CORN STALK ROT INCIDENCE, ETIOLOGY, AND CONTROL IN FLORIDA

By

THOMAS R. YOUNG

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1975

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Thomas A. Kucharek, Chairman of the Supervisory Committee, for his counsel, understanding, and encouragement. I also wish to thank the other members of the Supervisory Committee: Dr. D. J. Mitchell, Dr. D. A. Roberts, Dr. R. E. Stall, and Dr. J. W. Kimbrough for their valuable suggestions and criticisms of this work.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CORN STALK ROT INCIDENCE, ETIOLOGY, AND CONTROL IN FLORIDA

By

Thomas R. Young

June, 1975

Chairman: Thomas A. Kucharek
Major Department: Plant Pathology

Associations of fungi in stalks and roots of hybrid field corn occurred in a succession of five communities. These communities were associated with particular growth stages of the plants sampled. The first community, which consisted of Pyrenochaetae terrestris, Fusarium solani, Fusarium lateritium, Rhizopus sp., and Penicillium spp., appeared in the seedling stage, reached its maximum isolation frequency by silking and by the full dent stage was no longer recovered. The second community, consisting of Trichoderma viride and Fusarium moniliforme, appeared in the seedling stage and was isolated with increasing frequency throughout the remainder of the season. The third community, composed of Curvularia sp. and Mortierella sp., appeared at silking, reached its maximum frequency by the dough stage, and was not recovered after plants reached physiological maturity. The fourth community, which comprised Macrophomina phaseolina, Rhizoctonia solani, and Sclerotium rolfsii, appeared at the dough stage, increased in frequency up to physiological maturity and gradually declined to harvest time (15% kernel

moisture, 175 days after planting). The final community to appear was composed of Helminthosporium rostratum and Diplodia maydis. It appeared in the dough stage and increased in frequency until at harvest it was nearly as abundant as the second community.

The succession of fungi in corn stalks and roots could not be directly attributed to any of the following factors: seasonal fluctuations in temperature or moisture; effect of sucrose concentration on fungal growth and development; changes in 6-methoxy 2(3)-benzoxazolinone (MBOA) concentration; or the relative saprophytic ability of the fungi associated with corn stalks and roots.

Virulence tests indicated that the fungal complex of stalk rotting pathogens included F. moniliforme, H. rostratum, and D. maydis. In addition, T. viride and M. phaseolina, which were weak pathogens in living plants, were capable of extensive saprophytic decay activity in dead tissue.

Corn may be invaded by H. rostratum late in the season when airborne spores lodge between stalks and sheaths, germinate and penetrate the stalks. Helminthosporium rostratum and D. maydis were unable to survive in stalk residue buried in the field for 110 days, whereas F. moniliforme and T. viride were able to survive.

In Florida, the losses caused by stalk rot were estimated to be 8.5% of the value of the crop, or nearly three million dollars annually. Control of this syndrome through resistant varieties may be feasible since resistance to F. moniliforme and H. rostratum was demonstrated in some commercial hybrids and in some inbred breeding lines.

GENERAL INTRODUCTION

Stalk rot of corn (Zea mays L.) is of world wide significance. Christensen and Wilcoxson (15), who have most recently reviewed the subject of stalk rot, regard it as the most destructive disease of corn in the world. Losses may be direct through poor filling of ears or lightweight ears or indirect through harvest losses because of stalk breakage or lodging.

The disease is typically manifested by deterioration of senescent tissues, hastened by fungal invasion (109). Although specific fungi are frequently associated with the disease, it is generally accepted that a complex of organisms, including nematodes, insects, and bacteria may be involved (7, 27, 41). Among the stalk rot pathogens listed by Christensen and Wilcoxson (15) are: Gibberella roseum f. sp. cerealis (Cke.) Synd. & Hans. [Gibberella zeae (Schw.) Petch.] (A sexual stage, Fusarium roseum f. sp. cerealis 'Graminearum'), Diplodia maydis (Berk.) Sacc. [D. zeae (Schw.) Lev.], Fusarium moniliforme Sheld., Cephalosporium maydis Samra, Sabet and Hingorani, Nigrospora oryzae (Berk & Br.) Petch., Macrophomina phaseolina (Tassi) Goid., Pythium aphanidermatum (Eds.) Fitz., Pyrenochaeta terrestris (Hansen) Groenz, Walker & Larson, Pseudomonas alboprecipitans Rosen, and Erwinia stewartii (R.F.Sm.) Dye. These organisms

accentuate the reduction of physiological vigor and many are believed to continue their activities as saprophytes in dead plants leading to a loss in structural strength which contributes to plant lodging (3, 16, 23, 31).

Stalk rot of corn is not a new problem. Rotted and lodged stalks were common when open pollinated varieties were grown (23). Stalk rot became more conspicuous, however, when hybrid corn was adopted because hybrids have greater genetic uniformity, including disease reaction, and they are mechanically harvested. During the 1940's and early 1950's excellent progress was made in developing hybrids resistant to stalk rot (39). But the level of resistance has been partly offset by cultural practices, such as increased nitrogen fertilization and higher plant densities (91). Most breeding programs give high priority to stalk quality but high grain yield and stalk rot resistance are difficult to combine and intensive cultural practices designed for high yields also favor stalk rot development. Therefore, the need for stalk rot research continues to be as important today as it was 30 years ago.

Environmental factors such as temperature and moisture influence the development of stalk rot. Stalk rot is most destructive in regions which receive heavy rainfall in late summer or during the late growing season (63, 64, 96). But free moisture on the surface of the corn plant is not necessary germination of fungal spores since moisture accumulates between the leaf sheath and stalk. Drought may also cause greater susceptibility to stalk and root rotting

fungi because moisture stress accentuates senescence (15).

Chilling predisposes plants to stalk rot caused by Nigrospora oryzae (92). Hot dry weather favors the development of charcoal rot (107). Young et al. (120) reported that isolates of D. maydis differ in virulence with respect to their temperature requirements depending on the part of the United States in which the isolate originated.

It has long been recognized that soil fertility influences stalk rot severity (55). There is evidence that potassium fertilizers reduce the severity of stalk rot and that nitrogen fertilizers, especially if in excess compared with potash, increase the severity of stalk rot (1, 46, 77, 82, 97). A more thorough evaluation of stalk rot suppression by potassium revealed that the chloride ion present in the most commonly applied form of potash fertilizer (muriate of potash) was responsible for the observed disease reduction (74). Younts and Musgrove (121) demonstrated that the chloride ion competitively inhibited nitrate uptake and Nelson (74) showed that stalk rot was more severe with nitrate than ammoniacal nitrogen. The reduction in stalk rot observed with muriate of potash may result from the selective uptake of ammoniacal nitrogen by the plant in the presence of the chloride ion. Huber and Nelson (42) report that inhibition of nitrification of applied ammoniacal nitrogen by nitrapyrin, a nitrogen stabilizer, provides a practical control of stalk rot as well as a means to reduce over-winter nitrogen loss.

Certain hybrid varieties now grown in the corn belt seem fairly resistant to stalk rot, but no inbred or hybrid corn is immune to the disease (15). Resistance is inherited in a quantitative manner according to Kappelman and Thompson (47), but the resistance mechanism is unknown. Resistance to stalk rot is also related to sugar concentration (19, 70), soluble solids content (117), tissue density (19, 78, 84), level of fungistatic compounds (9), and stalk strength (43, 71). For plant breeders to make rapid progress and utilize inbred lines more effectively, definitive research on the nature of resistance to stalk rot must be conducted.

Stalk rot is one of the principal diseases of field corn grown in Florida. Despite the fact that corn is the most important field crop grown in Florida, from the standpoint of acreage, few Florida plant pathologists have investigated this syndrome. During the 1930's A. H. Eddins and R. K. Voorhees studied the etiology of several corn diseases including stalk rots caused by Diplodia macrospora Earle, F. moniliforme and M. phaseolina (24, 25, 26, 104, 105, 106, 107). No subsequent projects were reported until Kucharek (56) found that Helminthosporium rostratum Drechsl. caused stalk rot of field corn in Florida.

It was the purpose of this study to investigate the incidence, etiology and control of corn stalk rot as it exists under current cultural practices in Florida. Specifically, the objectives were:

1. to study the fungi associated with corn stalk and root tissue from seedling stage to maturity,

2. to study factors that affect the development of stalk rot,

3. to determine the incidence of stalk rot and its effect on corn yields in Florida,

4. to determine if stalk rot resistance is available in commercial corn varieties grown in Florida.

SECTION I
A FIELD STUDY OF FUNGI ASSOCIATED WITH HYBRID FIELD CORN GROWN IN FLORIDA

Occurrence of Fungi Associated with Corn

Introduction

Christensen and Wilcoxson (15) extensively reviewed the literature in relation to the organisms associated with corn stalk rot. They reported that in some cases specific fungi were incriminated at the cause of stalk rot while in other cases a complex of different organisms including fungi, bacteria and nematodes was involved. Eddins and Voorhees (24, 25, 105, 106, 107) studied corn stalk rot in Florida in the 1930's but their investigations were limited to the etiology of diseases incited by M. phaseolina, species of Diplodia, and F. moniliforme. Preliminary field studies in the fall of 1972 suggested that a complex of fungi was responsible for stalk rot in Florida. The present investigation was conducted to gather data on the kinds and frequencies of fungi which could be isolated from stalks and roots of modern hybrid field corn varieties grown under local cultural practices.

Materials and Methods

Field studies were carried out during 1973 and 1974 on the Agronomy farm of the University of Florida in Gainesville which has an Arredondo loamy fine sand/fine sand soil type.

In 1973 the test area was prepared for planting by plowing the field and broadcasting 700 lb./acre of 4-8-16 fertilizer. Two commercial varieties, which were yellow, full season hybrids, were each planted in six randomized plots of 200 plants. The variety 'McNair 508' had been reported to have excellent standability whereas 'PAG 751' did not have this characteristic. Of the six plots for each variety, three were fumigated with methyl bromide (2 lb./100 ft^2) prior to planting.

After planting, the test site was irrigated as needed, the nonfumigated plots were cultivated by hand, and all of the plots were sampled for nematodes 71 days after planting.

