Inoculum Potential in Relation to Biological Control of Fusarium Wilt of Peas

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Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree, Colorado State University.

Published with the approval of the Director of the Colorado State University Experiment Station, as Scientific Journal Series Paper No. 2131.

Accepted for publication 27 May 1976.

ABSTRACT

GUY, S. O., and R. BAKER. 1977. Inoculum potential in relation to biological control of Fusarium wilt of peas. Phytopathology 67: 72-78.

Addition of chitin or cellulose to soil induced changes in symptom development of Fusarium wilt of peas. In log-log transformations of inoculum density-disease severity data, however, curves were parallel regardless of treatment indicating constant relative changes in infection rates directly correlated with inoculum density. At any given inoculum density, chitin in soil decreased disease severity slightly but significantly compared with nonamended controls. Chitin in soil, however, had no influence on survival rate (in this case, decrease in inoculum density over time) of the pathogen in comparison with nontreated soil. A cellulose amendment increased disease severity slightly in comparison with controls but this increment could be explained by the increase of inoculum density of the pathogen as a result of adding the amendment. Log-log transformations of inoculum density-disease severity curves indicated slope values not significantly different from 0.67, which conformed to those predicted for fixed inoculum and moving infection courts.

Additional key words: Fusarium oxysporum f. sp. *pisi* Race 5, *Pisum sativum,* soil fungi.

The complex biological responses in a given host-pathogen system eventually will require detailed quantitative analysis for adequate evaluation of epidemiological interactions. Such a systems approach has been described for Rhizoctonia preemergence damping-off of radish (8, 9, 10). The present study extends this approach to provide a mathematical description of the impact of biological control systems on Fusarium wilt of peas (*Pisum sativum* L.) incited by *Fusarium oxysporum* Schlecht. emend., Snyd. & Hans. f. sp. *pisi* (Lindf.) Snyd. & Hans., race 5, a highly pathogenic race that has caused extensive losses in northwestern Washington (14).

Chitin and cellulose can induce biological control of plant pathogens (2). Chitin reduced root rot of bean (21). Maurer and Baker (17) were unable to confirm this observation but combinations of lignin and chitin gave small, significant increments of control. Chitin also reduced incidence of Fusarium wilts of radish (20) and peas (16). In the latter study, survival of the pathogen was reduced when chitin was added to soil. Schippers and DeWeyer (25) also observed poorer survival of *Fusarium solani* f. sp. *phaseoli* (added to soil as macroconidia) in chitin-amended soil compared to nonamended soil.

Addition of cellulose to soil in C:N ratios above 25/1 suppressed bean root rot (18), but inoculum densities in the rhizosphere did not change before and after control occurred (6). Chlamydospores of the pathogen germinated poorly in cellulose-amended soil, even in the presence of bean seed exudates (1). Control also was correlated with limiting nitrogen (6) which identified competition for this element as the mechanism of biological control (2, 27). Cellulose added to soil infested with *F. oxysporum* f. sp. *pisi,* however, resulted in a slight increase in symptoms of pea wilt compared with nonamended controls (16).

MATERIALS AND METHODS

A sandy clay loam soil collected near the Poudre River in Colorado had the following properties: pH 8.2 [determined colorimetrically in soil: 0.01 M $CaCl_2$ suspensions (1:2, v/v)] NO_3^--N, 21 μg/g; lime, high; P_2O_5, 15 μg/g; K_2O, 175 μg/g; Fe, 19.3 μg/g; Zn, 1.86 μg/g; total N, 1,600 μg/g; and organic matter 5.4%. The field was cropped previously with barley, but had been fallow for nearly 1 year.

A culture of *F. oxysporum* f. sp. *pisi* race 5 (kindly supplied by W. A. Haglund) that had been isolated from diseased peas in Washington was used in all experiments. New cultures were produced from single conidia every 4-5 weeks and selections were made for cultural characteristics resembling the original wild type (28). No decrease in virulence of inoculum from cultures was noted during these investigations.

