

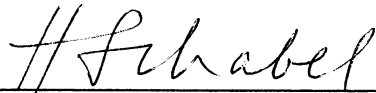
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VERTICAL MOVEMENT OF THE SPORES OF METARHIZIUM ANISOPLIAE  
(METSCH.) SOROKIN THROUGH SAND AND SOIL

by  
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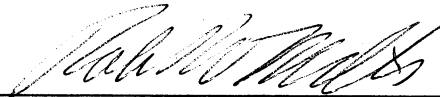
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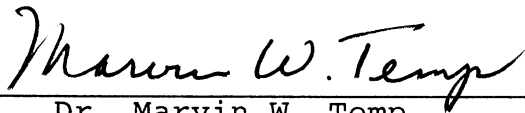
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## ABSTRACT

The purpose of this research was to determine whether spores of the green muscardine fungus, Metarhizium anisopliae (Metsch.) Sorokin would migrate through sand and soil by the action of water and a wetting agent under laboratory and field conditions. M. anisopliae is an entomogenous pathogen occurring naturally in soils and is widely used in experimental microbial control attempts. Knowledge of the migration of the spores of M. anisopliae could establish the fungus as an effective agent in controlling hard-to-reach soil inhabiting insect pests and encourage its use in the field as a biological control agent.

Laboratory trials consisted of placing a spore suspension of M. anisopliae on the surface of three types of sterile soils, i.e. coarse sand, sand-loam mixture, and soil of the Plainfield series which had been placed in plexiglas columns. This topical application was followed by 150ml of sterile distilled water. Samples of the soils were taken at 5cm intervals to a depth of 35cm. Spores were found to have traveled to an average depth of 30cm in the sand and Plainfield soil and 20cm in the sand-loam mixture. Unsterile Plainfield soil was placed in a stainless steel column and treated in the same manner as the sterile soils. In this soil, the spores migrated to a depth of 20cm.

The surface of eight one-quarter square meter field

plots were sprayed with a spore suspension of M. anisopliae. At the end of two months, the fungus was found at a depth of 18cm. M. anisopliae had migrated to a depth of 30cm after four months. There was no evidence that M. anisopliae occurred naturally in the soils of the test area.

A bioassay was devised to determine virulence of the spores after traveling through the soils. The larvae of the greater wax moth (Galleria mellonella) were inoculated with spores of M. anisopliae which were obtained from the cultures of the laboratory and field trials. All the larvae died of green muscardine disease.

Based on an evaluation of past research and this investigation, it is believed that M. anisopliae has a high potential as a microbial control agent against soil-inhabiting insects especially in conjunction with a pest management system such as an integrated control program.

## ACKNOWLEDGMENTS

I wish to thank Dr. Hans G. Schabel, my major professor and the members of my graduate committee, Dr. Robert Miller and Dr. Marvin Temp for having faith in me the last seven years. I also wish to thank my family for their understanding and patience when I ignored them with the excuse of study or work.

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## INTRODUCTION

The use of entomogenous pathogens, i.e. various fungi, bacteria, viruses, etc. as a means of microbial control of noxious insects, is gaining appeal as more chemical pesticides are being removed from the market due to their harmful effects against non-target organisms and their general pollution of the biosphere.

Entomogenous fungi have long played a significant role in the history of insect pathology and are important in the dynamics and natural regulation of insect populations (Steinhaus 1956). Despite their potential, there are very few economically successful programs which utilize fungal pathogens in general pest control procedures. Many of the problems lie in the lack of specific documentation such as dosage requirements, timing of applications, host insect life cycle, and the introduction and survival of fungal pathogens under field conditions.

Metarhizium anisopliae (Metsch.) Sorokin, the causal agent of green muscardine disease, is presently the second most widely used entomogenous fungus in experimental microbial control attempts and is continuously being appraised for its role in reducing pest populations. The fungus has been researched thoroughly under laboratory conditions but knowledge of the fate of the spores after topical application in the field is lacking. There is little information on the dispersal of the spores of M. anisopliae in soil

especially through the action of water. The present study was conducted to investigate this possibility that spores of M. anisopliae could migrate through sand or soil utilizing water as the transport medium. This could encourage its use in the field as a microbial control agent either by itself or in conjunction with an integrated pest management system.

M. anisopliae occurs naturally in the soil and attacks insects living in this environment. The fungus is particularly effective against the insect order Coleoptera (Urs and Govindu 1971). This order includes the white grub, Phyllophaga spp., which are serious pests in forest tree nurseries and plantations. These insects inhabit the soils of Central Wisconsin and are known to feed on the roots of red pine (Pinus resinosa) seedlings (Shenefelt and Simkover 1950). Red pine is extensively planted in soils which the grubs prefer and the insects have caused severe losses in forest tree nurseries in Central Wisconsin. In one instance, 60% of a new plantation of red pine were killed by white grubs in one year (Pers. Obs.).

Treatment with the soil insecticide Chlordane ® before planting the seedlings has proven successful (Speers and Schmiege 1971); however, the production of Chlordane ® has been suspended and no other method of reducing the grub population has since been developed. Since M. anisopliae is an effective agent against white grubs, biological con-

trol of this pest utilizing the fungus as a microbial insecticide was considered to be an alternative that would be more ecologically acceptable than chemical control.

If M. anisopliae was found to migrate through the soil profile with the action of water and then reach areas which white grubs inhabit, the fungus could in essence become an effective means of controlling this pest in forest nurseries and plantations.

## LITERATURE REVIEW

### History

M. anisopliae was first recorded as an insect pathogen in Russia in 1879 by Metschnikoff on the wheat cockchafer, Anisoplia austriaca, Herbst. The fungus belongs to the form-class Deuteromycetes, form-order Moniliales with a taxonomic position near Penicillium. The genus has been included in the Xerosporae because the spores of the fungus are dry and powdery (Wakefield and Bisby 1941). Names first ascribed to the fungus included Entomophthora anisopliae, Metsch.; Oospora destructor, Delacroix; and Penicillium anisopliae, Vuillemin (Rockwood 1950). In 1883, Sorokin described the fungus further and elected the genus Metarhizium, thus the final combination became Metarhizium anisopliae (Metsch.) Sorokin (Latch 1965).

The first attempts at using M. anisopliae as a biological control agent was by Krassiltschik in 1884 who mass-produced the fungus on beer mash. The fungus was then applied to small areas and it was reported to have killed 50-80% of the sugar beet curculio, Cleonus punctiventris Germ. (Latch 1965). Subsequently, M. anisopliae has been widely tested as a control agent in reducing various pest populations.

The cosmopolitan M. anisopliae is known to be highly pathogenic to over 200 species of insects representing seven different orders with Coleoptera (Scarabaeidae and

Curculionidae) being the most common host order (Yendol and Roberts 1970). The fungus is capable of attacking insects at any stage of development; however, the larval stage is generally the most susceptible.

#### Description

M. anisopliae is comprised of two forms; a short-spored form (forma minor) with conidia 5-8 $\mu$  long; and a long-spored form (forma major) with conidia 9-14 $\mu$  long (Latch 1965). It is thought that conidial length varies with the culture media suggesting that the difference between the forms are physiological (Tulloch 1976).

The conidia are one-celled, cylindrical to oval in shape, and usually truncate at both ends. Their color varies from light to dark olive green. The conidiophores are simple or branched and are closely compacted and individual chains of conidia are only distinguishable in the early stages of formation (Latch 1965).

#### Culture

M. anisopliae may easily be cultured on a wide variety of natural and synthetic media. Barnes et al. (1975) determined growth and sporulation of M. anisopliae on media containing various peptone sources. Latch (1965) described the appearance of fungal colonies grown on potato-dextrose agar (PDA) and media other than PDA. Roberts (1966) examined four media to determine the best source of spores for the production of toxic substances. Submerge and surface

cultivation for large scale field use was described by Adamék (1965) and Latch (1975).

A selective medium which acts against bacterial growth and minimizes the development of other fungi was formulated by Veen and Ferron (1966). This medium has been successful in the isolation of M. anisopliae from soil. Another selective medium which has been designated as CCE agar has recently been tested for estimating populations of Metarhizium in soil without interference from competing fungi (Pereira et al. 1979).

#### Germination

Relative humidities higher than 90% were found to be necessary for the germination of spores of M. anisopliae with the best germination occurring at humidities of 100% and no germination occurring at relative humidities below 90% (Walstad et al. 1970).

Optimum temperatures for spore germination generally are considered to be from 25°-30°C. with the temperature range for normal growth being 10°-30°C. (Walstad et al. 1970). M. anisopliae is relatively thermophilic with spores unable to germinate at temperatures below 10°C. The thermal death point is 49°C. (Schaerffenberg 1964).

The influence of the hydrogen ion concentration shows that mycelial growth curves are fairly level in a range of pH 4.7-10 with the optimum pH being 6.9-7.4 (Vouk and Klas 1931).

Light appears to be detrimental to mycelial growth and the viability of the spores of M. anisopliae (Roberts 1977).

Spores of M. anisopliae generally do not germinate on unsterile soil, duff, or leaf litter but remain viable there for days or weeks (Walstad et al. 1970). The spores will often germinate readily upon contact with the unsterile body wall of an acceptable host. Under optimum conditions, germination commences after 15 hours with the appearance of germ tubes (Latch 1965).

#### Viability

The length of a spore's life is measured by its ability to germinate after a period of time. The survival of the spores of M. anisopliae over an extended period of time is affected by storage temperature. Spore viability dropped to virtually zero in less than four months when the spores were stored above 21°C., while spores held below 8°C. had an 80% survival rate after 12 months (Walstad et al. 1970). There was no germination or mycelial growth of spores after being stored for 11 months at room temperatures of 15°-24°C., whereas those spores stored at 4° and -15°C. germinated and had vigorous mycelial growth (Latch 1976). Subzero temperatures are suitable for long term storage and spore suspensions held at -20°C. can remain viable for up to four years (Roberts and Campbell 1977).

Spore viability is also affected by relative humidities and spores survive longer at high or low than at median

humidities (Walstad et al. 1970). Maximum germination was retained when spores were stored at 8°C. and 75% relative humidity (Yendol and Hamlen 1973). Viability is also affected by light and spores survive longer in darkness than in light (Roberts and Campbell 1977).

Bell and Hamalle (1970) stated that conidia could survive and still be effective after 40 days in dry sand or soil. Infection of host insects dropped only 30-50% after two years in greenhouse experiments with M. anisopliae (Müller-Kögler 1976). Bell (1975) found that M. anisopliae was viable for six months when stored outside in a silica gel bag and concluded that if spore material is kept dry, it could survive temperature fluctuations and remain viable and pathogenic for at least one year.

#### Virulence

A high degree of virulence is important if the fungus is to be successful as a biological control agent. M. anisopliae is a facultative parasite and may be cultivated and raised for several generations on a variety of artificial media. If the parasitic phase of the life cycle is omitted there is some loss of virulence. Fox and Jaques (1958) found continued subculturing caused a decline in virulence which was indicated by failure of the inoculum to cause disease. Rockwood (1950), on the other hand, stated that virulence may be maintained in artificial substrates on approximately the same level through a number of generations

and up to seven years. Latch (1965) reported that isolates grown on artificial media showed no change in virulence after six months but that after 11 months, virulence had dropped by 24%. At this time, it is not known how many generations M. anisopliae can be cultivated on artificial substrates without loss of virulence. Therefore artificial subcultures of M. anisopliae are routinely interrupted by passage through host insects to insure virulence.

#### Mode of Infection

M. anisopliae initiates infection orally or percutaneously by germinating spores which produce germ tubes that typically grow over the surface of the host insect (Schabel 1976). The germ tube produces clavate, terminal dilations called appressoria at varying intervals which have been confirmed as origins for the penetrating hyphae (Schabel 1978). The invasive hyphae enter the host tissues and ramify throughout the hemocoel. Schabel (1978) also found that on adult pales weevils (Hylobius pales) hyphae grew extensively within the procuticle with a distinct preference for the interlamellar spaces. After filling the host with mycelium, emergence hyphae, will under proper conditions, grow through the integument to eventually produce spores on the external surface of the host. In the early stages of infection, the insect shows general ill effects, cessation of feeding, weakness, and disorientation. Soft-bodied hosts tend to change color and the cuticle often shows dark

melanotic spots.

#### Toxins

Two insecticidal substances have been isolated from filtrates of M. anisopliae. These toxins include the so-called destruxins A and B which are cyclic peptides. M. anisopliae is among those fungi which kill before extensive invasion of the organs takes place and it is thought that the production of toxic substances by the fungus is responsible. These toxins appear to be liberated into the host by intact hyphae and hyphal bodies. It has been suggested that M. anisopliae would prove to be more promising as a pathogen than as a source of toxins (Roberts 1965).

#### Soil Aspects

There is little information as to the persistence and mobility of M. anisopliae after it has been applied in the field. Latch and Falloon (1976) found little spread of the fungus from inoculated areas to adjacent areas and what migration occurred was probably from the infected host insects. Although the inoculum was only mixed into the top 10cm of soil, M. anisopliae spores were found to be present in material taken 20-30cm from the soil surface. The spores were presumed to have been washed down the soil profile by rain or through the activities of small organisms such as ants or millipedes. Burges (1950) and Hepple (1960) reported that fungi producing dry spores such as M. ani-

sopliae, have little likelihood of being washed to lower levels in soil.

#### Toxicity to Vertebrates

There are no current reports of allergic reactions or infections to vertebrates by M. anisopliae. Latch (1976) reported that animals which had been fed spores of M. anisopliae in their diet, showed no abnormal symptoms in their behavior and upon conclusion of the trial when the test animals were sacrificed and autopsied, no tissue or organic abnormalities were observed. Wasti et al. (1980) assessed the toxicity of M. anisopliae to birds and concluded that the fungus did not cause any mortality or abnormal behavior although he observed a small decline in body weight. Dosages were considerably higher than amounts normally used in programs in field trials and when extrapolated to man, were found to be greater than any possible exposure or contamination. M. anisopliae is considered neither toxic nor pathogenic to man or other warmblooded animals. However, as with any insecticide, microbial or chemical, proper precautions such as the use of protective clothing and face masks should be taken whenever the spores are handled.

## MATERIALS AND METHODS

### Fungus

A strain of M. anisopliae was isolated from a pales weevil obtained in Orange County, North Carolina, which had died of green muscardine disease. A culture was grown on dextrose-peptone-yeast agar (DPY), prepared according to the specifications of Schaerffenberg (1964) (App.2). Live white grubs approximately three centimeters in length were obtained in late April from a newly plowed field in Wood County, Wisconsin. These grubs were inoculated by brushing spores of the North Carolina isolate onto the integument of the larvae. The grubs subsequently died of green muscardine disease and formed the characteristic external fungal tufts of growth associated with M. anisopliae. Hyphal tip transfers were made from the grubs to the DPY agar and the resultant sporulating cultures (Fig. 1) were verified to be M. anisopliae. Subcultures were placed in slant tubes and used within a ten month period.

### Columns

#### Plexiglas Columns

Three plexiglas columns consisting of a back plate, two side plates, and a base plate all cemented together with epoxy glue were designed. Three one-quarter inch holes were drilled into the base plate to provide drainage. The front plate was constructed in such a manner that it would fit into the sides and base plates and yet be easily



Fig. 1. Typical growth and culture of Metarhizium anisopliae. This sample was obtained from field plot number 6 and placed on a selective medium then transferred to dextrose-peptone-yeast agar.

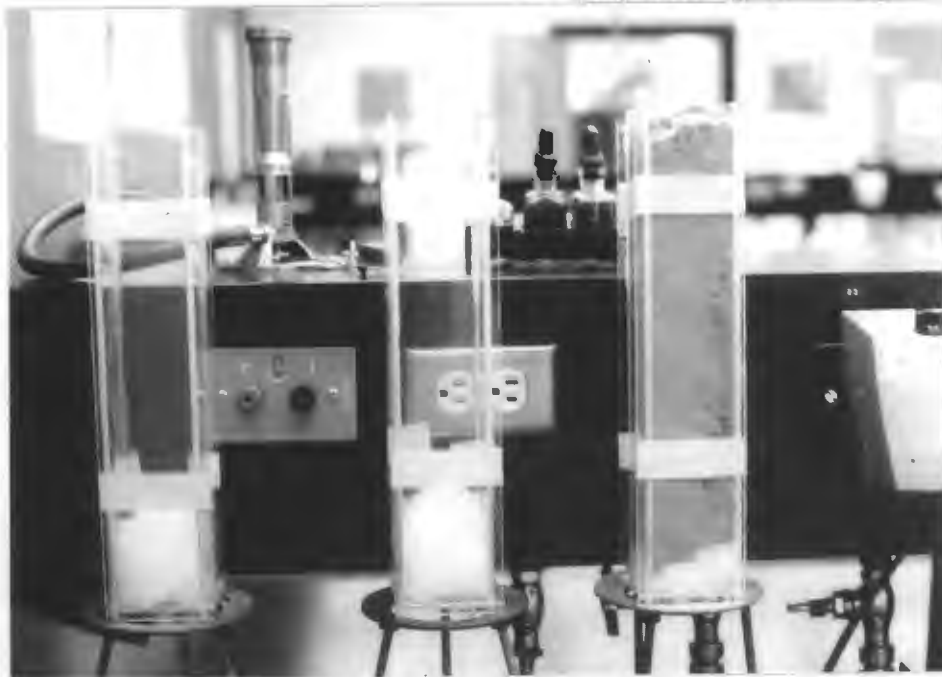


Fig. 2. The three columns constructed of plexi-glas. The right column is filled with sand and each column has a bottom filter plug of fiberglas. The columns are held together with glue and masking tape.

removed. An entire column measured 6x6x35cm and was firmly held together with masking tape (Fig. 2). The columns were rinsed in 95% ethyl alcohol for sterilization. A plug of fiberglas soaked in the alcohol was fitted into the base of the column. Other sterilization procedures were not found to be necessary as the number of contaminating colonies on the culture plates was minimal and they were easily recognized and discarded. The soil sample used for each trial was first sterilized in an autoclave for 15 minutes and then placed in the column. No special precautions were taken to keep the columns of sand or soil aseptic.

#### Stainless steel column

The stainless steel column was constructed by bending a single piece of stainless steel to form the back and side panels. A base plate was welded to this three sided column and three one-quarter inch holes were drilled into the base plate to provide drainage. A front plate fitted snugly over the sides and base plates and the whole column was held firmly together with three rigid stainless steel bands and wing nuts (Fig. 3). The column measured 7x7x35cm and could be sterilized together with the soil in an autoclave. The construction of a steel column was necessary in order to obtain intact soil profiles from soil pits.

#### Soil

Three different types of soil were used: coarse sand, a sand-loam mixture, and soil classified in the Plainfield series. The latter consists of "deep extensively drained

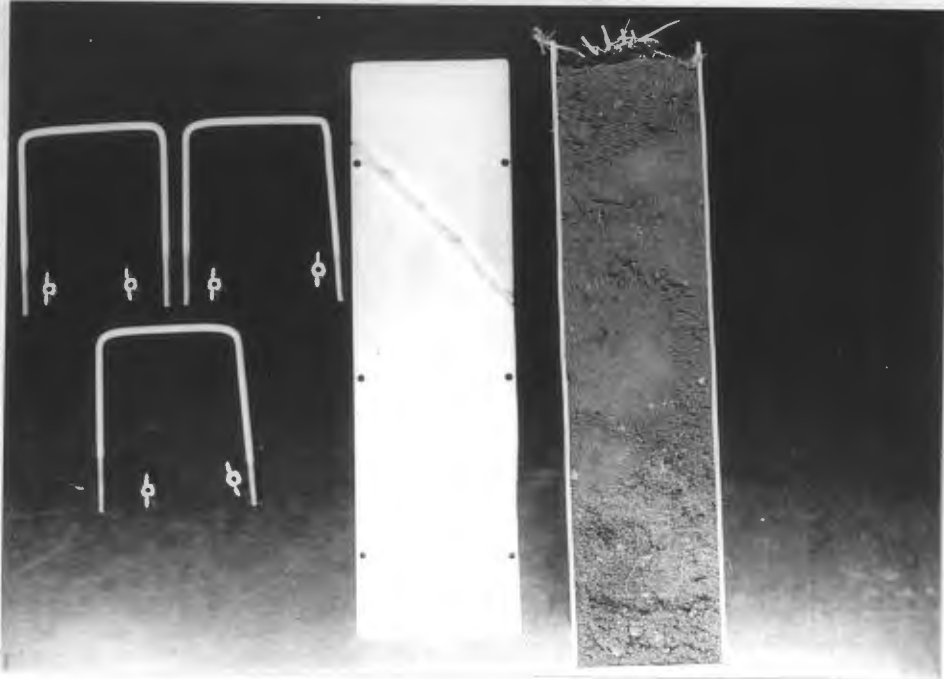


Fig. 3. Front view of the three sided column constructed of stainless steel and filled with a soil sample. To the left of the column, is the detachable front panel and its mounting hardware.

sandy soil on outwash plains" (USDA Soil Conserv. Serv. 1977). These soil types are characteristic of soils found in Central Wisconsin the area of this research.

