SOIL BIOSUPPRESSION OF WHEAT SEEDLING INFECTION BY HELMINTHOSPORIUM SATIVUM P. K. & B.

By

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CHAPTER I

INTRODUCTION

Common wheat (Triticum aestivum L.) is a very important crop in many parts of the world, constituting the principal foodstuff in all regions except in those areas where rice is the predominant food plant (55, 63). In Oklahoma, winter wheat is grown not only as a grain crop, but also as a forage crop for livestock and contributes significantly to the agricultural economy (58, 65).

Wheat plants, in all stages of their growth, are subjected to numerous diseases (22, 91). As an example, root rot diseases have long been a serious problem throughout many wheat growing regions in the U. S. and Canada (41, 66, 73, 75). In Oklahoma, wheat root rots are more severe when they occur as a disease complex which is the result of the interaction between several soil-borne fungi and nematodes than when they occur as individual diseases each of which is caused by a pathogen. Helminthosporium sativum P. K. & B. (Bipolaris sorokiniana [Sacc. in Sorok.] Shoemaker; perfect state Cochliobolus sativus [Ito and Kurib.] Drechsler ex Dastur.) is commonly found in association with this disease complex (92). The fungus can cause damage throughout the growing season such as seedling blight, root rot, leaf and head blight, and mature plant death. All phases of the disease reduce plant vigor and, therefore, forage and grain production (22, 41, 91, 92).

Various suggestions have been made for control of common root rot of wheat, for example, using clean or chemically disinfected seeds, clean cultivation, suitable fertlization, crop rotation, etc. Such programs have been observed to reduce the disease incidence significantly, but they do not control it completely (66, 91).

Helminthosporium sativum is a widespread, nondiscriminating pathogen on most cereals and numerous grasses (16, 30, 80). It is soil-borne and persists in/on soil, or in plant debris as conidia or mycelia (16, 41). Soil conditions, especially soil microorganisms, influence saprophytic and pathogenic activities of the pathogen (17, 23, 28, 29, 42, 74). Through management of soil conditions, soil-borne diseases may be controlled biologically. Naturally occurring biological control is recognized by conditions in which the plant pathogens can not persist, establish, or they establish but fail to cause disease in field soils (2).

The phenomenon of Fusarium-suppressive soils has been observed for decades (86). These soils can be separated into two distinct types: (a) classic type in which the pathogenic forms of the fungus are suppressed (78, 79), and (b) general type in which both pathogenic and nonpathogenic fusaria in soils are affected (77, 87). Stover (84), in a summary of the work of Volk on Fusarium wilt of banana in Guatemala, described a classification of the soils into 3 categories: (a) resistant -- those soils in which the spread of disease was so slow that the productive life of plantations of susceptible bananas extended for more than 20 years, (b) nonresistant -- those soils in which the susceptible varieties of banana succumbed to the disease at such a rate that the plantations had to be abandoned in 10 years or less, and (c) semiresis-

tant -- those soils which had an intermediate effect.

Monoculture of a crop plant species is generally thought to increase damage from soil-borne plant pathogens (31), yet the reverse is sometimes true (39, 71). The widespread demonstration of take-all decline (TAD) during cereal monoculture (71) could be greatly important if it can be exploited as an effective method of natural biological control for an intractable disease in which neither resistance nor chemical control is available and where extended crop rotations are not feasible (67, 75).

Ko (48) successfully developed an unusual use of suppressive soil in Hawaii to control root rot of papaya seedlings caused by Phytophthora palmivora. Healthy seedlings were planted in "islands" of virgin soil which was pathogen-free in Phytophthora-infested fields. The introduced virgin soil protected the young seedlings during the most susceptible stage. As the seedlings matured, they became more resistant to the pathogen, and less disease resulted when the mature root spread into the infested soil.

It was found that one field at Nash, Oklahoma, with at least 20 years continuous wheat cropping history is suppressive to H. sativum. The objectives of this study were (a) to evaluate soil suppressive characters of two soil types, (b) to determine the factors involved in the suppression, and (c) to determine how these factors affect the pathogen-host interaction.

CHAPTER II

LITERATURE REVIEW

There are several causal organisms associated with root rots of wheat. Some cause very characteristic field symptoms which can be readily diagnosed; others are completely and satisfactorily diagnosed only after suitable laboratory tests (66, 91). The term "common root rot" has been widely used to designate a group of diseases which are characterized by necrosis of roots, crowns, and stem bases, similar to but distinct from take-all of cereals. Common root rot tends generally to occur on individual plants growing in competition with healthy ones, while take-all occurs in small to large patches in which all plants are infested (19).

Common root rot has long been a problem in the major wheat and barley growing areas of North America and other parts of the world (22, 66, 91). Published information on this disease in the past 70 years is extensive. H. sativum P. K. & B. was known to be the predominant causal fungal pathogen of this disease complex (66). In 1909, Pammel (61) published a short article on a new disease of barley characterized by irregular brownish lesions on leaves. The next year, 1910, Pammel, King, and Bakke (62) attributed the spot disease on barley to H. sativum. The perfect state of the fungus is rarely found in nature, yet Tinline (85) obtained it in culture by pairing various isolates on suitable media, and the name "Cochliobolus sativus (Ito and Kurib.)

Drechsler ex Dastur" was established. Though C. sativus is generally accepted in some literature, the name H. sativum is more familiar to most plant pathologists and will be used here.

Helminthosporium sativum attacks every part of a susceptible host(16, 76, 91). Roots, stems, leaves, spikes, spikelets, and seeds all can become infected. Infection can occur at any stage of growth (34). The first apparent symptom of the disease in the field is seedling blight somewhat like damping-off. Darken subcrown internodes are an indication of attack by H. sativum (16, 91).

Wheat losses due to common root rot are difficult to estimate. Various methods have been used for disease ratings. McKinney (56) was the first to separate plants with common root rot into categories according to degree of infection on underground parts. Since, most investigators have used the categories CL (clean), SL (slight), MO (moderate), and SE (severe) to classify disease severity on plants or plant parts. A group of researchers in Western Canada recently developed a new method for disease rating which involved use of photographs to depict disease categories and classification of plants on the basis of lesioned area of subcrown internode. Thus, "clean plants" have no lesions, "slight" have 1-25 %, "moderate" have 25-50 %, and "severe" have over 50 % of total area of subcrown internode lesioned (51). Comparison of yields of individual healthy and diseased plants from the same population has been the method used most frequently to assess loss. In such studies, plants were sorted into disease classes and yield reductions were estimated. The losses varied in different growing areas (25, 66, 91). A yield loss of 3 to 4 % annually was

primarily attributed to common root rot damage in most parts of North America (91). In some wheat fields of New South Wales, Australia, a wheat loss due to H. sativum was reported to be as high as 85 to 95 % (37). Subsequent research results from Oklahoma soil fumigation studies have shown that reduction due to root rot disease complex in grain yield and forage were about 16 and 77 %, respectively (92). Losses are due to thinned stands, decreased tiller number, head size, and kernel weight (89, 91).

Helminthosporium sativum is a widespread pathogenic soil-inhabiting fungus (16, 17, 30). It has a very wide host range. Christensen (16) inoculated 134 species of cereals and grasses in the greenhouse and found that 98 became infected. Wheat, barley, and rye were susceptible, while corn and oats were usually immune.

Helminthosporium sativum has been considered as a group species (16, 23, 24). Christensen (17) showed that there were at least 50 apparent forms of this fungus. Of these, 37 were studied in detail. Wood (93) produced the evidence for physiological races of H. sativum as distinguished by their relative pathogenicities on wheat, barley, and oats.

In the absence of host plants, survival of H. sativum is through conidia or mycelia in plant debris or seeds, and in or on the surface soil over an extend period (16, 17, 41, 53, 76). These propagules thus serve as the primary inoculum of next crop (16). Longevity of conidia depends on environment. Moisture was found to shorten the viable period of conidia. Chinn and Ledingham (12) reported that in saturated soil, conidial viability dropped to 2 % in 2 months, whereas, in dry soil,

no decline occurred within the same period. Similarly, a successful transfer has been made from an old culture after it had been dormant and dry for about 5 years (16). Fenster et al. (27) found that conidia of H. sativum survived in fine sandy clay loam soil of southwestern Nebraska for over 4 years, but they quickly lost their viability in fine textured organic silty clay loam soils of southeastern Nebraska. Increased incubated temperature decreased conidial longevity (52).

Secondary infection results from a variety of sources: from conidia produced on primary lesions on cereals, from infected plants of the previous season, and from many infected common grasses (16). Secondary inocula are distributed by rain, water, or wind. The last probably is the most important. Stakman et al. (81) reported that conidia have been caught by spore-traps at elevation up to 10,000 ft. Once the fungus has sporulated on plants, repeated inoculation and infection may occur.

Environmental situations have great influences on growth, survival, and pathogenicity of the fungus, and subsequently, on development of disease. Predisposition plays an extremely important role in degree of infection by H. sativum (17). The disease is generally severe under conditions unfavorable to the growth of host plants. Generally, high temperatures are favorable to growth and conidial germination of, and infection by the fungus, and development of the disease (23, 56). The fungus grows at temperatures of 0-39 C, with optimum between 24 and 28 C (23). Wheat plants grow best at 20-24 C (56).

The effect of foot and root infection of wheat by H. sativum is more severe in extremely dry and extremely wet soils than in soils

containing optimum moisture for growth of wheat (17).

Many researchers have found that soil microorganisms greatly influence parasitic and saprophytic activities of cereal root rot pathogens. Henry (42) probably was the first to report that natural microflora of soil had a marked inhibitive effect on development of wheat root rot caused by H. sativum. Soil fungi, i.e., Cephalothecium (35), Aspergillus, Mucor, Absidia, Penicillum, Rhizopus, and Trichoderma (68); soil bacteria, i.e., Pseudomonas (11), Bacillus (1, 18); and many actinomycetes (68, 83) have been reported to reduce virulence of, or to be antibiotic to, H. sativum. More recently, giant soil amoebae were found to cause perforation of H. sativum conidia in soil (60).

Greaney (34) studied the influence of time, rate, and depth of seeding on the incidence of wheat root rot. He found that the higher the soil temperature at time of seeding the higher the degree of infection. Susceptibility of wheat varieties does not depend on the stage of wheat development. Also the incidence of root rot increased with increase in seeding rate and increased with depth of seeding. However, depth of seeding was important in some varieties, but not in others.

Ploughing reduced the incidence of disease, particularly in seedling stage, but the difference was lessened as the season advanced (54). Surface tillages had no marked difference in disease incidence (54). Long term crop rotation of wheat with nonhost crops probably would reduce the infection of wheat by H. sativum (50).

Effects of soil fertility on common root rot of cereals are not well understood. Dosdall (23) found no correlation between incidence

of wheat foot- and root-rot and any particular fertilizer. Butler (10) reported that H. sativum survived at a consistently higher level in nonfertile than in fertile test soil.

Helminthosporium root rot of wheat is difficult to control. Although many efforts have been made to control the disease, none have been able to control the disease completely. Using resistant cultivars probably is the most promising means of disease control (66, 91). Selection and breeding of desirable cultivars, however, takes a long time. In such situations, biological control by using antagonists may be of great value in "Integrated Pest Management" programs.