The pathogen was increased on sterilized leaves of carnation (*Dianthus caryophyllus* L.). After 4 weeks,

tissues containing the fungus were dried and powdered in a Micro-Mill (Chemical Rubber Co., Cleveland, Ohio). Microscopic examination of the powder revealed free microconidia and chlamydospores embedded in partially decomposed plant tissues.

For inoculum density-disease severity (ID-D) experiments, inoculum was blended with soil in a concrete mixer. Inoculum densities in this blend were determined using soil dilution plate counts or the selective medium of Nash and Snyder (22). One milliliter of a 1:1,000 or 1:10,000 soil dilution was applied to each plate and there were five replicate plates for each soil examined. Attempts were made to distinguish the added pathogen from background *F. oxysporum* already in the raw soil by obtaining mutants tolerant to dodine (15). Such "labeling" of a pathogen in soil has been accomplished for the fungus that causes Fusarium wilt of melon (19). In spite of repeated attempts, however, no stable mutants were obtained for *F. oxysporum* f. sp. *pisi* race 5. Thus, total counts were made of *F. oxysporum* making appropriate comparisons with colonies observed in noninfested controls; these were presumed

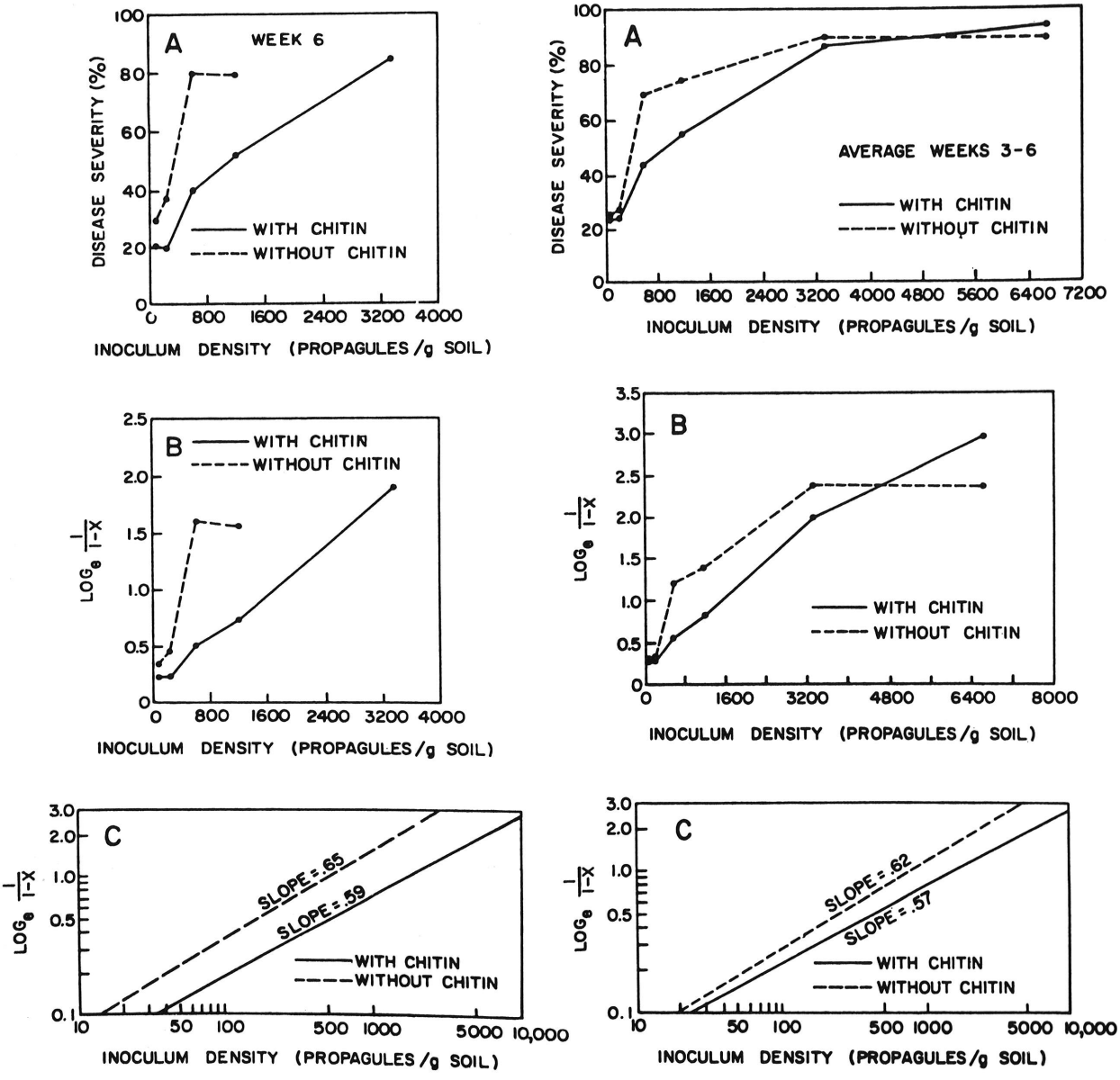


Fig. 1-(A to C). Effect of chitin added to soil on inoculum density-disease severity relationships for Fusarium wilt of peas 6 weeks after planting. Analysis: **A)** arithmetic plot; **B)** semilogarithmic transformation; and **C)** log-log transformation [x = disease severity (29)].