#### Sand

Sand was obtained from the substratum of a Plainfield type soil at a depth of five feet and consisted of a yellowish brown, single-grained, loose sand containing less than 95% quartz with an acidity of pH 5.9.

#### Sand-loam mixture

The sandy loam was obtained from the plow layer (soil ordinarily moved in tillage, about 5-8 inches in thickness) of a Plainfield type soil. The sand-loam mixture consisted of 50% coarse sand and 50% sandy loam. Both the sand and

sand-loam mixture were sifted through a number 35 soil sieve to remove lumps and debris before sterilization and placement in the plexiglas columns.

#### Plainfield soil

The Plainfield soil was a typical Plainfield type soil having a profile which included an Ap, A1, B2, and C1 to C3 horizons. A soil pit was dug and a column of soil as undisturbed as possible to a depth of 35cm was obtained by pressing the stainless steel column against the profile thus forcing the soil into the column. This soil was sterilized intact in the steel column. Control trials were conducted in which the column of soil was not sterilized in order to determine if there were any antibiotic or fungistatic effects occurring.

Three replicates of each of the above soils were taken.

#### Administration of the Inoculum

After the columns had been filled with the corresponding type of soil, sufficient amounts of sterile distilled water (SDW) were allowed to flow through the columns to pack the soil and bring the soil to field capacity. This helped to prevent channels through which the spores might readily wash.

Spores of M. anisopliae were suspended in a .4% solution of the wetting agent polyoxyethylene sorbitan mono-laurate (Tween 20 ®). A hemocytometer was used to calculate the spore suspension which contained an average of

$5 \times 10^7$  spores per ml. A standard volume (2ml) of spore suspension was pipetted carefully over the surface of the soil after the column had drained of excess water. This was immediately followed by 150ml of SDW pipetted in 10ml increments. Both the spore suspension and the additional water were slowly poured on the surface of the soil to prevent disturbance of the upper layers of the soil. No standing water was allowed to accumulate on the surface of the soils.

#### Culturing Techniques

Samples of the drainage water were taken after the addition of 100ml of SDW and again after another additional 50ml of SDW. These samples were cultured to determine whether any spores had migrated completely through the column. When the column had stopped draining, it was placed in a horizontal position and the face plate carefully removed. The outer 1cm of soil was discarded to avoid those spores which might have traveled more rapidly down the smooth surface of the columns. A sterile probe was used to remove .005-.015 grams of soil from the center of the column. Three samples were removed at 5cm intervals throughout the length of the column and placed in sterile Petri dishes (Warcup 1950). The nutrient agar medium (DPY) was carefully poured over the samples and the plates were incubated at 25° C. for three days. An initial count was made of any colonies found to be present on the medium after

the incubation period. Three replicates were made for each of the sterile soil types.

Samples of the non-sterile soil were taken at 5cm intervals and placed in sterile Petri plates. A selective medium for the isolation of M. anisopliae from soil prepared according to the specifications of Veen and Ferron (1966) was carefully poured over the sample. This medium eliminated many contaminants and slowed the growth of competitors thus allowing colonies of M. anisopliae to grow. Colonies of M. anisopliae were transferred through hyphal tips to the nutrient agar medium in order to confirm the actual presence of the fungus. The sporulating colonies were also examined microscopically for final verification that the fungus was M. anisopliae.

#### Field Procedure

Eight one-quarter square meter plots were prepared for the field trial. Four of these plots were scalped to the mineral soil (Fig. 4.) while the sod was left undisturbed on the other four plots (Fig. 5.). A plant water mister was used to spray each plot with 140ml of spore suspension containing an average of  $6 \times 10^7$  spores of M. anisopliae per ml. This topical application was immediately followed by three liters of distilled water.

The eight plots remained exposed to the full radiation of the sun. Complete weather data were provided by the National Weather Service in Madison, Wisconsin situated ap-



Fig. 4. One of four plots one-quarter square meter in size and scalped to mineral soil.



Fig. 5. One of four plots one-quarter square meter in size and left undisturbed.

proximately 100 miles south of the test site (App. 1). The plots were located in a field which had been planted to red pine the preceding spring (Fig.6). The soil was of the Plainfield series and was from the same area from which the soil samples had been taken for the laboratory investigation.



Fig. 6. Location of the plots used in the field trials. The area had been planted to red pine (Pinus resinosa) the preceding spring. Flags indicate plots.

Two samples each were taken of each plot at two and four month intervals after the initial application of the spore suspension. The sample obtained two months after application of the spores was taken at 2cm intervals to a depth of 20cm. The second sampling four months after the initial application was taken at 5cm intervals to a depth of 30cm. A section of soil from the plots was removed to provide

vertical access to a depth of 35cm. Two soil samples (.005-.015 g.) were removed with a sterile probe at each respective interval and placed on sterile Petri dishes. The selective medium formulated by Veen and Ferron (1966) was carefully poured over the soil samples and the samples were incubated at 25° C. until sporulation occurred. At this time, colonies which were thought to be M. anisopliae were transferred to the DPY agar and incubated to establish the identity of the fungus. Microscopic examination of the spores was also made to confirm this identity.

A control sampling was taken in the same area as the field trial to determine if M. anisopliae was occurring naturally in the soil. This sample was taken approximately 50 feet from the original plots to minimize any possible drift of spores from the prepared plots. A soil pit was dug and two samples each were obtained at 5cm intervals to a depth of 30cm. These samples were handled in the same manner as the samplings taken from the prepared plots.

#### Bioassay

A bioassay was devised to determine if the spores of M. anisopliae would lose their virulence while migrating through soil. Larvae of the greater wax moth were obtained from a live bait store for this portion of the research. Ten larvae were inoculated for each three samples and soil types. The spores were obtained from the cultures which had successfully produced colonies of M. anisopliae

from the different soils and depths. A camel's hair brush was used to brush the spores onto the integument of the larvae. The brush was dipped into 95% ethyl alcohol to sterilize it between each application of spores onto a larva. Each group of larvae were placed on moistened filter paper fitted into a sterile Petri dish (Fig. 7). The larvae were then stored at room temperature and checked every 24 hours for signs of mycosis.



Fig. 7. Group of ten larvae of the greater wax moth (Galleria mellonella) inoculated with spores of M. anisopliae and placed on moistened filter paper in a sterile Petri plate.

A bioassay was also made of the positive cultures taken of the samples from the field plots, to determine if any inhibitory effects due to fungistasis would affect spore virulence.

A control group of ten larvae each were placed in ten sterile Petri dishes. These larvae were handled and kept in the same manner as the larvae which were inoculated with the spores of M. anisopliae.

## RESULTS

### Laboratory

Spores of M. anisopliae migrated downward through the columns of all three types of soils. Fig. 8 shows the variability of migration of spores in the three types of soil, i.e. coarse sand, sand-loam mixture, and Plainfield soil.

#### Composite soil type data

The rate of migration observed in the columns of sterile sand indicated a high retention of spores on the sand surface followed by a sharp decrease at the 5cm level. At 10cm the number of colonies had again increased to a level almost that found on the surface. The number of colonies then declined as the depth increased. No spores traveled completely through any of the columns of sterile sand.

The sterile sand-loam mixture as indicated in Fig.8 had a gradual decrease in the number of spores to the 5cm depth where an average of 145 colonies per sample were plated out. By 10cm level, the number of spores had decreased sharply to less than 20 colonies per sample. Few spores traveled past the 20cm depth and no spores migrated completely through the columns of the sterile sand-loam mixture.

The sterile Plainfield soil (Fig. 8) showed a higher variability in its curve than the other two soil types.

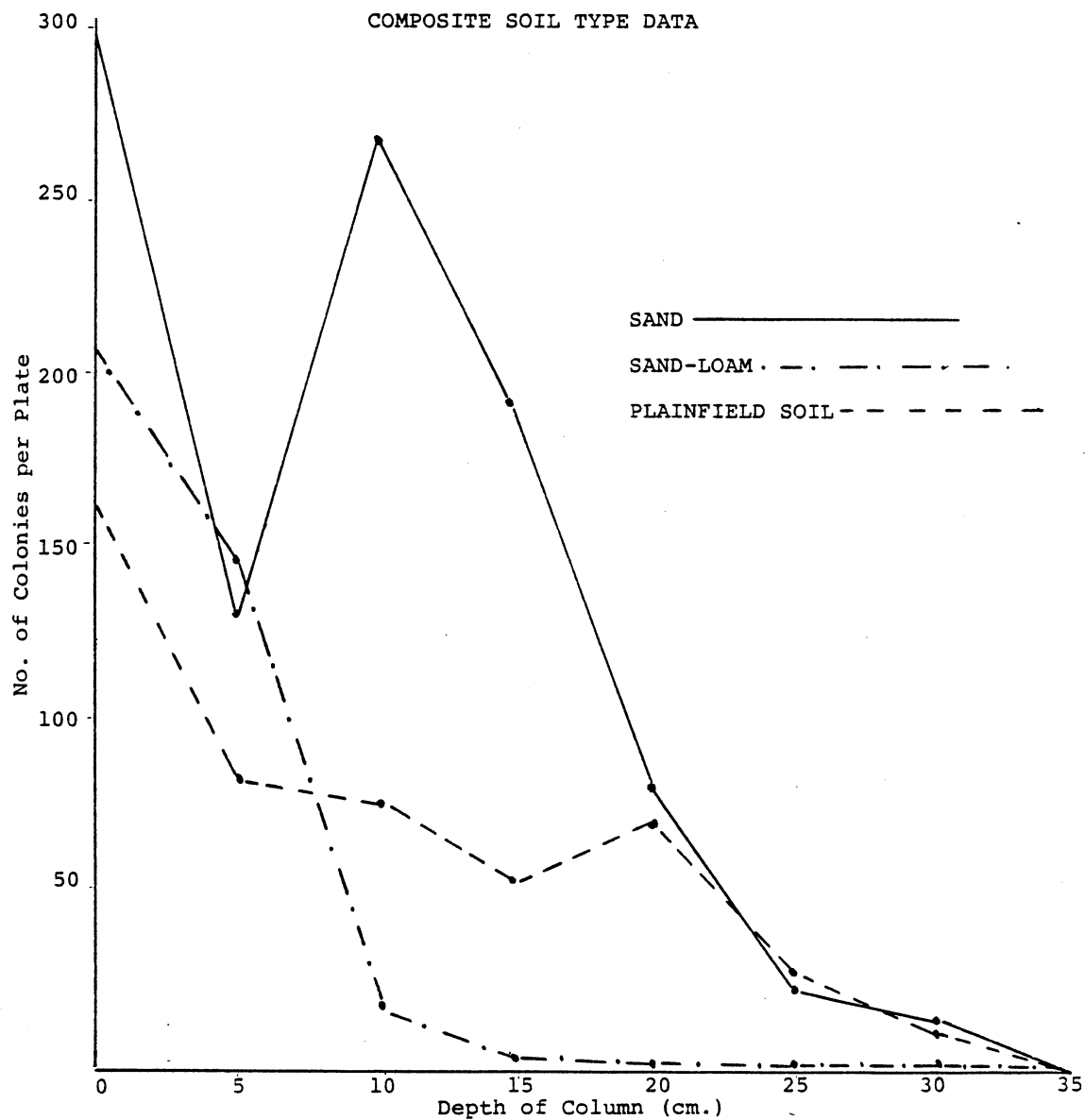


Fig. 8 Distribution of spores of *Metarhizium anisopliae* versus depth in columns of sterile sand, sand-loam mixture, and Plainfield soil after topical application of 2ml of spore suspension and 150ml of sterile distilled water. Each plotted value represents an average of nine replicates.

There was a drop in the number of spores at the 5cm depth similar to the curve represented by the sterile sand. After 5cm the concentration of spores was relatively constant down to 20cm indicating spore migration through this depth. From the 20cm depth, the number of colonies decreased and approached zero by 35cm. No spores traveled completely through the columns of sterile Plainfield soil.

#### Sterile sand

Fig. 9 shows the migration of the spores of M. anisopliae versus column depth for the three columns of sterile sand. All three columns show a decrease in the number of colonies to 5cm followed by a sharp increase at 10cm. In columns A and C the number of spores declined after 10cm as depth increased approaching zero by 25cm. Column B however, maintained a constant concentration of colonies from 10cm through 15cm before declining and approaching zero at 35cm. Fig. 9 indicates the variability of the migration of spores between the three columns while Fig. 10, 11, and 12 show the variability within Columns A, B, and C. Each set of graphs demonstrates the repeatability of the trials with the sterile sand and indicates the same general migration trend.

#### Sterile sand-loam mixture

Fig. 13 represents the concentration of colonies versus depth in the three columns of sterile sand-loam mixture. This graph shows the variability of migration of spores

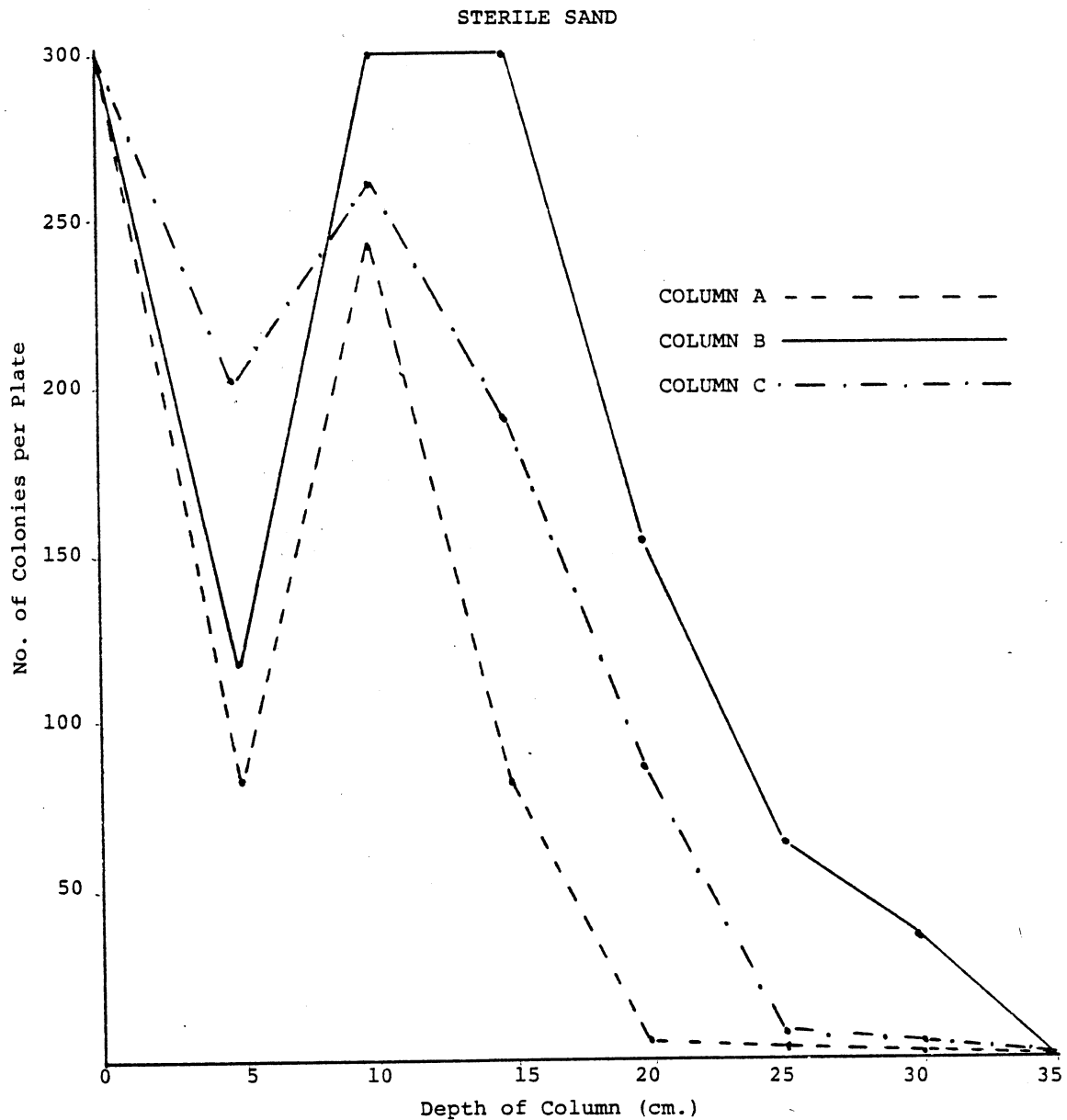


Fig. 9. Distribution of spores of *Metarhizium anisopliae* versus depth in three columns of sterile sand after topical application of 2ml of spore suspension and 150ml of sterile distilled water. Each plotted value represents an average of three replicates obtained from each column.

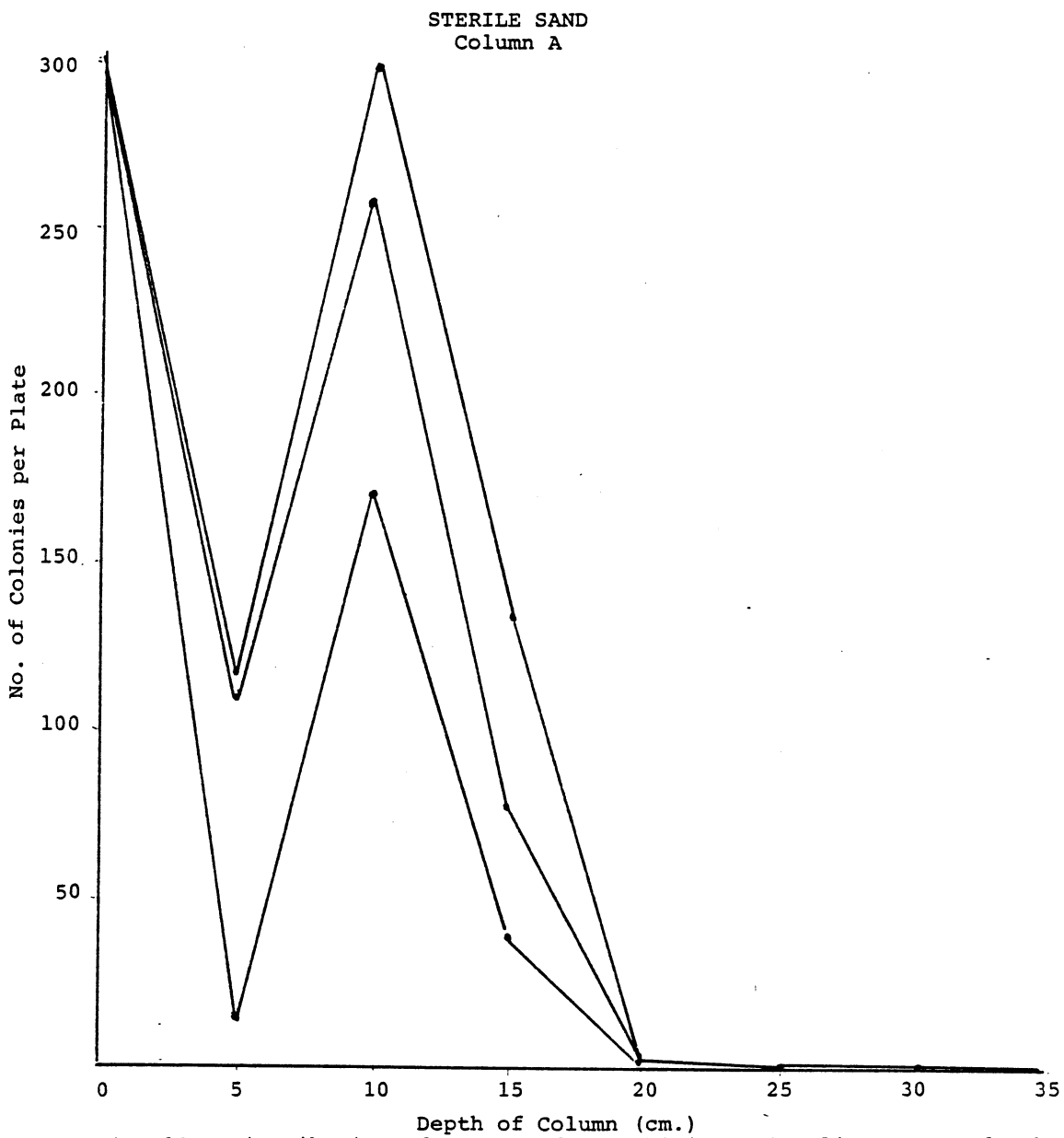


Fig. 10. Distribution of spores of *Metarhizium anisopliae* versus depth in Column A of the sterile sand after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