Baker and Cook (2) suggest that pathogen-suppressive soils may greatly contribute to biological control management. They classified suppressive soils into 3 categories: (a) The pathogen fails to become established -- this is illustrated by Fusarium wilt pathogens in numerous soils. Walker and Snyder (90) reported that in Wisconsin, Fusarium wilt of pea, caused by F. oxysporum f. sp. pisi, progressed little from year to year in a Colby silt loam, was never observed, and could not be established in Lake Superior red clay. In California, soils of the coastal area near Castroville are conducive to Fusarium wilt of susceptible crops grown there, while soils from the Salinas and San Joaquin Valleys are suppressive to Fusarium wilt of susceptible crops (78). Smith and Snyder (79) showed that many soils are permanently suppressive to pathogenic F. oxysporum, but saprophytic F. oxysporum are not suppressed in these soils. Usually if a soil is suppressive to one pathogenic forma specialis of F. oxysporum, it is suppressive to all 50-75 % of the wilt pathogens of this species.

Germination of chlamydospores of pathogenic F. oxysporum was less than that of saprophytes in wilt-suppressive soils (79). (b) The pathogen becomes established but fails to produce disease -- suppression of Phytophthora cinnamomi in certain soils of eastern Australia is an example of this type. Broadbent and Baker (5) noted that avocado trees growing in some clay soil infested with virulent P. cinnamomi had high yields under favorable climatic conditions. Trees in nearby groves sustained slight to severe root rot during the experimental period. Suppressive soil had more bacteria and actinomycetes than did conducive soils (4). The soil suppressiveness could be overcome by water-logging, addition of subterranean clover meal, addition of excessive amount of P. cinnamomi inoculum, or eliminated by steam treatment at 100 C for 30 minutes (6). (c) The pathogen diminishes with continued monoculture -- the best example of this type is take-all decline (TAD) of wheat. The progressive but gradual disappearance of take-all in wheat has been widely observed in the world (71). Reports for years showed that severity of take-all reaches a maximum in 2 or 3 years of monoculture, and declines subsequently (26, 32). Many workers suggested that the most likely explanation for TAD is that it is brought about by a microbiological interaction between pathogen and other microorganisms in the soil associated with the increase in fungal inoculum during monoculture (7, 32, 64, 72). The extent and speed of development of antagonism depended on soil type and previous cropping history.

CHAPTER III

MATERIALS AND METHODS

Culture Maintenance and Inoculum Preparation

A culture of H. sativum P. K. & B. was obtained from a diseased, field collected wheat plant. Infected subcrowns were surface sterilized with a 1:1 solution of Clorox (active ingredient sodium hypochlorite 5.25 %) and ethanol (95%) for 30 seconds, blotted dry on a paper towel, placed on modified Czapek Dox agar (appendix), 5 per plate, and incubated at 25 C. After several days, isolates of H. sativum-like colonies were transferred to Czapek Dox agar (appendix), incubated at 25 C, and subsequently identified as H. sativum. Stock cultures were kept on wheat kernel medium (whole wheat kernels : water= 1 : 1 (v/v), autoclaved at 121 C, 1.1 atms, for 30 minutes) in test tubes.

Conidial suspensions for soil infestation were obtained by flooding 10-day old cultures of H. sativum grown on Czapek Dox agar plates at 25 C with 10 ml of sterilized distilled water followed by gently scraping the culture surface with a sterilized spatula. Clumps of conidia and mycelial fragments were removed by filtering the suspensions through a double layer sterilized cheesecloth. Number of conidia per ml of conidial suspension was determined by pipetting 1 ml of conidial suspension into a nematode counting dish, and counting under a dissecting microscope. The resulting conidial suspensions

were then adjusted to a suitable concentration and added to soils to be tested to give appropriate numbers of conidia per gram of soil (on dry weight basis).

Field Soil Sampling and Processing Procedure

Soil samples were collected from two locations (Nash and Cherokee, Oklahoma), one field at each location. In each field, three soil sites were randomly selected; and at each soil site, a soil section was vertically exposed to a depth of at least 30 cm. Soil samples were then removed in 10 cm increments (0-10, 10-20, and 20-30 cm) to a horizontal depth of approximate 20-30 cm. Each sample was bagged and labeled by depth, site, and location for transport to the laboratory for further processing. Considerable care was exercised so as not to intermix soils from different depths. Each soil sample was air-dried for about 10 days at room temperature (21±2 C), stored in cold room (4 C) until used, and sieve with a screen with 2 mm opening prior to use to remove straw and break down soil clumps.

Bioassay Procedure for Soil-Borne Inoculua of *H. sativum*

Seedling tests were conducted to determine amounts of viable inocula of *H. sativum* in tested soils. Twenty seeds of TAM 101, a wheat cultivar susceptible to *H. sativum*, were seeded at a depth of about 3.5 cm in the soil in each plastic pot (5 x 5 x 5 cm). Pots were incubated at room temperature (21 ± 2 C) with an illumination of 280 lux and top watered individually as needed. After 14 days of incubation,

seedlings were washed under tap water to remove soil from roots; the exposed subcrown internodes (the underground portions of seedling stems from soil surface to the point of attachment to seed) were individually excised, collected in Petri dishes, surface sterilized with a 1:1 solution of Clorox (active ingredient sodium hypochlorite 5.25 %) and ethanol (95 %) for 30 seconds as previously described, placed on modified Czapek Dox agar, and incubated at 25 C for 3-4 days. Each subcrown internode was individually rated for preincubation coloration and lesion size, and for postincubation infected quadrants and colony area. Preincubation color indexing (C) was done by color grading each subcrown internode according to the scale shown in Table I. Similarly, lesion area determination was made and expressed as a percentage determination of the total area of each individual subcrown internode. After 3-4 days of incubation, microscopically the number of infected quadrants (I) was determined for H. sativum. This was done by visually bisecting each subcrown vertically and horizontally into 4 equal parts (quadrants). Then ratings were made on scale of 0-4 with 0= no quadrants with H. sativum growing, 1= one infected quadrant with H. sativum growing, 4= all quadrants showing growth of H. sativum, and so on. Colony area (CA) was measured as the percentage of the total area of subcrown internode and medium around subcrown internode which was colonized by H. sativum after 3-4 days of incubation.

Analyses of Soil Chemical and Physical Properties

Chemical properties of individual soil samples were determined by

TABLE I

COLOR INDEXING OF SUBCROWN INTERNODES

Grade	Description
0	Normal (clear, no lesion)
1	Light Brown
2	Brown
3	Dark Brown
4	Light Black or Dark Grey
5	Black

the Soil and Water Service Laboratory of Oklahoma State University, Agronomy Department. About 500 g of each soil sample were analyzed for soil reactions, i.e., pH and buffer index (B.I.), nitrate-nitrogen, available phosphous and potassium, and % of organic matter.

Soil types and texture classes were determined in our laboratory with a hydrometer following those procedures of Nesmith and Averre (57). The relationship between soil moisture content (%) and soil total water potential (bars) in each soil type was determined with a HR-33 T dew point microvoltmeter. The moisture contents of 3 samples from the depth of 0-10 cm in each field were adjusted to 1, 3, 5, 7, 7.5, 8, 8.5, 9, and 10 % moisture contents prior to incubation. Soil moisture contents were calculated according to the formula:

$$\% \text{ soil moisture} = \frac{Mw}{Ms} \times 100$$

where Mw is the mass of soil water and Ms is the mass of soil particles (43). Each moistened soil with a soil moisture and temperature monitoring probe was put into 1.5x15 cm test tubes and filled to within 3 cm from the lip of the test tube. The test tubes were then sealed with "parafilm", incubated in 25 C water bath, and allowed to equilibrate for 24 hours . Dew point (DP) readings in microvolts in the soil of each test tube were taken and converted to total water potential according to the formula:

$$\text{Total water potential (bars)} = \frac{\text{DP (microvolts)}}{\text{-0.75 (microvolts/bar)}} \text{ (44).}$$

Evaluating Soil Biosuppression of *H. sativum*

Comparisons of soil suppressive characters in two soils were made

by comparing the effects of increasing inoculum densities on infection and disease severity of seedlings in sterilized and nonsterilized soils.

Individual soil samples collected from 3 profiles of each field at two locations (Nash and Cherokee) were equally divided and received one of the following treatments: fumigated with methyl bromide at rate of 1.5 lbs/fumigation, steam-sterilized in autoclave at 121 C, 1.1 atms, for 30 minutes, and nontreated (natural). Each soil treatment was artificially infested with H. sativum by adding suitable amount of conidial suspension to give rates of 0, 10, 100, and 1000 conidia/g soil (on dry weight basis); each treatment was replicated 3 times. Infested soils were subjected to the bioassay as previously described.

Transfer of Suppressive Factors to Conducive Soils

Natural soils collected from the depth of 0-10 cm were used in this experiment. Natural soil from Nash was added to steam sterilized soil of same location, and to natural soil of Cherokee at rates of 0, 1, 10, 50, 90, and 100 % (on dry weight basis), and uniformly mixed in plastic bags. The mixed soils were artificially infested with conidial suspension of H. sativum at rates of 0, 10, and 100 conidia/g soil (on dry weight basis), and were immediately subjected to bioassay as previously described.

Effects of Temperatures and Soil Suppression on Survival of H. sativum in Soil in the Absence of Host Plant

Natural soils collected at depth of 0-10 cm at Nash and Cherokee

fields, and steam sterilized soil from same depth of Nash (incubated at 20 C only) were infested with a conidial suspension of H. sativum at rate of 100 conidia/g soil (on dry weight basis), put in plastic bags (each plastic bag then was sealed with a cotton plug), and incubated at temperatures of 0, 20, 25, 30, and 40 C. Before incubation the soil moisture contents were adjusted to 5 and 7.5 % for Cherokee and Nash soils, respectively, which resulted in both soils having an equal soil total water potential of about -1 to -2 bars. During incubation the soil moisture contents were periodically (about every two weeks) readjusted to preincubation levels by weighing and adding suitable amounts of sterilized water. Survival of H. sativum conidia in soils were determined by bioassay as previously described at the end of 0, 30, 60, and 120 days of incubation.

Estimates of Soil Total Microflora

Soil microbial populations were estimated by "Plate-Dilution Frequency Method" (38). Ten grams of each soil sample were placed in a 250-ml graduated Erlenmeyer flask. A 0.1-0.2 % solution of sterilized water agar was added so that a total volume of 100 ml was reached. The flask containing the suspension was shaken for several minutes; one ml of this suspension was immediately drawn into a sterilized 1-ml pipette and transferred into 9 ml of 0.1-0.2 % sterilized water agar in another test tube. This procedure was repeated several times until a final dilution of $1:10^8$ was obtained. Dilution levels from $1:10^2$ to $1:10^7$ and from $1:10^3$ to $1:10^8$ were used for estimation of total fungi and total bacteria and actinomycetes, respectively. Eight replicates

of 0.01 ml sample of each dilution level were spotted onto $\frac{1}{2}$ of a Petri plate with appropriate growth medium; the other $\frac{1}{2}$ was similarly spotted with another dilution level as described by Harris and Sommers (38). Rose bengal-Streptomycin-Chloramphenicol (RBC) medium (40) and M-4 glucose medium (82) were used for culturing fungi and bacteria and actinomycetes, respectively. The inoculated plates were incubated at room temperature (21 ± 2 C) for 2-3 days; the number of positive responses of each dilution level were recorded and converted into most probable numbers of microbial population per gram of soil using the table provided by Harris and Sommers (38).