Fig. 2-(A to C). Effect of chitin added to soil on inoculum density-disease relationships when symptom expression rating of Fusarium wilt of peas over 3-6 weeks were averaged. Analysis: **A)** arithmetic plot; **B)** semilogarithmic transformation; and **C)** log-log transformation [x = disease severity (29)].

nonpathogenic and, indeed, did not incite wilt of peas.

After inoculum had incubated for 2 weeks, raw soil was mixed with the infested soil in a twin-shell blender for 5 minutes to secure the various inoculum densities desired. Powdered cellulose (18) or chitin (17) was mixed at the same time at the rate of 3 g/ kg soil (oven dry basis) in the amended treatments. Four or 6 kg of soil were placed in either 20.3- or 25.4-cm diameter pots, respectively, in three replications for each treatment and inoculum density. Over this, a 3-cm layer of sand was added and 10 or 15 pea seeds (cultivar Little Marvel) were planted in each pot at a depth of about 2 cm. Thus, the seed germinated in an environment that was free of inoculum and the roots penetrated the soil infested with the pathogen. This conforms to the interaction phenomena of infection court and inoculum under field conditions (5, 24).

Pots were placed in a greenhouse. Temperatures varied from a low of 15 (at night) to 21-30 C (during the day) and plants were watered as required. Wilt symptoms usually appeared 5 weeks after planting and disease readings were taken at weekly intervals for 4 weeks beginning at the time symptoms appeared. Disease was assessed by determining the ratio of the number of wilted leaves to the total number of leaves for each plant. This ratio was converted to the percentage of wilted leaves and used as an indicator of relative disease severity. For certain data