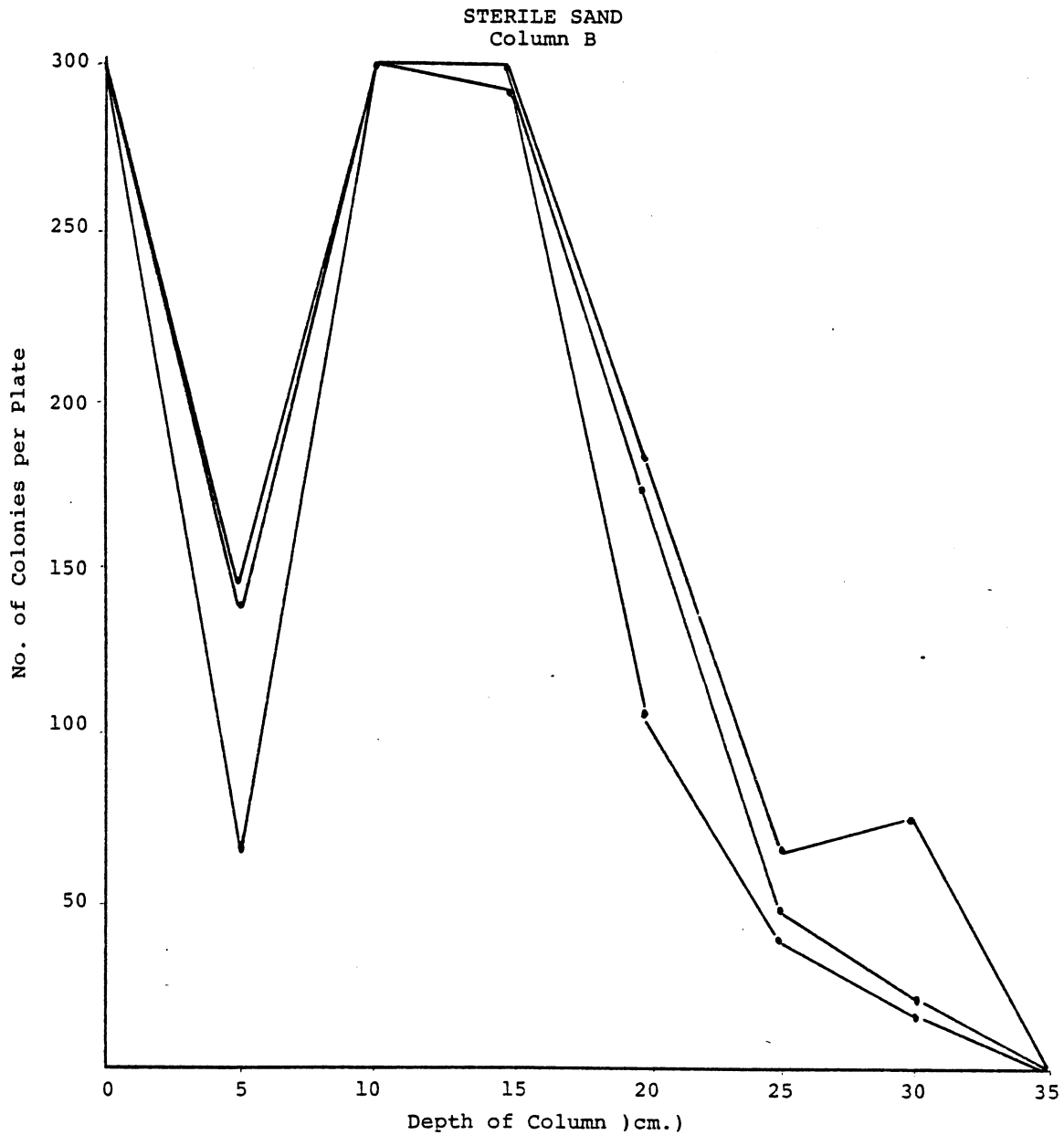


Fig. 11. Distribution of spores of *Metarhizium anisopliae* versus depth in Column B of sterile sand after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

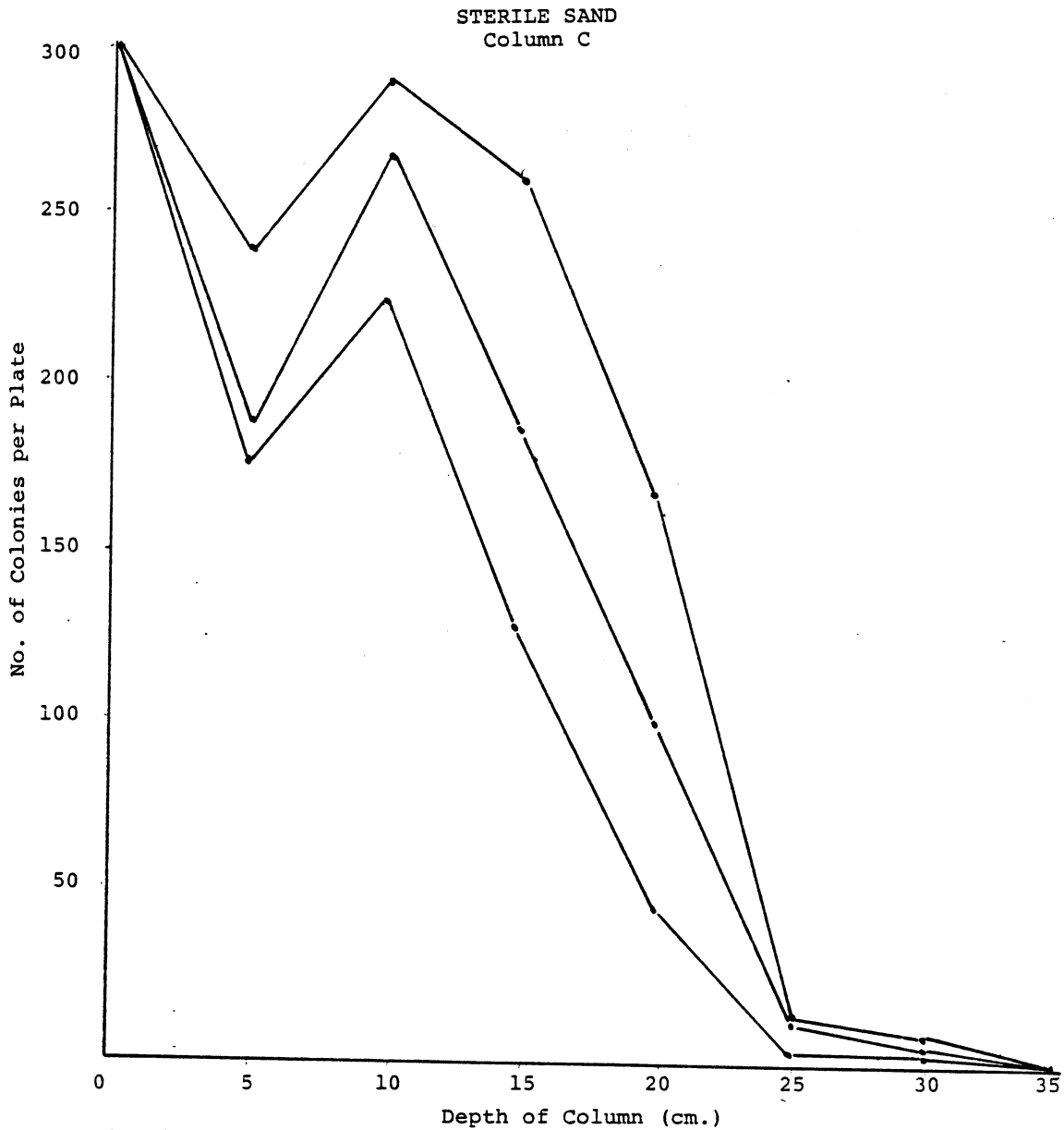


Fig. 12. Distribution of spores of Metarhizium anisopliae versus depth in Column C of sterile sand after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

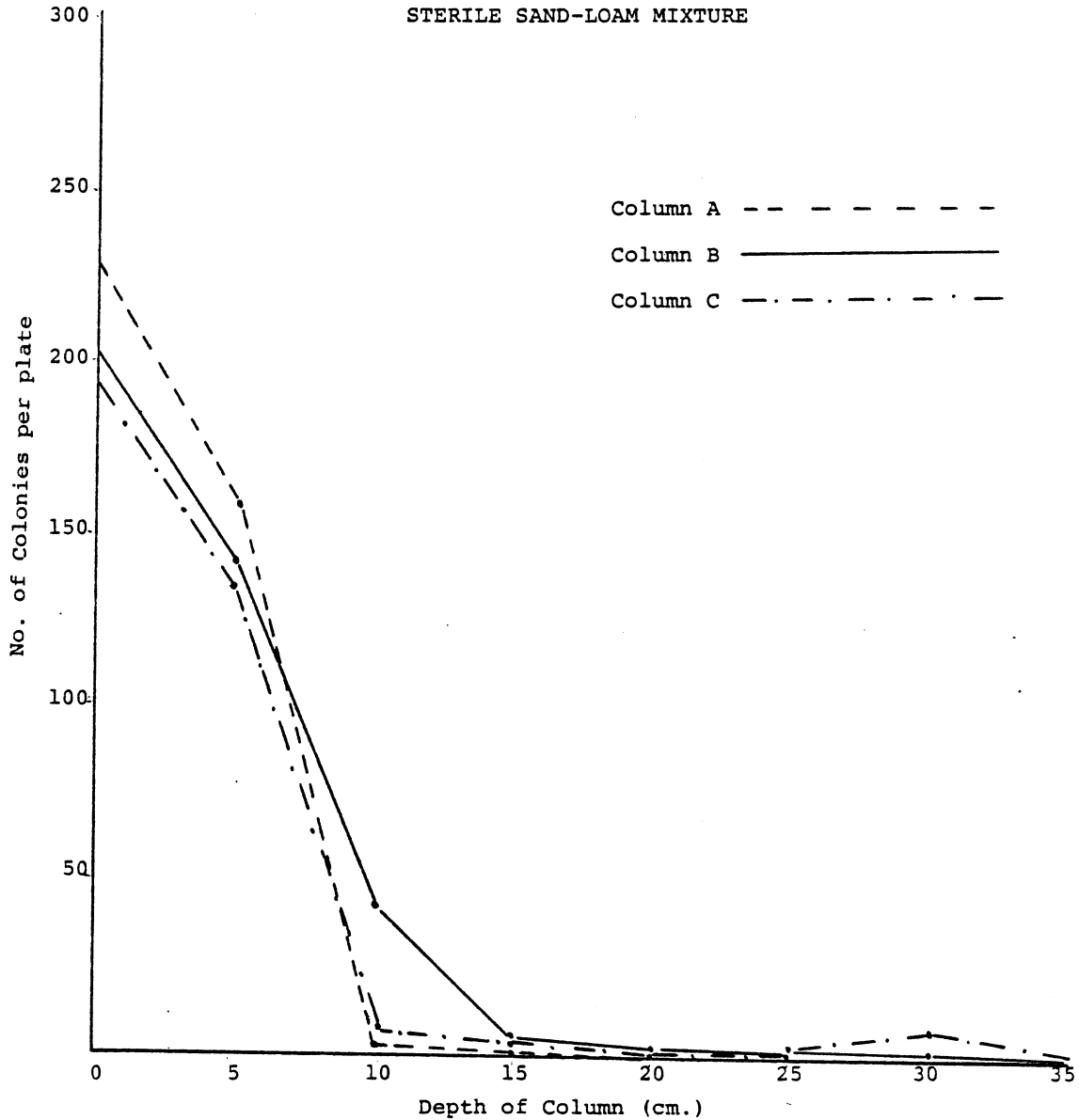


Fig. 13. Distribution of spores of *Metarhizium anisopliae* versus depth in three columns of sterile sand-loam mixture after topical application of 2ml of spore suspension and 150ml of sterile distilled water. Each plotted value represents an average of three replicates obtained from each column.

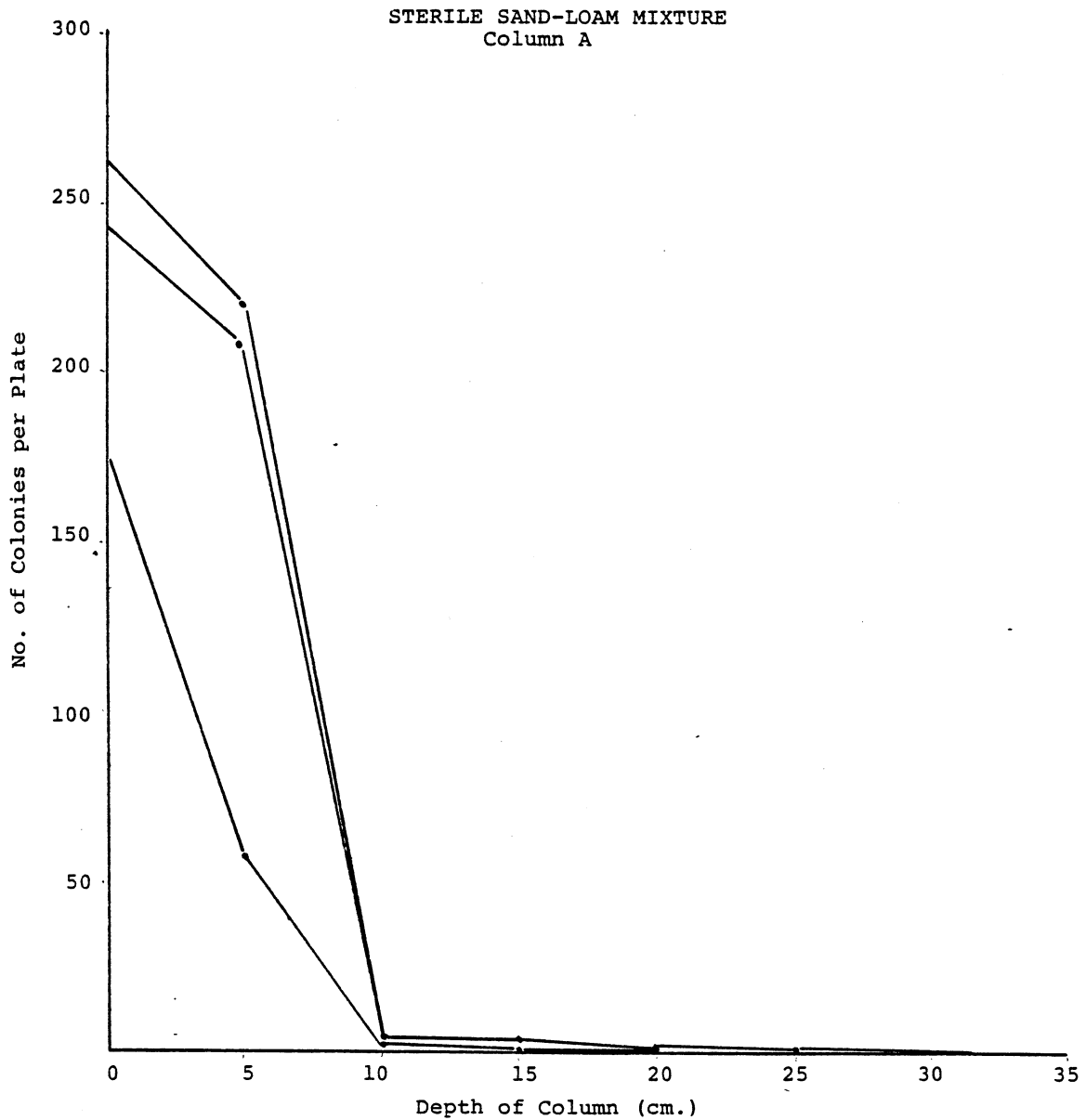


Fig. 14. Distribution of spores of Metarhizium anisopliae versus depth in Column A of sterile sand-loam mixture after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

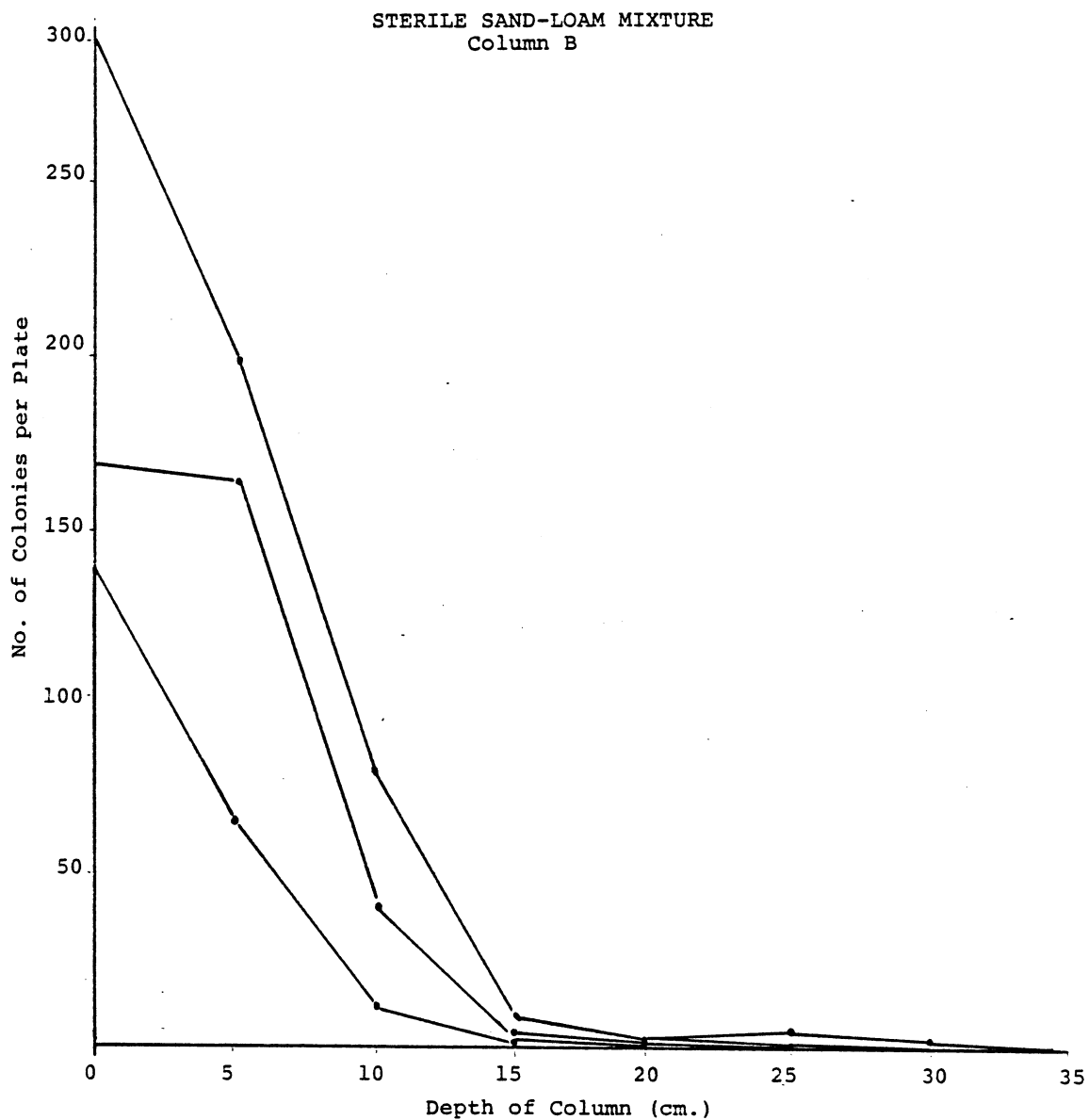


Fig. 15. Distribution of spores of *Metarhizium anisopliae* versus depth in Column B of sterile sand-loam mixture after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