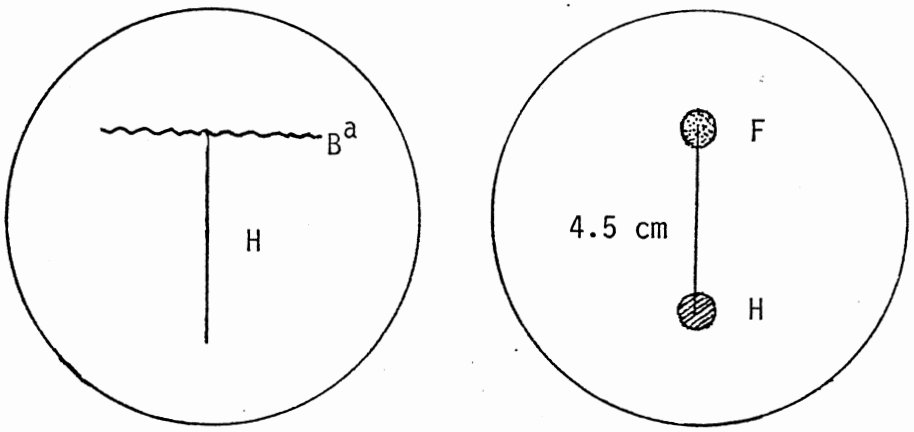
Isolation of Candidate Antagonists

Candidate antagonists were isolated from the soil which was collected from the depth of 0-10 cm at Nash field and had been subjected to one crop (14 days) of wheat in laboratory just prior to the isolating procedure. Ten grams of this soil (on dry weight basis) were used to prepare a 10-fold dilution series following the procedure as described previously. The media used for isolating soil fungi, and bacteria and actinomycetes were the same as those used for estimating total soil microbial populations in this report. Aliquots from dilution levels of $1:10^3$-$1:10^5$ and $1:10^5$-$1:10^7$ were inoculated on solid media to isolate potential antagonistic fungi, and bacteria and actinomycetes, respectively. One ml of each dilution level was transferred onto appropriate media which was poured into Petri dishes on a level surface and allowed to harden overnight, and partially air-dried at room temperature for 2-3 days prior to inoculation. Isolates were randomly selected from

inoculated plates and transferred to slants of media in test tubes. Potato dextrose agar (PDA) (88) and nutrient agar (NA) (88) were used for pure cultures of fungi and bacteria and actinomycetes, respectively.

Microorganisms isolated from soil-dilution plates were then tested individually for antagonism to H. sativum in pure culture. Both H. sativum and a potential antagonistic fungus were inoculated on the surface of modified V-8 juice agar medium (appendix) in Petri dishes as shown in Figure 1. Each potential antagonistic bacterium or actinomycetes was streaked on modified V-8 juice agar at the periphery of Petri dish; the conidial suspension of H. sativum was then streaked at the right angle to the original streak of the potential antagonistic bacterium or actinomycetes one day later (Figure 1). Inoculated plates were incubated at 25 C for 10 days and checked periodically during the incubation period. At the end of the incubation period, production of an inhibition zone between H. sativum and the antagonist was recorded.

Figure 1. Diagram of the technique used for testing antagonism of soil microorganisms to *H. sativum*.



[a] B -- Soil Bacteria of Actinomycetes

F -- Soil Fungi

H -- H. sativum

CHAPTER IV

RESULTS

Chemical and Physical Properties of Nash and Cherokee Soils by Depth

Some of the chemical and physical properties of Nash and Cherokee soils are listed in Table II by depth. With respect to chemical properties, the pH values ranged from 5.2 to 5.9 and from 5.0 to 5.4 among three soil depths for Nash and Cherokee, respectively. Similarly, the buffer index values ranged from 6.9 to 7.1 and from 7.2 to 7.4 among soil depths for Nash and Cherokee, respectively. Thus the soils did not differ greatly with respect to pH and buffer index values. In general, the nitrate-nitrogen, the available phosphorus and potassium trends were similar among soil depths in each soil type. However, the amounts of available potassium in the soil from Nash were 2-3 fold higher than those of the Cherokee's. The greatest observed difference between the soil types was in the percent organic matter which ranged from 0.9 to 1.2 and from 0.2 to 0.3 for Nash and Cherokee, respectively. Thus Nash soil had a 3-6 fold greater organic matter content than Cherokee soil.

With respect to soil physical properties, the sand, silt, and clay percentages ranged from about 20, 60, 20, and 90, 5, 5 for Nash and Cherokee, respectively. Although there were no great differences

TABLE II

CHEMICAL AND PHYSICAL PROPERTIES OF NASH AND CHEROKEE SOILS

	LOCATION					
	Nash			Cherokee		
	Soil Depth Increment (cm)			Soil Depth Increment (cm)		
	0-10	10-20	20-30	0-10	10-20	20-30
CHEMICAL						
pH	5.4	5.2	5.9	5.0	5.1	5.4
Buffer Index	6.9	7.0	7.1	7.3	7.4	7.2
Nitrate-Nitrogen	13.45[a]	25.33	19.05	25.78	20.18	12.33
Available Phosphorus	59.40[a]	69.49	30.26	79.58	53.80	49.32
Available Potassium	750.96[a]	722.94	359.79	212.96	102.00	127.78
Organic Matter (%)	1.2	1.2	0.9	0.3	0.2	0.3
PHYSICAL (Soil Texture)						
Sand (%)	21.90	22.92	16.74	89.79	89.58	87.09
Silt (%)	64.23	63.15	66.20	4.67	5.09	6.43
Clay (%)	13.87	13.93	17.24	5.54	5.33	6.48
Classification	Silty Loam	Silty Loam	Silty Loam	Sand	Sand	Sand

[a] Kilograms per hectare (kg/ha)

in soil texture among soil depths of each soil type, the soils were greatly different with respect to their particle composition and were classified as silty loam and sand for Nash and Cherokee, respectively. These physical differences were further contrasted in terms of standard curve determinations of soil water potential vs. soil moisture content. As shown in Figure 2, the response curves of soil water potential to soil moisture content of both soil types were not the same. Water potential of soil from Nash (silty loam) declined gradually while that of soil from Cherokee (sand) declined much faster as the soil moisture content decreased from 10 to 1 %. These curves are a direct reflection of the soil physical property differences in that the Nash with higher clay content can retain water more tenaciously than can the Cherokee with a higher percent sand content.

Characters of Soil Suppression of *H. sativum* in Nash and Cherokee Soils

As shown in Figures 3-6, soil suppressive differences were characterized as preincubation (color indexing and percent lesion area) and postincubation (infected quadrants and percent colony area) disease indices as the inoculum density was increased from 0 to 1000 conidia/g soil in Nash and Cherokee soils. Within each soil type and by soil depth, the treatments were steam sterilized (ST), methyl bromide treated (MB), and natural (NT) nontreated soils. Regardless of the indexes used, as the inoculum level increased there was a corresponding increase in preincubation subcrown discoloration and percent lesion area, and in postincubation infected quadrant index and colony area index irrespective

Figure 2. Relationship between soil moisture content and soil total water potential in two soil types (Nash and Cherokee).

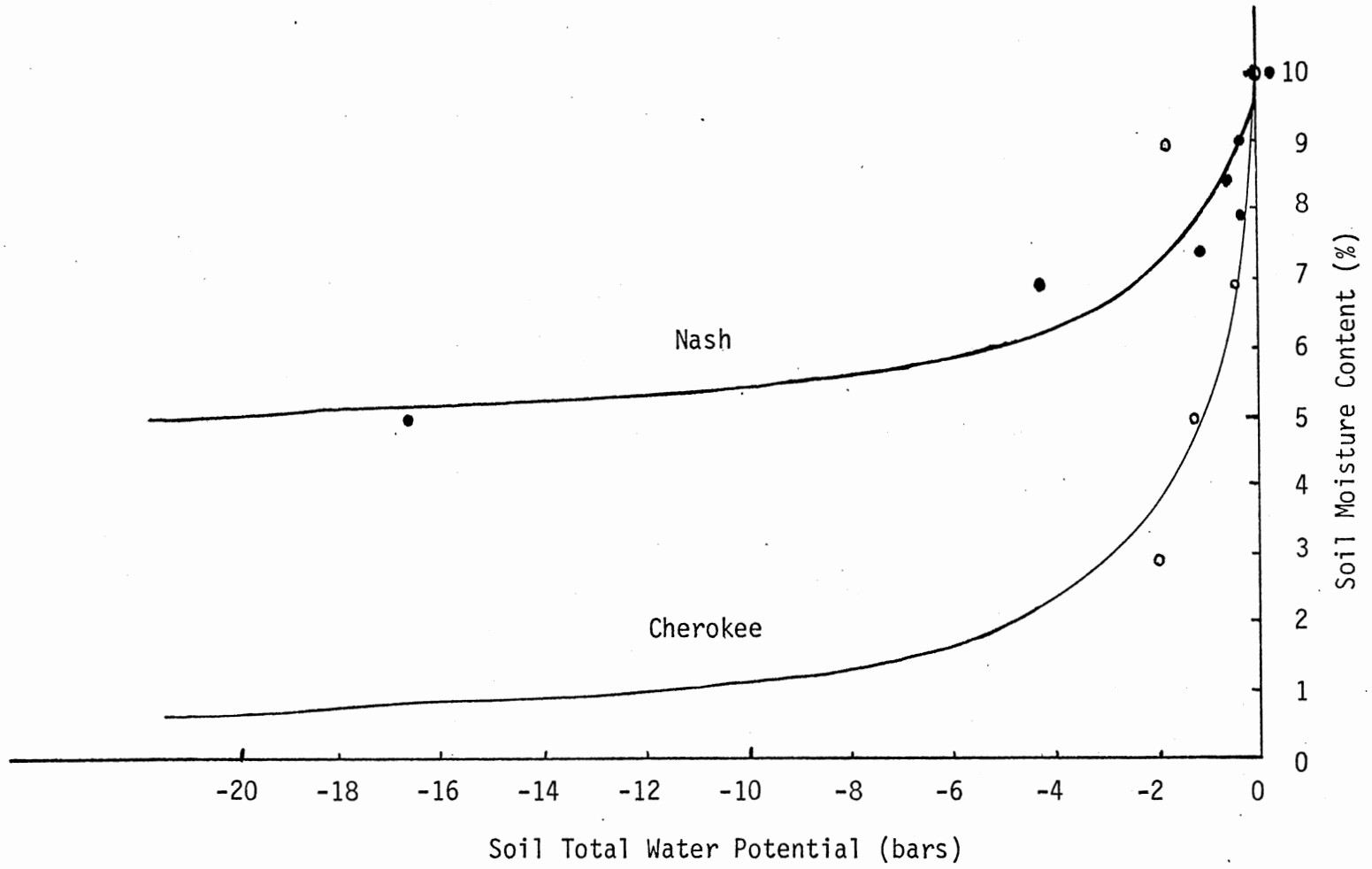

Nash
Cherokee
10
9
8
7
6
5
4
3
2
1
0
Soil Moisture Content (%)
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
Soil Total Water Potential (bars)

Figure 3. Preincubation Color Indexes of TAM 101 wheat seedling subcrown internodes grown in biocidal agent treated and nontreated soils of Nash and Cherokee. The soils were collected at depths of 0-10, 10-20, and 20-30 cm at each location, and each soil sample was subjected to the treatments of steam air (ST) at 121 C for 30 minutes, methyl bromide (MB), and nontreated natural (NT), and infested with H. sativum conidia at rates of 0, 10, 100, and 1000 conidia/g soil (on dry weight basis).