Five plants were randomly collected every 14 to 37 days from each of the 12 plots beginning 41 days after planting. Two cubes of tissue, ca. 5 mm on each side, were excised from the roots, crown, first, second, and third nodes and internodes from each plant. Tissue pieces were surface disinfested with a 0.5% NaOCl solution, placed on acidified (pH 5.5) potato dextrose agar (APDA) and wheat straw agar in petri dishes, and incubated at 25 C under 12 hours of flourescent light per day for up to 14 days. Fungal cultures were identified and if a fungus was isolated from any point in a stalk, the stalk was considered infected by that fungus. The isolation frequency of fungi from root tissue was determined in a similar manner, however the results were recorded separately.

In the spring of 1974 this study was repeated with the following modifications. Only 'McNair 508' was planted

since 1973 data revealed no difference between the varieties relative to their colonization by fungi. In addition to fertilization with 10-5-10 with micronutrients (600 lb./acre), the field was treated with Sutan (0.5 gal/acre), Atrazine (2 lb./acre), Dasinit (0.5 gal/acre), and Nemagon (2 gal/acre) on a broadcast basis. The soil was not fumigated with methyl bromide. The crop was sprayed as needed with Lannate for insect control and mechanically cultivated. Samples, which were collected six times during the season at 17 to 32 day intervals, were composed of 50 plants randomly collected from the field at each sampling date.

Results

As a result of sampling over 7,500 pieces of tissue over the 1973 and 1974 growing seasons, the following general statements can be made. First, there was an extensive mycoflora associated with developing corn plants grown in Florida, because over 30 fungal species were recovered from stalks and roots in the course of a growing season. Second, the fungi did not all inhabit the stalks at the same time, each particular species was recovered only during a particular growth stage of the plants sampled. Third, the fungi were recovered in groups that were associated with the plants during particular stages of development. These groups will be termed communities.

Isolation frequencies of fungi appeared to demonstrate the existence of five communities. The fungi within each community have three characteristics in common: (1) they

each invaded plants about the same time; (2) they reached their maximum frequency within plants at ca. the same time; (3) all the species in one community declined in frequency at ca. the same time. The times referred to can be expressed in terms of the chronological age of the plant (days after planting) or the physiological development of a plant. From 0-60 days the corn plant is in a vegetative phase of growth. Tasseling, beginning at about 65 days, marks the transition of the plant into the reproductive phase. Silking is completed by 80 days and senescence of pith parenchyma has begun. By day 100 the grain is in the dough stage, by 150 days the plant is physiologically mature, the grain has reached the full dent stage, and stalk and leaf tissue have senesced. However, many Florida farmers do not harvest their corn crop until the moisture content of the grain declines to 15%. This may require an additional 30 days or longer.

The first community of fungi was detected in the seedling stage. This community was composed of Fusarium solani (Mart.) App. & Wr. em. Synd. & Hans., species of Penicillium Link ex Fr., a species of Rhizopus Ehrenb. ex Corda, Pyrenochaeta terrestris (Fig. 1, 2, 3, 4) and to a minor extent Fusarium lateritium (Nees) em. Synd. & Hans. Fungi in this community were recovered with increasing frequency until tasseling commenced, and with declining frequency after tasseling until the plants reached physiological maturity (Fig. 5).

All fungi in the first community were recovered from root samples as well as from stalk samples. Recovery of these fungi from root tissue paralleled their occurrence in stalks

with the exception of the Penicillium spp. which persisted in the roots of several corn plants after they could no longer be recovered from stalks.

Fungi in the second community were Trichoderma viride Pers. and Fusarium moniliforme (Fig. 6, 7). Trichoderma viride appeared before flowering but F. moniliforme was detected only at low levels until the corn tasseled. This is in accord with the findings of other workers (30, 35, 95) who have not found F. moniliforme in stalks until after flowering. Miller (67), however, found that both F. moniliforme and T. viride colonized corn roots early in their development. This community gradually increased in the population until the fungi had invaded over 90% of the plants sampled at physiological maturity (Fig. 8). Trichoderma viride was isolated from roots as frequently as it was from stalks. However, F. moniliforme was not isolated from roots as frequently as from stalks, although the association of the fungus with roots followed the same pattern as with stalks.

The third community, which includes two common soil saprophytes, a species of Curvularia Boedijn and a species of Mortierella Coemans, appeared at flowering and by the full dent stage could no longer be recovered (Fig. 9, 10, 11). In the roots, Curvularia sp. followed the same pattern as in the stalks. Mortierella sp. was quite transitory in the roots compared to stalks. It was not detected in 1973 and was recovered at only a single sampling date in 1974.

The fourth community was composed of Macrophomina phaseolina, Rhizoctonia solani Kuhn, and Sclerotium rolfsii

Sacc. (Fig. 12, 13, 14). It appeared during the dough stage, increased in frequency up to 150 days and gradually declined to harvest time (15% kernel moisture, 175 days). Fungi in this community were never recovered from more than 25% of the plants sampled (Fig. 15). The occurrence of R. solani and M. phaseolina was the same in the roots as in the stalks. However, S. rolfsii was recovered from the roots of only a single plant at a single sampling date in 1973. This may be an indication that this fungus penetrated stalks at the crown rather than entering via the roots, a situation similar to its invasion of peanuts (32).

The final community to appear was composed of Helminthosporium rostratum and Diplodia maydis (Fig. 16, 17). This community appeared at the dough stage (day 100) and increased in frequency until at harvest it was nearly as abundant as the second community (Fig. 18).

Diplodia stalk rot has been recognized in Florida since 1930 when Eddins (24) pointed out that it occurred after silking and is caused by airborne spores which lodge between the stalk and sheath, germinate and penetrate the stalks at the nodes. The fungus frequently associated with D. maydis was H. rostratum, which was recently cited as a stalk rotting pathogen by Kucharek (56). Neither fungus in this community was recovered from the roots of diseased plants.

In the course of this study, numerous fungi were recovered sporadically, or in such low amounts that they were not incorporated into this scheme. Genera which were

occasionally recovered included: Aspergillus, Alternaria, Cunninghamella, Monotospora, Neurospora, Nigrospora, Pestilotia, Pythium, and Zygorhynchus.

The soil samples assayed for nematodes had no plant parasitic nematodes except in one replication of 'McNair 508' in which there was a light infestation of ring nematode (Criconemoides sp.). Although bacteria were frequently isolated from stalk and root tissue, none belonged to a genus known to be pathogenic to corn. Most workers consider bacteria found in rotted stalks to be secondary invaders (15). Bacterial stalk rot may be a problem on some sweet corn varieties but field corn is generally not susceptible to the rots incited by these organisms (85).

As a result of these studies it is proposed that five fungal communities (Fig. 19) exist in stalks and roots of hybrid field corn grown in Florida. Although nematodes could accentuate or modify these communities, they apparently occur in the absence of nematode damage.

Discussion

Corn stalk rot is regarded as the most destructive disease of corn in the world (15). The importance of this disease has prompted a large volume of research, most of which has dealt with organisms of known or suspected pathogenic capabilities. Certain workers (35, 49, 53) have studied the succession of pathogenic fungi in roots or stalks throughout a growing season and their work, therefore has, predominantly autecological implications. As the initial step

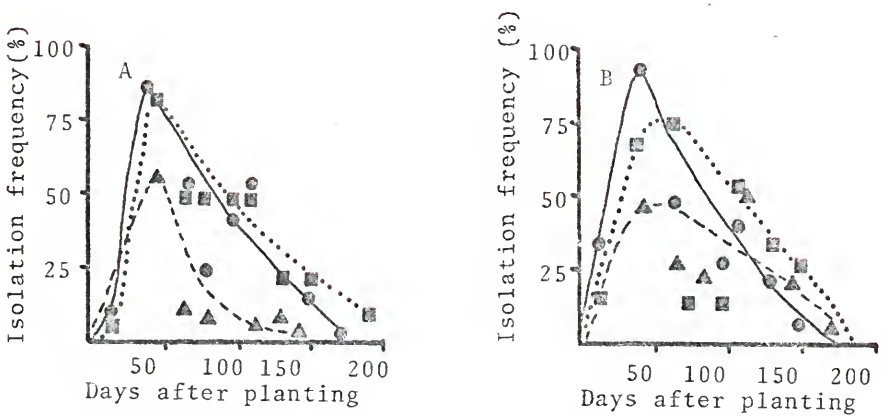


Fig. 1-A,B. Isolation frequency of Fusarium solani as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

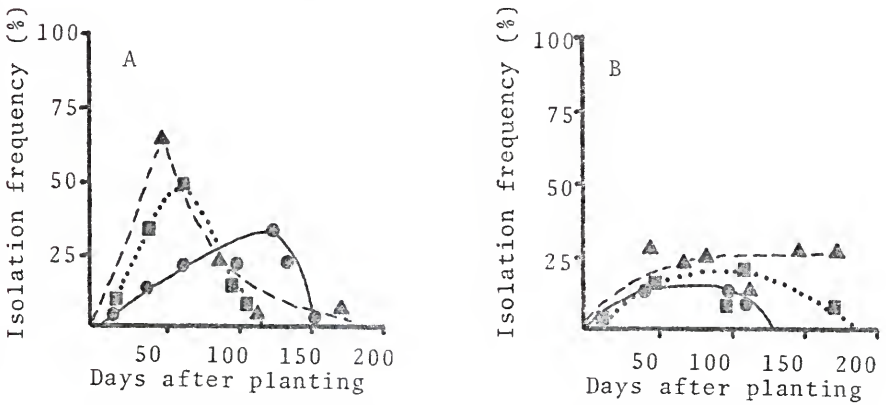


Fig. 2-A,B. Isolation frequency of Penicillum spp. as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

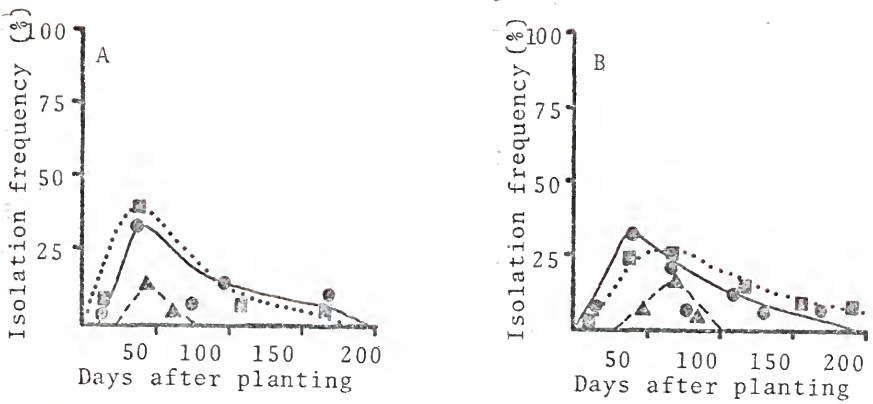


Fig. 3-A,B. Isolation frequency of Rhizopus sp. as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲- -▲), PAG 751 in 1973 (■·····■).