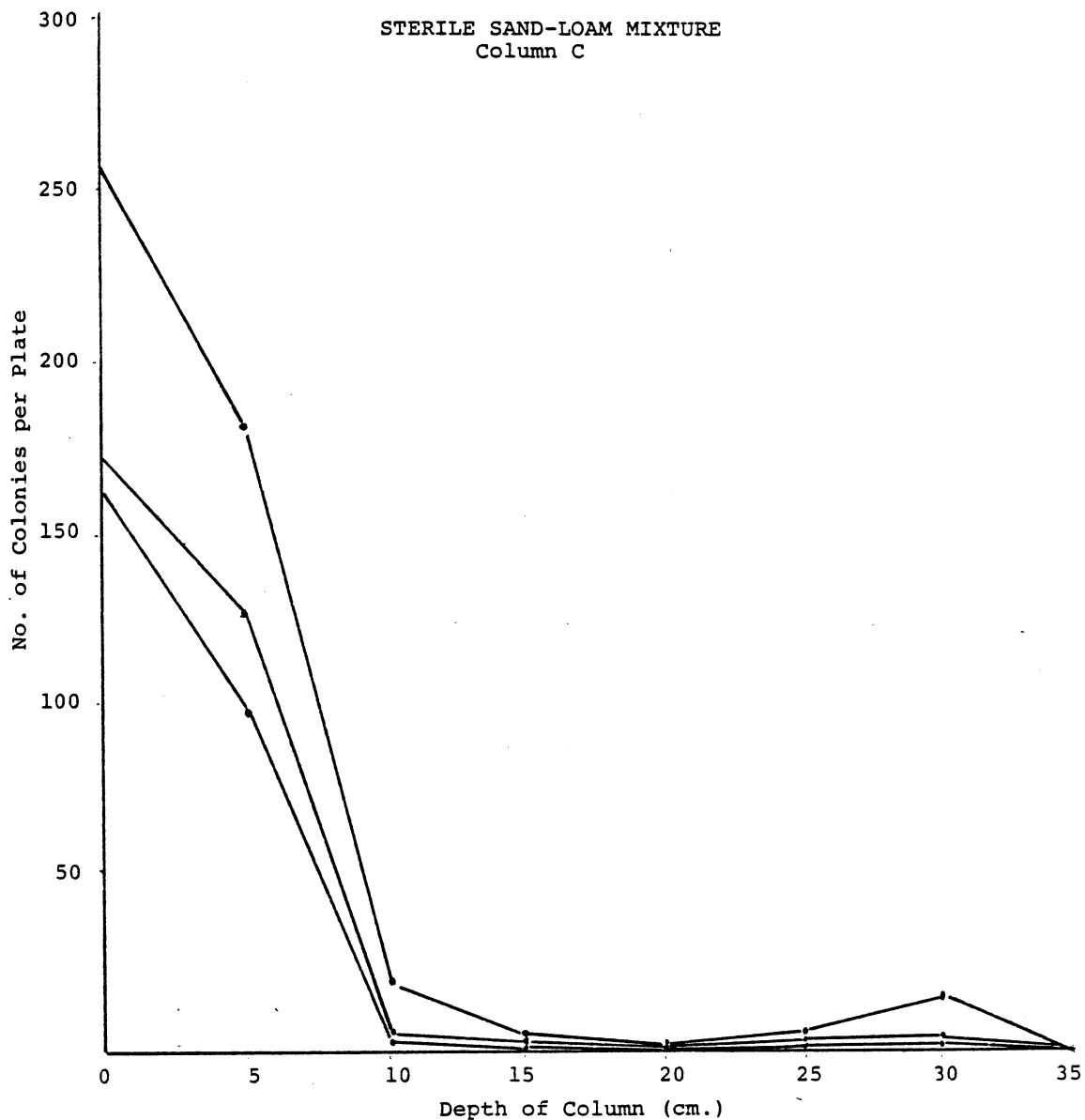


Fig. 16. Distribution of spores of *Metarhizium anisopliae* versus depth in Column C of sterile sand-loam mixture after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

between the columns while Fig. 14, 15, and 16 show the variabilities observed within the columns. These curves indicate that the spores of M. anisopliae migrated to a depth of 5cm. At increased depths the number of colonies decreased sharply approaching zero between 10 and 15cm. The pattern of scatter between the three columns were relatively similiar showing the repeatability of the migration of spores through columns containing a sterile sand-loam mixture under laboratory conditions.

#### Sterile Plainfield soil

Fig. 17 shows the variability of migration of spores versus depth for the three columns containing the sterile Plainfield soil. Fig. 18, 19, and 20 represent the variability within the columns of soil. These graphs have a much wider range of variation than those representing the sterile sand and sand-loam mixture.

Column A (Fig. 18) shows a gradual decrease in the number of spores to the 10cm depth followed by a slight increase in concentration of spores between 15 and 20cm. This was followed by a decrease in spore concentration which approached zero by 35cm.

Column B (Fig. 19) shows a decrease in the number of spore concentration at 10cm similiar to the increase noted in Columns A and C. By 30cm, all replicates had few if any colonies plating out.

Column C (Fig. 20) shows a general decrease in con-

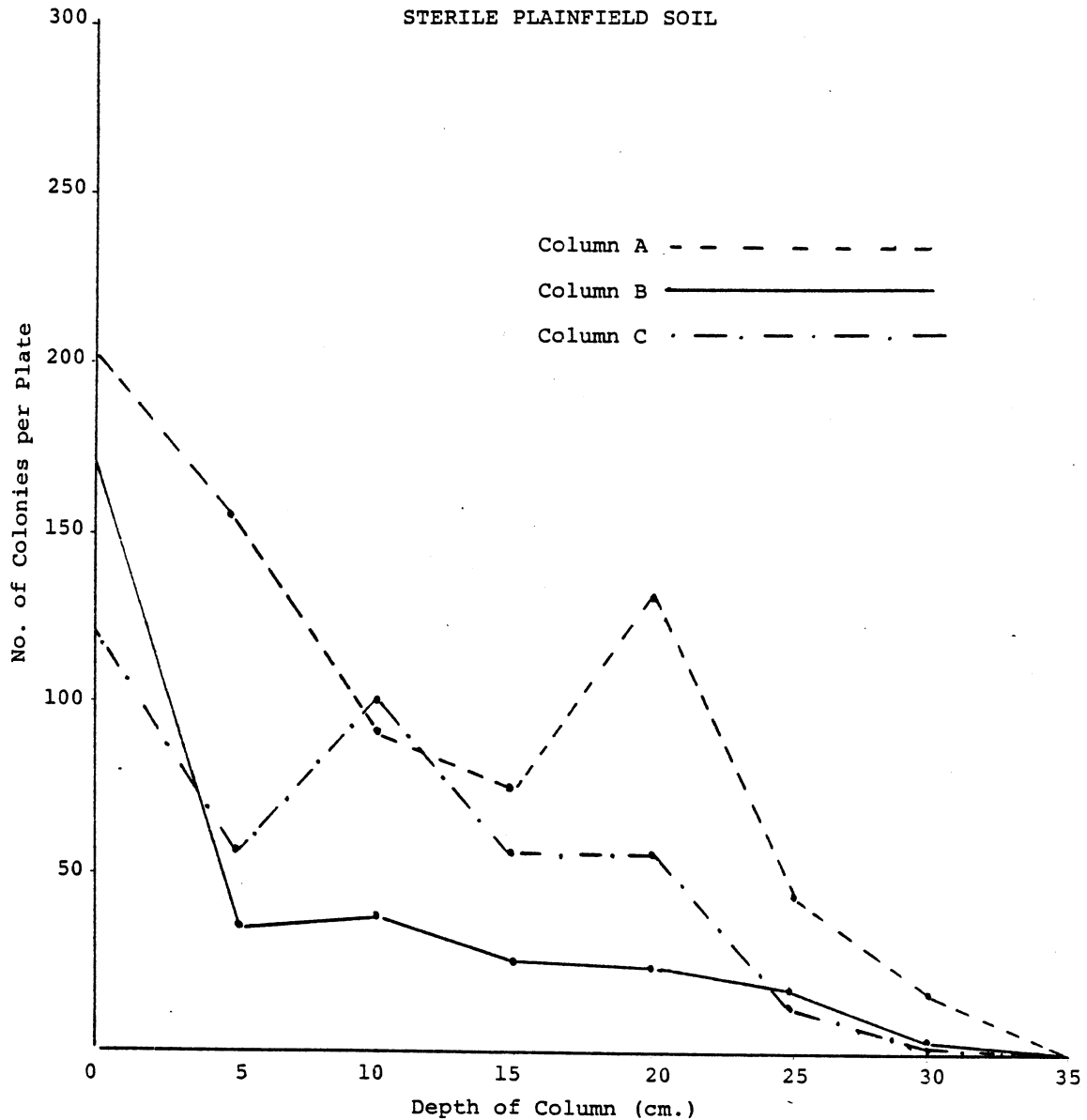


Fig. 17. Distribution of spores of *Metarhizium anisopliae* versus depth in three columns of sterile Plainfield soil after topical application of 2ml of spore suspension and 150ml of sterile distilled water. Each plotted value represents an average of three replicates obtained from each column.

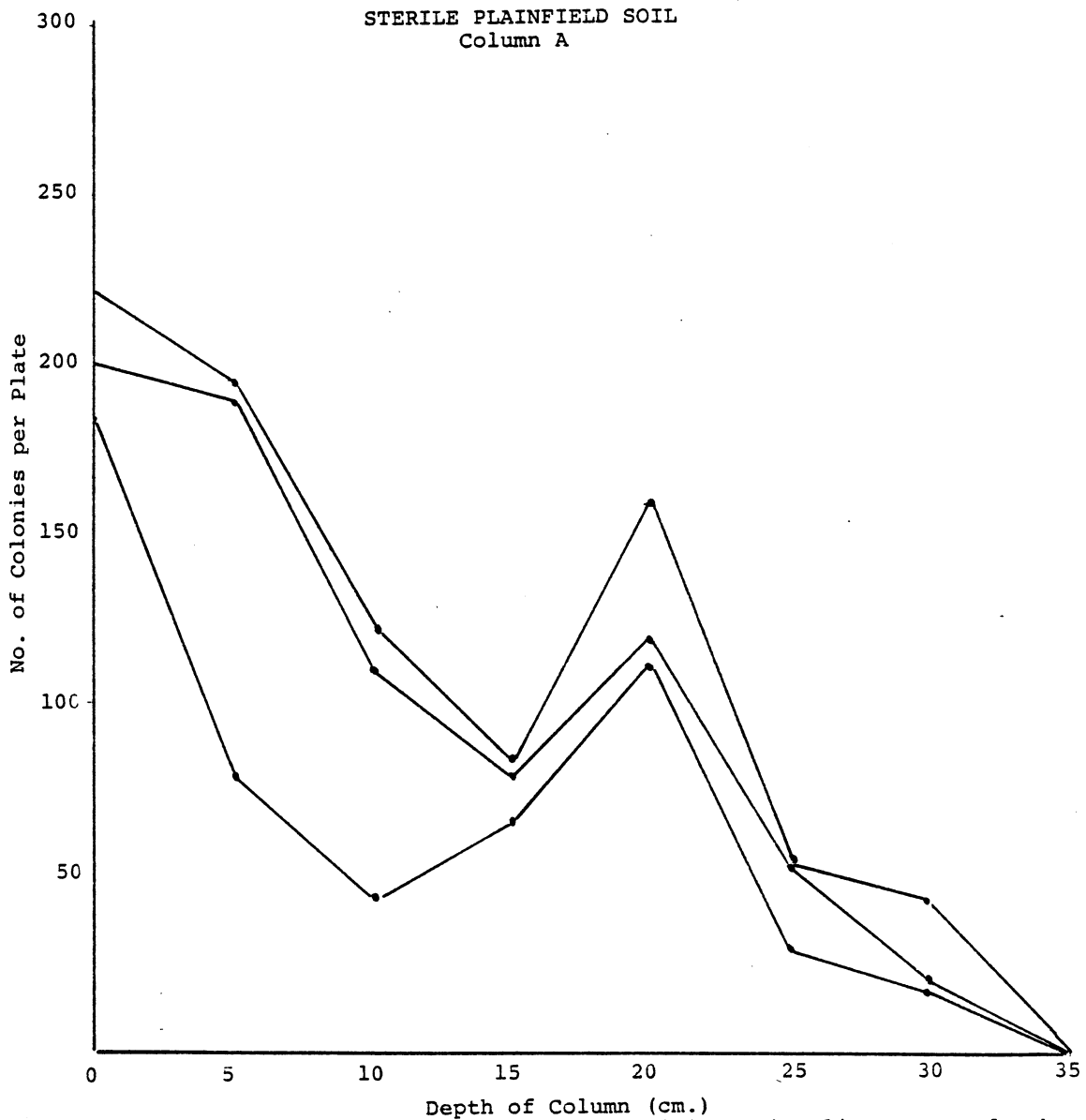


Fig. 18. Distribution of spores of *Metarhizium anisopliae* versus depth in Column A of sterile Plainfield soil after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

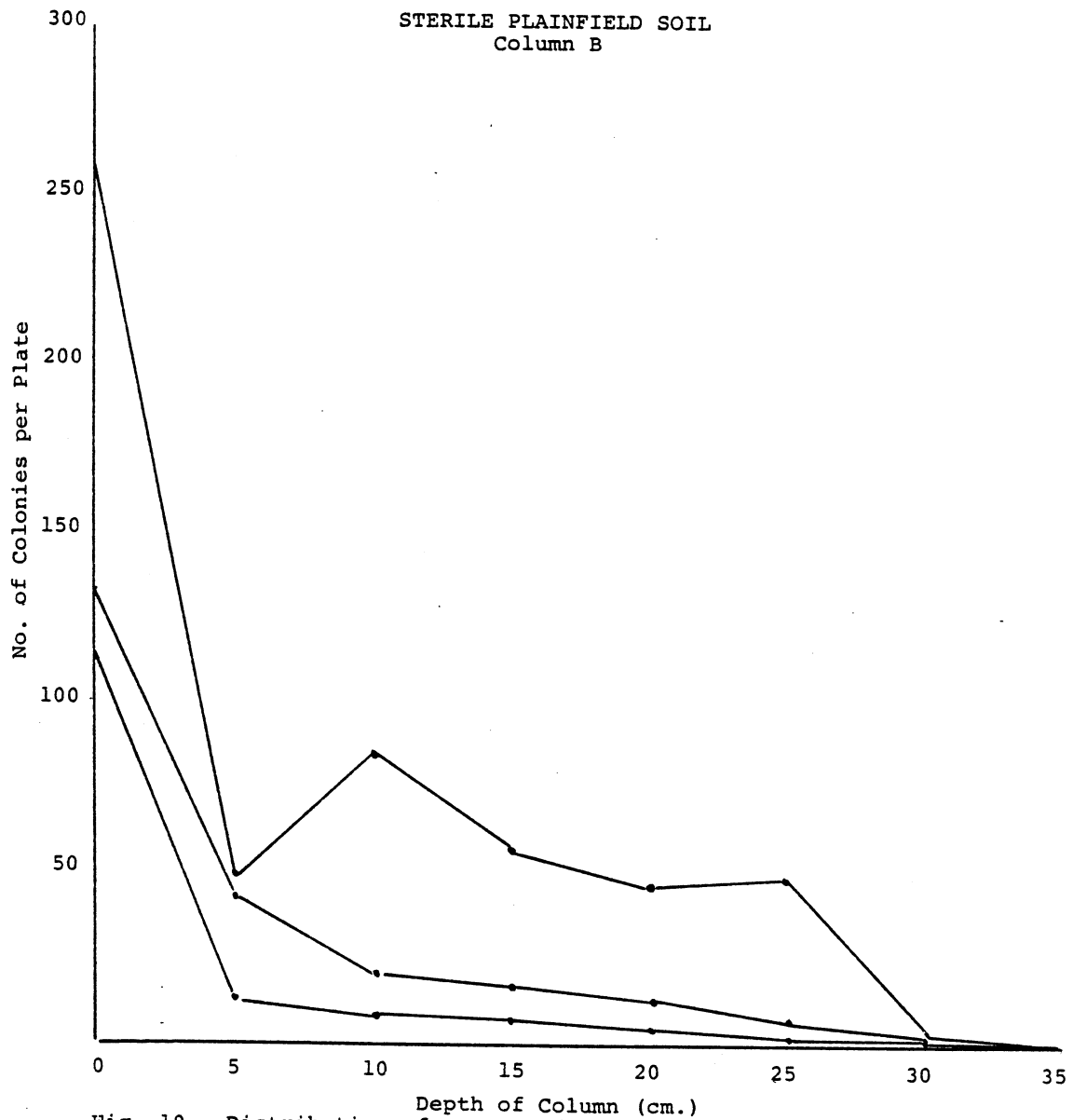


Fig. 19. Distribution of spores of *Metarhizium anisopliae* versus depth in Column B of sterile Plainfield soil after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

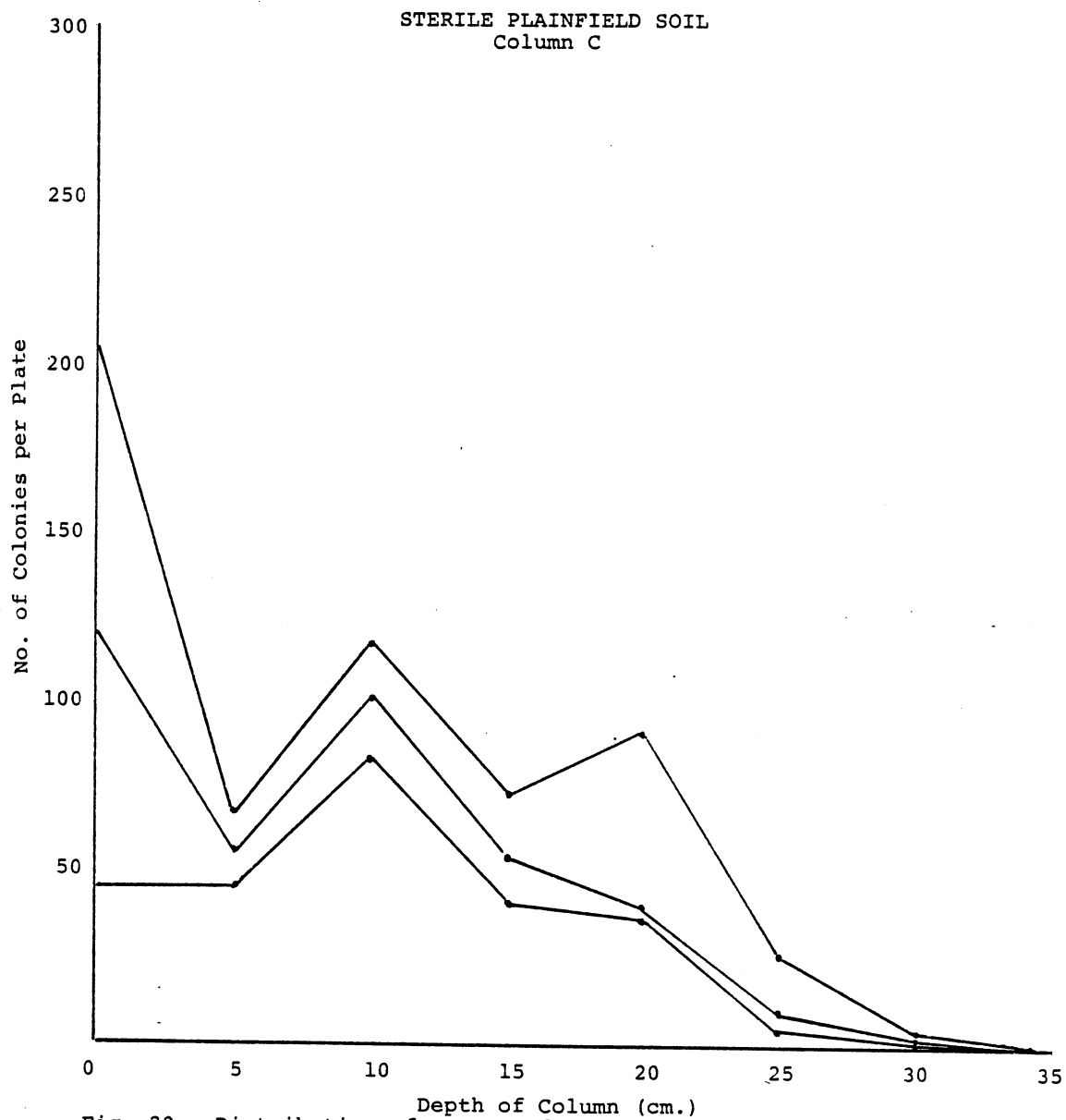


Fig. 20. Distribution of spores of *Metarhizium anisopliae* versus depth in Column C of sterile Plainfield soil after topical application of 2ml of spore suspension and 150ml of sterile distilled water.

centration of spores to the 5cm depth followed again by an increase in spore count at 10cm. After 10cm, spore concentration gradually diminished with depth to virtually zero by 35cm.