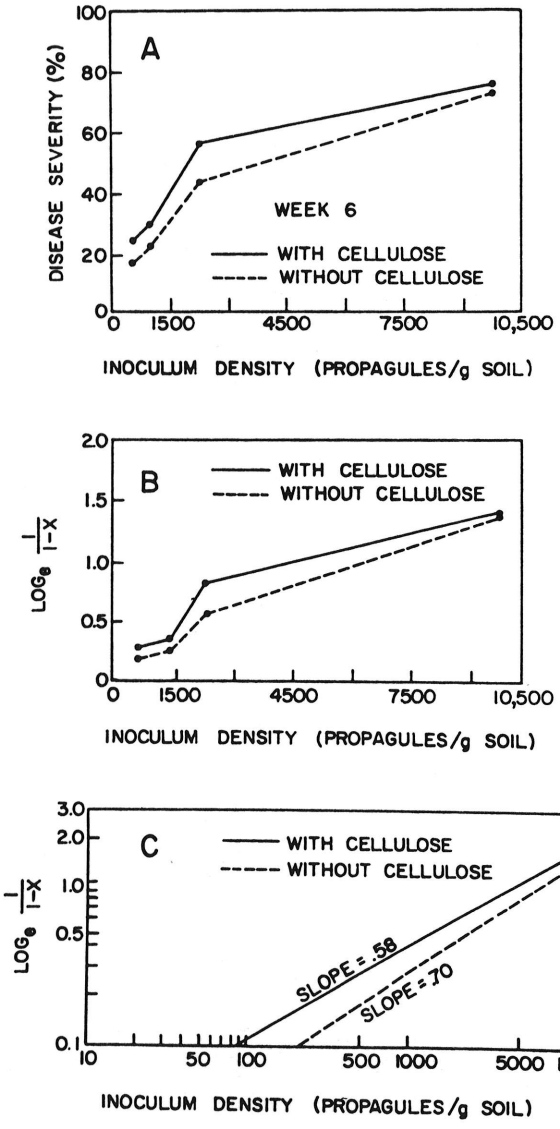


Fig. 3-(A to C). Effect of cellulose added to soil on inoculum density-disease relationships for Fusarium wilt of peas 6 weeks after planting. Analysis: **A)** arithmetic plot; **B)** semilogarithmic transformation; and **C)** log-log transformation [x = disease severity (29)].