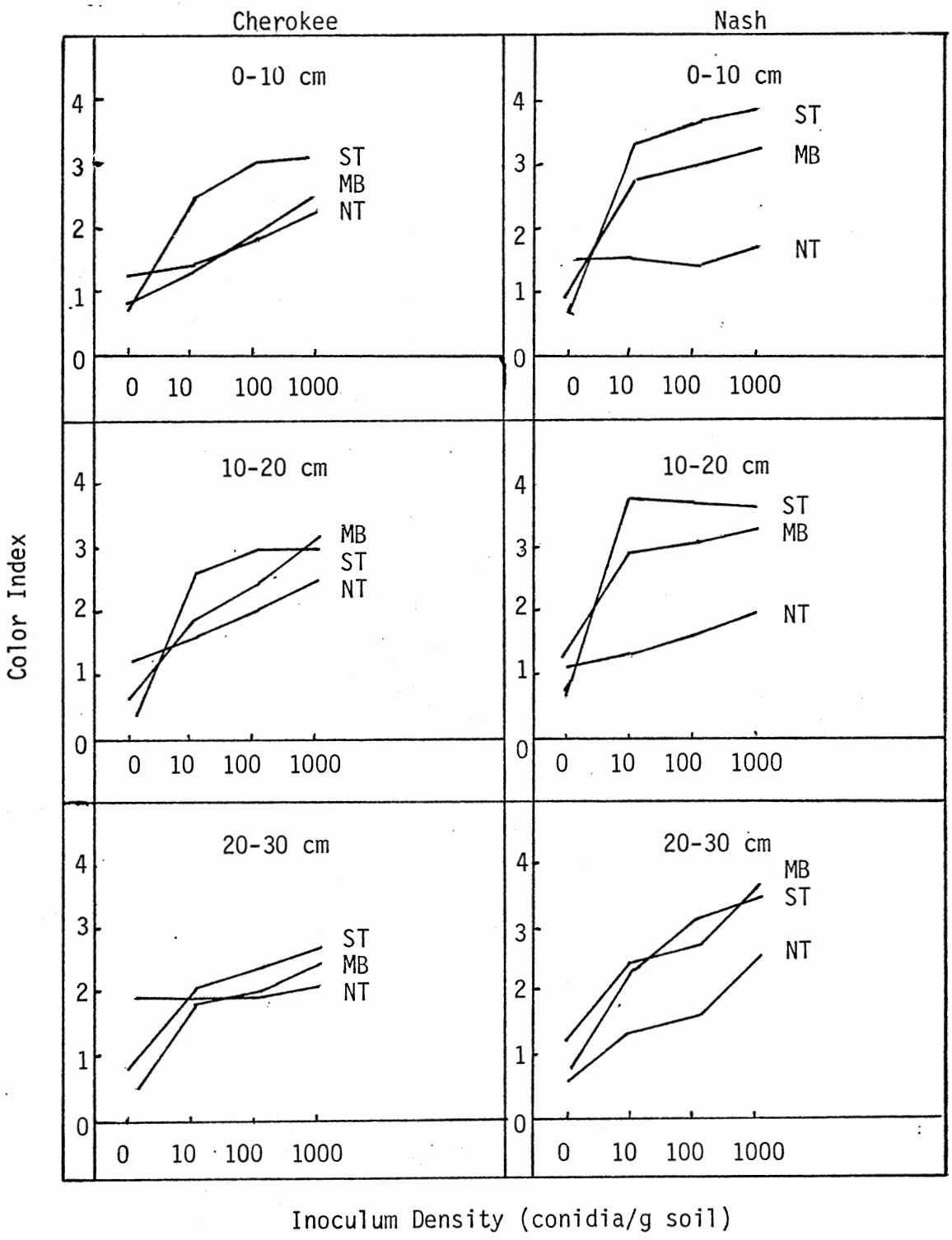
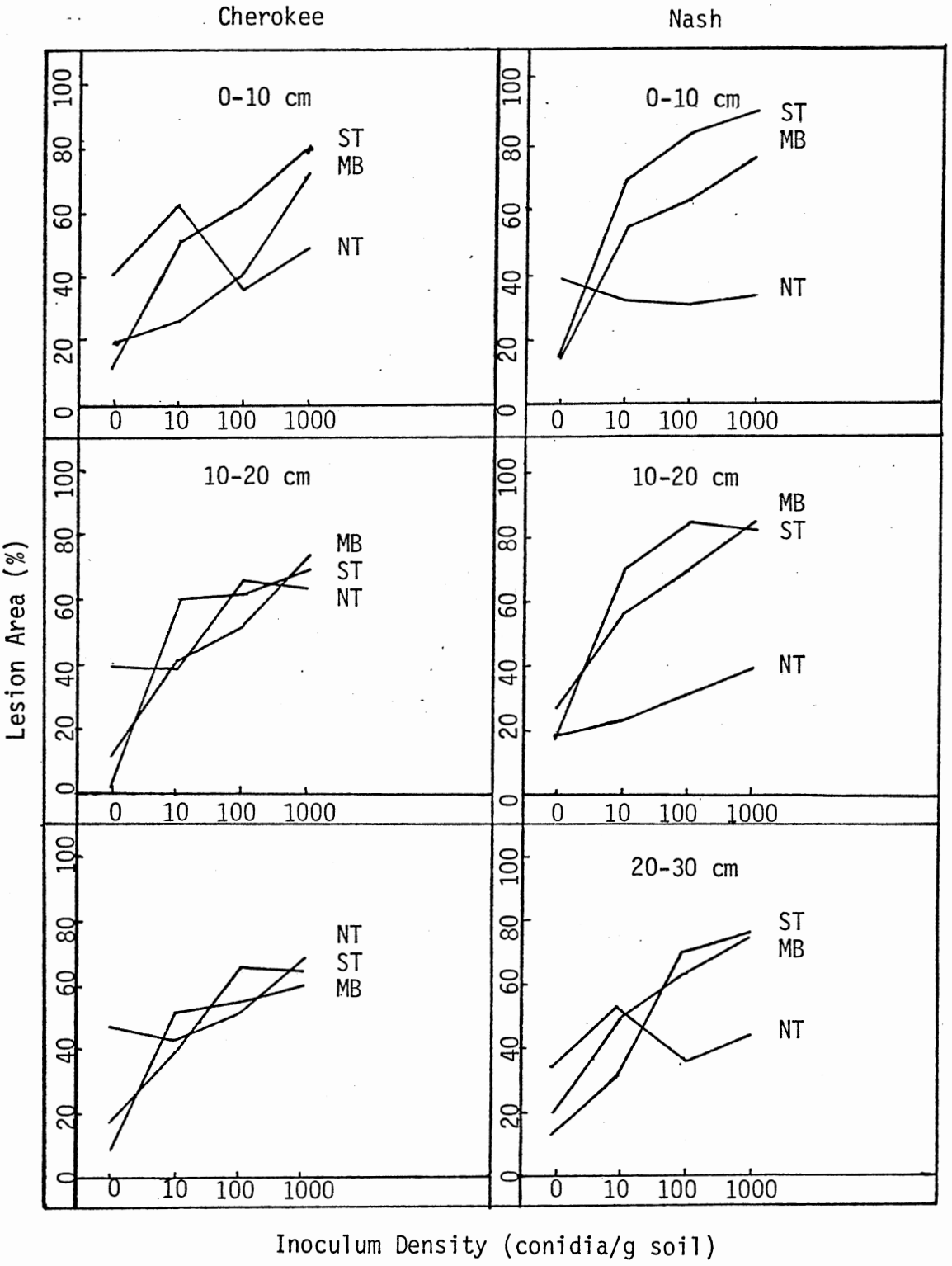

Cherokee
Nash
0-10 cm
10-20 cm
20-30 cm
ST
MB
NT
Color Index
0 10 100 1000
Inoculum Density (conidia/g soil)

Figure 4. Preincubation Lesion Area of TAM 101 wheat seedling subcrown internodes grown in biocidal agent treated and nontreated soils of Nash and Cherokee. The soils were collected at depths of 0-10, 10-20, and 20-30 cm at each location, and each soil sample was subjected to the treatments of steam air (ST) at 121 C for 30 minutes, methyl bromide (MB), and nontreated natural (NT), and infested with H. sativum conidia at rates of 0, 10, 100, and 1000 conidia/g soil (on dry weight basis).


Cherokee
Nash
0-10 cm
10-20 cm
20-30 cm
ST
MB
NT
Lesion Area (%)
0
20
40
60
80
100
0
10
100
1000
Inoculum Density (conidia/g soil)

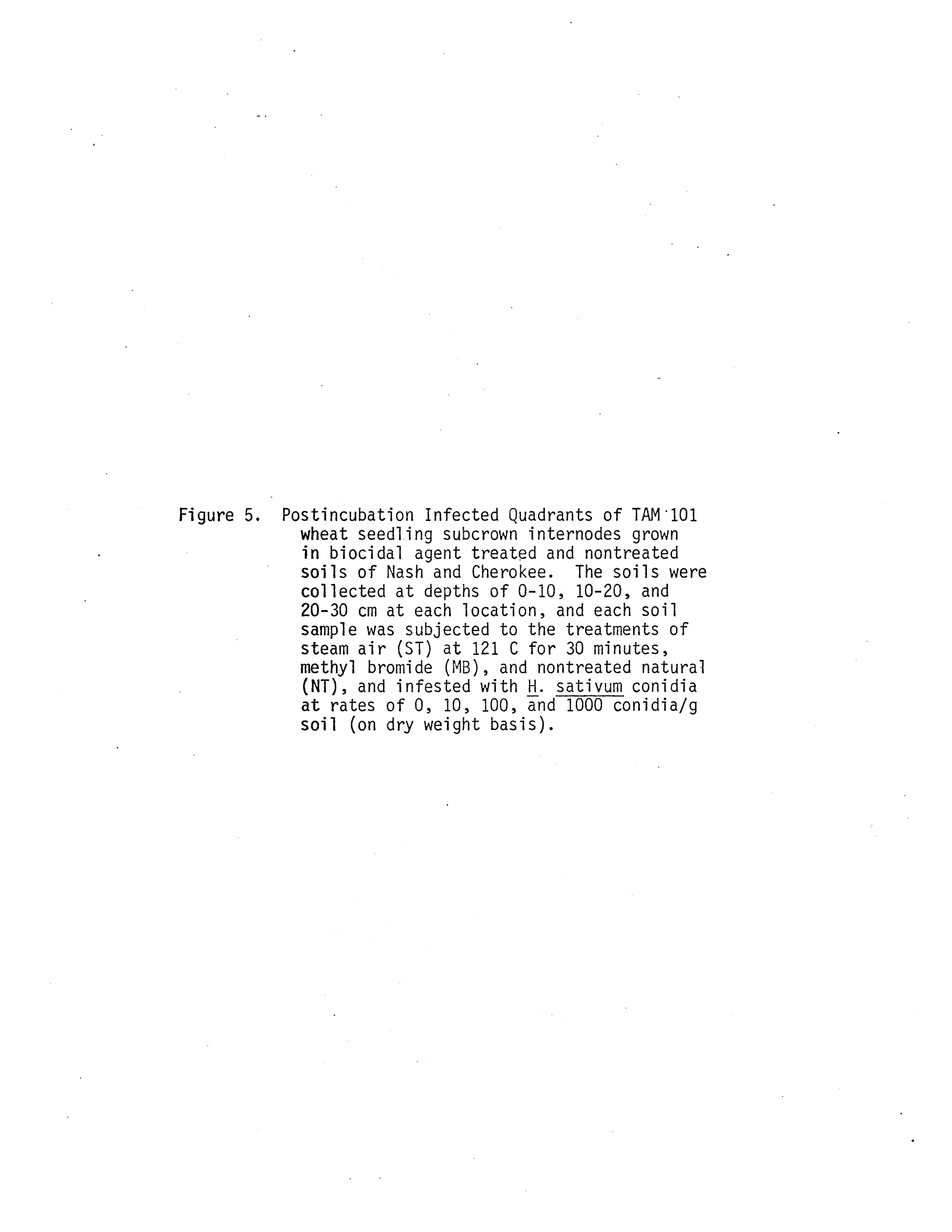


Figure 5. Postincubation Infected Quadrants of TAM 101 wheat seedling subcrown internodes grown in biocidal agent treated and nontreated soils of Nash and Cherokee. The soils were collected at depths of 0-10, 10-20, and 20-30 cm at each location, and each soil sample was subjected to the treatments of steam air (ST) at 121 C for 30 minutes, methyl bromide (MB), and nontreated natural (NT), and infested with H. sativum conidia at rates of 0, 10, 100, and 1000 conidia/g soil (on dry weight basis).