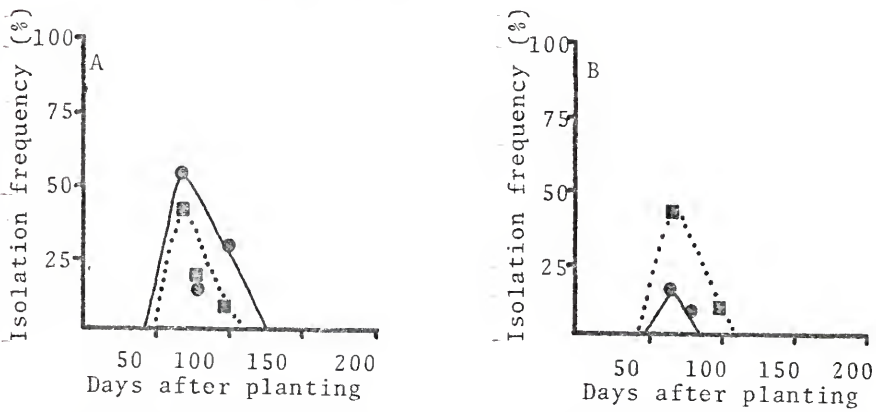


Fig. 4-A,B. Isolation frequency of Pyrenochaeta terrestris as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars samples were: McNair 508 in 1973 (●——●), PAG 751 in 1973 (■···· ■).

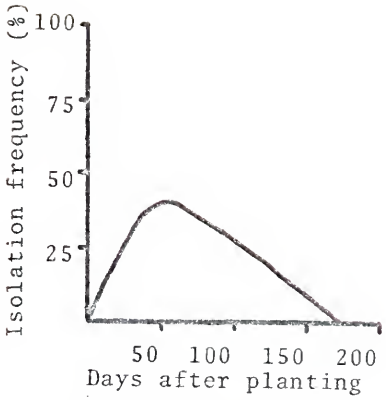


Fig. 5. Proposed representation of a community based on the isolation frequency of Fusarium solani, Penicillium spp., Rhizopus sp., Pyrenochaeta terrestris.

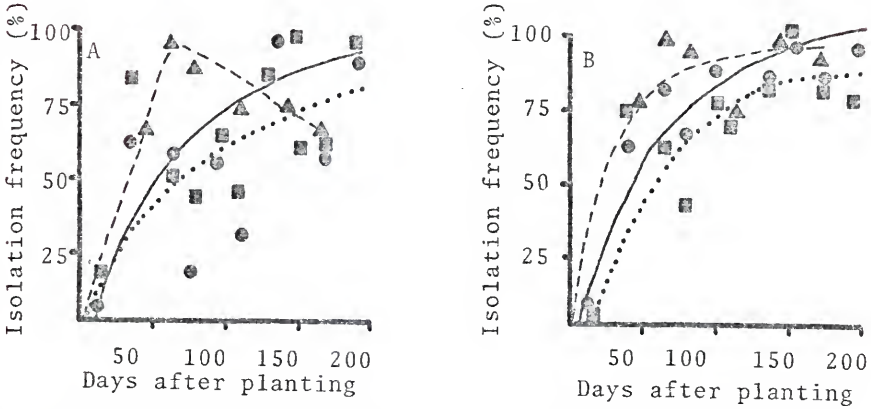


Fig. 6-A,B. Isolation frequency of Trichoderma viride as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲--▲), PAG 751 in 1973 (■····■).

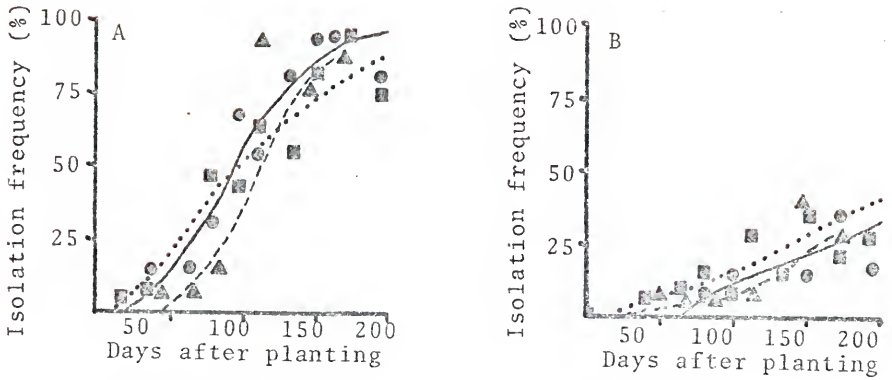


Fig. 7-A,B. Isolation frequency of Fusarium moniliforme as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●—●), in 1974 (▲--▲), PAG 751 in 1973 (■·····■).

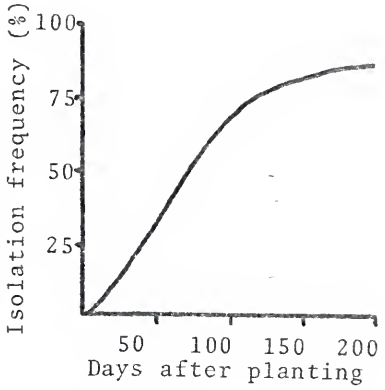


Fig. 8. Proposed representation of a community based on the isolation frequency of Trichoderma viride and Fusarium moniliforme.

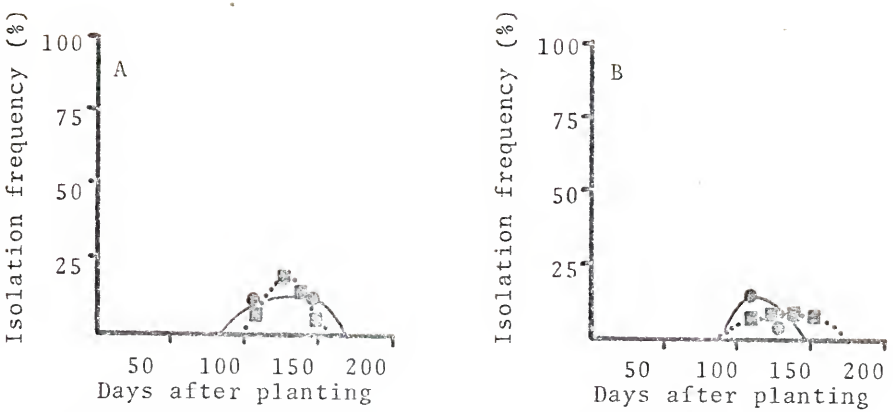


Fig. 9-A,B. Isolation frequency of Curvularia sp. as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), PAG 751 in 1973 (■·····■).

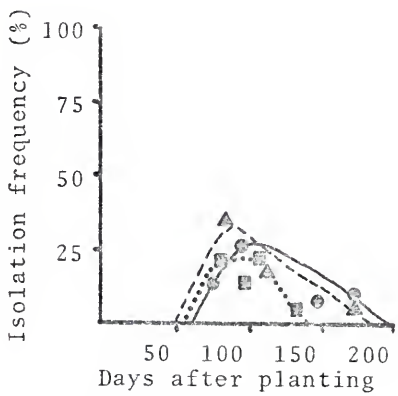


Fig. 10. Isolation frequency of Mortierella sp. as percentages of stalks from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

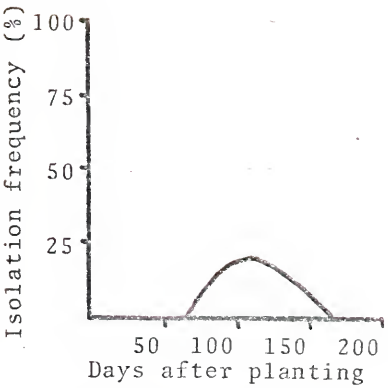


Fig. 11. Proposed representation of a community based on the isolation frequency of Curvularia sp. and Mortierella sp.

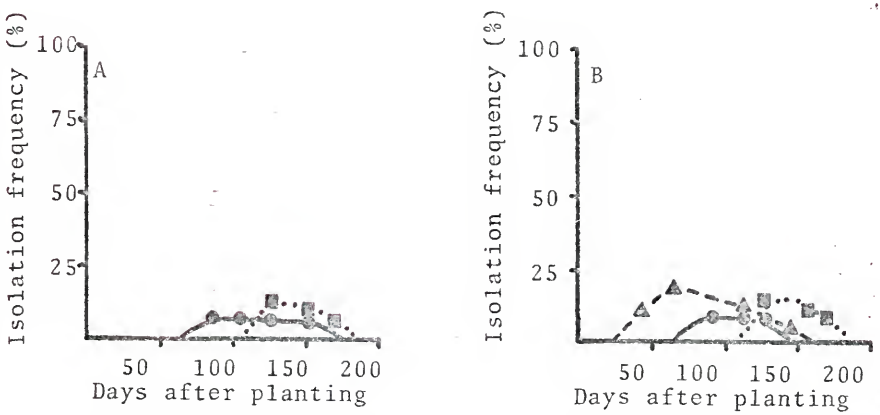


Fig. 12-A,B. Isolation frequency of Macrophomina phaseolina as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

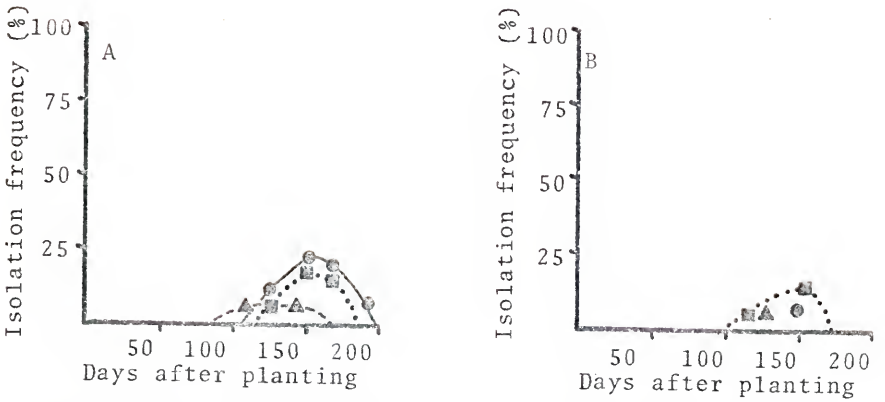


Fig. 13-A,B. Isolation frequency of Rhizoctonia solani as percentages of stalks (A) and roots (B) from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

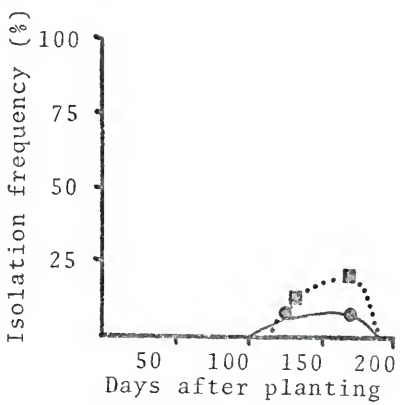


Fig. 14. Isolation frequency of Sclerotium rolfsii as percentages of stalks from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), PAG 751 in 1973 (■·····■).