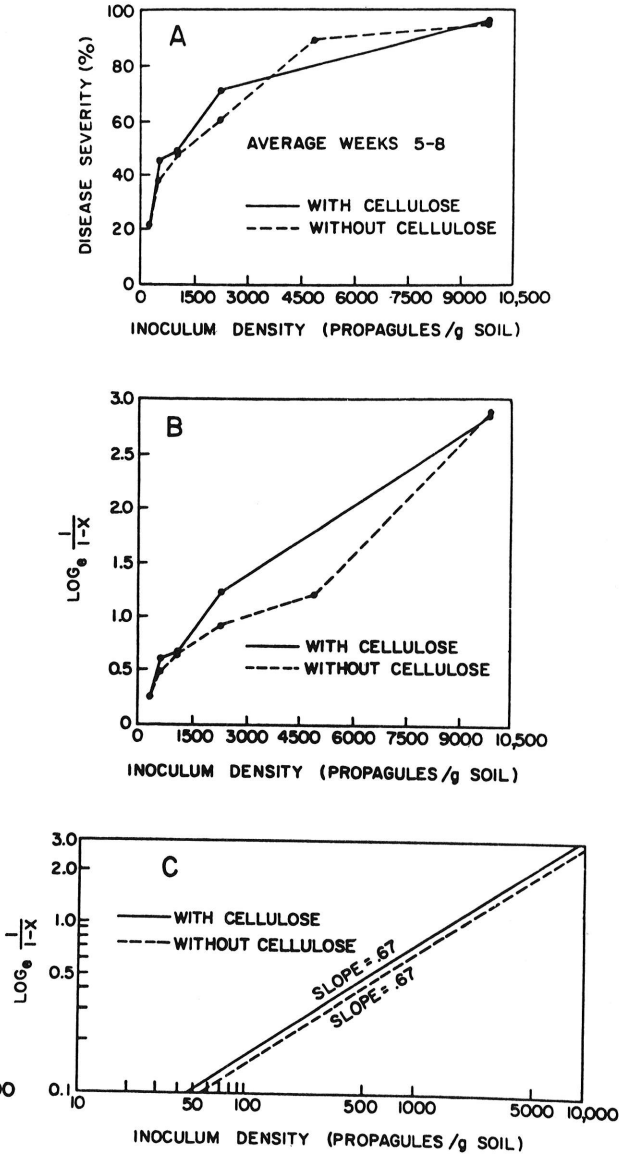
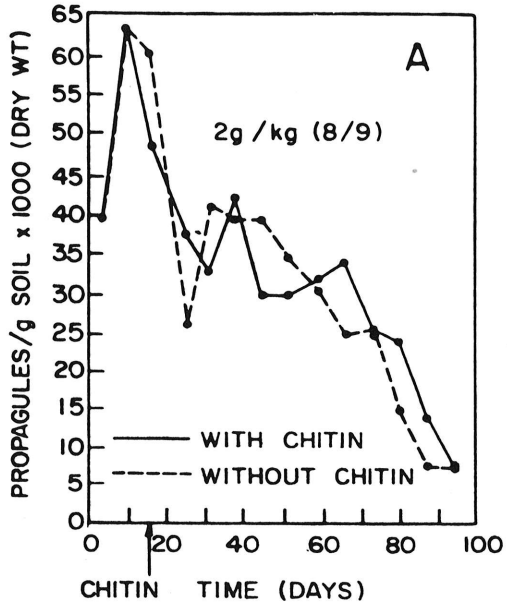
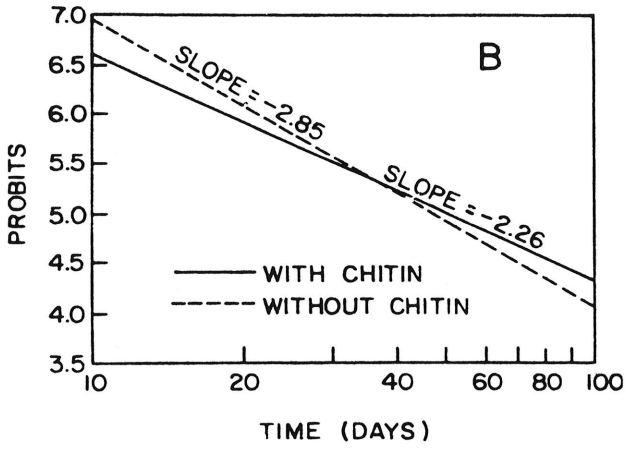
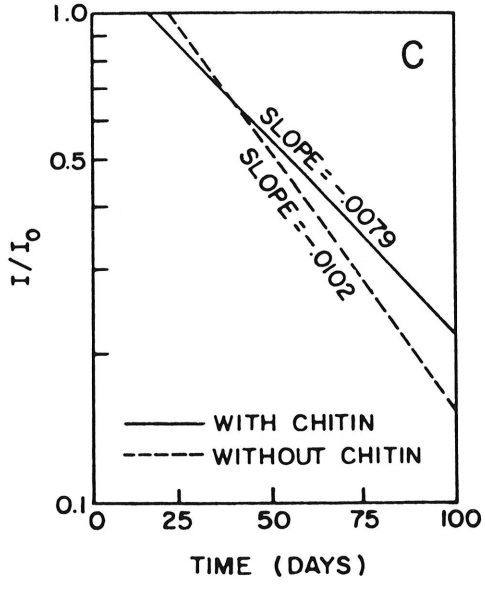


Fig. 4-(A to C). Effect of cellulose added to soil on inoculum density-disease relationships when symptom expression ratings of Fusarium wilt of peas over 3-6 weeks were averaged. Analysis: **A)** arithmetic; **B)** semilogarithmic transformation; and **C)** log-log transformation [x = disease severity (29)].







transformations, this disease index (x) was corrected for multiple infections (13) by the formula $\log_e[1/(1-x)]$ (29). All experiments were repeated once.

Death rates of inoculum in nonamended and amended soils were obtained using the procedures described by Benson and Baker (10). Positions of survival curves were determined by interpolating a T_s50 value (time required for 50% of the propagules to die in soil). Inoculum densities at various times again were determined by the method of Nash and Snyder (22) and experiments were repeated once.