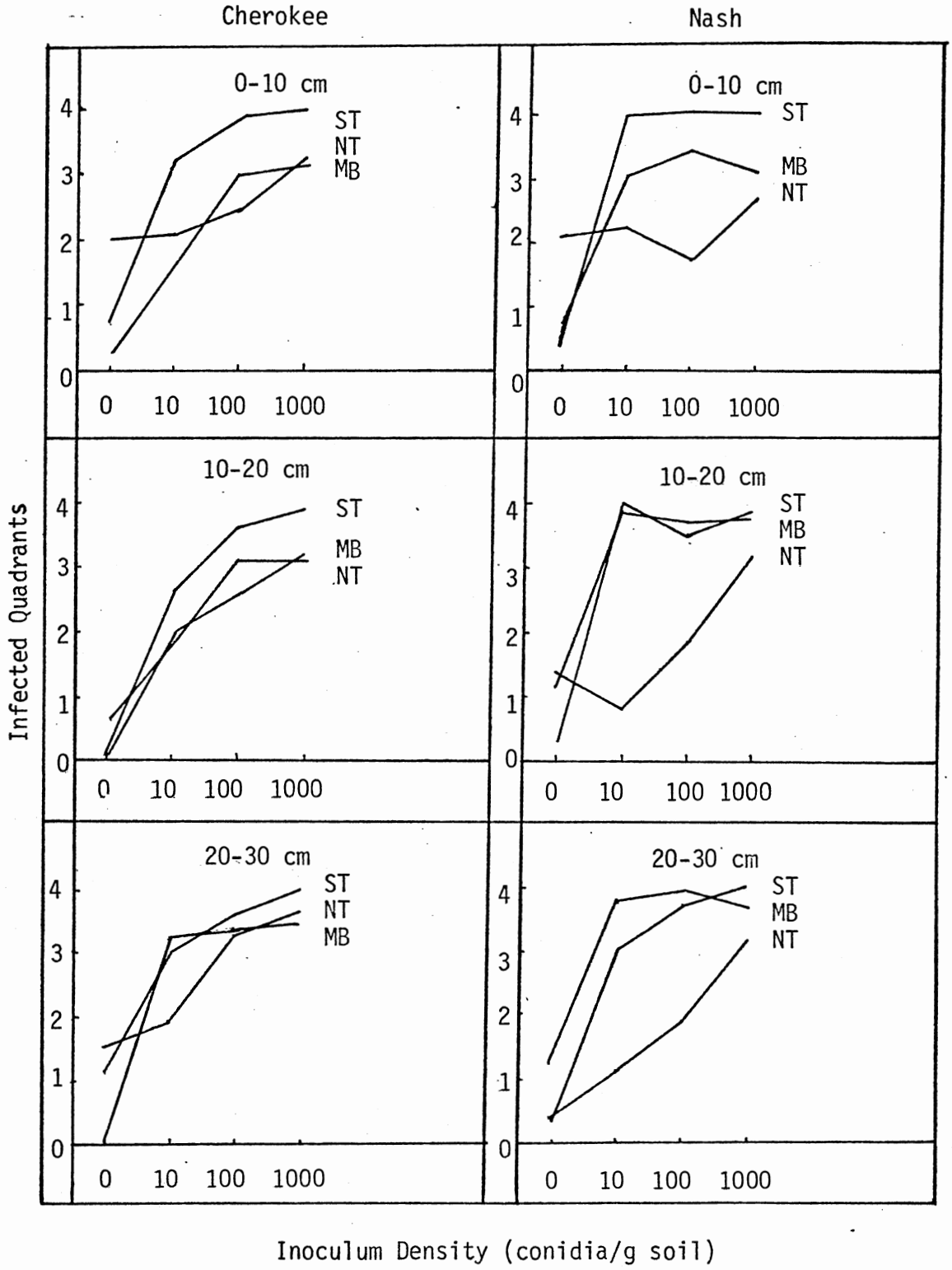
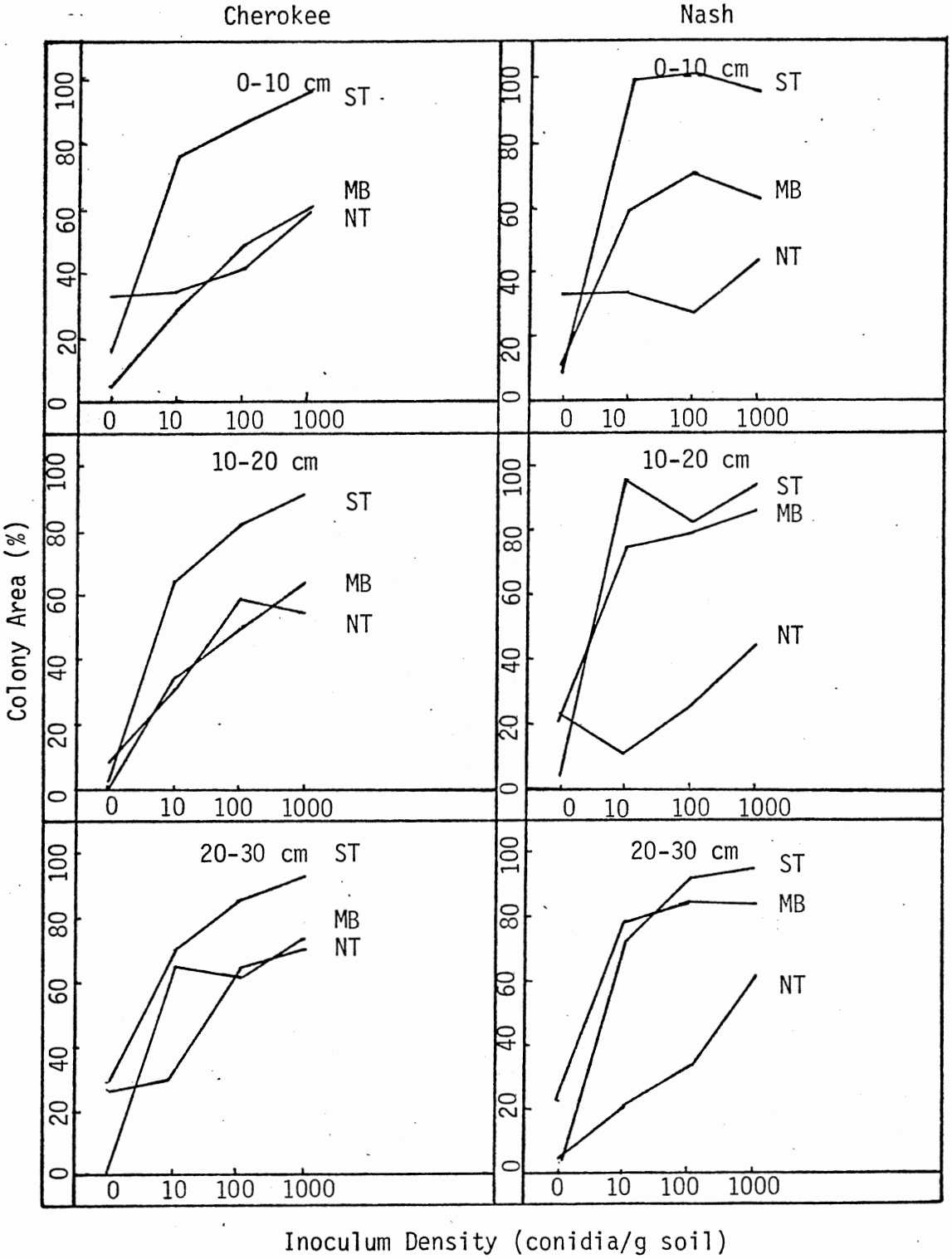

Cherokee
Nash
0-10 cm
10-20 cm
20-30 cm
ST
NT
MB
Infected Quadrants
0
1
2
3
4
0 10 100 1000
Inoculum Density (conidia/g soil)

Figure 6. Postincubation Colony Area of TAM 101 wheat seedling subcrown internodes grown in biocidal agent treated and nontreated soils of Nash and Cherokee. The soils were collected at depths of 0-10, 10-20, and 20-30 cm at each location, and each soil sample was subjected to the treatments of steam air (ST) at 121 C for 30 minutes, methyl bromide (MB), and nontreated natural (NT), and infested with H. sativum conidia at rates of 0, 10, 100, and 1000 conidia/g soil (on dry weight basis).