#### Unsterile Plainfield soil

The trial initiated with the columns of unsterile soil taken from the field test area showed a general downward migration of spores similiar to those columns containing the sterile soil. Table 1 represents the depths at which colonies of M. anisopliae were present on the culture plates. The number of colonies of M. anisopliae from each sample could not be counted as there were other contaminants growing on the plates even though a selective medium for the isolation of M. anisopliae was utilized. It was only determined if M. anisopliae was present or absent at the depths sampled. The cultures indicated that spores were present in relatively high numbers to the 15cm depth showing a general downward migration trend. By 20cm, the number of colonies on the culture plates had decreased and by 30cm had virtually approached zero. Cultures of the drainage water indicated no evidence of any colonies of M. anisopliae migrating completely through any of the columns containing the unsterile soil.

#### Field Trial

Soil samples were taken from eight test plots which had had spores of M. anisopliae sprayed on the surface.

Column A    Column B    Column C

Surface	+++	+++	+++
5cm	+++	+++	+++
10cm	++	++	+++
15cm	+	+++	+
20cm	0	+	+
25cm	0	0	+
30cm	0	0	0

Table 1. Vertical distribution of spores of Metarhizium anisopliae in three columns of unsterile Plainfield soil after topical application of a spore suspension and 150ml of sterile distilled water. +++ = 70-100% coverage on the culture plates; ++ = 30-70% coverage; + = 1-30% coverage; 0 = no colonies observed on the culture plates. Initial observation taken two weeks after inoculation.

Two months after the initial application of spores, it was observed that the spores had migrated downward to 18cm (Table 2). Fungal colonies were present from the surface to 18cm. By 20cm, no colonies were observed for any of the eight plots. The soil samples were placed on the selective medium to reduce some of the contaminants. Accurate counts of the number of colonies found at each depth could not be taken due to other contaminants, however, the cultures did indicate that colonies of M. anisopliae could be grown from samples of soil taken from the test plots down to a depth of 18cm. Viable inoculum were found in 68% of all samples taken from the plots which were scalped to mineral soil and 75% of the plots which were left undisturbed after two months.

Two samples were taken at 5cm intervals from the field plots and placed on the selective medium four months after the initial application of spores (Table3). The percent of plots with viable inoculum was 75% in samples taken from the plots which had been scalped to mineral soil, and 50% on the undisturbed plots. Seventy five percent of the plots showed evidence that the inoculum had migrated to 20cm while 38% indicated migration to 25 and 30cm. Table 3 shows the results of the positive cultures of M. anisopliae at the various depths in samples obtained from the field plots four months after initial application of spores.

Plot #	Sur- face	2cm	4cm	6cm	8cm	10cm	12cm	14cm	16cm	18cm	20cm	Ave. %
1	X	X	X	X	0	X	X	X	X	X	0	
2	X	X	X	X	X	X	0	0	0	X	0	
3	X	X	X	0	0	0	X	X	0	X	0	
4	X	X	X	X	X	0	X	X	X	0	0	
% Present	100	100	100	75	50	50	75	75	50	75	0	68
5	X	X	X	X	X	X	X	X	X	X	0	
6	X	X	X	X	0	X	X	0	X	X	0	
7	X	X	X	X	X	X	X	X	X	X	0	
8	X	X	X	X	0	0	0	X	X	0	0	
% Present	100	100	100	100	50	75	75	75	100	50	0	75

Table 2. Soil samples obtained from field plots two months after initial application of a spore suspension containing  $6 \times 10^7$  spores per ml of Metarhizium anisopliae. X indicates presence, 0 indicates absence of the fungus. Plots 1-4 were scalped to mineral soil, plots 5-8 were left undisturbed. The soil is classified in the Plainfield series.

Plot #	Sur- face	5cm	10cm	15cm	20cm	25cm	30cm	Ave. %
1	X	0	X	X	X	X	X	
2	0	X	X	X	0	0	0	
3	X	X	X	X	X	X	X	
4	X	X	X	X	X	0	0	
% Present	75	75	100	100	75	50	50	75
5	X	0	0	X	X	0	0	
6	0	X	X	X	X	X	X	
7	X	0	0	0	0	0	0	
8	X	0	X	X	X	0	0	
% Present	75	25	50	75	75	25	25	50

Table 3. Soil samples obtained from field plots four months after initial application of a spore suspension containing  $6 \times 10^7$  spores/ml of Metarhizium anisopliae. X indicates presence, 0 indicates absence of the fungus. Plots 1-4 were scalped to mineral soil, plots 5-8 were left undisturbed. The soil is classified in the Plain-field series.

### Control Trials

None of the control samples indicated that M. anisopliae was naturally present in the soil. A few colonies that appeared suspiciously like M. anisopliae were transferred to the DPY agar and allowed to sporulate. However, these colonies turned out to be Penicillium. Metarhizium was not found to be present in any of the control samples.

### Bioassay

The larvae of the greater wax moth which were inoculated with the spores of M. anisopliae were examined every 24 hours. Thirteen percent of the larvae were dead after 24 hours. Forty eight hours after inoculation, 78% of the larvae were dead and after 72 hours, there was 100% mortality. All larvae showed flaccidity and shrinkage. Within two weeks, the larvae were covered with the characteristic fungal growth diagnostic of M. anisopliae (Fig. 21 and 22).

The control group of larvae showed no sign of any fungal infection after 14 days.



Fig. 21. Larvae of the greater wax moth (Galleria mellonella) 10 days after inoculation with spores of Metarhizium anisopliae originating from cultures obtained from spores which had migrated through 15cm of sterile sand. The larvae show signs of external fungal growth characteristic of M. anisopliae



Fig. 22. Larva of the greater wax moth (Galleria mellonella) two weeks after inoculation with spores of Metarhizium anisopliae originating from cultures obtained from spores which had migrated through 20cm of sterile sand-loam mixture. The insect is covered with the fungal growth diagnostic of M. anisopliae.

## DISCUSSION

The objective of this research was to determine whether M. anisopliae would migrate vertically through soils following topical application of spores. This factor would be essential if the fungus is to be considered for field application as a microbial insecticide against soil-inhabiting insects. This research revolved around the possibility of M. anisopliae reaching white grubs, Phyllophaga spp. which attack the roots of various nursery and seedling stock including red pine. Red pine grows best on well-drained sandy soils and has been extensively planted in Central Wisconsin as a source of pulpwood for the area's paper mills. Since light sandy soil is also a favorite habitat of white grubs, red pine seedlings are often planted in areas of high grub infestation.

The three types of soil tested in this experiment are commonly found in Central Wisconsin, the area of this research. Thirty percent of the soils in Central Wisconsin are classified in the Plainfield series (Hole 1976). This research was designed to determine the migration of spores of M. anisopliae through coarse sand and sand-loam mixture of which the Plainfield soils consist. If spores traveled through these soils, it was assumed they would also travel through the soil most commonly associated with red pine and white grubs. Because of the reduction of chemical spraying for the control of white grubs due to enforcement by environmental groups, a biological agent such as M. anisopliae which is capable of existing in soils and is able

to migrate through soils would be of importance.

The laboratory tests indicate that spores of M. anisopliae are capable of dispersing in soil through the direct action of water. Water may affect dispersal through splash, run-off, and percolation under natural conditions. Dispersal by water is also dependent on interstitial pore space, spore shape, and consistency of spore coat. Spore dispersal by water is considered effective in a vertical mode rather than a lateral one.

Fungi which produce a mucilaginous spore mass are more capable of dispersing in the soil through the action of water than fungi which produce dry and powdery spores (Burgess 1950, Hepple 1960). Spores of M. anisopliae were suspended in a wetting agent (Tween 20) to help alleviate the antagonistic action between the spores and water by artificially changing the behavior of the spores to lessen their resistance to wetting. The concentration of Tween 20 was .4%, which is known not to interfere with the viability or pathogenicity of the spores.

The trials undertaken in the laboratory were concerned only with the dispersal of spores by the influence of water combined with Tween 20. The SDW poured on the columns and the distilled water sprayed on the field plots after the addition of the spore suspension, were equivalent to approximately one and one-quarter inches of rain per acre. This amount was derived as an amount of rain that can be expected in the spring when red pine seedlings are planted.

If tree planting could coincide with a forecast of heavy rain, the spores would have a good chance of being washed into the upper layers of soil thus protecting them from direct exposure to the elements.

The results from the trials conducted in the laboratory were fairly constant due to the fact that the variables were kept to a minimum. The columns filled with the sterile sand and sand-loam mixture were consistent in particle size and compaction to each other and the methods of experimentation were the same. The results indicated this by the repeatability of the trials. Some variations could have occurred due to human inconsistency as to speed in which the SDW was added and the length of time elapsed between the final addition of SDW and the taking of the samples.

The spores were moved rapidly downward to the 10cm level in the columns filled with sterile sand. The reason for this retention of spores at that level is not clear, but it is possible that it may be due to the strong adhesion of the spores to the sand grains. An insignificant number of spores did migrate all the way down the column although not completely through the column. This research was concerned only with the possibility of spores migrating to a certain depth and not with the quantity of spores that reached that depth. Although spores did migrate to the 35cm depth, the amount of spores probably would not be significant in the incidence of infection in insects.

The columns containing the sterile sand-loam mixture

also showed a general downward migration of spores but the spores migrated only to a depth of 20cm with the majority of spores retained at the 5cm depth. This higher retention of spores at the upper levels of the columns might be attributed to the finer consistency of the soil. Small bodies such as spores also may carry electrical charges and it is possible that this ionization between the spores and the soil might be responsible for the retention of spores after traveling only a short distance. Spomer (1980) has shown that soils in containers develop a perched water table with the bottom 8-10cm of the soil having a 100% pore saturation. This water distribution exhibits a sharp gradient in sand and silty clay loam soils and may block access of the spores to lower column levels.

Results from samples of soil taken from the field and then sterilized showed the most variation. This was due partly to the method of obtaining the sample. Each sample was taken separately and on different days as only one stainless steel column was used in this part of the experiment. Only one column was constructed because of the difficulty and expense in making the column. In comparison, the sterile sand and sand-loam mixtures were placed in three columns and the trials for all three replicates were accomplished at one time. Since each soil sample was obtained on different days, the soil sample would have been in a different state of moisture content. One sample could

have been more tightly packed in the column itself than another. Each sample was sterilized intact in the column and the trial then proceeded in the same manner as those conducted with the sand and sand-loam mixtures. The results indicated that the spores moved through the column of soil without any of the sharp drops or peaks associated with the other types of soils. The majority of spores reached the 20cm depth before dropping off to insignificant levels. This further penetration of spores could have been the result of the roots of the grasses providing channels through which the spores could more easily pass. The smaller number of spores at the surface of the columns of soil could have been a result of a looser consistency of the soil due again to the vegetative growth. Different horizons could act as a filter bed for the spores washed down from the soil surface, or the nature of the soil horizon itself could result in the migration or retention of the spores.

The trial consisting of the three replicates of unsterile soil was procured and conducted in the same fashion as the trial in which the soil was sterilized. One column was again utilized and each trial was obtained and conducted on different days. The same variables would thus enter into the results. The effect of unsterile soil on the spores of the fungus has to be considered. Microorganisms which are naturally found in the upper layers

of soil and especially associated with the roots of grasses could have had an antibiotic or fungistatic effect which might account for the fewer number of spores observed on the surface and upper layers of the columns of unsterile soil.

A selective medium for the isolation of M. anisopliae was poured over the soil samples. An accurate count of the colonies of M. anisopliae could not be taken due to other fungal and bacterial contaminants. Fungistasis or antibiosis could have influenced the results. There did appear to be fewer colonies of M. anisopliae present on the plates than those samples taken from the soils which had been sterilized. The reduced number of colonies could also have been due to samples being placed in the selective medium which includes various antibiotic substances acting as bacterial and fungal inhibitors. Although these antibiotics are not supposed to inhibit the growth of M. anisopliae, they might reduce the germinating power of the spores. Fungistasis is an occurrence that must be taken into consideration when attempting to employ a fungus as a microbial insecticide.

There is apparently no clear evidence of the origin or nature of soil fungistasis. Jackson (1965) reviews some of the possible sources of soil fungistasis with the conclusion that the inhibiting conditions in the soil could

be ascribed to a complex of different inhibitory factors including antibiotic reaction, a form of staling between like and unlike organisms, and/or toxic metabolites. Roberts and Campbell (1977) report that other microorganisms found in the soil could also produce fungistatic compounds. These interrelating factors probably act together in a synergistic fashion to produce conditions which are unsuitable for spore germination. Fungistasis has been described from tropical to subarctic soils and in a wide range of soil types. Lockwood (1964) reviews the theory, origins, and work conducted on soil fungistasis. Fungistasis would appear to be a factor in the germination of the spores of M. anisopliae under natural conditions as Walstad et al. (1970) state that the spores do not germinate in unsterile soil.

No attempt was made to determine whether other factors such as soil structure, soil microorganisms, wind, erosion, etc. would have an effect on the migration of spores of M. anisopliae. In establishing M. anisopliae as a microbial control agent, these dispersal factors must be taken into consideration. Once the inoculum is placed in the field, it is subject to the vagaries of the environment. The importance of the dispersion of spores has been emphasized by Tanada (1963) in his statement that an insect pathogen lacking a high dispersal capacity may have a low potential of developing an epizootic even though it may possess a high virulence for the host coupled with efficient survival

capacities.

Dispersal in nature occurs in various ways which include both passive and active movement. Passive dispersal employs such agents as wind, water, animals, and other vectors. Active dispersal depends on either the mobility of the organism itself or on its growth. This growth can exhibit a definite search pattern for the host. Since the spores of M. anisopliae are not motile and have not been found to germinate and grow in soil (Walstad et al. 1970), the fungus must depend on passive mechanisms for its spores to be dispersed throughout the soil.

Vectors such as earthworms, nematodes, mites, and various microorganisms are important biotic agents contributing to the dispersal of fungi. Insects are other important agents, especially the primary hosts since they have the closest association with the particular pathogen. These insects may contract the disease and transmit infective material either externally or through the digestive system in the excretion of spores. Non-susceptible insect species may carry spores adventitiously through the soil. Mites have also been associated with the distribution of entomopathogenic soil fungi in a horizontal and vertical mode (Samsišňáková and Samsišňák 1970). Schabel (in press) reports on the possible role of several species of phoretic mites as carriers of the inoculum of M. anisopliae against soil-inhabiting insects. Small animals, birds, and man

also contribute to the dispersal of fungal spores.

The dispersal of spores of M. anisopliae under natural conditions would depend on too many external and uncontrollable factors for dependable and repeated success in the field. Spore dispersal therefore should rely primarily on manipulated dispersion. This would involve direct introduction of the inoculum in an area which is in close approximation to the susceptible host. M. anisopliae could be used as a microbial insecticide where thorough coverage through repeated applications may place infective material in contact with the pest species.

The soil which was used for this research was designated as a Plainfield sand having a 2-4% slope. The hazard to water erosion is slight and that of blowing soil severe. Permeability is rapid and the available water is low. Plainfield sand is suitable for forestation with species such as red pine, Eastern white pine (Pinus strobus) and Jack pine (P. banksiana) with red pine being the preferred species. Red pine has a high potential productivity with a yearly growth per acre of 210 board feet (USDA Soil Cons. Serv. 1977). While seedling mortality is low on Plainfield soils, white grubs are a serious pest of red pine seedlings especially when the pine transplants are planted in soils that have been idle for a year or more and have a heavy grass or weed cover (Rose and Lindquist 1973). Damage is most severe on light soils such as

Plainfield sand. The white grub has been recognized as a problem for more than 50 years. In 1953, white grubs caused the principal injury to young plantations on 8000 acres in South Carolina (Speers and Schmiede 1971) while in Central Wisconsin a loss of 10-15% of nursery stock is not unusual especially in the age class below 2-2 (Shenefelt and Simkover 1950). The white grub was thus targeted as the insect used for this research. The insect is susceptible to green muscardine disease and M. anisopliae has been an important factor in grub control on several continents (Ritcher 1958). There are more than 100 species in the genus Phyllophaga and most cause some type of damage to crops, grass pastures, and nursery plantings. The larvae of all species inhabit the soil and have a one to three year life cycle. Those species having a three year life cycle are considered the most injurious and happen to be commonly found in Wisconsin. The larvae cause damage by severing or girdling the lateral and tap roots of young seedlings causing injury or death. Populations of white grubs can build up on open land and populations of two or more per two square feet can cause high mortality (Speers and Schmiede 1971).

The adults lay eggs in the soil at a depth of 5-15cm. These hatch in two to three weeks and the larvae feed on soil organic matter. The larvae hibernate in the soil at various depths depending on frost levels. The second summer

they feed on roots and are most injurious at this stage. The grubs again hibernate in the winter and return to the surface the following spring where they feed on roots until June. At this time, they make earthen cells at depths ranging from 8-30cm and change to the inactive pupal stage (Ritcher 1958). They remain in this stage until the following spring when they emerge as adults.

The larvae are very sensitive to moisture conditions and move up and down in the soil as moisture conditions change (Ritcher 1958). Therefore, it could be assumed that M. anisopliae would not be effective during dry years as the grubs would be at a depth to which the spores would be unlikely to migrate.

The effect of M. anisopliae on the insect family Scarabaeidae (to which the genus Phyllophaga belongs) has been studied by other researchers in its possible use as a biological control agent against these insects. Latch (1965) reported on the possible use of M. anisopliae for controlling pasture-inhabiting grubs. Urs and Govindu (1971) experimented with M. anisopliae and infected several species of Scarabaeidae to observe disease incidence and found a number of species were susceptible to the fungus. Latch (1976) tested the pathogenicity of M. anisopliae to the coconut rhinoceros beetle and attempts to control this pest with the fungus have been made by spraying a spore suspension on the beetle's breeding sites. These studies

have found M. anisopliae to be effective against the insect family Scarabaeidae in the field.

Several white grubs obtained from the soils of Central Wisconsin were inoculated with spores of M. anisopliae to determine the effectiveness of green muscardine disease on this particular insect. All the larvae subsequently died of mycosis and were covered with the characteristic fungal tufts of growth diagnostic of M. anisopliae within three weeks. The identity of the fungus was confirmed by transferring mycelial growth on the larvae to a nutrient agar medium and also by microscopic examination of the spores. It was assumed that the grubs which are native to the soils of Central Wisconsin are susceptible to M. anisopliae.

The spores which were applied to the soil were exposed to changes in temperature and moisture conditions and subjected to direct radiation. The weather following the application of spores in mid-June was warm, sunny, and dry. No water was applied to the plots despite the dry conditions except the three liters of distilled water initially sprayed on the surface of the plots. The first sampling of the plots was taken after two months had elapsed from the initial application of spores. In this time, 7.24 inches of rain had fallen. Most of that precipitation came in the month of August with June recording only .63 inches of precipitation. The average temperature for the two month period was 70.2°F. with the sunshine averaging 66% (App. 1).

This first sampling produced colonies of M. anisopliae on the selective medium to a depth of 18cm. This was verified by transferring suspected colonies of M. anisopliae to the DPY agar, allowing these cultures to sporulate, and observing the spores under a microscope. A few other contaminants including Fusarium, Penicillium, Aspergillus, and Streptomyces were also found growing on the medium.

The rains began in earnest shortly after the first sampling of the plots had been taken in mid-August and continued through September. Between mid-August and mid-October when a second sampling was taken, an average of 10.09 inches of precipitation had fallen, the average temperature was 59.9°F. and the possible sunshine was 57% (App. 1). This second sampling indicated that the spores of M. anisopliae had migrated to a depth of 30cm. The environmental conditions in this portion of the research could not be controlled. It is not known whether the spores would have migrated further if the application of spores had been followed by wet conditions, either through natural means or through artificial irrigation.