RESULTS

Inoculum density-disease severity interactions.—Chitin or cellulose was added to soil infested with the pathogen at various inoculum densities. Symptom expression (6 weeks after planting) was reduced for any given inoculum level when chitin was added to soil (Fig. 1). Slope values for the log-log transformation of data on symptom expression (Fig. 1-A) were 0.65 without chitin and 0.59 with chitin added to soil. Effective dosage (ED_{50}) values were 287 and 895 propagules per gram of soil, with and without chitin, respectively. Analysis of covariance indicated that the positions of the ID-D curves ($P = 0.02$), but not their slopes, were significantly different. Results from disease readings taken 3-6 weeks after planting (Fig. 2) produced a slope of 0.62 without and 0.57 with chitin; ED_{50} values were 427 and 760 propagules per gram of soil, respectively. Again, slopes were not significantly different but difference in position was questionable ($P = 0.09$).

Symptoms were more severe on plants growing in soil amended with cellulose than in nonamended soils at many of the inoculum levels (Fig. 3 and 4). Indeed, positions of ID-D curves in the log-log transformations of data taken 6 weeks after planting (Fig. 3-C) were somewhat different ($P = 0.07$): ED_{50} values for cellulose treatment were 1,920 and 2,980 propagules per gram for cellulose-amended and the control soils, respectively. Data averaged over 5-8 weeks gave ED_{50} values of 973 and 1,080 propagules per gram of soil (Fig. 4-C) for soils with and without cellulose, respectively; and positions of the curves were not significantly different. Slope values for all treatments varied from 0.58 to 0.70 but were not significantly different.

Survival.—No differences in survival of *F. oxysporum* f. sp. *pisi* were observed between soils treated or not treated with chitin (Fig. 5). Colony counts declined logarithmically at first (hereafter, this is referred to as the initial survival curve), but became relatively stable after approximately 100 days. When data were plotted on a log-probit or semilog basis, only points on the initial survival curve were used. There was no significant difference in position or slope of these plots of points. Values for T_s50 in the log-probit transformation (Fig. 5-B) were 52 days for soils amended with chitin and 48 days for nontreated soil. In the semilog transformation (Fig. 5-C) T_s50 values were 54 and 50 days, respectively.

Survival of the fungus was better in soil supplemented

Fig. 5-(A to C). Effect of chitin on survival of *Fusarium oxysporum* in soil. Analysis: **A)** arithmetic plot; **B)** log-probit transformation; and **C)** semilogarithmic transformation.

with cellulose than in nontreated soil; however, slope values were not significantly different (Fig. 6). Values for T_s50 in the log-probit transformation (Fig. 6-B) were 80 days for soil amended with cellulose and 22 days in the control; for the semilog transformation (Fig. 6-C), they were 81 and 19 days, respectively. Positions of regression lines were significantly different ($P \leq 0.005$) in both cases.