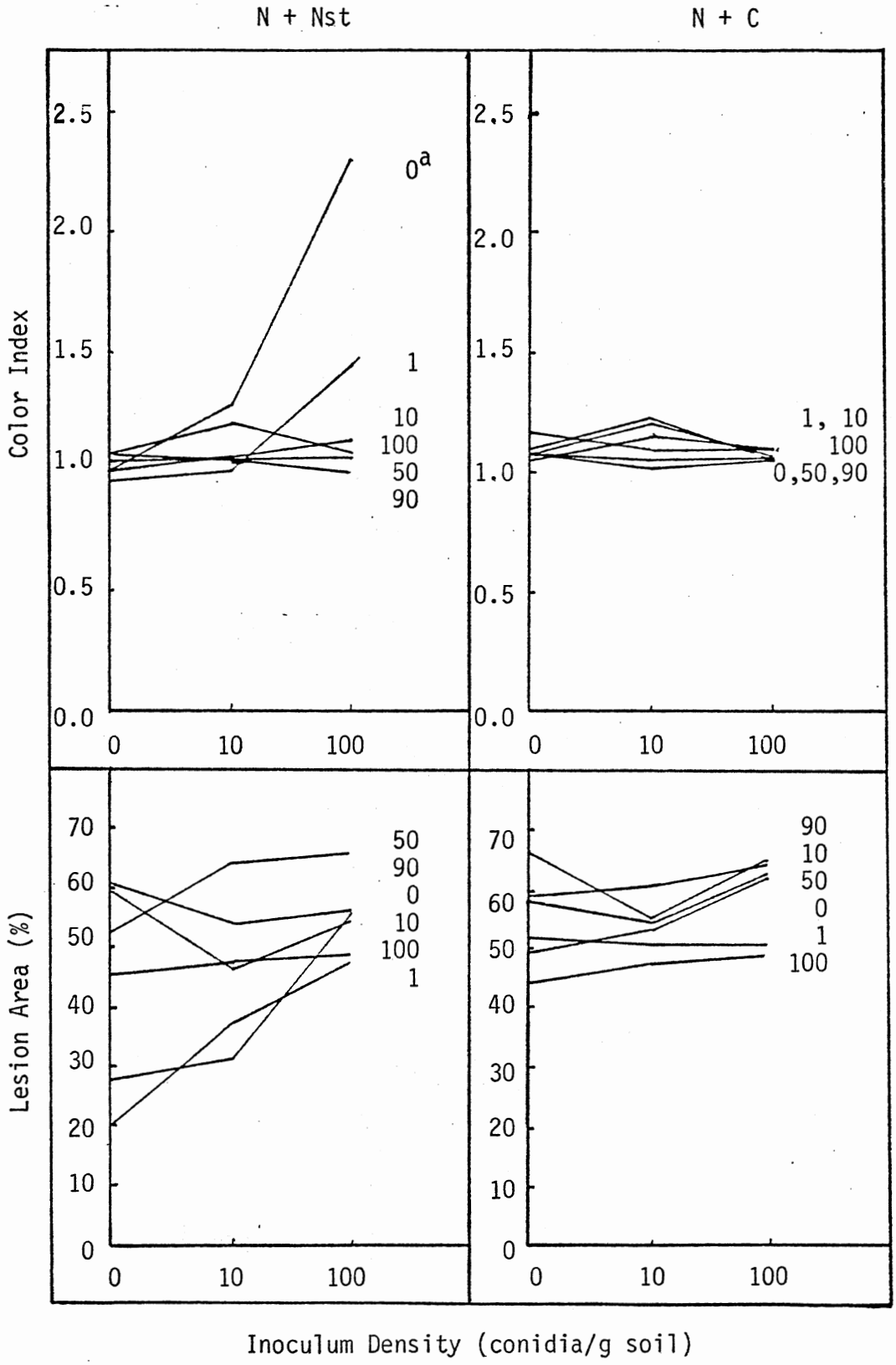

Cherokee
Nash
0-10 cm
10-20 cm
20-30 cm
ST
MB
NT
Colony Area (%)
0
20
40
60
80
100
0
10
100
1000
Inoculum Density (conidia/g soil)

of soil type and/or soil treatment. The disease index responses to increasing inoculum density were similar regardless of soil depth and will be discussed collectively with regard to soil treatment differences and soil type differences only. In the Cherokee soil, the disease indexes increased similarly regardless of soil treatments, i.e., the natural soil response to inoculum level increase was similar to steam sterilized and methyl bromide treated soils. In the Nash soil, the steam sterilized and methyl bromide treated soils reacted the same to increasing inoculum density; however, in almost every case, the natural soil component with the exceptions of the inoculum levels of 0 and 1000 conidia/g soil in some instance, the indexes were significantly less than the indexes for steam sterilized and methyl bromide treated soils.

Transfer of Suppressive Factors from Nash Natural Soil to Nash Sterilized and Cherokee Natural Soils

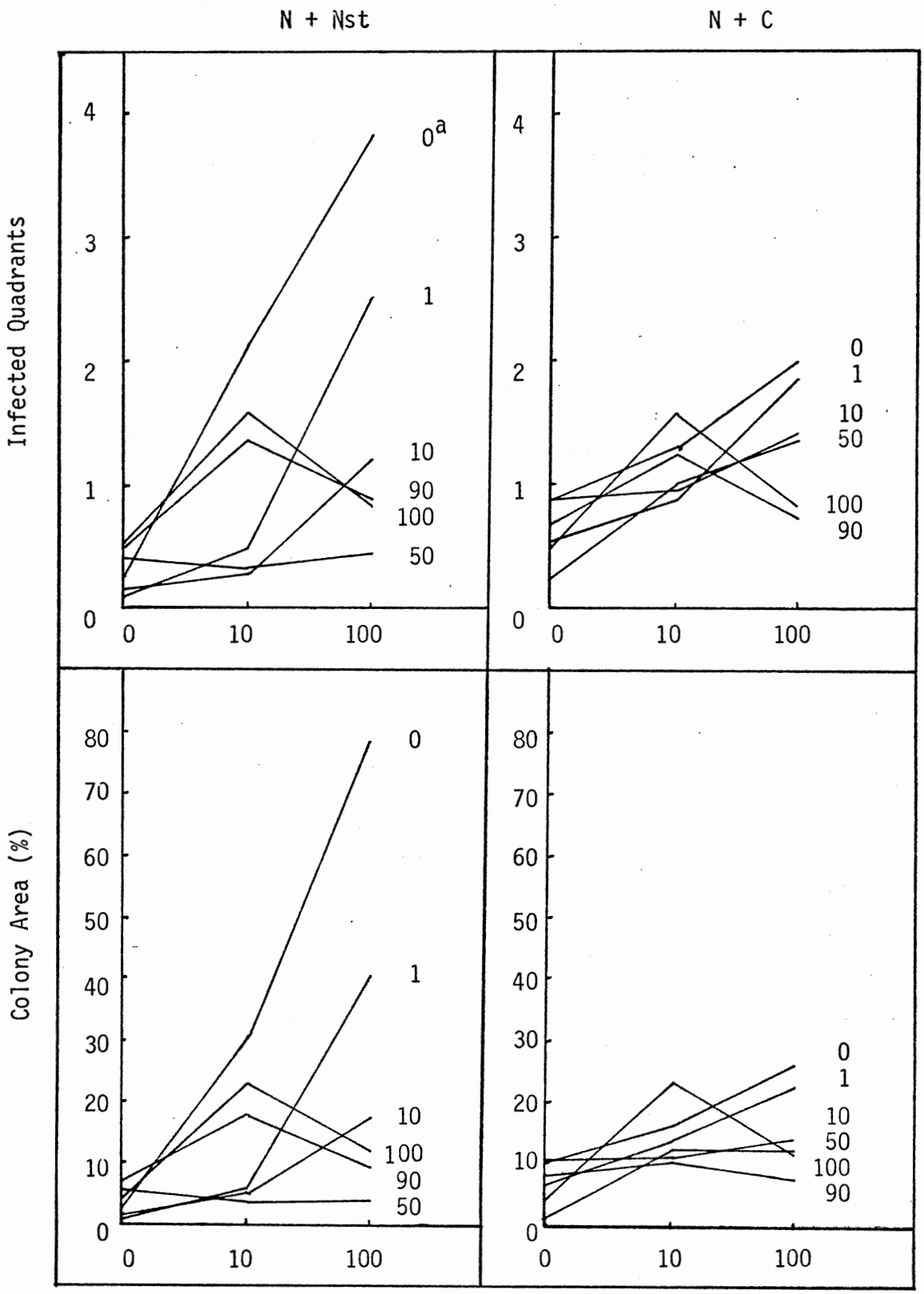
As shown in Figures 7 and 8, Nash natural soil was added in percentage increments of 1, 10, 50, and 90 % (w/w, dry weight basis) to Nash sterilized soil and Cherokee natural soil. The preincubation and post-incubation index responses were evaluated at 3 inoculum density levels (0, 10, and 100 conidia/g soil) for each soil mixture. The zero percentage represented Nash sterilized check and Cherokee natural check in respective cases, and the 100 percentage represented the response in Nash natural soil. With respect to Nash natural-Nash sterilized soil mixtures, in general, the disease indexes increased as the inoculum density increased. The only significant effects, however, were at the

Figure 7. Preincubation Color Indexes and Lesion Area of TAM 101 wheat seedling subcrown internodes grown in mixed soils. The soil mixtures were made by adding Nash natural soil (N) to Nash sterilized soil (Nst) or to Cherokee natural soil (C) at rates of 0, 1, 10, 50, 90, and 100 % (w/w), mixed uniformly, and infested with H. sativum conidial suspension at rates of 0, 10, and 100 conidia/g soil (on dry weight basis).



[a] Percentage of Nash natural soil in soil mixtures.

Figure 8. Postincubation Infected Quadrants and Colony Area of TAM 101 wheat seedling subcrown internodes grown in mixed soils. The soil mixtures were made by adding Nash natural soil (N) to Nash sterilized soil (Nst) or to Cherokee natural soil (C) at rates of 0, 1, 10, 50, 90, and 100 % (w/w), mixed uniformly, and infested with H. sativum conidial suspension at rates of 0, 10, and 100 conidia/g soil (on dry weight basis).



[a] Percentage of Nash natural soil in soil mixtures.

0 and 1 % Nash natural soil levels at 100 conidia/g soil for preincubation color index and postincubation infected quadrants and colony area. Thus as the percentage of Nash natural soil increased in Nash natural-Nash sterilized soil mixtures the soil suppression decreased the response to increasing inoculum density. In the Cherokee natural soil there was little if any response with the addition of Nash natural soil to the increasing of inoculum density.

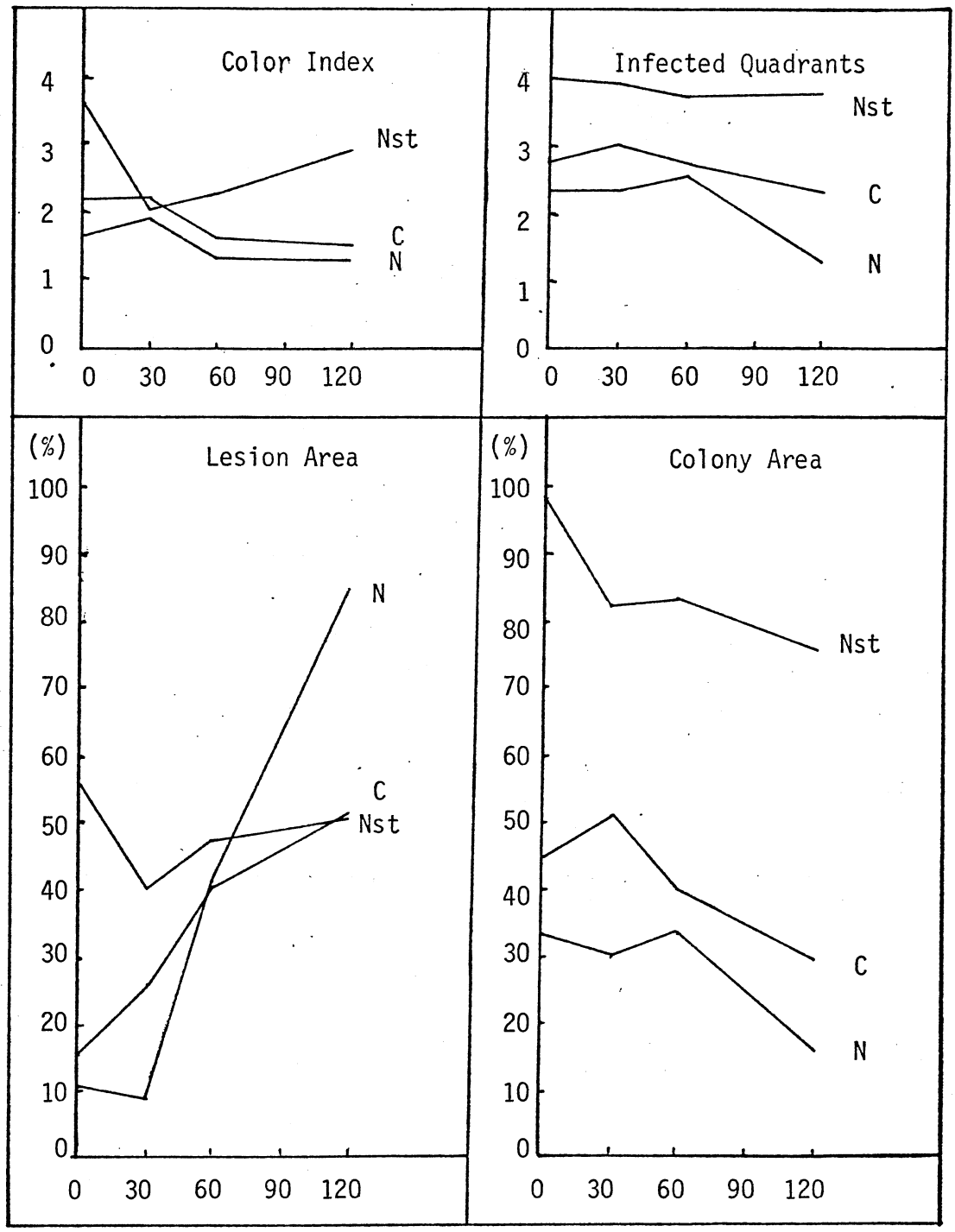
It seemed that the nonsterilized soil of Nash greatly decreased the infection of wheat seedlings by H. sativum when it was added to sterilized soil of the same source at the ratios of 0.1, 0.5, and 0.9 but did not greatly reduce the disease incidence when it was added to Cherokee natural soil as compared with 100 % of Cherokee natural soil.