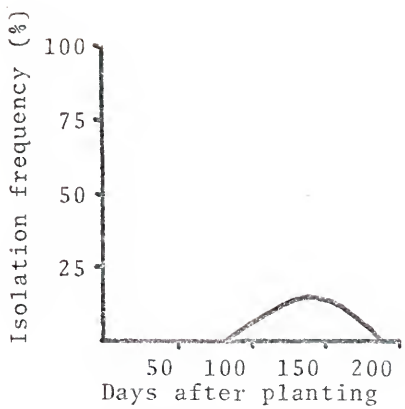


Fig. 15. Proposed representation of a community based on the isolation frequency of Macrophomina phaseolina, Sclerotium rolfsii, and Rhizoctonia solani.

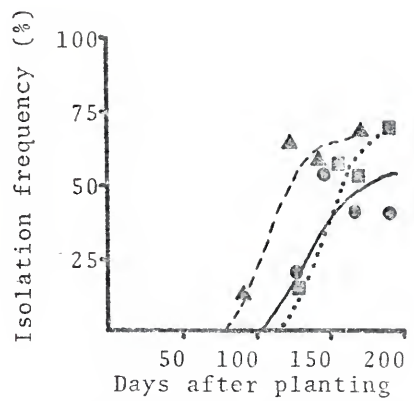


Fig. 16. Isolation frequency of Helminthosporium rostratum as percentages of stalks from which it could be isolated as related to days after planting. Cultivars sampled were: McNair 508 in 1973 (●——●), in 1974 (▲- -▲), PAG 751 in 1973 (■·····■).

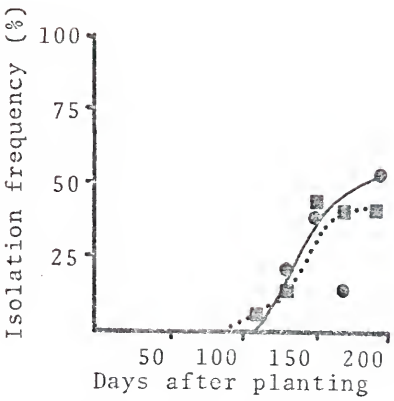


Fig. 17. Isolation frequency of Diplodia maydis as percentages of stalks from which it could be isolated as related to days after planting. Cultivars were: McNair 508 in 1973 (●——●), in 1974 (▲– –▲), PAG 751 in 1973 (■·····■).

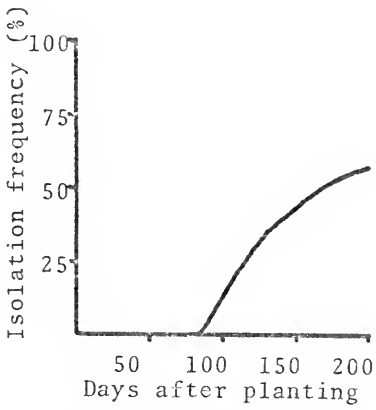


Fig. 18. Proposed representation of a community based on the isolation frequency of Helminthosporium rostratum and Diplodia maydis.

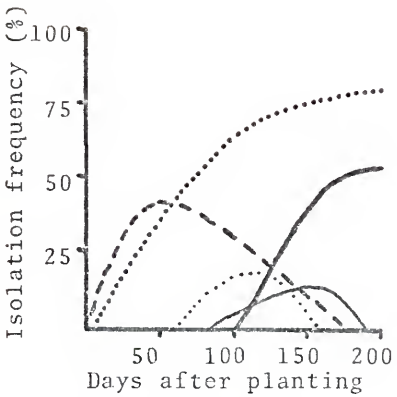


Fig. 19. Representation of five proposed communities based on the isolation frequency of the following fungi: Trichoderma viride and Fusarium moniliforme (.....); Fusarium solani, Penicillium spp., Rhizopus sp., and Pyrenochaeta terrestris (- - - - -); Curvularia sp. and Mortierella sp. (.....); Macrophomina phaseolina, Sclerotium rolfsii and Rhizoctonia solani (——————); Diplodia maydis and Helminthosporium rostratum (——————). Fungi were isolated from the stalks and roots of the following corn cultivars: McNair 508 in 1973 and 1974, PAG 751 in 1973.

in understanding the stalk rot syndrome in Florida, this investigator undertook a synecological approach to the study of fungal associations with field corn. The intent to describe the total mycoflora during this study was subject to the restrictions imposed by sampling procedures and laboratory methods. This author knows of no previous report of such a study on corn but the approaches taken by Garren (33) and Jackson (44) in the study of mycoflora of peanut fruit served as examples.

The purpose of this study was to provide a basis for a general description of the stalk and root mycoflora of developing field corn. As a result, several communities were distinguished (Fig. 19). These communities constitute a miniature succession, or serule.

A serule (110) is a series of communities composed of minute or microscopic organisms, especially bacteria, fungi, and insects. Such successions are characteristic of forests, where they serve to return fallen organic matter to the soil by decomposition. The determination of whether or not succession has occurred is largely a subjective matter hinging on the decision of what constitutes a change in the community. In this study, minor fluctuations in the density of taxa have not been interpreted as evidence of a change in the basic nature of a community. In graphing the results of this study, an effort has been made to depict the simplest trend consistent with the data. For example, a strict interpretation of the isolation frequencies from 'McNair 508' in 1974 would yield a bimodal curve with peaks at 70 and 210

days, but there were no sudden changes in environmental conditions or laboratory methods that could be correlated with the abrupt change in frequency (Fig. 1B). Therefore, the results were depicted as a simple curve and the variation was ascribed to sampling error.

In this discussion, fungi from corn stalks and roots have been conceived as constituting communities that may exhibit successional changes. The validity of this assumption is supported by the similarities in composition and changes of fungal associations with two varieties, grown for two years, and under differing cultural regimes. Furthermore, the proposed patterns were consistent with spot checks made throughout central and west Florida on diverse varieties in 1973 and 1974. However, the isolation of fungi from plant tissue demonstrates associations, but does not prove the fungi present are responsible for physiological dysfunctions or morphological abnormalities. Virulence data are a requisite for such assertions.

Relative Virulence of Fungi Associated with Corn

Introduction

In the Southeast Region of the United States, F. moniliforme is generally thought to be an important cause of stalk rot (39, 105). Other fungi known to be associated with the stalk rot complex in Florida included Diplodia spp. (24, 25) and H. rostratum (56). The purpose of this field test was to compare the virulence of the numerous fungi isolated from corn stalks and roots in order to determine their roles in the pathogen complex.

Materials and Methods

Field experiments were conducted in the spring of 1973 and in the fall of 1974. In 1973, the field was prepared for planting as described in the 1974 ecology study. On April 5 it was planted with 'Funks G795W', a hybrid of poor standability. Seed was hand-planted in 16 rows 180 feet long, with row centers of 36 inches and plant spacing of 12 inches.

Plants in the soft dough stage of development were inoculated on June 26 using the toothpick method (19). A single toothpick, infested with the test fungus, was inserted into a puncture in the first expanded internode above the brace roots. Several isolates of each of the following fungi were tested: F. moniliforme, F. solina, F. lateritium, D. maydis, Curvularia sp., Aspergillus niger van Tiegh. Rhizopus sp., Mortierella sp., M. phaseolina, and H. rostratum. The test fungi were isolated from corn growing in the following Florida counties: La Fayette (L), Alachua (A), Suwanee (S and SA), Calhoun (C), and Jackson (JM and JL). Non-infested toothpicks served as a control. Fifteen plants were inoculated with each isolate.

The relative virulence of the isolates was judged 31 days after inoculation. Stalks were split longitudinally at a right angle to the long axis of the toothpick and scored for stalk rot using a modification of a scheme described by Hooker (38): 0.5 = 12.5% or less of the inoculated internode discolored, 1 = 12.6-15% discolored, 2 = 26-50% discolored, 3 = 51-75% discolored, 4 = 76-100% discolored,

5 = discoloration spreading into the adjacent internodes. Following the rating procedure, tissue from at least five stalks per isolate was assayed for the test fungus by the culture plate technique described in the ecology study. In addition pith tissue from internodes inoculated with H. rostratum was sectioned, mounted in distilled water, and examined with a microscope.

Trichoderma viride was not evaluated in the 1973 test because it is generally regarded as a saprophyte (15). However, this fungus was commonly isolated in 1973 and 1974. Peterson (86) working in New Jersey, and Sumner (95) in Nebraska, isolated cultures of T. viride that they reported were as pathogenic as F. moniliforme. Consequently, a virulence test was made comparing four isolates of T. viride to isolates of H. rostratum, F. moniliforme, and D. maydis. The test, conducted in the fall of 1974, varied from the 1973 study in that the cultivar planted was 'Pennington Florida 200A' and plants were inoculated on October 4 (midsilk stage).