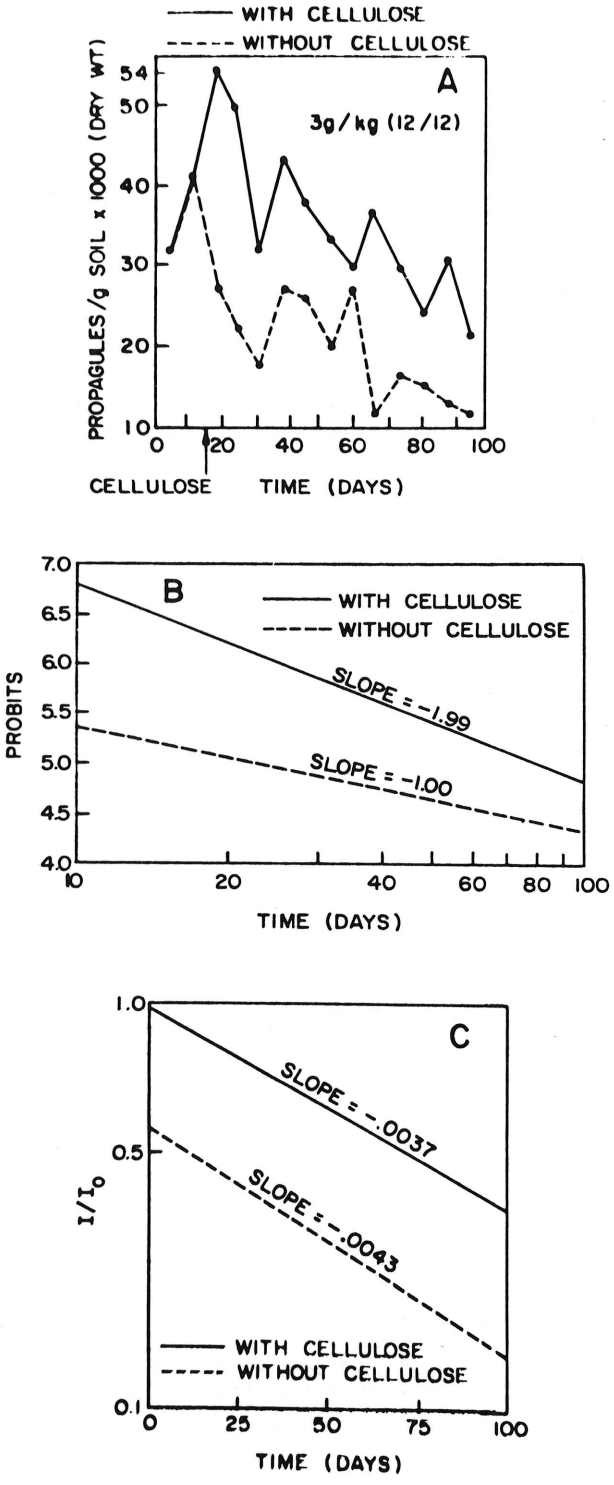


Fig. 6-(A to C). Effect of cellulose on survival of *Fusarium oxysporum* in soil. Analysis: **A)** arithmetic plot; **B)** log-probit transformation; and **C)** semilogarithmic transformation.

DISCUSSION

The number of wilted leaves, which is the visible and measurable consequence of infection, was assumed to be the best evidence of pathogenicity. This indirect measurement of plant reaction (7) provided a reasonably accurate quantitative analysis of host reaction even though the causal agent was systemic. As long as analyses are made on a per unit basis (4, 29), numbers of infections can be determined using the multiple-infection transformation (13); and severity of symptoms is directly proportional to numbers of infection sites in Fusarium wilt of peas (24).

Nyvall and Haglund (24) concluded that numerous independent infections of roots are necessary to produce severe wilt in peas. In our experiments, the possibility of infection of pea cotyledons and atypical infection courts (e.g. cortical tissues) was obviated by planting seeds in sand above infested soil and allowing the roots to invade the infested soil. This conforms to the previously described (5) Model II [i.e., a motile infection court (the root tip) invading a matrix with fixed inoculum (chlamydospores) in a three-dimensional volume (soil)].

The predicted slope of a log-log transformation of the ID-D curve (4) for this model is 0.67 (5). Slope values measured in our experiments ranged from 0.57 to 0.70 and in no instance were values significantly different within experiments.

The moving infection courts of Model II (5) should encounter a relatively high proportion of the inoculum contained in the volume of soil occupied by the roots of a host. Thus, extrapolation predicts that thresholds of infection should occur at relatively low population levels in comparison to models involving fixed infection courts given reasonably efficient inoculum capacity for penetrating and infecting host tissue (3, 5). In these experiments, however, the range of ED_{50} values was 287-1,080 propagules per gram of the gross population of *F. oxysporum* in soil. This contrasts with ED_{50} values of 5.2-50 propagules per gram reported by Benson and Baker (9) for Rhizoctonia damping-off of radish.