Effects of Temperatures and Soil Suppression on Survival of H. sativum in Soil in the Absence of Host Plant

As affected by time and temperature, the amount of H. sativum conidia surviving in the soils was evaluated indirectly by bioassay method and measuring the pre- and postincubation disease indexes of wheat seedlings grown in the inoculum infested soils at the ends of 0, 30, 60, and 120 days of incubation.

Figure 9 shows the tendency of H. sativum conidia to survive in Nash natural, Cherokee natural, and Nash sterilized soils incubated at room temperature (21±2 C). Preincubation disease indexes suggested that the color indexes for Nash natural and Cherokee natural soils declined only slightly over 120 days of incubation. In the contrast,

Figure 9. Survival of H. sativum in Nash natural (N), Nash sterilized (Nst), and Cherokee natural (C) soils. The soils were infested with H. sativum conidia at rate of 100 conidia/g soil (on dry weight basis) and incubated at room temperature (21 ± 2 C). The viability of conidia was measured indirectly by preincubation Color Indexes and Lesion Area, and postincubation Infected Quadrants and Colony Area in the bioassay experiments at the end of 0, 30, 60, and 120 days of incubation period.


Color Index
Nst
C
N
Infected Quadrants
Nst
C
N
(%)
Lesion Area
N
C
Nst
(%)
Colony Area
Nst
C
N
0 30 60 90 120
Incubation Period (days)

the lesion area index increased rapidly in both natural soils over 120 days of incubation period, yet it was more or less constant in Nash sterilized soil. Postincubation disease indexes decreased gradually in both natural soils with the greatest decline in Nash natural soils. Colony area index declined most rapidly in the natural soils suggesting a reduction in viability of H. sativum conidia. The inverse relationship in preincubation lesion area index and postincubation colony area index suggested that H. sativum is being displaced by some other lesioning entities, however.

Figures 10 and 11 show the effects of temperature on survival of H. sativum conidia in Nash and Cherokee natural soils. Preincubation color index declined more rapidly as the incubation temperatures increased. Lesion area again increased more rapidly at temperatures below 40 C in both natural soils. For the postincubation disease indexes, the infected quadrants and colony area declined rapidly as temperatures increased over 120 days of incubation period in both natural soils. For a specific incubation temperature, the tendency of reducing conidial viability when incubated in two soil types seemed to be only slightly different during the experimental period.

Total Counts of Microflora of Nash and Cherokee Soils

The estimates of total fungi and total bacteria and actinomycetes in 3 soil depths of each field were listed in Table III. In general, total counts of soil microorganisms of Nash soil were much higher than those of Cherokee soil. The numbers of soil microorganisms declined

Figure 10. Effects of incubation temperatures (0, 20, 25, 30, and 40 C) and soil types (Nash and Cherokee) on survival of H. sativum conidia as measured by preincubation Color Indexes and Lesion Area in the bioassay experiments. The soils were infested with conidia at rate of 100 conidia/g soil (on dry weight basis) and incubated at various temperatures for 0, 30, 60, and 120 days.

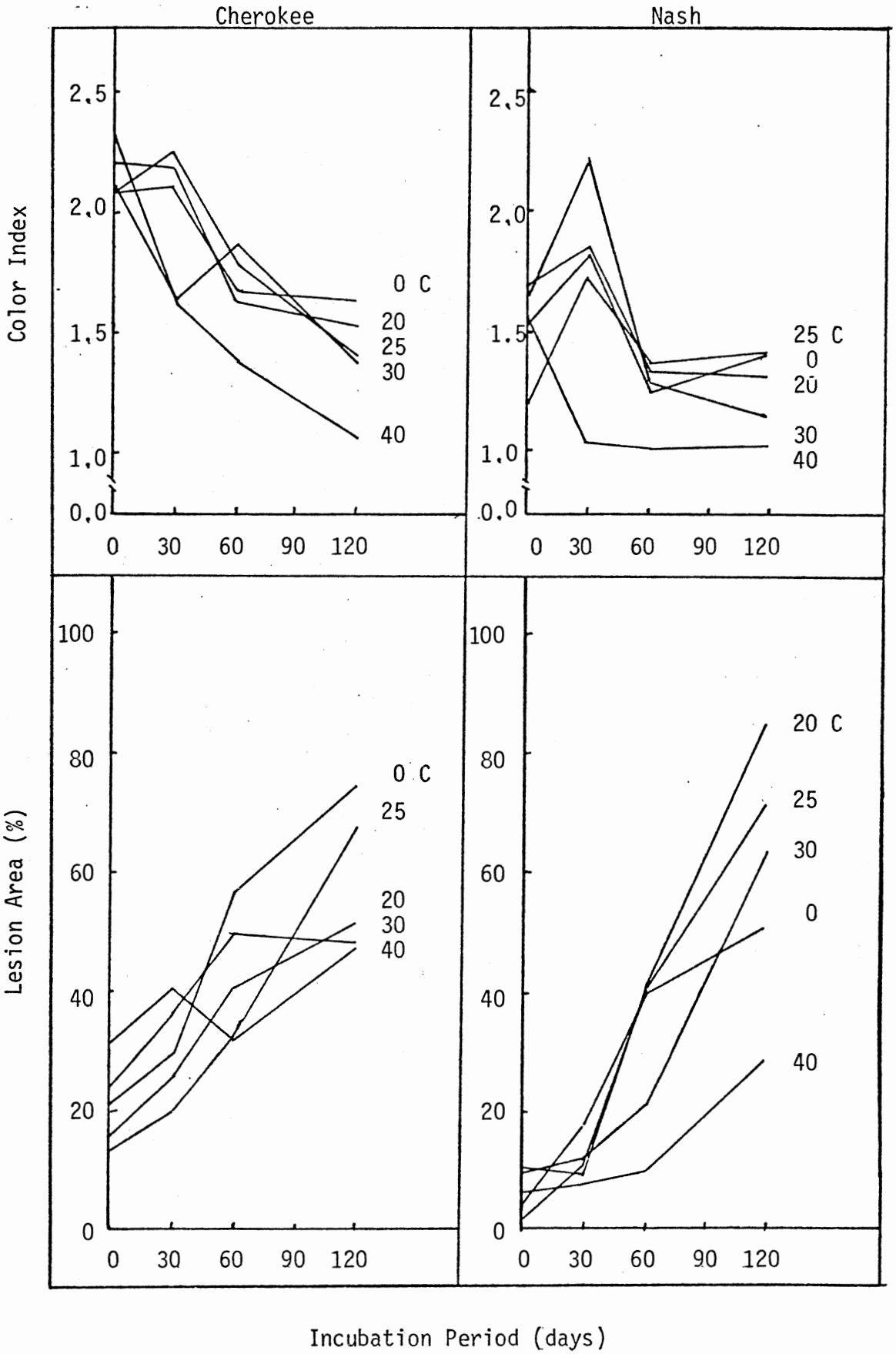
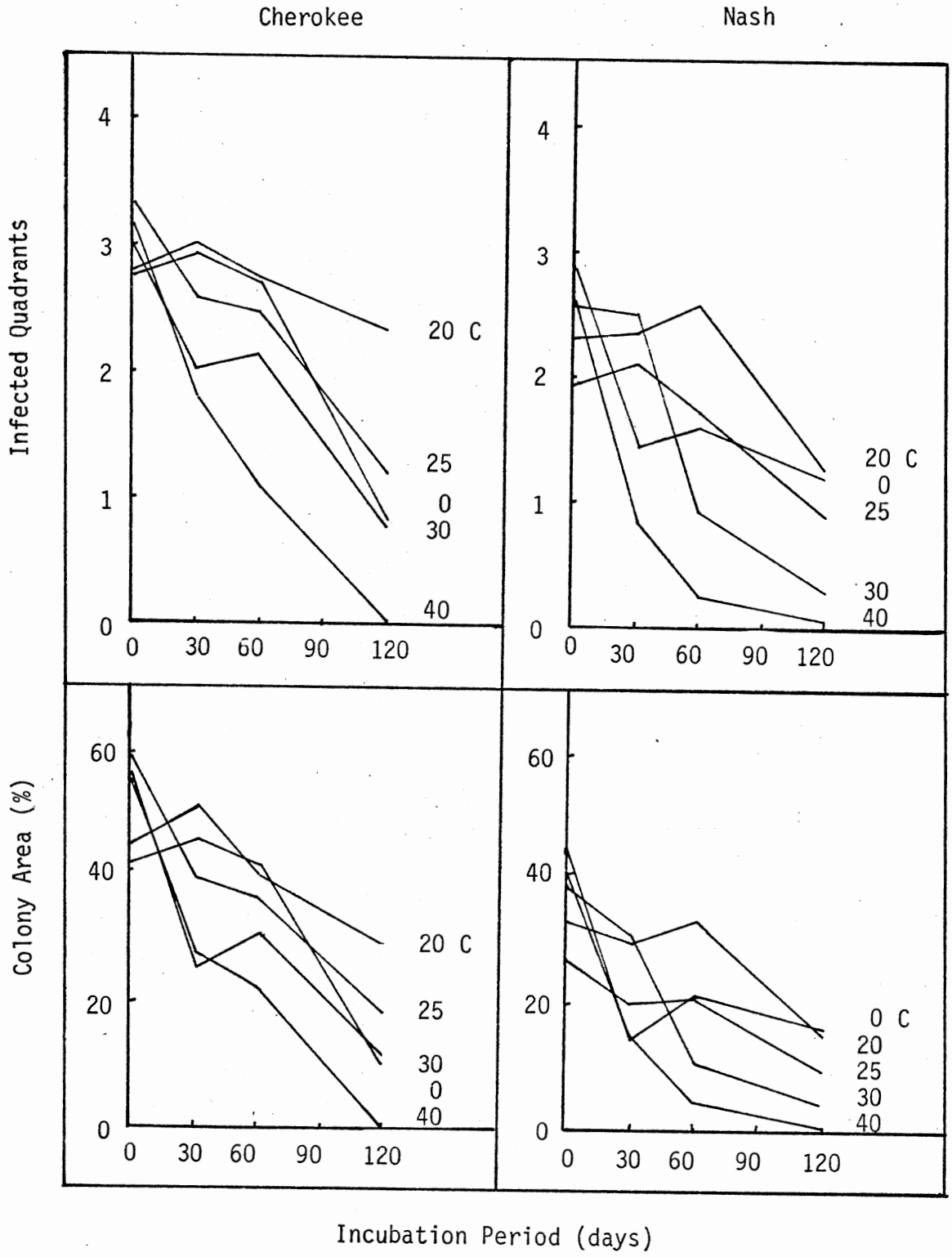

Cherokee
Nash
Color Index
2.5
2.0
1.5
1.0
0.0
0 C
20
25
30
40
25 C
0
20
30
40
0 30 60 90 120
Lesion Area (%)
100
80
60
40
20
0
0 C
25
20
30
40
20 C
25
30
0
40
Incubation Period (days)

Figure 11. Effects of incubation temperatures (0, 20, 25, 30, and 40 C) and soil types (Nash and Cherokee) on survival of H. sativum conidia as measured by postincubation Infected Quadrants and Colony Area in the bioassay experiments. The soils were infested with conidia at rate of 100 conidia/g soil (on dry weight basis) and incubated at various temperatures for 0, 30, 60, and 120 days.