Results

A high degree of variability in virulence among species and among isolates of species occurred in the 1973 virulence test (Table 1). The most virulent isolates were those of F. moniliforme, H. rostratum and D. Maydis. Helminthosporium rostratum caused discoloration of ground parenchyma and vascular tissue and occlusion of xylem and phloem tissue (Fig. 20). Fusarium solani, F. lateritium, Curvularia sp., Rhizopus sp.,

A. niger and M. phaseolina were less virulent than the control. The test fungi were recovered from internodes inoculated with H. rostratum, F. moniliforme, D. maydis, or M. phaseolina. However, in stalks inoculated with Curvularia sp., Mortierella sp., and Rhizopus sp., the principal fungi isolated from tissue pieces were Fusarium spp. Stalks inoculated with A. niger yielded T. viride and those inoculated with blank toothpicks yielded cultures of Fusarium spp., T. viride, D. maydis, and Penicillium sp. The check treatment apparently provided an excellent infection court for secondary fungi.

In the 1974 virulence test, isolates of T. viride incited relatively little stalk rot. Their mean stalk rot ratings ranged from 0.6 to 1.2 (Table 2). The virulence of most of the Trichoderma isolates was significantly less ($P = 0.01$) than that of H. rostratum or D. maydis. The test fungi were recovered from all inoculated stalks and there were few secondary invaders. Although the results of the two tests may not be directly comparable because different cultivars and environmental conditions were involved, the results strongly suggest that T. viride is not a virulent component of the stalk rot complex.

Discussion

The most virulent test fungi were isolates of H. rostratum and D. maydis, which belong to the final community, and F. moniliforme, which is a member of one of the initial communities. The vascular occlusions induced by H. rostratum

Table 1. Corn stalk rot ratings with 'Funks G795W' 31 days after inoculation with fungi in the first expanded internode above the brace roots

Fungus	Isolate	Stalk Rot Rating[a]
Fusarium lateritium	A 19	1.2[b,c]
Fusarium lateritium	A 17	1.2
Macrophomina phaseolina	JL 4	1.2
Fusarium solani	A 16	1.3
Aspergillus niger	A 5	1.4
Curvularia sp.	JM 9	1.6
Diplodia maydis	C 5	1.7
Macrophomina phaseolina	L 1	1.7
Fusarium solani	A 15	1.9
Fusarium lateritium	A 20	1.9
Macrophomina phaseolina	A 9	1.9
Macrophomina phaseolina	JM 5	1.9
Macrophomina phaseolina	C 8	2.0
Curvularia sp.	JL 1	2.0
Fusarium moniliforme	JM 16	2.2
Helminthosporium rostratum	S 1	2.3
Rhizopus sp.	---	2.3
Blank toothpick	---	2.4
Fusarium moniliforme	L 3	2.7
Helminthosporium rostratum	JL 2	2.7
Helminthosporium rostratum	L 5	2.7
Diplodia maydis	L 2	2.8
Mortierella sp.	A 6	2.9
Fusarium moniliforme	S 1	2.9
Fusarium moniliforme	A 13	3.0
Helminthosporium rostratum	A 2	3.0
Diplodia maydis	JM 3	3.1
Diplodia maydis	SA 2	3.4
Fusarium moniliforme	A 14	3.6
Fusarium moniliforme	JM 12	3.7
Helminthosporium rostratum	JM 11	3.8

[a]Rating scale was: 0.5 = 0-12.5%, 1 = 12.6-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of inoculated internode discolored.

[b]Mean of 15 plants.

[c]Means followed by the same bar are not significantly different (P = 0.01) according to Duncan's multiple range test.

Table 2. Corn stalk rot ratings from 'Pennington Florida 200A' inoculated 31 days previously with certain fungi in the first expanded internode above the brace roots

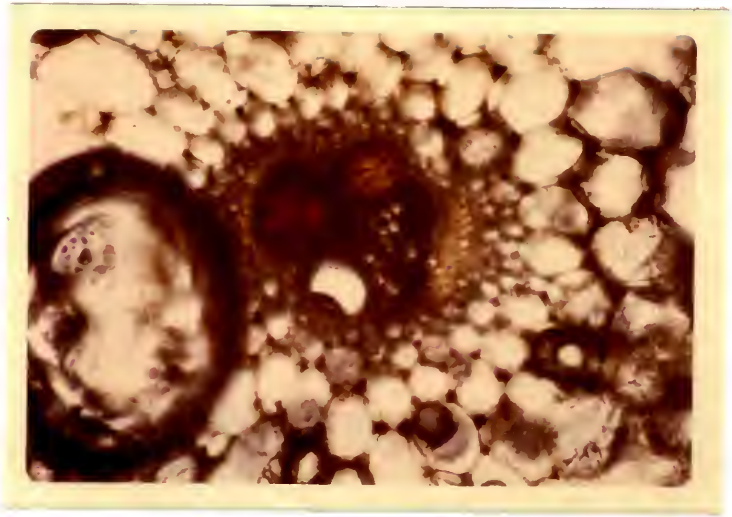
Test Fungus	Isolate	Stalk Rot Rating[a]
Blank toothpick	---	0.5[b,c]
Trichoderma viride	A 1	0.6
Trichoderma viride	C 2	0.6
Fusarium moniliforme	A 14	1.0
Trichoderma viride	SA 1	1.0
Trichoderma viride	JM 4	1.2
Helminthosporium rostratum	JM 11	1.9
Diplodia maydis	SA 2	2.7

[a]Rating scale was 0.5 = 0-12.5%, 1 = 12.6-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of inoculated internode discolored.

[b]Average of 15 plants.

[c]Means followed by the same bar are not significantly different (P = 0.01) according to Duncan's multiple range test.

A



B

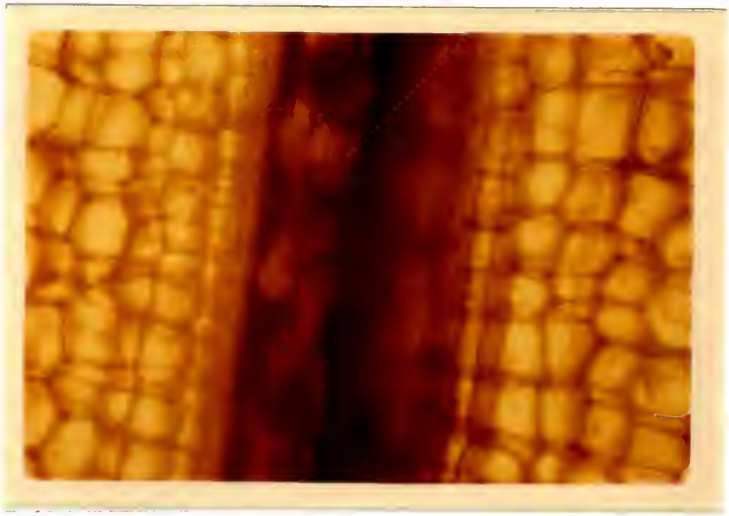


Fig. 20-A,B. Cross section (A) and tangential section (B) of a vascular bundle in a stalk rot lesion developing in the pith of a corn plant inoculated with Helminthosporium rostratum. Note in A, the dark walls of parenchyma cells and the accumulation of dark substances in the intercellular spaces, vessels, and protoxylem lacunum. Note in B, the dark occlusions in the vessels.

were similar to those reported to be induced by F. moniliforme (57) and F. graminearum (59). The other fungi tested, including T. viride, were less virulent.

The role of T. viride in the stalk rot complex does not seem to be that of a virulent pathogen. The possibilities of synergistic interactions or predisposition to infection were not studied in this test and therefore cannot be ruled out. It is unclear whether virulent isolates of T. viride tested by Sumner (95) and Peterson (86) were reisolated from inoculated stalks or whether stalk rot was due to secondary infection by other (undetected) pathogens as happened in the 1973 field test reported here.

The isolates of Macrophomina phaseolina tested in this study had a low degree of virulence but this fungus can cause extensive stalk rotting (15). Moisture stress along with high temperatures (35 C) favor charcoal rot in sorghum (28) and corn (53). Since the site of inoculation (young, vigorous tissue) and growing conditions (irrigated field, moderate temperatures) were inimicable to charcoal rot, the comparative virulence of M. phaseolina was likely underestimated by the procedures under which it was tested. During hot, dry conditions this fungus could be a significant stalk-rotter in Florida.

SECTION II
EFFECT OF CERTAIN SUSCEPT, PATHOGEN, AND ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF STALK ROT AND THE SUCCESSION OF FUNGI IN ROOTS AND STALKS

Various fungi associated with corn plants reach maximum frequencies at particular stages in the development of the plants (Section I). The following series of experiments tested the correlation between certain factors that occurred during particular growth stages of corn and the fungi associated with plants at those stages. The factors studied were: air and soil temperature, airborne inoculum, survival of fungi in infected tissue, effect of sucrose concentration on fungal growth, relative saprophytic ability of certain fungi, and sensitivity of fungi to inhibitory substances extracted from corn.

Effect of Temperature on Fungal Succession

Introduction

Weather conditions affect the prevalence and severity of various stalk rot pathogens. Stalk rot caused by M. phaseolina is favored by hot, dry weather, whereas stalk rot caused by D. maydis is enhanced by abundant rainfall in the latter part of the growing season (53). Warm, wet weather two or three weeks after silking favors the development of stalk rot incited by F. moniliforme (2). The purpose of this study was to evaluate the influence of seasonal changes in

the average monthly maximum and minimum air and soil temperatures on the occurrence of the fungi associated with corn stalks and roots.

Materials and Methods

Weather data, collected at a standard meteorological station at the Agronomy Farm, University of Florida, Gainesville, was tabulated for the 1973 and 1974 growing seasons. Maximum and minimum soil temperatures were measured four inches below centipede grass sod. Rainfall data are not included in this study because the test sites were irrigated.

Results

The average monthly air temperatures for 1973 and 1974 are depicted in Fig. 21. When the changes in temperature between 50 and 150 days after planting are compared with the changes in frequency of fungal communities over the same period of time, it appears unlikely that temperature is directly responsible for the changes observed. Referring to Fig. 19 (Section I), it can be seen that at 50 days after planting no differences between the frequencies of the two communities occurred. By 150 days after planting all communities had appeared, three were approaching their maximum frequencies, one was declining in frequency, and the fungi in one could no longer be recovered. The maximum monthly air temperatures between these two reference dates ranged from 30-33 C. The minimum monthly air temperatures ranged from 19-21 C and it seems unlikely that air temperature shifts amounting to 2-3 C could account for the abrupt appearance and disappearance of fungi in corn stalks.