The first sampling indicated that the plots which were left undisturbed had a higher percentage of viable spores than those plots which were scalped to the mineral soil. This could have been a result of the protection the sod provided. The grasses could have created a more favorable microenvironment by producing slightly lower soil temper-

atures and a higher available moisture content. Spores were readily cultured from samples taken at the surface and upper layers of soil. Four months after the initial application of spores, the samples indicated a different percentage between the two types of field plots. A higher percentage of viable spores was cultured from those plots which had been scalped and the percent of viable spores from the undisturbed plots had decreased by 25%. Roots are associated with their own microorganisms and these could have caused a fungistatic or antibiotic effect on the spores creating unfavorable conditions for their survival. There were fewer colonies of spores observed from the surface and upper layers of soil.

A bioassay was devised to determine whether M. anisopliae remains virulent as it migrates through soils. Greater wax moth larvae were used for this bioassay. This insect was well suited for the bioassay because of its ready availability and its sensitivity to small amounts of the toxins produced by M. anisopliae. The larvae had been treated with insect growth regulators to prevent them from entering the pupal stage. Spores were brushed on the head and integument of the larvae. No attempt was made to determine the number of spores applied. A few of the larvae did spin a cocoon but not enough to affect the results. Roberts (1965) used the greater wax moth in his studies on the production of toxins produced by M. aniso-

pliae.

Is M. anisopliae then a suitable organism for microbial control of white grubs and other noxious insects? The outlook for practical application of M. anisopliae as a microbial insecticide in reducing pest populations is considered favorable for a number of reasons. The inoculum is easily prepared in bulk on natural or synthetic media from conidia. The spores can be disseminated over large areas either in an aqueous solution mixed with a wetting agent or in combination with dry fillers such as sand, silt, or talc. The conidia are able to withstand cold and temperature fluctuations and can adjust to either a saprophytic or parasitic mode of existence. Virulence can be maintained through a number of generations on artificial substrates. It has been shown that M. anisopliae can survive under field conditions for a period of time (Bell and Hamalle 1970, Walstad et al. 1970, Bell 1975, Müller-Kögler 1976).

M. anisopliae has a wide host range and cross infection capabilities and has long been studied as a control agent against major insect pests. Adamek (1965), Schaerf-fenberg (1964), Latch (1965, 1976), Bell and Hamalle (1970), and Schabel (1976, 1978), have indicated that M. anisopliae indeed has potential and successfully infects a number of insect pests under laboratory conditions and in a few instances with small field trials. However, results of large scale field tests (Latch 1965, 1976; Fox and Jaques

1958), have ended with only minor successes and often with negative results. These results support the theory that control is dependent on proper conditions of temperature, humidity, and light which affect fungal spore viability and competitive ability. Fox and Jaques (1958) state that the quantity of spores applied is of secondary importance to environmental conditions which become the limiting factor. Even though M. anisopliae was found to migrate through the soil profile, environmental conditions would appear to prevail in control measures. This does not imply that M. anisopliae could not be successfully employed in large scale pest control. Much of this research is not recent and the unsuccessful attempts could be due to a lack of knowledge on the background of the fungus (dosage requirements, biochemical relationship between the fungus and insect, microenvironmental requirements, dispersal capabilities).

The factors that do prevail are the environmental conditions. Environmental conditions can be altered to favor the fungus. Changing the soil by modifying cultural practices or including a selective fungicide with the fungus to suppress agents in the soil that might inhibit it could be implemented. Repeated applications of the fungus in the role of a biological insecticide may also counteract the effect of the microenvironmental conditions.

M. anisopliae by its natural existence in the soil,

has been involved in naturally occurring biological control. Natural control can be defined as "the maintenance of a more or less fluctuating population density of an organism within certain definable upper and lower limits over a period of time" (DeBach 1964). Classical biological control on the other hand, is commonly understood as the regulation of a pest population by manipulating resident natural enemies to increase their regulating capabilities (Caltagirone 1981). These pests usually reach a high population density and present economic problems to man. Biological control is dependent upon a foundation of ecological principles and is implemented by manipulating biological materials.

A successful control program for the use of M. anisopliae against insect pests probably lies in an integrated program. Basically integrated control is a comprehensive systems approach to pest control that is ecologically sound and which utilizes a variety of control technologies compatible in a pest management system with the ultimate objective to produce optimum return (crop, comfort, recreation) at minimum cost (Falcon 1973). It would appear that none of the traditional methods of control; chemical, biological, genetic, or cultural, alone can provide the final answer to pest control. However, a combination of two or more practices can often produce favorable results. The prime requisite for integrated control is basic ecological

knowledge, a thorough understanding of the target pest, and information about the pathogen in its use, safety, selectivity, persistence, and dissemination.

An example of a successful program utilizing the integrated approach in a combination of practices including the application of M. anisopliae was established in Northeast Brazil in the sugarcane fields. This program involved 1) chemical insecticide which was combined with 2) cultural control with the burning of the sugarcane straws at the end of the season, 3) biological control using insect parasites, and 4) microbial control with the mass dissemination of spores of M. anisopliae. In this combination of integrated control, it was found that M. anisopliae was the most important mortality factor in the regulation of the pest species in the sugarcane fields. M. anisopliae used alone in this case, would probably have met with little success (Falcon 1974).

An integrated program for the control of white grubs in potential plantation or nursery sites is envisioned to include a three point attack, involving 1) cultural, 2) chemical, and 3) microbial practices. Preventive measures should be taken before seedlings are planted such as discing or plowing up the sod the spring preceding planting. This would expose the insects to the environment and predators such as various mammals and birds. The field should be kept free of grasses and weeds so that the adult insect

## CONCLUSION

Mankind is in constant competition with many pest species that interfere with his ability to produce food and fiber. There will be a continual need to provide means for effective control and management of these pests. Metarhizium anisopliae appears that it could be an effective candidate as a microbial agent of certain soil-inhabiting insects. It can be easily mass-produced on artificial media, it is fairly stable under field conditions, and it remains virulent and effective against a number of soil-inhabiting insects. Spores can migrate downward through soils with the aid of a wetting agent (Tween 20) and water and thus may be dispersed to areas inhabited by pest populations. Therefore, M. anisopliae should be effective in pest control management, especially in an integrated aspect and in habitats where optimum environmental conditions exist.

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APPENDIX 1.

Local Climatological Data - June 1980 through October 1980.

JUNE 1980  
MADISON, WISCONSIN  
NATIONAL WEATHER SERVICE OFC  
TRUAX FIELD

# Local Climatological Data

## MONTHLY SUMMARY



JUNE 1980

MADISON, WISCONSIN

LATITUDE 43° 08' N LONGITUDE 89° 20' W ELEVATION (GROUND) 958 FT. STANDARD TIME USED: CENTRAL MOAN #14837

DATE	TEMPERATURE °F			DEPARTURE FROM NORMAL	AVERAGE DEW POINT	HEATING INDEX	COOLING INDEX	WEATHER TYPES OR DATES OF OCCURRENCE	SNOW, ICE OR SLEET	PRECIPITATION		WIND	SUNSHINE	SET COVER PERCENTS												
	MAXIMUM	MINIMUM	AVERAGE							WATER	SNOW				RESULTANT DIR.	RESULTANT SPEED	AVERAGE SPEED	FASTEST WIND	PERCENT POSSIBLE	SUNSHINE TO	PERCENT					
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22					
1	65	55	60	-2	53	5	0	1	0	0	0	0	28	84	11	7.5	9.1	19	SE	85	9	10	10			
2	77	52	65	3	55	0	0	0	0	0	0	0	26	81	09	1.8	6.2	19	N	526	58	6	7			
3	73	52	63	1	52	0	0	2	0	0	0	0	29	09	06	1.9	3.7	10	SE	711	78	5	5			
4	74	52	63	0	50	0	0	0	0	0	0	0	29	26	16	7.0	8.2	22	S	653	72	8	8			
5	79	56	68	5	56	0	0	3	1	0	0	0	28	17	15	7.9	9.9	24	NW	248	27	9	9			
6	79	61	70	7	60	0	0	5	3	0	0	0	28	87	21	2.4	8.5	19	S	322	35	7	9			
7	82	53	68	4	59	0	0	3	3	5	0	0	1.01	0	28	85	31	3.7	12.1	44	NW	568	62	6	7	
8	69	45	57	-7	42	0	0	0	0	0	0	0	0	29	20	30	12.4	12.7	24	NW	777	85	3	3		
9	71	42	57	-7	47	0	0	0	0	0	0	0	0	28	95	30	11.1	11.4	22	NW	463	50	8	9		
10	68	37	53	-12	37	12	0	0	0	0	0	0	0	29	07	33	4.5	5.8	15	NW	876	95	5	4		
11	77	49	59	-4	43	0	0	0	0	0	0	0	0	29	28	25	2.2	3.7	10	SW	920	100	0	0		
12	81	51	66	1	50	0	0	0	0	0	0	0	0	29	23	18	9.8	10.2	19	S	709	77	9	7		
13	81	59	70	8	55	0	0	1	1	0	0	0	0	29	04	18	9.3	7.1	18	SW	505	55	9	9		
14	87	60	74	8	60	0	0	0	0	0	0	0	0	28	42	20	4.6	6.5	24	SW	932	92	3	5		
15	63	44	54	-12	48	11	0	0	0	0	0	0	0	05	0	29	33	25	11.9	12.2	19	NE	110	13	10	9
16	71	37	54	-12	39	11	0	0	0	0	0	0	0	0	29	26	01	3.5	4.6	12	S	922	100	0	0	
17	75	45	60	-4	48	5	0	0	0	0	0	0	0	0	29	18	21	8.4	9.5	16	SW	912	99	3	2	
18	74	51	63	-4	52	3	0	0	0	0	0	0	0	0	29	06	03	1.3	7.2	16	NE	326	35	9	9	
19	68	48	58	-9	46	7	0	3	0	0	0	0	0	0	29	06	34	7.7	9.6	20	N	603	65	6	6	
20	79	43	61	-4	46	4	0	0	0	0	0	0	0	0	29	21	25	3.4	4.2	10	SW	849	92	4	20	
21	92	56	69	2	49	0	4	0	0	0	0	0	0	0	29	12	22	7.0	7.3	16	SW	800	87	5	6	
22	88	60	74	6	56	0	0	0	0	0	0	0	0	0	29	05	20	5.9	6.5	14	SW	512	55	9	9	
23	91	58	75	7	60	0	0	10	0	0	0	0	0	0	29	04	16	3.7	4.0	14	S	467	51	10	9	
24	90	58	74	6	62	0	0	9	1	0	0	0	0	0	29	05	10	3.2	4.6	16	SE	746	81	5	5	
25	93	62	78	10	65	0	0	13	2	0	0	0	0	0	29	06	22	4.7	4.9	14	SW	815	88	3	25	
26	96	64	80	12	68	0	0	15	1	0	0	0	0	0	29	03	25	5.0	8.2	16	N	608	66	9	8	
27	79	58	65	1	55	0	0	4	3	0	0	0	0	0	29	03	08	11.4	11.7	18	SW	299	32	9	9	
28	88	62	75	6	61	0	0	10	1	3	0	0	0	0	28	91	28	3.5	7.6	23	E	830	90	2	3	
29	72	55	64	-5	52	1	0	0	0	0	0	0	0	0	28	49	24	10.1	11.8	22	NW	489	53	5	4	
30	78	51	65	-4	54	0	0	1	0	0	0	0	0	0	29	08	25	3.4	5.9	10	SW	830	90	5	5	

SUM	SUM	TOTAL		TOTAL	TOTAL	FOR THE MONTH:		TOTAL	SUM	SUM	
2350	1507	34	100	0	43	0	29 05 122 1 2 7 9 44	18313	182	179	
116	116	116	262	116	262	262	PRECIPITATION	262	DATE: 37	MONTHLY	
78.3	52.2	55.3	-3.5	52	12	4	2.01 INCH	11	-0.90	125801	
NUMBER OF DAYS		TOTAL		TOTAL		GREATEST IN 24 HOURS AND DATES		GREATEST DEPTH OR GROUND OF SNOW		ICE POLLETS IN ICE AND DATE	
4	0	0	0	4	20	0	0	0	0	0	

EXTREME FOR THE MONTH - LAST OCCURRENCE IF MORE THAN ONE.  
 T TRACE AMOUNT  
 + ALSO ON AN EQUALER DATE, OR DATES.  
 HEAVY FOG - VISIBILITY 1/4 MILE OR LESS.  
 FIGURES FOR WIND DIRECTIONS ARE TENS OF DEGREES CLOCKWISE FROM TRUE NORTH, OR ° CALCL. DATA IN COLS. 6 AND 12-15 ARE BASED ON 7 OR

MORE OBSERVATIONS PER DAY AT 3-HOUR INTERVALS.  
 FASTEST WIND SPEEDS ARE FASTEST OBSERVED ONE-MINUTE VALUES WHEN DIRECTIONS ARE IN TENS OF DEGREES. THE / WITH THE DIRECTION INDICATES PEAK GUST SPEED.  
 ANY ERRORS DETECTED WILL BE CORRECTED AND CHANGES IN SUMMARY DATA WILL BE INDICATED IN THE ANNUAL SUMMARY

### SUMMARY BY HOURS

HOUR	LOCAL TIME	SET COVER PERCENT	STATION PRESSURE IN.	TEMPERATURE				RELATIVE HUMIDITY %	WIND SPEED M.P.H.	DIRECTION	RESULTANT WIND SPEED M.P.H.
				AIR °F	WET BULB °F	SHADE °F	WIND CHILL °F				
001	6:29:04	58	54	51	77	7.2	19	1	3		
021	6:29:33	56	53	50	81	5.8	18	1	8		
041	6:29:06	58	54	51	80	5.8	16	4	6		
061	6:29:08	67	59	61	92	2.5	2.7				
121	6:29:06	72	62	54	52	10.2	23	2.5			
181	6:29:03	76	63	54	47	9.7	24	2.8			
181	6:29:02	73	62	54	54	8.4	21	1.4			
211	6:29:04	63	58	53	70	6.2	12	4			

### HOURLY PRECIPITATION (WATER EQUIVALENT IN INCHES)

HOUR	1 2 3 4 5 6 7 8 9 10 11 12											
	1	2	3	4	5	6	7	8	9	10	11	12
1												
2												
3												
4												
5												
6												
7	.10	.14	T	T	.01	T	T	.06	.01	.29	T	
8												
9												
10												
11												
12												
13												
14	T	T	T									T
15												
16												
17												
18												
19	T											
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21												
22												
23												
24												
25												
26												
27												
28	.03											
29												
30												

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*Daniel B. Mitchell*  
 DIRECTOR, NATIONAL CLIMATIC CENTER

# OBSERVATIONS AT 3-HOUR INTERVALS

HOUR	SKY COVER	CEILING HDS. OF FT.	VISIB- ILITY MILES	WEATHER	TEMPERATURE					WIND					SKY COVER	CEILING HDS. OF FT.	VISIB- ILITY MILES	WEATHER	TEMPERATURE					WIND							
					AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.	DIR	SPEED MPHS	AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.					DIR	SPEED MPHS	AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.	DIR	SPEED MPHS					
DAY 01																															
00	10	100	10		64	63	62	93	00	0	10	90	10		64	62	60	87	00	0	2	UNL	10		61	60	60	97	00	0	
03	3	UNL	10		58	57	57	96	06	3	10	50	10		68	62	59	73	09	8	0	UNL	10		61	60	60	97	00	0	
06	2	UNL	10		59	58	58	95	03	3	10	80	10		63	61	60	90	36	5	0	UNL	10		59	58	58	97	00	0	
09	2	UNL	10		79	67	60	53	23	6	10	65	9		63	62	61	83	06	8	0	UNL	10		73	67	63	71	34	3	
12	0	UNL	10		89	70	59	36	20	7	10	150	7		75	66	61	62	07	9	1	320	9		77	66	59	54	18	4	
15	2	UNL	10		92	71	61	36	27	5	10	80	10		74	66	62	66	05	5	3	UNL	10		85	66	55	36	28	5	
18	4	UNL	10		87	70	61	42	31	6	8	UNL	10		76	68	63	64	32	6	0	UNL	10		80	65	56	44	20	5	
21	4	UNL	10		65	61	59	81	00	0	1	UNL	10		64	62	61	90	00	0	0	0	UNL	10		66	62	59	78	00	0
DAY 02																															
00	6	250	10		66	64	63	90	17	5	10	9	10		68	67	66	93	17	6	0	UNL	10		61	60	59	93	02	3	
03	5	UNL	10		64	62	61	90	13	6	2	UNL	10		65	65	65	100	19	4	10	210	9		61	60	60	97	00	0	
06	10	200	10		65	62	61	87	18	9	10	UNL	6		65	64	63	93	19	5	10	140	7		67	66	65	93	00	0	
09	10	140	9		74	66	62	66	18	11	2	UNL	7		73	67	63	71	22	6	10	75	7	RM	73	70	68	84	17	5	
12	10	140	9		84	75	71	65	19	13	0	UNL	10		82	68	61	49	22	7	3	UNL	10		85	73	68	37	19	5	
15	10	140	6		84	75	71	65	19	7	0	UNL	10		85	69	61	45	21	11	7	60	10		86	75	70	59	18	2	
18	10	65	6		81	75	72	74	24	5	0	UNL	10		82	68	65	38	29	6	0	UNL	10		86	75	70	59	18	7	
21	10	80	4		69	68	67	93	19	18	1	UNL	10		68	64	61	78	19	6	0	UNL	10		75	72	70	85	18	5	
DAY 03																															
00	6	250	10		66	64	63	90	17	5	10	9	10		68	67	66	93	17	6	0	UNL	10		61	60	59	93	02	3	
03	5	UNL	10		64	62	61	90	13	6	2	UNL	10		65	65	65	100	19	4	10	210	9		61	60	60	97	00	0	
06	10	200	10		65	62	61	87	18	9	10	UNL	6		65	64	63	93	19	5	10	140	7		67	66	65	93	00	0	
09	10	140	9		74	66	62	66	18	11	2	UNL	7		73	67	63	71	22	6	10	75	7	RM	73	70	68	84	17	5	
12	10	140	9		84	75	71	65	19	13	0	UNL	10		82	68	61	49	22	7	3	UNL	10		85	73	68	37	19	5	
15	10	140	6		84	75	71	65	19	7	0	UNL	10		85	69	61	45	21	11	7	60	10		86	75	70	59	18	2	
18	10	65	6		81	75	72	74	24	5	0	UNL	10		82	68	65	38	29	6	0	UNL	10		86	75	70	59	18	7	
21	10	80	4		69	68	67	93	19	18	1	UNL	10		68	64	61	78	19	6	0	UNL	10		75	72	70	85	18	5	
DAY 04																															
00	6	250	10		66	64	63	90	17	5	10	9	10		68	67	66	93	17	6	0	UNL	10		61	60	59	93	02	3	
03	5	UNL	10		64	62	61	90	13	6	2	UNL	10		65	65	65	100	19	4	10	210	9		61	60	60	97	00	0	
06	10	200	10		65	62	61	87	18	9	10	UNL	6		65	64	63	93	19	5	10	140	7		67	66	65	93	00	0	
09	10	140	9		74	66	62	66	18	11	2	UNL	7		73	67	63	71	22	6	10	75	7	RM	73	70	68	84	17	5	
12	10	140	9		84	75	71	65	19	13	0	UNL	10		82	68	61	49	22	7	3	UNL	10		85	73	68	37	19	5	
15	10	140	6		84	75	71	65	19	7	0	UNL	10		85	69	61	45	21	11	7	60	10		86	75	70	59	18	2	
18	10	65	6		81	75	72	74	24	5	0	UNL	10		82	68	65	38	29	6	0	UNL	10		86	75	70	59	18	7	
21	10	80	4		69	68	67	93	19	18	1	UNL	10		68	64	61	78	19	6	0	UNL	10		75	72	70	85	18	5	
DAY 05																															
00	6	250	10		66	64	63	90	17	5	10	9	10		68	67	66	93	17	6	0	UNL	10		61	60	59	93	02	3	
03	5	UNL	10		64	62	61	90	13	6	2	UNL	10		65	65	65	100	19	4	10	210	9		61	60	60	97	00	0	
06	10	200	10		65	62	61	87	18	9	10	UNL	6		65	64	63	93	19	5	10	140	7		67	66	65	93	00	0	
09	10	140	9		74	66	62	66	18	11	2	UNL	7		73	67	63	71	22	6	10	75	7	RM	73	70	68	84	17	5	
12	10	140	9		84	75	71	65	19	13	0	UNL	10		82	68	61	49	22	7	3	UNL	10		85	73	68	37	19	5	
15	10	140	6		84	75	71	65	19	7	0	UNL	10		85	69	61	45	21	11	7	60	10		86	75	70	59	18	2	
18	10	65	6		81	75	72	74	24	5	0	UNL	10		82	68	65	38	29	6	0	UNL	10		86	75	70	59	18	7	
21	10	80	4		69	68	67	93	19	18	1	UNL	10		68	64	61	78	19	6	0	UNL	10		75	72	70	85	18	5	
DAY 06																															
00	6	250	10		66	64	63	90	17	5	10	9	10		68	67	66	93	17	6	0	UNL	10		61	60	59	93	02	3	
03	5	UNL	10		64	62	61	90	13	6	2	UNL	10		65	65	65	100	19	4	10	210	9		61	60	60	97	00	0	
06	10	200	10		65	62	61	87	18	9	10	UNL	6		65	64	63	93	19	5	10	140	7		67	66	65	93	00	0	
09	10	140	9		74	66	62	66	18	11	2	UNL	7		73	67	63	71	22	6	10	75	7	RM	73	70	68	84	17	5	
12	10	140	9		84	75	71	65	19	13	0	UNL	10		82	68	61	49	22	7	3	UNL	10		85	73	68	37	19	5	
15	10	140	6		84	75	71	65	19	7	0	UNL	10		85	69	61	45	21	11	7	60	10		86	75	70	59	18	2	
18	10	65	6		81	75	72	74	24	5	0	UNL	10		82	68	65	38	29	6	0	UNL	10		86	75	70	59	18	7	
21	10	80	4		69	68	67	93	19	18	1	UNL	10		68	64	61	78	19	6	0	UNL	10		75	72	70	85	18	5	
DAY 07																															
00	3	UNL	10		73	72	71	93	18	5	4	UNL	10		67	66	66	97	18	5	6	25	5	DAY 09	64	64	64	100	00	0	
03	10	80	6		64	64	64	100	16	13	10	60	8		67	66	66	97	05	4	0	UNL	7	F	62	62	62	100	00	0	
06	10	38	10		64	64	64	100	01	16	10	55	7	TRM	64	63	63	97	01	7	10	250	7	F	62	62	62	100	00	0	
09	10	40	7		65	64	64	97	09	5	7	250	7		69	67	66	90	18	13	10	80	7		69	68	67	93	02	4	
12	7	250	7		79	73	70	74	18	13	7	250	10		81	74	71	72	25	9	10	250	7		77	70	67	71	11	5	
15	9	50	8		73	72	72	97	14	10	10	250	10	RM	77	71	68	74	28	7	10	80	10		78	71	68	71	06	6	
18	10	30	6		69	68	67	93	20	7	10	250	10		74	71	70	87	27	5	10	47	10		73	69	67	82	07	6	
21	10	70	6</																												