Cherokee
Nash
Infected Quadrants
Colony Area (%)
Incubation Period (days)
20 C
25
0
30
40
0 C
20
25
30
40
0
30
60
90
120

as the soil depth increased in the field of Nash. At Cherokee, the numbers of soil microorganisms were much lower and more variable among soil depths than those of Nash. These data tend to agree with the soil test data in that Nash soils were more fertile and can support much more microorganisms than those of Cherokee.

Isolation of Antagonists

Candidate antagonists were randomly isolated from the plates inoculated with soil dilutions of the soil collected from depth of 0-10 cm in the field of Nash. Sixty-eight isolates of bacteria and actinomycetes were obtained; twenty of these showed antagonistic to H. sativum when inoculated simultaneously with H. sativum in one plate. Fifty isolates of fungi were obtained; none of these were significantly antagonistic to H. sativum in vitro.

TABLE III

ESTIMATED TOTAL COUNTS OF MICROORGANISMS PER GRAM OF SOIL IN NASH AND CHEROKEE SOILS

Soil Depth (cm)	Nash		Cherokee	
	Total Bacteria and Actinomycetes	Total Fungi	Total Bacteria and Actinomycetes	Total Fungi
0-10	2.36×10^9	2.33×10^6	0.01×10^9	0.04×10^6
10-20	0.41×10^9	1.30×10^6	0.10×10^9	0.02×10^6
20-30	0.01×10^9	0.03×10^6	0.03×10^9	0.01×10^6

CHAPTER V

DISCUSSION

Seedling tests have been utilized for measuring the amount of effective inoculua in the soil (9). The severity of infection of seedling by H. sativum has been shown to be directly proportional to the amount of inoculum added to the soil in seedling tests (15, 70).

Of four variables recorded to grade the extent of seedling infection in this study, the preincubation color index and lesion area of subcrown internodes were not as reliable for quantifying infection by H. sativum as the postincubation infected quadrants and colony area. This is true because other pathogens, i.e., Fusarium spp., Pythium spp., and Rhizoctonia spp., etc. also could affect preincubation discoloration and lesion production on the subcrown internodes of wheat seedlings. However, the postincubation infected quadrants and colony area data were a more direct reflection of the presence of H. sativum.

Root rots of winter wheat in Oklahoma represent a disease complex that is very important to wheat production. The incidence of wheat root rot caused by H. sativum, a major pathogen of this disease complex, usually increases in conjunction with minimum tillage practices (unpublished data of Oklahoma Extension Service, 3). However, the preliminary investigations suggested that some soils were biologically suppressive in reducing wheat seedling infection by H. sativum (Singleton

and Russell personal communication, 1978). Thus in one field at Nash, Oklahoma, the soil exhibited naturally suppressive effects on H. sativum infection. In deed, other investigators have found that some natural soils were inhospitable to soil-borne plant pathogens such that the disease could not develop on the plants grown in these soils (2).

The results of this study showed that naturally occurring inocula of H. sativum were detected virtually in all soil samples collected from either Nash or Cherokee, Oklahoma. However, the soils collected from Nash showed a greater suppressive effect on seedling infection of wheat by H. sativum than those collected from Cherokee in bioassay experiments. The degree of infection and the severity of seedling disease increased more rapidly when inoculum levels increased in the soils of Cherokee than in those of Nash. The naturally occurring inocula of H. sativum also existed at all soil depths but the differences among the surveyed soil depths (0-10, 10-20, and 20-30 cm) were not significant. However, the suppressiveness of even the Nash soil could be overcome by increasing inoculum density level, or by sterilizing soil with biocidal agents, i.e., steam air (at 121 C for 30 minutes) and methyl bromide (a broad spectrum soil fumigant). In sterilized soils, an inoculum level of 10 conidia/g soil would result in a high degree of seedling disease.

The soils from Nash and from Cherokee differed in soil texture, also they differed in some chemical and physical properties, especially the amounts of available potassium and organic matter. As others have found, antagonism is most pronounced in the soils with high organic matter and high microorganism numbers, and least in unfertile soils (8).

All these findings suggested that the factors involved in the soil suppression of H. sativum are biological in nature.

Common root rot of wheat, caused by H. sativum, is not easy to control because the fungus is ubiquitous and nondiscriminating on most cereals and numerous grasses (66). Specific resistance in wheat to this pathogen is still not well understood (66). Nevertheless, H. sativum was known to be a poor competitor in soil under some conditions, and germination lysis and antagonism by soil microorganisms have been suggested as the control mechanisms of the disease (13, 14). Henry (42) found that the natural microflora of some soil had a marked inhibitive effect on infection of wheat seedlings by H. sativum. The addition of very small amounts of natural soil or the simultaneous infestation of H. sativum with a number of other fungi and bacteria could completely inhibit the H. sativum infection of wheat seedlings in naturally conducive soils.

Biological antagonism is affected by various factors that modify the soil environment. Some of these are soil type, soil moisture content, soil temperature, degree of sterilization and subsequent treatment, and kind of inoculum (29). Soil conditions may affect a soil-borne disease by acting directly upon the pathogen in both parasitic and nonparasitic phases of its life in soil, or by modifying the resistance to attack of host plant. In this report, we dealt with the effects of soil conditions upon the pathogen only.

Soil water content, more precisely, soil water potential has great influences on root diseases (21). Each microorganism has its optimal and minimal water potential requirements for growth (36, 69). Water

potentials that prevent the growth of most root-infecting fungi are about -30 to -50 bars or less (21). Bacterial activity may be minimized at water potentials below -10 to -50 bars (20). Actinomycetes can grow in fairly dry soils (33, 49). Conidia of H. sativum are highly resistant to desiccation (52). The percentages of soil water in the survival tests of this study were adjusted to 7.5 and 5.0 % for Nash and Cherokee soils, respectively. These gave a soil water potential of about -1 to -2 bars in each soil, and should have been beneficial to both pathogen and antagonists.

An organism must adjust itself to its new environment when it is introduced artificially into soil and if it is to survive and multiply (47). Successful inoculation is dependent primarily on the persistance and development of the inoculum in the soil. As it would be expected, the transfer of biological suppressive factors to conducive soil would be more successful in Nash natural-Nash sterilized soil mixtures, since the antagonists were introduced to the similar and noncompetitive soil environment. The antagonists introduced to Cherokee natural soil, i.e., in Nash natural-Cherokee natural soil mixtures, must compete with the existing microflora in natural soil environment. In addition, the overall lack of available nutrients and much lower organic matter resulted in fewer success for transfers of the biological suppressive factors from Nash to Cherokee natural soil.

A knowledge of viability of soil-borne plant pathogens under varied conditions is important in designing cultural practices useful in disease control. Old (59) reported that death of H. sativum conidia was accelerated at high incubation temperature, and that conidia of the same fungus

remained viable throughout incubation period at room temperature in steamed soils, but some factors in natural soils caused the death of most conidia within 50 days. The survival of H. sativum conidia varied considerably with soil type. Results of the experiment of effects of temperature on survival of H. sativum conidia in this study coincided with that of Old's report. The fact that conidia of H. sativum survived longer in Nash sterilized soil probably is because sterilization of soil brought about favorable conditions for the fungus such as absence of an active competing microflora and an increase in available nutrients (46). Preliminary characterization was made of probable antagonists associated with this suppressive soil response.

When two microorganisms are inoculated at a distance from each other on an agar medium plate, they grow toward each other and several reactions may occur: (a) mutual intermingling of the two organisms; (b) inhibition of one organism on contact, the other organism continues to grow through the colony of the inhibited organism; (c) mutual inhibition on contact; (d) inhibition of one organism at a distance, the antagonist continues to grow through the resulting clear zone; and (e) mutual inhibition at a distance (45). In this report, we considered only the ones which resulted in a clear zone of inhibition between two organisms which were inoculated on the same plate at a distance from each other. As recorded in the results, about 29.4 % of bacterial and actinomycetes isolates were found to be antagonistic to H. sativum in vitro; none of the fungal isolates were found to be antagonistic to the same fungus. This consideration probably was not exact because antagonism occurs in many ways (94). The only possible conclusion here

is that there are antagonists present in the Nash soil, primarily of bacterial and actinomycetes in nature.

CHAPTER VI

SUMMARY

1. The soils from Nash and Cherokee differ in texture (silty loam and sandy soil, respectively); also they differ in some chemical properties, especially the amounts of available potassium and organic matter.

2. Naturally occurring inocula of H. sativum were detected in all soil samples from both Nash and Cherokee; the differences of naturally occurring inocula among soil depths of each location were not significant.

3. The soil from Nash showed a greater suppressiveness of wheat seedling infection by H. sativum than that from Cherokee.

4. The soil suppressiveness could be eliminated by sterilizing the soil with biocidal agents, i.e., steam air (at 121 C for 30 minutes), and methyl bromide (a wide spectrum soil fumigant); also it could be overcome by increasing the inoculum density.

5. The suppressive factors could be transferred to conducive soils by whole soil amendments. As low as 1 % (w/w) of Nash natural soil could significantly reduce disease extent at inoculum level of 10 conidia/g soil; 10 % of Nash natural soil greatly reduced the disease development even at the inoculum level of 100 conidia/g soil.

6. The viability of H. sativum conidia declined greatest in Nash

natural soil and least in Nash sterilized soil; also it declined much rapidly as the incubation temperature increased.

7. Total counts of soil microorganisms of the soil from Nash were much higher than those from Cherokee.

8. The possible conclusions here are that the factors involved in the soil suppression of H. sativum are biological in nature, and the antagonists present in the Nash soil are possibly bacteria and actinomycetes.

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APPENDIX

Czapek Dox Agar

Ingredients:

Czapek Dox Broth (Difco Bacto, dehydrated) 35 g

Agar 15 g

preparing:

1. Mix the above materials in a 2-liter container; add distilled water to make the total volume to be 1 liter.

2. Sterilized in an autoclave at 1.1 atms (121 C) for 15 minutes.

Modified Czapek Dox Agar

The principal ingredients of this medium are the same as those of Czapek Dox Agar, except that this medium is amended with the following antibiotic and fungicide after autoclaving and cooling to 45-50 C:

Streptomycin 0.1 g/l

Benomyl 0.004-0.008 g/l

Modified V-8 Juice Agar

Ingredients:

V-8 Juice 177 ml

$CaCO_3$ 2.7 g

Agar 15 g

preparing:

1. Mix V-8 juice and $CaCO_3$ well with a small amount of distilled water, pour into a 1-liter graduated cylinder, add distilled water again to make a total volume to be 1 liter, and set the cylinder in a refrigerator overnight.

2. Pour out the upper portion of clear solution into another container, add 15 g of agar and adjust the total volume to be 1 liter with distilled water again.

3. Autoclave at 1.1 atms (121 C) for 15 minutes.

VITA

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