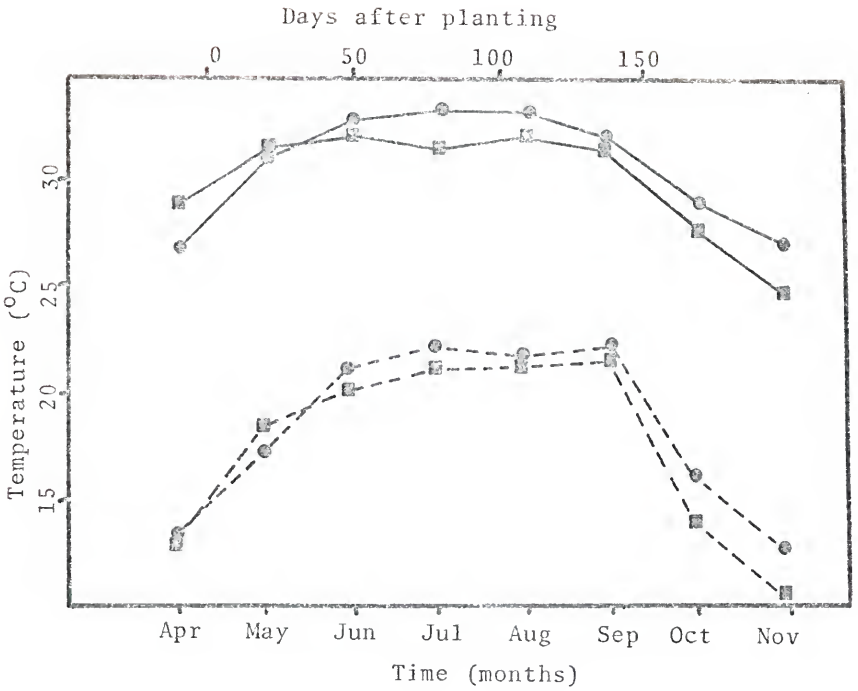


Fig. 21. Average monthly air temperature measured five feet above ground on the Agronomy Farm, University of Florida, Gainesville. Average monthly maximum temperature in 1973 (●——●). Average monthly maximum temperature in 1974 (■——■). Average monthly minimum temperature in 1973 (●- - - -●). Average monthly minimum temperature in 1974 (■- - - -■).

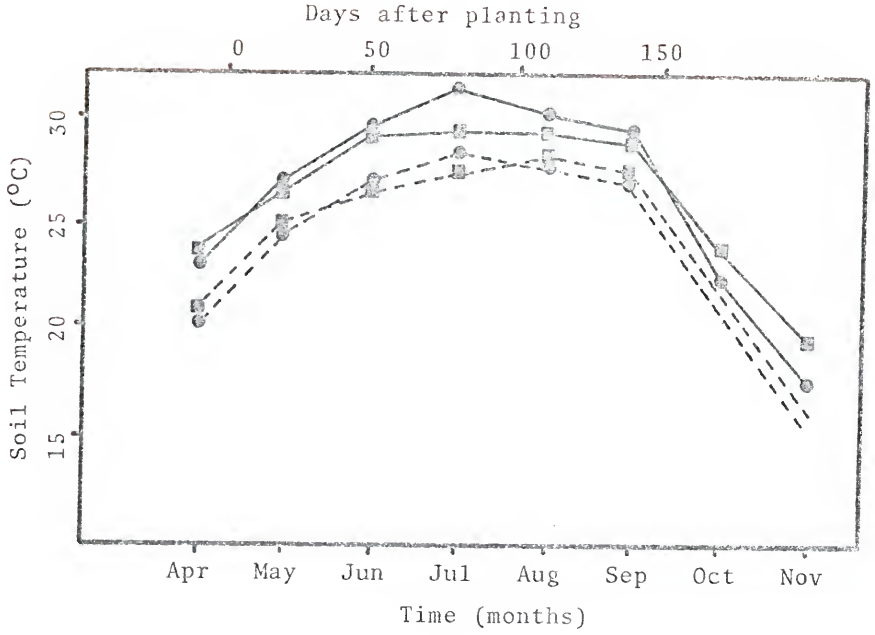


Fig. 22. Average monthly soil temperature measured four inches below centipede grass sod on the Agronomy Farm, University of Florida, Gainesville.
Average monthly maximum soil temperature in 1973 (●——●).
Average monthly maximum soil temperature in 1974 (■——■).
Average monthly minimum soil temperature in 1973 (●- - - -●).
Average monthly minimum soil temperature in 1974 (■- - - -■).

The shifts in soil temperature, depicted in Fig. 22, were of similar magnitude. Maximum monthly soil temperatures between 50 and 150 days after planting varied from 29-32 C, while the minimum monthly soil temperatures were 25-28 C. The minimum soil temperature were extrapolated since these data were not available for the months of October and November. The range in both the maximum and the minimum soil temperatures, between 50 and 150 days after planting, was 3 C and therefore shifts in the composition of fungal populations within corn roots or stalks cannot be attributed to temperature shifts of this magnitude.

Discussion

Average temperatures for most of the season were within the optima reported for the stalk rots caused by many of the fungi under study (2, 15, 53). The abundance of soil moisture was not a variable, since the field plots were irrigated throughout the season. Therefore, the succession of fungi in corn stalks cannot be directly attributed to seasonal fluctuations in temperature or moisture.

The Role of Airborne Inoculum

Introduction

H. rostratum contributed significantly to the stalk rotting complex in 1973 and was isolated exclusively from the aerial portions of the plants at the end of the growing season. The exclusive occurrence of H. rostratum in stalks suggested that airborne inoculum might be epidemiologically important. According to Koehler et al. (54), invasion of

nodes may occur with G. zeae, D. maydis, and F. moniliforme from spores that have fallen between the stalk and leaf sheath. The purpose of this study was twofold: (1) to determine if certain fungi penetrate and infect a plant through its aerial parts, and (2) to determine if there are seasonal fluctuations in airborne spore populations of H. rostratum which can be correlated with the occurrence of the fungus in stalks.

Materials and Methods

The site and mode of invasion was studied by placing inoculum in the space between leaf sheaths and stalks and by inoculating wounded internode rind tissue with spore suspensions. Twenty plants per treatment were inoculated at the second expanded internode above the brace roots. Leaf sheath inoculations were made by puncturing through the sheath with a hypodermic syringe and injecting one ml of spore suspension. Inoculum consisted of spores washed from a Difco PDA culture of the test fungus. The following fungi were tested by this method: F. solani; isolates JM12, A14, and A19 of F. moniliforme; Curvularia sp.; R. solani; isolates S1 and JL2 of H. rostratum; M. phaseolina; and Mortierella sp. Each spore suspension was standardized to 10^6 spores/ml with the following exceptions: H. rostratum (S1), 2×10^4 spores/ml; Curvularia sp., 2×10^4 spores/ml; M. phaseolina, 5×10^3 sclerotia/ml; R. solani, mycelial fragments. The control treatment consisted of plants inoculated with sterile water. Following inoculation the

puncture was wrapped with tape in order to reduce contamination and to aid in the location of the inoculated internode when stalk rot ratings were made 30 days later.

The wounding treatment was done the same way except that the rind[1] was wounded to a depth of three to four mm with a sterile needle after inoculation. Only H. rostratum (isolate S1) at 2 x 10^4 spores/ml and F. moniliforme (isolate A14) at 1 x 10^6 spores/ml were used in this treatment. The wounded area was wrapped with tape and stalk rot ratings were made 30 days after inoculation.

In order to determine when airborne spores of H. rostratum appeared during the growing season, two spore traps were set up in conjunction with the 1974 ecology study (Section I). The spore trap was a rod sampler as described by Roelfs et al. (87). One trap was placed in the center of the field and the other on the margin or the field. The samplers were exposed for 48 hours, once a week, throughout the growing season and at a later time they were microscopically examined for spores of H. rostratum. No attempt was made to monitor the number of spores on the sampler; rather, the sampling date at which they could be first detected was the primary concern.

---

[1]Within this paper the terms rind and pith are used to indicate general regions of the corn stem. Rind tissue is the exterior portion of the stem, composed of the epidermis, sclerified parenchyma, and closely grouped vascular bundles. Pith tissue is the soft spongy tissue in the interior of the stem composed of thin walled parenchyma cells and widely scattered vascular bundles.

Results

When inoculum was placed in the space between the leaf sheath and stalk no visible browning developed in nodes or internodes adjacent to the inoculated leaf sheaths. However, when the internodes were wounded after inoculation, rotting occurred with both H. rostratum and F. moniliforme. Fusarium moniliforme caused slight browning in 30% of the inoculated stalks since six of the 20 stalks were rated 0.5 and 14 stalks were rated 0. Helminthosporium rostratum caused rot in 45% of the stalks since four stalks rated 0.5, four stalks rated 1.0, one stalk rated 4.0, and 11 stalks rated 0. Fusarium moniliforme and H. rostratum were isolated from 100% and 50%, respectively, of wounded, rotted stalks. In addition, F. moniliforme was isolated from 50% of wounded stalks inoculated with H. rostratum.

Spore samplers were checked weekly throughout the summer of 1974. No spores of Helminthosporium spp. were observed until July 1. The initial Helminthosporium spp. spores were identified as those of H. maydis and H. turcicum. On August 2 (116 days after planting) the first spores of H. rostratum were observed, and they appeared thereafter on all spore samplers examined. In the 1973 and 1974 ecology studies, H. rostratum was isolated from stalk tissue beginning in early August (Section I).

Discussion

The mode of infection of corn stalks by H. rostratum may be similar to that which Eddins (24) described for D.

maydis; in which airborne spores lodge between stalks and sheaths, germinate and penetrate stalks. Fungal organisms may progress rapidly within internodes; whereas their progress is retarded at the nodes (15). Nodal tissue remains alive and resistant to infection after internodes have senesced and become susceptible (79, 81). Thus, the occurrence and severity of infection may be increased by providing an infection portal into the internode, such as was done when inoculated stalks were wounded.