# OBSERVATIONS AT 3-HOUR INTERVALS

HOUR	WIND			TEMPERATURE			WIND			TEMPERATURE			WIND			TEMPERATURE			WIND					
	DIR	SPEED	KNOTS	AIR °F	WET BULB °F	DEW PT. °F	DIR	SPEED	KNOTS	AIR °F	WET BULB °F	DEW PT. °F	DIR	SPEED	KNOTS	AIR °F	WET BULB °F	DEW PT. °F	DIR	SPEED	KNOTS			
DAY 01																								
00	10	UNL	10	55	51	47	78	08	8	10	1	0	12	PM	60	58	57	90	19	7	9	6	4	
03	10	UNL	10	56	51	47	72	10	10	7	UNL	6	6	F	56	55	54	93	28	5	8	90	1	
06	10	UNL	10	55	53	53	86	10	12	3	UNL	11	7	F	57	55	54	90	34	3	4	UNL	5	
09	10	UNL	10	59	56	53	81	07	10	10	11	7	F	61	56	53	75	01	5	4	UNL	8		
12	10	UNL	10	65	59	55	70	11	8	3	UNL	7	8	F	68	61	56	66	11	5	4	UNL	10	
15	10	UNL	10	64	59	55	73	13	5	4	UNL	7	7	TRW	76	63	55	48	17	4	6	UNL	10	
18	10	UNL	10	64	60	57	78	18	5	10	47	6	TRW	68	62	58	71	06	5	0	UNL	10		
21	4	UNL	4	60	58	56	87	17	4	10	250	10		59	57	55	87	08	9	0	UNL	10		
DAY 02																								
00	10	UNL	10	60	56	53	78	18	6	10	100	10		58	53	49	72	18	11	10	20	3		
03	8	UNL	10	53	51	49	96	00	0	10	100	10		57	51	46	67	11	8	10	100	10		
06	10	UNL	10	54	51	49	83	06	4	10	28	7		57	53	50	78	11	8	8	31	10		
09	10	UNL	10	64	55	47	54	18	8	10	16	6	TRMM	61	57	55	81	18	11	10	23	10		
12	10	UNL	10	69	59	51	53	16	7	10	140	6	H	67	63	60	78	18	13	10	22	10		
15	8	UNL	10	73	61	51	48	18	7	10	12	4	H	76	68	64	66	18	6	10	14	10		
18	1	UNL	10	70	60	53	55	13	14	8	45	5	H	75	68	67	71	09	8	10	17	10		
21	10	UNL	10	61	53	47	60	15	7	10	38	5	H	67	63	61	81	11	4	0	UNL	10		
DAY 03																								
00	10	UNL	10	63	59	57	81	32	15	63	59	57	81	32	15	63	59	57	81	32	15	63	59	57
03	8	UNL	10	63	59	56	78	17	14	63	59	56	78	17	14	63	59	56	78	17	14	63	59	56
06	10	UNL	10	63	59	57	81	30	7	63	59	57	81	30	7	63	59	57	81	30	7	63	59	57
09	10	UNL	10	66	61	58	76	18	3	66	61	58	76	18	3	66	61	58	76	18	3	66	61	58
12	10	UNL	10	72	65	60	66	19	4	72	65	60	66	19	4	72	65	60	66	19	4	72	65	60
15	8	UNL	10	77	68	63	62	18	5	77	68	63	62	18	5	77	68	63	62	18	5	77	68	63
18	1	UNL	10	77	68	64	64	17	6	77	68	64	64	17	6	77	68	64	64	17	6	77	68	64
21	10	UNL	10	69	65	63	81	06	5	69	65	63	81	06	5	69	65	63	81	06	5	69	65	63
DAY 04																								
00	10	UNL	10	67	64	62	84	08	6	2	UNL	10			57	50	44	62	28	10				
03	10	UNL	10	66	62	59	78	13	6	0	UNL	10			56	50	44	64	28	9				
06	10	UNL	10	65	62	60	84	10	10	0	UNL	10			56	50	45	67	29	8				
09	10	UNL	10	69	65	62	79	19	8	1	UNL	10			63	54	46	54	30	11				
12	5	UNL	10	79	68	62	56	23	8	1	UNL	10			67	56	48	51	30	14				
15	3	UNL	10	80	67	59	49	31	11	0	UNL	10			67	57	50	55	31	12				
18	8	UNL	10	70	62	56	61	31	16	2	UNL	10			59	55	51	75	31	10				
21	10	UNL	10	63	57	52	68	33	20	0	UNL	10			56	53	51	83	30	5				
DAY 05																								
00	2	UNL	10	42	39	36	79	06	4	0	UNL	10			52	48	45	77	09	3				
03	4	UNL	10	39	38	36	89	00	0	0	UNL	10			51	47	44	77	18	4				
06	10	UNL	10	38	37	35	89	00	0	0	UNL	10			56	52	48	75	18	6				
09	2	UNL	10	53	44	33	47	26	4	0	UNL	10			50	45	40	58	10	4				
12	7	UNL	10	63	50	38	40	35	10	0	UNL	10			58	49	39	49	30	11				
15	3	UNL	10	79	68	62	56	23	8	1	UNL	10			65	53	43	45	30	12				
18	9	UNL	10	80	67	59	49	31	11	0	UNL	10			66	53	42	42	30	13				
21	10	UNL	10	70	62	56	61	31	16	2	UNL	10			66	55	45	47	29	13				
DAY 06																								
00	2	UNL	10	54	47	40	59	35	4	0	UNL	10			58	51	45	62	28	6				
03	4	UNL	10	42	40	38	86	00	0	0	UNL	10			46	44	41	83	32	8				
06	10	UNL	10	50	45	40	69	31	10	6	UNL	10			50	45	40	69	31	10				
09	10	UNL	10	58	49	39	49	30	11	9	UNL	10			58	49	39	49	30	11				
12	5	UNL	10	65	53	43	45	30	12	10	UNL	10			65	53	43	45	30	12				
15	3	UNL	10	66	53	42	42	30	13	7	UNL	10			66	53	42	42	30	13				
18	8	UNL	10	66	55	45	47	29	13	10	UNL	10			66	55	45	47	29	13				
21	10	UNL	10	58	51	45	62	28	6	0	UNL	10			58	51	45	62	28	6				
DAY 07																								
00	4	UNL	10	62	59	57	84	06	13	62	59	57	84	06	13	62	59	57	84	06	13	62	59	57
03	8	UNL	10	60	54	49	67	18	8	10	80	10			58	56	55	90	06	13				
06	10	UNL	10	62	56	51	67	18	8	7	250	10			68	60	57	78	17	4				
09	7	UNL	10	70	61	54	57	18	10	0	UNL	10			76	65	59	56	19	7				
12	4	UNL	10	72	63	58	43	19	10	1	UNL	10			83	69	62	49	21	6				
15	10	UNL	10	74	65	59	60	18	10	2	UNL	10			87	70	61	42	29	6				
18	8	UNL	10	71	65	61	71	17	7	6	UNL	10			84	71	64	51	25	5				
21	10	UNL	10	65	61	59	81	18	5	6	UNL	10			71	65	62	73	01	3				
DAY 08																								
00	0	UNL	10	45	43	41	86	07	4	0	UNL	10			52	47	41	66	18	9				
03	0	UNL	10	40	39	38	93	31	4	0	UNL	10			47	44	40	77	18	5				
06	0	UNL	10	45	43	40	83	34	4	1	UNL	10			53	49	45	74	18	5				
09	0	UNL	10	59	49	35	48	02	7	8	250	10			63	56	50	63	26	12				
12	0	UNL	10	66	51	35	32	34	7	1	UNL	10			72	60	52	50	23	9				
15	0	UNL	10	67	55	37	31	04	3	0	UNL	10			74	61	52	46	22	10				
18	0	UNL	10	68	62	37	32	04	3	0	UNL	10			70	60	52	53	22	9				
21	0	UNL	10	52	47	41	66	08	3	0	UNL	10			63	57	52	68	20	7				
DAY 09																								
00	10	UNL	10	50	47	44	80	00	0	8	UNL	10			50	47	44	80	00	0				
03	10	UNL	10	52	48	45	77	00	0	3	UNL	10			52	48	45	77	00	0				
06	10	UNL	10	70	59	51	51	23	6	6	250	10			70	59	51	51	23	6				
09	10	UNL	10	76	58	44	32	29	5	3	UNL	10			76	58	44	32	29	5				
12	8	UNL	10	78	59	45	31	24	6	2	UNL	10			78	59	45	31	24	6				
15	4	UNL	10	75	59	47	37	23	5	6	UNL	10			75	59	47	37	23	5				
18	0	UNL	10	65	57	50	59	30	4	8	UNL	10			65	57	50	59	30	4				
DAY 10																								
00	6	UNL	10	61	57	55	81	00	0	3	UNL	6	H		65	61	58	78	06	3</				

# Local Climatological Data



## MONTHLY SUMMARY

LATITUDE 43° 08' N LONGITUDE 89° 20' W ELEVATION (GROUND) 958 FT. STANDARD TIME USED: CENTRAL GCMAN 114837

SEPTEMBER 1980 MADISON, WISCONSIN

DATE	TEMPERATURE °F			DEGREE DAYS BASE 59°		WEATHER TYPES ON DATES OF OCCURRENCE	SNOW INDEX	PRECIPITATION		WIND STATION	WIND			SUNSHINE		SKY COVER PERCENT		DATE		
	MAXIMUM	MINIMUM	AVERAGE	DEPARTURE FROM NORMAL	AVERAGE DEW POINT			WATER	SNOW		RESULTANT DIR.	RESULTANT SPEED M.P.H.	AVERAGE SPEED M.P.H.	FASTEST WIND M.P.H.	DIRECTION	MINUTES	PERCENT OF POSSIBLE		SUMMERS TO SUNSET	MIDNIGHT TO MIDNIGHT
1	77	63	70	5	65	0	0	0.09	0	028.95	30	6.3	7.2	17	SM	105	13	10	9	1
2	76	55	66	2	60	0	0	0	0	029.11	30	4.8	5.5	15	NM	757	96	2	3	2
3	83	54	69	5	60	0	0	0	0	029.16	17	8.2	8.3	18	S	772	98	4	4	3
4	78	49	64	1	59	1	0	0.16	0	029.13	26	5.0	5.6	17	N	326	42	7	5	4
5	80	46	63	0	55	2	0	0	0	029.22	21	4.2	4.5	12	SM	732	94	4	3	5
6	78	52	65	2	57	0	0	0.02	0	029.23	04	1.0	6.0	15	SE	512	66	6	6	6
7	74	58	66	4	62	0	0	1.80	0	029.13	17	6.0	7.1	17	SE	138	18	9	9	7
8	87	58	73	11	66	0	0	0	0	029.12	17	3.6	3.6	11	S	587	76	4	5	8
9	75	50	63	1	58	2	0	0.80	0	029.18	31	6.3	8.3	20	N	538	70	8	7	9
10	71	43	57	-4	48	8	0	0	0	029.20	29	3.5	3.6	10	N	766	100	0	0	10
11	64	46	56	-5	52	9	0	0	0	029.11	08	1.9	4.3	10	NE	50	7	10	8	11
12	71	58	65	4	61	0	0	1.53	0	028.96	13	6.2	8.3	16	SE	57	8	10	10	12
13	79	60	70	10	64	0	0	0.23	0	028.99	24	4.3	5.9	17	SM	473	62	5	6	13
14	61	53	57	-3	56	8	0	0.02	0	029.16	34	5.2	6.8	13	NE	0	0	10	10	14
15	59	52	56	-4	53	9	0	0	0	029.11	14	3.2	6.3	14	SE	17	2	10	9	15
16	60	46	54	-5	52	11	0	0.61	0	028.84	28	2.2	6.8	15	NM	57	4	10	9	16
17	63	36	49	-11	48	16	0	0	0	029.01	33	2.5	5.2	13	NM	553	74	4	5	17
18	71	45	58	-5	58	7	0	0	0	029.11	22	5.4	9.5	15	SM	478	64	5	4	18
19	76	43	60	-2	52	5	0	0	0	029.11	16	7.1	8.8	20	S	501	68	8	7	19
20	77	62	70	12	64	0	0	1.00	0	028.91	21	1.6	8.6	26	S	191	26	8	8	20
21	74	59	67	9	60	0	0	0.48	0	028.82	25	5.0	8.6	24	S	306	42	6	6	21
22	68	47	58	0	56	7	0	0	0	028.91	31	4.9	9.2	20	NM	276	38	8	6	22
23	61	38	50	-7	40	15	0	0	0	029.21	01	2.9	5.3	9	N	689	95	2	1	23
24	62	42	52	-5	44	13	0	0	0	029.13	19	3.6	3.9	14	S	304	42	8	7	24
25	60	43	52	-5	45	13	0	0.09	0	029.06	30	6.4	6.8	21	NE	171	24	9	8	25
26	57	31	44	-13	36	21	0	0	0	029.40	28	3.8	6.5	11	N	708	98	2	4	26
27	69	45	57	1	44	8	0	0	0	029.25	21	6.5	7.1	17	SM	671	94	4	3	27
28	60	44	52	-4	51	13	0	0.16	0	029.22	10	3.6	5.0	11	SE	0	0	10	8	28
29	72	44	58	2	50	7	0	0	0	029.09	18	6.4	6.6	14	S	677	95	3	3	29
30	75	48	62	7	56	3	0	0	0	028.98	25	1.8	4.5	10	SM	257	36	8	6	30

SUM	SUM	TOTAL	TOTAL	TOTAL	TOTAL	FOR THE MONTH:	TOTAL	%	SUM	SUM		
2119	1472	119	31	7.84	0.24	10.22	2.3	6.51	26	5	116.29	FOR 1980 12.9
AVG	196	196	AVG	SEP	SEP	SEP	PRECIPITATION	SEP	DATE: 20	MONTH: 1980	AVG	AVG
70.6	49.1	49.3	3	2	54	5	3.21	14	4.48	224.94	52	6.3

\* EXTREME FOR THE MONTH - LAST OCCURRENCE IF MORE THAN ONE.  
 † TRACE AMOUNT  
 ‡ ALSO ON AN EARLIER DATE, OR DATES.  
 § HEAVY FOG - VISIBILITY 1/4 MILE OR LESS.  
 ¶ FIGURES FOR WIND DIRECTIONS ARE TENS OF DEGREES CLOCKWISE FROM TRUE NORTH, 08 = CALM.  
 DATA IN COLS. 8 AND 12-15 ARE BASED ON 7-30

WIND OBSERVATIONS PER DAY AT 3-HOUR INTERVALS. FASTEST WIND SPEEDS ARE FASTEST OBSERVED ONE-MINUTE VALUES WHEN DIRECTIONS ARE IN TENS OF DEGREES, THE / WITH THE DIRECTION INDICATES PEAK GUST SPEED.  
 ANY ERRORS DETECTED WILL BE CORRECTED AND CHANGES IN SUMMARY DATA WILL BE INDICATED IN THE ANNUAL SUMMARY

### SUMMARY BY HOURS

HOUR	MAX. WIND	SKY COVER	STATION PRESSURE	TEMPERATURE				RELATIVE HUMIDITY %	WIND SPEED M.P.H.	DIRECTION	SPEED M.P.H.
				AIR °F	NET BUB °F	DEW PT.	WIND CHILL				
00	6	29	09	54	54	53	90	5.4	20	2.1	
03	5	29	09	53	52	52	94	5.3	20	1.5	
06	7	29	10	53	52	52	96	4.8	20	1.0	
09	7	29	12	61	58	55	81	7.8	23	2.9	
12	7	29	13	66	59	56	80	8.4	24	4.4	
15	6	29	09	68	61	55	68	7.8	25	3.6	
18	5	29	08	63	59	55	76	6.8	22	1.1	
21	4	29	09	57	55	54	90	5.2	14	1.8	

### HOURLY PRECIPITATION (WATER EQUIVALENT IN INCHES)

DATE	1980 SEP 1												1980 SEP 2											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
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**noaa** NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION / ENVIRONMENTAL DATA AND INFORMATION SERVICE  
*David B. Mitchell*  
 DIRECTOR, NATIONAL CLIMATIC CENTER

# OBSERVATIONS AT 3-HOUR INTERVALS

HOUR	SKY COVER % OVER HORIZ. OF FL.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND		SKY COVER % OVER HORIZ. OF FL.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND		SKY COVER % OVER HORIZ. OF FL.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND					
				AIR °F	WET BULB °F	DEW PT. °F	REL. HUM. %	DIR	SPEED KNOTS				AIR °F	WET BULB °F	DEW PT. °F	REL. HUM. %	DIR	SPEED KNOTS				AIR °F	WET BULB °F	DEW PT. °F	REL. HUM. %	DIR	SPEED KNOTS	AIR °F	WET BULB °F	DEW PT. °F	REL. HUM. %
DAY 01																															
00	0	UNL	7	53	53	53	100	18	4	0	UNL	7	46	44	43	89	30	6	10	27	10	0	UNL	7	38	37	25	89	33	10	
03	0	UNL	7	59	58	58	97	18	7	0	UNL	7	45	43	40	83	27	6	6	45	10	0	UNL	7	34	33	31	89	33	8	
06	10	250	7	58	56	55	90	18	6	0	UNL	7	41	40	38	89	14	4	5	45	10	0	UNL	7	31	31	30	96	34	5	
09	10	250	7	57	55	54	88	18	6	0	UNL	7	41	40	38	89	14	4	5	45	10	0	UNL	7	31	31	30	96	34	5	
12	10	120	10	53	56	51	65	32	12	8	UNL	10	50	51	44	60	30	10	10	27	10	0	UNL	7	42	37	31	65	33	8	
15	10	80	10	58	53	48	70	31	7	5	42	10	55	48	41	59	30	12	10	45	10	0	UNL	7	44	38	29	56	32	8	
18	3	UNL	7	52	49	45	74	26	5	10	13	7	45	44	43	93	31	11	10	50	10	0	UNL	7	40	37	32	73	100	0	
21	5	UNL	7	53	48	45	77	28	7	10	12	7	49	48	38	37	93	01	7	10	55	10	0	UNL	7	36	35	33	89	06	0
DAY 02																															
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DAY 31																															

NOTES  
CEILING  
UNL INDICATES UNLIMITED

- WEATHER
- T TORNADO
  - TS THUNDERSTORM
  - Q SQUALL
  - R RAIN
  - RM RAIN SHOWERS
  - ZR FREEZING RAIN
  - L DRIZZLE
  - ZL FREEZING DRIZZLE
  - S SNOW
  - SP SNOW PELLETS
  - IC ICE CRYSTALS
  - SM SNOW SHOWERS
  - SG SNOW GRAINS
  - IP ICE PELLETS
  - A HAIL
  - F FOG
  - IF ICE FOG
  - GF GROUND FOG
  - BD BLOWING DUST
  - BN BLOWING SAND
  - BS BLOWING SNOW
  - BY BLOWING SPRAY
  - K SMOKE
  - H HAZE
  - D DUST

WIND  
DIRECTIONS ARE THOSE FROM WHICH THE WIND BLOWS, INDICATED IN TENS OF DEGREES FROM TRUE NORTH; I.E., 09 FOR EAST, 18 FOR SOUTH, 27 FOR WEST. ENTRY OF 00 IN THE DIRECTION COLUMN INDICATES CALM.

