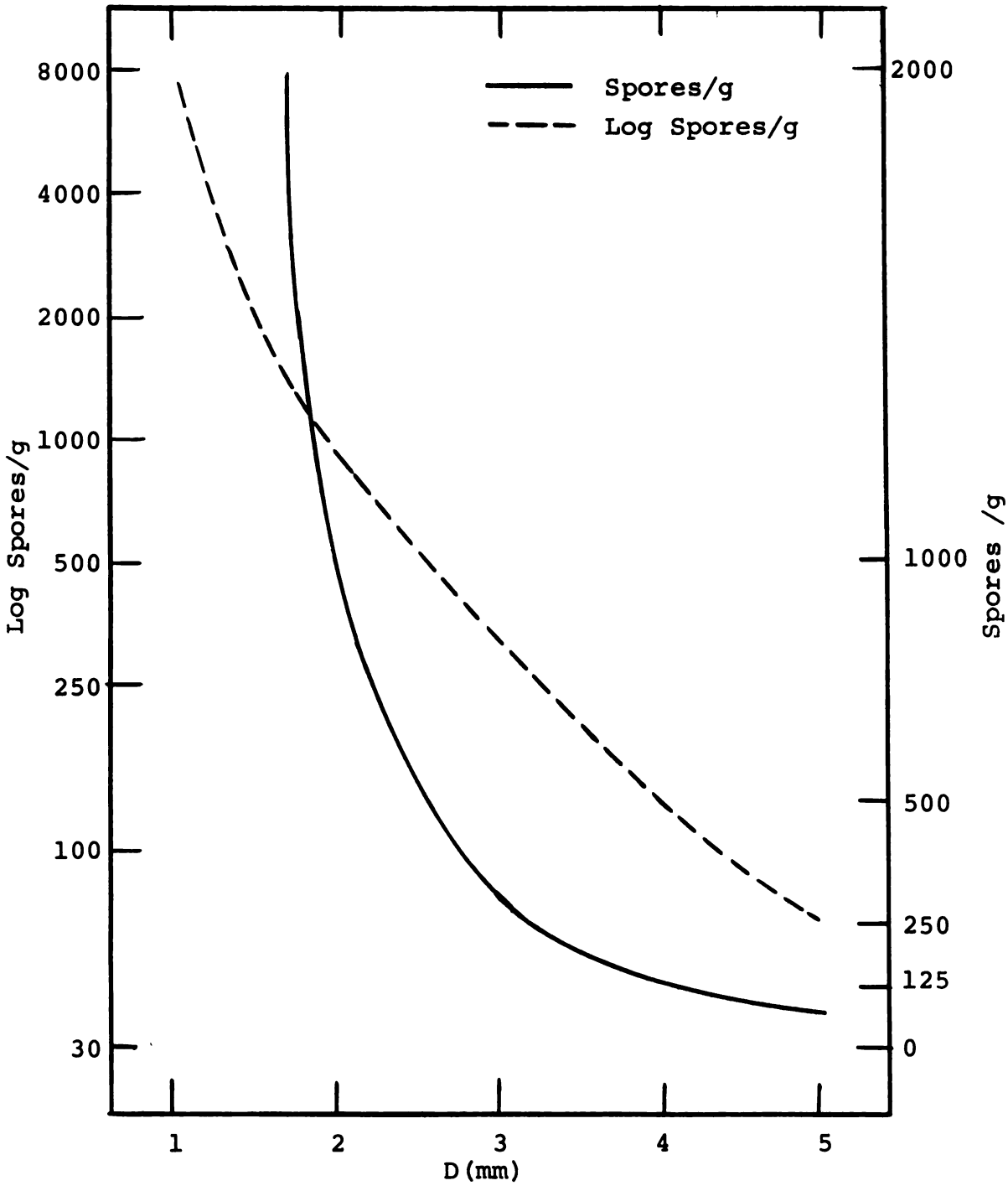

Figure 12. The relation between calculated distance (D) between spores in soil and number of spores or log number of spores/g of soil.

first suggested that this factor could be important in limiting the density of natural pathogen populations in soil.

Transformations using the log number of propagules/g of soil when relating disease and inoculum densities below 1,000 propagules/g of soil are linear because the relation between the log number of propagules/g of soil and distance between propagules is linear in this range (Figure 12). However, above 1,000 propagules/g the relation is not linear and the disease response curve flattens when plotted against the log number of propagules/g of soil. This may explain the common, so-called logarithmic effect in natural phenomena, and the linearity of curves using logarithmic transformations.