Because H. rostratum was isolated from stalks only after the spores of this fungus were observed on spore traps and because H. rostratum is not known to infect roots (Section I) it seems likely that airborne spores of this fungus serve as primary inocula for stalks. The source of such spores was not determined, but numerous grass hosts are known to be susceptible to this fungus (111).

Leaf sheaths act as reservoirs for inoculum, and provide fungi with suitable conditions for germination. The following fungi were isolated from the space between leaf sheaths and stalks of plants in the mid-silk stage of maturity: A. niger, Penicillium spp., F. moniliforme, Fusarium spp., Mortierella sp. and T. viride. Wounding, in the form of insect injuries and growth cracks or natural points of entry such as sheath and brace root junctions with the stalk, may facilitate penetration, as was demonstrated in this test. However, stalk rot may also develop from airborne inoculum through unbroken tissue, as was demonstrated by Kucharek and Kommedahl (58).

Survival of Fungi in Naturally Infected Plants in the Field

Introduction

The longevity of fungi associated with residues of corn stalks and roots has not been reported for corn grown in Florida. In Nebraska, M. phaseolina survived as sclerotia in corn residue for 18 months (17). Nyvall and Kommedahl (75, 76) demonstrated that F. moniliforme can survive in the field as thickened hyphae in infested stalks, and that roots of corn plants often grow into and through buried corn stalks. Several workers have obtained viable conidia of D. maydis from pycnidia in host tissue that had remained in the soil for several seasons (13, 22, 36).

This test was conducted to determine whether the fungi associated with naturally infected corn plants could survive in stalk and root residue buried at varying depths in the field.

Materials and Methods

The survival of fungi in naturally infected plants was studied in the winter of 1974. Complete plants were removed from the field on December 1, when they were 233 days old, the date of the first mild frost. The fungi associated with the plants were determined by the tissue plating technique described previously (Section I). Plants were stored inside until January 4, 1974 when they were buried in a corn field immediately after it had been cultivated. Roots on two opposite sides of the stalk were removed so that the remaining tissue was in plane ca. 3 cm thick. Twelve plants were

buried at each of the following depths: 30.4 cm, 15.2 cm, 7.5 cm, 2.5 cm, and on the surface.

The plants were retrieved on April 24, 1974, after a total of 110 days, approximately the length of time a field would lie fallow before spring planting, and fungi associated with the stalks and roots were identified from tissue placed on APDA and wheat straw agar.

Results

Visible assessment revealed plant tissue decomposition was greatest at soil depths of 7.5 and 15.2 cm. There was less decomposition at 2.5 cm and the plants buried at 30.4 and zero cm showed very little decomposition. In general, fungi did not survive as long in buried stalks and roots as in those left unburied, T. viride and F. moniliforme being exceptions (Table 3). Trichoderma viride increased in buried stalks and remained at pre-burial levels in roots. The survival of F. moniliforme in stalks was unaffected by burial at any depth, but burial of roots at depths of 2.5 cm or greater reduced recovery of F. moniliforme to 10% or less. Helminthosporium rostratum was reduced when plants were buried at any depth. This fungus was isolated from 10% of the stalks buried at 7.5 cm or more, while 70% of those left on the surface contained the fungus. Diplodia maydis survived poorly in surface litter and was not recovered from buried material. The remaining fungi isolated from the buried plants were not present when the stalks were buried, and only at low levels when the plants were recovered.

Table 3. Survival of fungi in corn debris as judged by their isolation frequencies from corn cultivar 'McNair 508', 110 days after 233 day old plants were buried at varying depths in the soil in the field

	Trichoderma viride	*Fusarium moniliforme*	*Helminthosporium rostratum*	*Diplodia maydis*	*Sclerotium rolfsii*	*Rhizoctonia solani*	*Curvularia* sp.	*Macrophomina phaseolina*	*Rhizopus* sp.	*Fusarium solani*
Stalks										
Before burial	67[a]	67	53	47	7	0	0	0	0	0
After burial at:										
0 cm	90[b]	70	70	10	0	10	10	0	0	0
2.5 cm	100	50	20	0	0	0	10	0	0	0
7.5 cm	100	50	10	0	0	0	0	0	0	0
15.2 cm	90	70	10	0	10	0	10	0	0	0
30.4 cm	90	50	0	0	0	0	0	0	0	10
Roots										
Before burial	67[a]	40	7	0	0	0	0	0	0	0
After burial at:										
0 cm	60[b]	30	0	0	0	0	30	10	0	0
2.5 cm	60	10	0	0	0	0	0	10	10	10
7.5 cm	10	0	0	0	0	0	0	0	0	0
15.2 cm	40	0	0	0	0	0	0	0	0	0
30.4 cm	10	0	0	0	0	0	0	0	0	0

[a]Isolation frequency percentages are based on 15 plants.

[b]Isolation frequency percentages are based on 10 plants.

Discussion

When corn growers employ minimum or no-tillage combined with monocropping, plant residue is not buried. This delays decay and extends the time that certain fungi are protected within the residues. Under such conditions, infested debris could serve as an inoculum source for H. rostratum early in the growing season since spores of H. rostratum normally do not appear until after anthesis. Furthermore if plants were placed under stress, as by foliage diseases or drought, resulting in early senescence of stalk tissue, epiphytotics of stalk rot might ensue.

The inability of H. rostratum and the ability of F. moniliforme to survive when buried in the soil offers at least a partial explanation for the former fungus being exclusively a stalk invader and the latter fungus a root invader in addition to being a stalk invader.

Effect of Sucrose Concentration on In Vitro Growth of Certain Fungi

Introduction

Considerable evidence has been obtained that the level of sugar in stalks may greatly influence the severity of stalk rot. Craig and Hooker (19) have shown that an increase in sucrose is associated with resistance to stalk rot. Wysong and Hooker (117) reported that stalk rot development was inversely associated with changes in soluble solids content of stalks. Holbert and Hoppe (37) concluded that low carbohydrate content of the stalks increased susceptibility to Diplodia stalk rot. Craig and Hooker (19) advanced the

theory that a decrease in the sugar level of the stalk causes senescense of pith tissue and susceptibility to Diplodia maydis. In addition to supporting a steady respiration rate, sucrose may directly affect invading fungi. Seasonal changes in sucrose concentration occur in corn stalks (69, 89) and these changes may contribute to the succession of fungi in stalks and roots. The objective of this study was to determine the influence of sucrose concentration on the growth of certain fungi in vitro.

Materials and Methods

Several isolates of ten fungal species were grown in petri plates containing Pridham and Gottlieb's carbon utilization medium with 1% sucrose (99). Uniform discs (5 mm in diameter) from these cultures were used to inoculate petri plate cultures. Culture plates contained 20 ml carbon utilization medium which contained 0.1, 1.0, 5.0, or 10.0% sucrose. There were five replications of each sucrose concentration for each isolate tested. Cultures were incubated in the dark at 25 C until colonies growing on any one of the sucrose concentrations covered 3/4 of the plate (usually 10-14 days). Growth of the isolates was quantitatively evaluated by measuring colony diameter and qualitatively evaluated by comparing mycelial density and sporulation.

Results

Increasing the concentration of sucrose in the medium generally resulted in colonies of a larger diameter and more vigorous growth (Table 4). Fungi typically isolated from

Table 4. Growth of certain fungi *in vitro* on varying concentrations of sucrose in carbon utilization agar medium

Fungus	Isolate	Colony Diameter in mm, % Sucrose 0.1	1.0	5.0	10.0	Colony Characteristics
Macrophomina phaseolina	JM 5	76.8[a]	70.6	85.0	85.0	Density and sclerotia formation increase with the sugar concentration for all four isolates.
"	L 1	75.4	79.0	85.0	85.0	
"	A 9	55.4	52.2	64.4	60.0	
"	C 8	85.0	85.0	85.0	85.0	
Fusarium moniliforme	A 14	75.8	73.8	77.8	85.0	Density increased with sugar concentration. Sporulation was similar at all concentrations for all isolates.
"	S 1	78.2	75.6	75.8	85.0	
"	JM 12	61.8	63.6	66.0	71.3	
"	L 3	75.8	79.0	80.0	85.0	
Helminthosporium rostratum	JM 11	66.4	61.0	60.2	73.0	Density increases with sugar concentration. Sporulation best at 0.1 and 1.0% sucrose.
"	A 2	62.4	64.0	43.8	75.0	
"	L 5	85.0	73.0	80.0	85.0	
"	JL 2	71.8	83.0	72.5	85.0	
Trichoderma viride	C 2	65.2	79.6	79.6	70.4	Density similar at all concentrations. Sporulation most vigorous at 1 and 5% for all isolates.
"	A 1	70.2	79.5	69.0	70.4	
"	JM 4	68.6	77.4	75.3	72.6	
"	SA 1	78.0	80.0	77.6	76.2	
Fusarium solani	A 16	56.0	55.6	57.2	58.6	Density increases with conc. and sporulation best at 0.1%.
"	C 5	67.8	59.4	60.2	67.2	
Curvularia sp.	JL 1	61.0	64.4	66.8	69.9	Density increases with conc. sporulation only at 0.1%.
"	JM 9	75.0	70.0	69.2	83.0	
Diplodia maydis	SA 2	73.4	74.4	84.0	68.0	Most dense 5 and 10%, no sporulation.

Table 4 - Continued

Fungus	Isolate	Colony Diameter in mm % Sucrose 0.1	1.0	5.0	10.0	Colony Characteristics
Mortierella sp.	A 6	62.0	75.4	79.4	78.6	Sporulation increases with conc.
Fusarium lateritium	A 18	68.4	35.8	55.4	85.0	Density = at 1, 5 and 10%, spor. at 0.1%.
Pestilotia sp.	A 7	57.6	66.6	71.6	85.0	Density equal at 1, 5 and 10%.

[a]Each figure is the average of five replications.

corn stalks at the end of a season (F. moniliforme, T. viride, D. maydis, and H. rostratum) were not adversely affected by high sucrose concentrations, nor was their growth favored by low sucrose concentrations.

Discussion

Sucrose concentrations of 0.1 to 10.0% were selected because of their similarities to reported concentration within stalks. Sucrose accumulates rapidly after tasseling until it constitutes 6 to 10% of the green weight of pith (14, 117). However, sucrose concentration may fall to 0.1% or less by physiological maturity (117).