SPEED IS EXPRESSED IN KNOTS; MULTIPLY BY 1.15 TO CONVERT TO MILES PER HOUR.

STATION  
MADISON WISC

YEAR & MONTH  
80 10

U.S. DEPARTMENT OF COMMERCE  
NATIONAL CLIMATIC CENTER  
FEDERAL BUILDING  
ASHEVILLE, N.C. 28801

AN EQUAL OPPORTUNITY EMPLOYER

POSTAGE AND FEES PAID  
U.S. DEPARTMENT OF COMMERCE

COM-210



FIRST CLASS

# Local Climatological Data



## MONTHLY SUMMARY

LATITUDE 43° 08' N LONGITUDE 89° 29' W ELEVATION (GROUND) 658 FT. STANDARD TIME USED: CENTRAL MEAN 414837

OCTOBER 1980 MADISON, WISCONSIN

DATE	TEMPERATURE °F					WIND		PRECIPITATION		WIND		WIND		WIND		WIND		WIND		WIND		WIND	
	MAXIMUM	MINIMUM	AVERAGE	DEPARTURE FROM NORMAL	AVERAGE DEW POINT	HEATING INDEX	COOLING INDEX	WIND CHILL INDEX	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION
1	66	46	54	1	51	9	0	0	T	028	82	27	4.7	8.1	16	NM	152	22	9	6	1	1	
2	60	38	49	-6	41	16	0	0	0	028	51	30	8.4	10.5	20	NM	353	50	10	6	2	3	
3	45	31	38	-17	32	27	0	0	0	029	04	33	6.6	7.1	14	N	54	5	7	6	4	3	
4	48	37	40	-14	28	27	0	0	0	029	10	03	4.0	4.6	13	N	385	55	10	7	5	4	
5	56	37	45	-10	36	25	0	0	0	029	21	23	6.8	9.2	18	SW	693	100	0	0	0	6	
6	65	37	51	-3	38	14	0	0	0	029	21	23	6.8	9.2	18	SW	577	84	6	6	6	6	
7	75	37	56	3	45	4	0	0	0	029	06	20	6.2	6.5	16	SW	630	92	0	0	1	7	
8	77	46	62	9	47	3	0	0	0	028	06	29	3.0	7.9	12	N	685	100	0	0	0	8	
9	65	35	50	-3	40	15	0	0	0	029	09	07	4.8	6.9	15	E	682	100	0	0	0	9	
10	67	43	55	2	37	10	0	0	0	028	09	25	8.0	10.5	28	N	474	70	3	5	10	10	
11	47	35	41	-11	31	24	0	0	0	028	05	30	14.6	14.7	24	NM	3	0	10	9	11	11	
12	50	27	39	-13	27	26	0	0	0	029	23	32	6.9	7.3	14	N	523	78	3	1	12	12	
13	54	23	39	-12	29	26	0	0	0	029	27	08	5.3	6.5	14	SE	421	63	5	1	13	13	
14	51	41	46	-5	41	19	0	0	0	029	08	07	12.9	13.2	19	NE	0	0	10	10	14	14	
15	46	41	44	-7	42	21	0	0	0	029	15	05	7.8	7.9	13	NE	0	0	10	10	15	15	
16	63	44	54	4	52	11	0	0	0	028	08	13	5.8	8.6	18	S	0	0	10	10	16	16	
17	67	46	57	7	48	8	0	0	0	028	04	21	15.5	18.0	28	SW	422	64	4	6	17	17	
18	50	39	45	-4	33	20	0	0	0	028	06	27	13.1	14.1	22	N	80	12	9	10	18	18	
19	52	35	44	-5	33	21	0	0	0	028	17	27	8.1	9.1	17	SW	293	45	7	1	19	19	
20	54	34	45	-4	38	20	0	0	0	028	06	27	6.5	8.3	17	NM	303	47	6	5	20	20	
21	55	31	42	-5	33	22	0	0	0	029	07	30	8.3	8.5	19	NM	520	90	4	3	21	21	
22	53	23	38	-10	31	27	0	0	0	029	29	10	5.3	6.5	15	SE	261	40	18	8	22	22	
23	61	40	51	4	39	14	0	0	0	029	26	17	12.4	13.1	21	SE	319	50	10	10	23	23	
24	35	23	29	-13	26	32	0	0	0	028	06	30	4.1	10.4	16	NM	0	0	10	10	24	24	
25	35	31	33	-13	26	32	0	0	0	028	06	30	14.7	14.7	23	NM	0	0	10	10	25	25	
26	37	20	29	-17	31	26	0	0	0	029	15	20	11.3	11.4	23	NM	100	21	10	9	26	26	
27	35	20	28	-17	23	37	0	0	0	029	16	04	2	1.6	10	NE	0	0	10	9	27	27	
28	43	18	31	-14	19	34	0	0	0	029	23	32	9.9	10.2	15	NM	528	100	0	0	28	28	
29	49	16	31	-13	17	34	0	0	0	029	17	28	3.0	3.5	9	N	626	100	5	2	29	29	
30	53	29	41	-3	29	24	0	0	0	029	20	22	11.5	12.2	20	SW	465	75	5	2	30	30	
31	59	31	45	2	26	20	0	0	0	029	17	28	10.4	10.6	19	N	529	85	5	3	31	31	

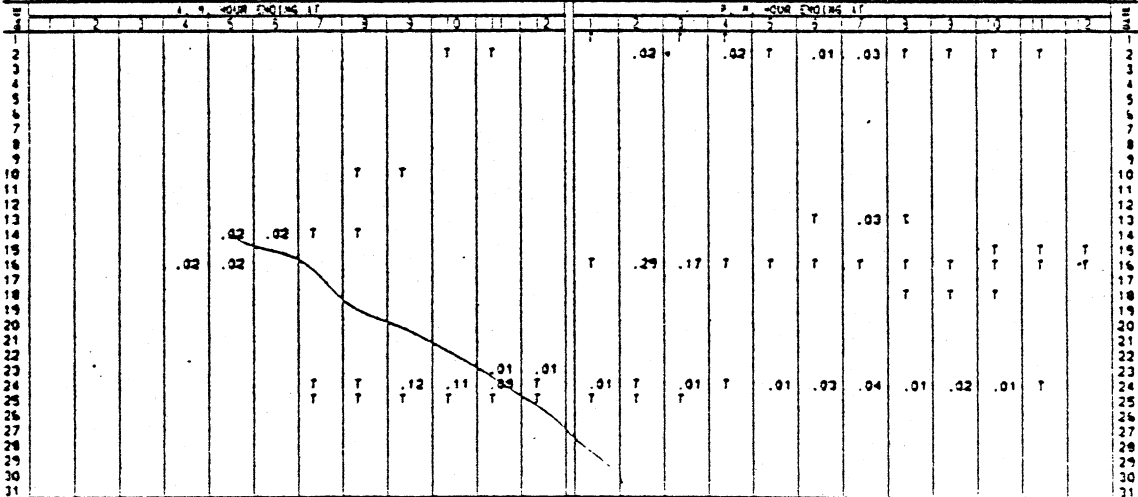
\* EXTREME FOR THE MONTH - LAST OCCURRENCE IF MORE THAN ONE.  
 † TRACE AMOUNT.  
 \* ALSO ON AN EARLIER DATE, OR DATES.  
 HEAVY FOG: - VISIBILITY 1/4 MILE OR LESS.  
 FIGURES FOR WIND DIRECTIONS ARE TENS OF DEGREES CLOCKWISE FROM TRUE NORTH. 00 = CALM.  
 DATA IN COLS. 6 AND 12-16 ARE BASED ON 7 OR

MORE OBSERVATIONS PER DAY AT 3-HOUR INTERVALS. FASTEST WIND SPEEDS ARE FASTEST OBSERVED ONE-MINUTE VALUES WHEN DIRECTIONS ARE IN TENS OF DEGREES. THE / WITH THE DIRECTION INDICATES PEAK BUST SPEED.  
 ANY ERRORS DETECTED WILL BE CORRECTED AND CHANGES IN SUMMARY DATA WILL BE ANNOTATED IN THE ANNUAL SUMMARY.

SUMMARY BY HOURS

HOUR	TEMPERATURE	WIND SPEED	WIND DIRECTION	WIND		WIND SPEED	WIND DIRECTION	WIND SPEED	WIND DIRECTION	
				RELATIVE HUMIDITY	WIND DIRECTION					
00	51	29	07	40	38	35	82	7	27	2.4
03	51	29	06	39	37	34	83	7	27	2.3
06	51	29	07	37	35	33	84	7	27	2.0
09	67	29	09	45	41	36	71	10	6	3.7
12	67	29	08	51	44	35	56	12	0	4.8
15	77	29	05	52	44	34	54	11	6	6.3
18	67	29	06	45	41	35	67	8	27	3.7
21	61	29	08	42	39	34	76	9	27	1.5

HOURLY PRECIPITATION (WATER EQUIVALENT IN INCHES):



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 Daniel B. Mitchell DIRECTOR, NATIONAL CLIMATIC CENTER  
 USCOMH--NOAA--ASHEVILLE 10/31/80 500

# OBSERVATIONS AT 3-HOUR INTERVALS

HOUR	SKY COVER	CEILING HDS. OF FT.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND		SKY COVER	CEILING HDS. OF FT.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND		SKY COVER	CEILING HDS. OF FT.	VISI- BILITY MILES	WEATHER	TEMPERATURE				WIND	
					AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.	DIR	SPEED KNOTS					AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.	DIR	SPEED KNOTS					AIR °F	WET BULB °F	DEW PT. °F	REL. HUM.	DIR	SPEED KNOTS
00	10	300	4	DAY 01	63	63	63	100	09	3	6	UNL	10	DAY 02	66	65	65	97	23	4	2	UNL	7	DAY 03	59	58	57	93	15	3
03	10	20	2	F	58	57	56	93	18	6	10	250	10	66	65	65	100	28	3	6	80	10	59	58	57	93	17	3		
06	10	170	10	TRNF	65	65	65	100	18	4	1	UNL	7	66	65	65	100	28	3	6	80	10	59	58	57	93	17	3		
09	10	250	10		67	65	64	90	23	6	0	UNL	10	67	65	65	100	28	3	6	80	10	59	58	57	93	17	3		
12	10	250	10		72	67	65	79	22	7	4	UNL	10	73	64	58	59	29	3	4	UNL	10	80	68	61	52	18	9		
15	10	UNL	10		76	70	67	74	21	7	6	UNL	10	74	64	57	55	30	5	0	UNL	10	83	69	62	49	18	13		
18	8	28	8	RM	72	68	66	82	19	10	1	UNL	10	72	64	59	64	36	4	0	UNL	10	77	67	62	60	18	9		
21	3	UNL	8		68	67	66	93	19	7	4	UNL	10	58	57	57	96	00	0	0	UNL	10	69	64	61	76	17	8		
00	10	250	10	DAY 04	66	62	59	78	18	0	0	UNL	10	DAY 05	49	49	49	100	00	0	2	UNL	2	DAY 06	57	57	57	100	32	6
03	10	90	10	RM	63	61	60	90	00	0	0	UNL	10		49	48	48	96	18	6	1	UNL	1	F	53	53	53	100	31	5
06	10	120	10		62	61	61	97	00	0	3	UNL	10		49	49	49	100	00	0	0	UNL	3	F	53	53	53	100	32	5
09	9	120	10		65	65	63	81	23	8	9	UNL	10		49	49	56	58	23	5	0	UNL	3	F	69	61	59	61	06	4
12	9	16	10		69	66	64	84	29	10	2	UNL	10		75	62	53	46	19	9	8	UNL	10		76	64	57	52	18	5
15	4	UNL	10		77	67	62	60	29	9	2	UNL	10		78	67	61	54	23	6	10	250	10		77	64	56	48	19	3
18	0	UNL	10		70	60	52	53	24	6	1	UNL	10		75	68	65	71	22	5	10	270	10		69	64	61	76	07	8
21	0	UNL	10		58	55	53	84	29	5	0	UNL	10		62	61	61	97	00	0	10	90	10	TRN	66	63	61	84	12	6
00	10	80	10	DAY 07	63	60	58	84	18	9	7	250	10	DAY 08	62	62	62	100	00	0	10	250	7	DAY 09	75	72	71	87	19	5
03	10	50	6	T	60	59	58	93	15	6	2	UNL	10		58	58	58	100	00	0	10	40	7	TRN	67	67	67	100	13	3
06	10	70	4	TRNF	60	59	59	97	18	9	6	250	3	F	58	59	58	100	00	0	7	100	10		65	64	64	97	32	8
09	10	40	5	TRNF	61	60	60	97	20	8	0	UNL	5	H	71	65	62	73	17	5	9	250	10		63	60	58	84	21	11
12	10	55	10	TRNF	65	63	62	90	18	4	1	UNL	5	H	80	72	68	67	18	5	9	250	10		66	60	55	68	31	9
15	3	UNL	10	T	72	65	67	84	18	4	4	UNL	6	H	87	78	75	68	16	5	9	UNL	10		67	57	50	55	32	12
18	10	47	10		67	66	66	97	11	7	7	UNL	4	H	86	76	74	82	18	6	4	UNL	10		62	56	51	67	31	6
21	10	30	10		65	64	64	97	09	4	10	250	7	H	74	73	73	97	18	5	1	UNL	10		52	50	49	40	35	3
00	0	UNL	10	DAY 10	51	49	48	90	00	0	0	UNL	10	DAY 11	49	48	48	96	00	0	10	70	10	DAY 12	60	57	55	84	11	7
03	0	UNL	10		45	45	45	100	31	4	3	UNL	10		50	48	47	89	04	3	10	50	10		60	58	57	90	10	7
06	0	UNL	10		44	44	44	100	00	0	8	UNL	10		47	47	47	100	00	0	10	11	1	TRNF	59	59	59	100	08	9
09	0	UNL	10		61	54	49	65	30	6	10	100	10		62	57	53	73	18	5	10	4	2	RMF	59	59	59	100	08	9
12	0	UNL	10		66	54	44	45	29	4	9	90	10		65	53	52	63	23	5	9	13	5	H	66	53	61	84	16	13
15	0	UNL	10		71	59	50	48	29	7	10	30	10		65	60	57	75	06	4	8	14	5	H	69	65	63	81	18	9
18	0	UNL	10		65	57	50	59	27	4	10	75	7	RM	62	60	58	87	06	7	10	250	4	FM	67	66	65	93	09	4
21	0	UNL	10		54	52	50	86	00	0	10	80	10		58	56	55	90	07	6	10	250	5	FM	67	66	65	93	19	4
00	10	30	4	DAY 13	66	65	64	93	19	8	2	UNL	10	DAY 14	61	60	60	97	30	5	10	30	10	DAY 15	53	52	51	93	03	5
03	10	80	7	TRNF	64	63	62	93	22	7	9	14	10		59	58	58	97	30	5	10	8	10		52	51	51	96	05	4
06	10	120	7	RM	60	60	60	100	00	0	10	19	10		59	58	57	93	31	6	10	8	10		52	51	51	96	05	4
09	4	UNL	10		70	66	63	79	20	9	10	8	10		58	57	57	96	31	7	10	34	8		56	54	53	90	11	6
12	4	UNL	10		75	69	64	74	28	10	10	5	2	LF	58	58	58	100	32	5	10	26	10		59	58	58	100	17	5
15	2	UNL	10		77	69	64	64	29	5	10	12	10		57	55	54	90	02	8	10	17	8		59	58	54	84	19	5
18	4	UNL	10		72	68	66	82	31	3	10	20	7		55	53	52	90	03	6	9	26	10		59	56	54	84	18	7
21	5	UNL	10		63	63	63	100	00	0	10	23	10		54	52	51	90	04	5	6	250	10		57	55	54	90	16	9
00	6	UNL	10	DAY 16	56	55	55	96	18	7	10	80	10	DAY 17	48	46	44	86	32	10	9	90	10	DAY 18	48	44	40	74	18	10
03	0	UNL	7		56	55	55	96	18	5	0	UNL	10		40	40	39	96	32	6	7	100	10		48	45	43	83	18	3
06	10	7	3	F	58	57	57	96	15	5	0	UNL	10		37	37	37	100	00	0	10	70	10		49	47	45	86	19	8
09	10	6	2	F	60	60	60	100	29	5	1	UNL	10		54	48	41	62	25	6	7	90	7		58	53	48	70	21	10
12	10	30	7	TRN	56	52	48	75	31	6	3	UNL	10		59	46	32	36	24	5	7	90	7		65	57	51	61	24	10
15	10	42	5		53	48	46	84	28	10	10	20	10		58	46	31	36	28	8	10	26	10		60	56	53	79	32	9
18	10	75	10		51	49	47	86	33	8	5	UNL	10		59	44	39	69	14	5	0	UNL	10		70	61	54	72	30	9
21	10	75	10		49	47	46	89	32	6	7	100	10		49	44	39	69	14	5	0	UNL	10		49	48	47	93	05	3
00	0	UNL	10	DAY 19	46	45	45	96	00	0	10	36	10	DAY 20	68	61	57	68	31	10	6	250	7	DAY 21	73	68	66	79	18	13
03	0	UNL	10		44	43	43	96	03	3	10	36	5	TRN	63	62	62	97	20	9	0	UNL	10		72	66	63	73	23	12
06	3	UNL	7		45	44	44	96	07	4	10	7	1	RMF	62	62	62	100	10	10	10	17	10		42	40	39	90	28	10
09	8	UNL	7		60	57	55	84	14	10	10	17	4	F	67	66	65	93	30	3	10	14	10		61	59	59	90	30	7
12	5	UNL	7		72	65	60	64	17	11	8	90	5	H	71	67	65	81	00	0	9	24	10		65	61	58	78	29	9
15	10	250	7		75	65	59	59	18	10	10	21	6	H	73	70	68	84	07	6	2	UNL	10		72	66	63	59	25	4
18	10	200	7		69	66	64	84	28	10	10	20	10	FM	69	68	67	93	29	10	2	UNL	10		65	61</				

APPENDIX 2.

Formulation of Media

Dextrose-Peptone-Yeast Agar (DPY) as formulated by Schaerf-fenberg (1964).

30g	dextrose
10g	meat peptone
5g	yeast extract
20g	agar
1000ml	distilled water

Selective medium for the isolation of Metarhizium anisopliae as formulated by Veen and Ferron (1966).

10g	dextrose
10g	meat peptone
15g	oxgall
.068g	rose bengale
30g	agar
1000ml	distilled water
0.5g	Chloramphenicol R
0.01g/ml	Actidione R with 25ml of distilled water placed in the medium after sterilization.