The relatively high ED_{50} values for pea wilt could be construed as indicating that high inoculum densities may be required for symptom expression. Environmental factors involved with disease proneness, however, may modify symptom expression of hosts of vascular wilt pathogens (e.g., 12, 23). This restricts formulation of principles for this model based on inoculum density alone. There also were some experimental limitations, bearing on interpretations. First, for example, because stable mutants of *F. oxysporum* f. sp. *pisi* tolerant to dodine (19) could not be used as markers for the pathogen, total populations of *F. oxysporum,* both the added pathogen and the nonpathogenic forms found in raw soil, were counted; nonpathogens represented 15-35% of *F. oxysporum* in inoculated treatments as estimated by the number of "background" colonies recovered from noninfested control soil. Second, actual inoculum densities were lower (based on survival curves)

by the time infection courts invaded infested soil since ID-D curves were based on initial densities. Third, the soils were infested with inoculum grown on plant tissue to simulate natural inoculum (10, 26), and chlamydospores were observed, but there were also microconidia, perhaps able to produce colonies on the selective medium (22) but unable to infect. Finally, the 8-week experimental period may have been long enough only for plants with multiple infections to exhibit symptoms (24). Longer incubation may be required for single or low numbers of infections. These factors all would affect the proportions of propagules actually participating in the infection process.

Adding chitin to soil significantly reduced expression of Fusarium wilt in peas (Fig. 1). There was no indication, however, that survival of the pathogen in soil was influenced by this amendment (Fig. 5). Khalifa (16) found survival level of the pathogen in the rhizosphere remained relatively constant in chitin-amended soils but increased in nonamended controls. It is likely that a high proportion of the inoculum used by him was in the form of macroconidia. Macroconidia may be adversely affected by chitin during their conversion to chlamydospores (25). Thus, death rate of macroconidia per unit of time, r (11), is different in chitin-amended soils compared with nonamended controls; but this would not necessarily be true for chlamydospores.

When cellulose was added to soil, there was a small increase in disease severity compared to that of nonamended soil (Fig. 3). Initial populations in both nonamended and cellulose-amended soils were the same; but by 20 days, the population of *F. oxysporum* had increased in cellulose-amended soil to more than twice that of nonamended soil (Fig. 6). When this difference in population level was used in plotting the inoculum-density disease curves, rather than the initial inoculum densities, there was no significant difference in position of the curves for soils with or without cellulose. Thus, any effect on disease attributed to cellulose amendment can be explained by increased inoculum density.

Cellulose added to soil results in biological control of Fusarium root rot of beans as evidenced by reduction in hypocotyl lesions caused by *F. solani* f. sp. *phaseoli* (2), an example of Model I (5). Why not for Fusarium wilt of peas? The answer may be in the different distribution of infection courts of these two host pathogen systems in soil; i.e., hypocotyl vs. root tips. Evidence suggests biological control of *F. solani* f. sp. *phaseoli* using cellulose is due to competition (2, 27); chlamydospore germination is very low in soils amended to produce high C:N ratios and thus are deficient in available nitrogen. However, absolute numbers of propagules germinating and infecting the host can be increased in this system by raising the initial inoculum density (3); biological control is nullified because the increase in successful infections compensates for the effect of the cellulose amendment. For Model II (5), the amount of inoculum encountered by the motile infection courts is much more than for the fixed infection court of Model I (3). This, in effect, increases the proportion of inoculum (per unit area of infection court) in soil participating in the infection process for Fusarium wilt in comparison to bean root rot. Even if inoculum is less efficient in inciting disease in cellulose-amended soil, continuous contacts with propagules as the root tips move through soil may compensate for any decrease in activity caused by the amendment.

In the candidate biological control treatments applied in these investigations, positions but not slopes of the inoculum-density-disease curves were altered in log-log transformations. This indicates constant relative changes in infection rates directly correlated with inoculum density regardless of treatment (4).

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