The position of a disease response curve with respect to inoculum density may change if: (1) spore germination was less than 100%; (2) if spore viability was different from that assumed; (3) if more than one spore were required for infection; or (4) if host resistance was different; however, the curve would remain linear. A change in position was illustrated by the planting depth experiment (Figure 7).

The response to inoculum present in the top 5 mm of soil was not increased by planting deeper; however, if inoculum was placed in the zone 5 mm above the seed a significant increase in infection occurred as planting depth

was increased (Table 2). The length of exposure to inoculum contained in the top 5 mm of soil was not increased by deeper planting. However, the length of exposure to inoculum in the 5 mm zone immediately above the seed was increased by deeper planting. This increase in disease is probably due to increased time of exposure of susceptible tissue to inoculum placed in the immediate vicinity of the seed. It appears that the cotyledon is uniformly susceptible and that the increase in disease at deeper plantings was due to increased time of exposure.

The primary soil factors influential in disease incited by soil-borne pathogens are: temperature, moisture, soil type, pH, and fertility (44). The effect of temperature on the relation between inoculum density and disease was best shown at 16°C. The difference in amounts of disease with 1,000 or 2,000 spores/g may be explained, in part, by the effect of temperature on spore germination *in vitro*. Spore germination data (23) indicate 48% germination at 16°C and 84% at 24°C after eight days. Assuming that approximately half of the spores in soil would germinate at 16°C that would germinate at the optimum temperature, as was shown *in vitro* (23) the reduction in disease at 16°C might have been predicted, because the effective inoculum level was reduced by nearly half. Thus 2,000 spores were reduced to 1,000 effective spores/g and 1,000 to 500 effective spores/g because of incomplete germination at the

below-optimum temperature. The temperature effect on rate of emergence (Table 3) and fungus growth at 16 and 20°C was reported to be small (12, 47). The change in response of a population of plants to a decrease in inoculum density from 2,000 to 1,000 spores/g was very small (Figure 5); however, the change in response to a decrease from 1,000 to 500 spores/g was much greater. In the past, the effect of sub-optimal temperature on amount of disease may have been masked by using a high inoculum level (46).

Although the effect of temperature on amount of disease at 16°C may be explained by a reduction of spore germination, other factors may become 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 (12, 23); therefore amount of disease would be limited (Figure 7). Spore germination and vegetative growth of the fungus were reported to be optimum at 24°C (12, 23), but were nearly as good at 20°C where optimum infection occurred. The mean length of exposure of the cotyledon to inoculum was six days at 24°C and eight days at 20°C. The first true leaf appearance was three days earlier at 24°C 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, 46); 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 restricted at 28°C (12, 23, 47). Therefore, even when exudation was greatest, the amount of disease was low. There was general agreement, with results reported earlier (46), that infection can occur at either high or low temperature, but that at above 25°C a decided reduction in disease occurs.

Germinating onion seeds and developing onion cotyledons were demonstrated to exude soluble organic nutrients. The amount of material exuded was not abundant, but present in sufficient quantity to support teliospore germination. Seeds with young emerging radicles were the major source of nutrients. This early exudation pattern was also suggested from inoculum studies where infested soil was placed in zones. The amount of disease was always greatest when inoculum was placed in the zone in which germination began. However the zone in which germination began always was exposed to inoculum for a longer time.

Exudation at temperatures of 12, 16, 20, and 24°C was not significantly different, but the amount of material exuded at 28°C was higher than the amounts recovered at 12-24°C. Increased exudation at 28°C would not affect smut disease severity because both germination and growth of the fungus were limited at this temperature (12, 23, 47).

Onion seedlings also develop rapidly at 28°C, shortening the time of exposure to inoculum. The amounts of ninhydrin- and anthrone-positive materials exuded from sterile onion seedlings were much higher than the amounts recovered from nonsterile seedlings. This may have been due to: (a) lowering of amounts of recoverable materials by contaminating microorganisms, (b) absorption of nutrients from agar by the seed and emerging radicle which were later recovered in exudates, or (c) increased release of nutrients due to the shock of transplanting into the sand system.

Although no direct evidence that specific substances were exuded to selectively cause pathogen spores to germinate, onion seeds and exudates from germinating onion seeds were shown to be sufficient to support teliospore germination, which could lead to successful infection.

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