This test failed to demonstrate a relationship between the linear growth of certain fungi as a function of sucrose concentration with the isolation frequency of those fungi from stalks as a function of plant maturity. The succession of fungi in stalks cannot be directly attributed to changing sucrose concentrations; however, sugar levels may be indirectly associated with resistance to stalk rot.

The indirect association of sugar concentration with resistance is a concept favored by several researchers (19, 70). According to them, resistance appears to depend upon the maintenance of a certain degree of physiological vigor, particularly in the stalks during the long post-maturity period in the fall. Conversely, susceptibility is due to a lack of plant vigor. Physiological vigor depends upon a steady respiration rate supported by a continuous supply of carbohydrates. Under ideal conditions the corn

plant produces sufficient carbohydrate material to meet all the requirements of both ears and vegetative parts. Under any condition of stress which restricts photosynthesis or alters any of the subsequent processes of carbohydrate metabolism, the amount of carbohydrate produced may be insufficient to satisfy all demands. Consequently, the requirements of the developing kernels are met first which results in a reduction in the levels of sugar in the stalk (61). When the vigor of a plant drops below a certain level it becomes susceptible to invasion by saprophytes and weak pathogens (19, 70).

Relative Saprophytic Ability of Fungi Associated with Corn Stalks

Introduction

Pappelis (80) reported a relationship between parenchyma cell death in stalks of corn and the spread of stalk rotting fungi in those stalks. It was later reported that living stalk cells resist the spread of D. maydis, and that cell death is related to susceptibility to the spread of D. maydis (83). Pappelis et al. (83) reported that parenchyma cell death in stalks and ears was similar (83). Because of the uniformity of cell types, cob parenchyma may be more suitable tissue than stalk pith in which to study host-pathogen interactions with various stalk rot organisms (83). The objective of this study was to utilize cob pith to determine the relationship between the saprophytic ability of certain fungi and the succession of those fungi in stalks and roots.

Materials and Methods

Ten fungi were tested for their relative saprophytic ability based on their utilization of corn cob pith as their sole nutrient substrate. The weight loss of cob sections inoculated with each fungus was used as a measure of pith decomposition. Cob pith from 'Pennington Florida 200A', and breeding line #5580 was used because these varieties had large ears and therefore more cob pith would be available. Corn plants had completely senesced when taken from the field 150 days after planting.

Pith was extracted from the cobs with a #2 cork borer, dried for 12 hours at 77 C, cooled 24 hours in a dessicator, weighed to one tenth of a milligram, and sterilized with propylene oxide for 72 hours. Plastic petri dishes (60 mm in diameter) each held one piece of pith (40-80 mg, initial weight), 3 ml sterile, distilled, demineralized water and the fungal inoculum. Inoculum was grown on APDA, transferred to water agar (Difco) and incubated at 27 C for three weeks in the dark. Five mm diameter plugs were taken from water agar cultures of each fungus and placed next to each pith piece. The following fungi were tested: D. maydis (two isolates), M. phaseolina (three isolates), H. rostratum (three isolates), Mortierella sp., Rhizopus sp., F. moniliforme (three isolates), F. lateritium, F. solani, T. viride (three isolates), and R. solani.

The pith cultures were incubated in the dark at 28 C for 21 days, after which the pith was removed from the plates with forceps, washed clean of external mycelium by a stream

of water and dried and weighed as before. Reduction in dry weight was expressed as a percentage of initial weight. The check treatment was pith inoculated with a water agar plug. There were five replications of each treatment.

Results

Highly significant differences in ability to cause decay occurred among the different fungal isolates on pith of both varieties (Tables 5 and 6). However, considerable variation in decomposition among isolates of the same species also occurred. For example, the decomposition of 'Florida 200A' pith was greatest by M. phaseolina isolate A9 and least by isolate L1 of the same fungus (Table 5). Also, H. rostratum isolates A2 and JM11 were significantly different in decomposition ability.

When ranked according to the amount of decay they caused, the ordinal positions of the isolates fluctuated within the two cultivars. This interaction was typified by the decay activity of D. maydis (SA2) which was very effective in decomposing tissue of breeding line #5580 but much less effective in rotting tissue of 'Florida 200A' (Tables 5 and 6).

Discussion

Hypothetically, the ability to decay pith tissue should have been greatest for those fungi which are associated with senesced stalks rather than those associated with young, vigorous stalks. However, the results do not show a consistent correlation between the saprophytic ability of a

Table 5. Mean weight loss of corn cob pith sections of 'Pennington Florida 200A' incubated for 21 days with fungi associated with corn stalks

Fungus	Isolate	Mean percentage of Original Weight[a]
None--control	---	94.9
Macrophomina phaseolina	L 1	91.2
Helminthosporium rostratum	A 2	90.6
Mortierella sp.	A 6	89.8
Rhizopus sp.	---	89.7
Fusarium solani	A 16	86.6
Diplodia maydis	SA 2	86.0
Fusarium lateritium	A 17	85.8
Fusarium moniliforme	A 14	85.0
Helminthosporium rostratum	S 1	84.1
Fusarium moniliforme	JM 16	83.8
Trichoderma viride	A 1	83.2
Macrophomina phaseolina	JL 4	82.2
Fusarium moniliforme	L 3	81.0
Rhizoctonia solani	---	79.2
Trichoderma viride	C 2	79.0
Trichoderma viride	JM 4	76.0
Helminthosporium rostratum	JM 11	72.6
Macrophomina phaseolina	A 9	69.2

[a]Means are the average of five replications, arranged in the order of rank. Means not underscored by the same line are significantly different at the 1% level.

Table 6. Mean weight loss of corn cob pith sections of 'breeding line 5580' incubated for 21 days with fungi associated with corn stalks

Fungus	Isolate	Mean percentage of Original Weight[a]
None--control	---	91.3
Helminthosporium rostratum	S 1	88.2
Fusarium moniliforme	A 14	88.1
Trichoderma viride	A 1	86.3
Helminthosporium rostratum	A 2	86.1
Fusarium Moniliforme	JM 16	85.9
Mortierella sp.	A 6	85.2
Fusarium lateritium	A 17	84.3
Macrophomina phaseolina	L 1	84.2
Macrophomina phaseolina	JL 4	83.7
Fusarium moniliforme	L 3	83.5
Helminthosporium rostratum	JM 11	83.2
Trichoderma viride	JM 4	83.0
Fusarium solani	A 16	82.8
Trichoderma viride	C 2	80.0
Diplodia maydis	SA 2	77.8
Macrophomina phaseolina	A 9	76.4

[a]Means are the average of five replications, arranged in the order of rank. Means not underscored by the same line are significantly different at the 1% level.

particular fungus and the stage of growth of the host plant during which that fungus was isolated. Fungal succession in corn stalks cannot be solely attributed to differences in the relative saprophytic ability of the fungi involved, although this may be one of the contributing factors.

Although the results did not resolve the basis for the colonization of corn stalks, they may aid in understanding the role of certain fungi in the stalk-rot complex. In one respect, these results complement the results of the virulence test (Section 1) in that they show that T. viride and M. phaseolina which were weak parasites in living plants were capable of extensive saprophytic decay activity in dead tissue. These fungi are quite prominent in mature stalks and their activities as saprophytes in dead plants could lead to a loss of structural strength thereby contributing to plant lodging.

The experimental method used in this study was a modification of the technique of Amosu and Hooker (3). They measured the saprophytic ability of 12 fungi and found that D. maydis and G. zeae, organisms that commonly cause stalk rots of living corn plants, were less effective than T. viride, Penicillium urticae Bainier, and Helminthosporium pedicellatum Henry in decomposing dead stalk tissue. Although Amosu and Hooker (3) found significant differences ($P = 0.05$) among fungal spp. in ability to cause weight loss of pith tissue, they tested only a single isolate of each species. In addition to testing several isolates of most species, this study differed from theirs in other respects:

1) The plant tissue utilized in this test was cob pith versus stalk pith; 2) Pith tissue in this test was completely senesced, versus metabolically active (collected 10 weeks versus 2 weeks after flowering); 3) Tissue in this test was sterilized by propylene oxide rather than autoclaving; and 4) The sole nutrient source of the test fungi in this test was pith tissue, whereas Amosu and Hooker (3) incubated fungi on a substrate of pith tissue and potato dextrose agar (PDA).

The system used in this study is highly artificial and cannot be equated with conditions as they might occur in the field. Furthermore in the field, a combination of several organisms may be involved in the decay of corn stalks, and the activity of any one organism may follow a prior invasion by one or more pathogens that have killed the plant.

Relation of Antifungal Substances in Corn Hybrids to the Succession of Fungi in Stalks

Introduction

Corn plants in stages of growth preceding anthesis and kernel development are generally highly resistant to stalk-rotting pathogens (37, 53). Whitney and Mortimor (112) suggested that stalks of young corn are not attacked by F. moniliforme or G. zeae because of the presence of an antifungal substance in the young stalks. Benzoxazolinones are fungitoxic compounds that exist in maize at different levels depending on the inbred line and stage of development of the plant (9, 52).

The 1, 4 benzoxazolinones are a group of compounds found in graminaceous plants that have fungitoxic properties. In

1955 Virtanen and Heitala (101) reported an anti-Fusarium factor from rye seedlings which they determined to be 2(3)-benzoxazolinone (BOA). Ether or acid extracts from rye inhibited cultures of Fusarium nivale (Fr.) Cesati and Sclerotinia trifolium Eriks. Four years after finding BOA, Virtanen and Heitala (102) isolated a precursor of BOA from rye seedlings. Glucose was attached to it and enzymatic activity resulted in the formation of BOA. Virtanen and Whalroos (103) described an intermediate, 2,4-dihydroxy-1,4-(2H)-benzoxazin-3-one (DIBOA) that had only half the anti-Fusarium activity of BOA. BOA is formed as a result of the hydrolysis of DIBOA glucoside to glucose and DIBOA. The aglucon decomposes in water to form BOA.

Turner (100) reported a substance in oat root and leaf meristem which inhibited the take-all fungus Gaeumannomyces graminis (Sacc.) Arx & Olivier. He found that G. graminis var avenae was able to infect oat plants resistant to G. graminis because it produced a specific glucosidase capable of destroying the biological activity of the inhibitory glucoside of DIBOA.

Virtanen and Whalroos (103) found an antifungal compound in wheat seedlings which was shown to be 6-methoxy 2(3)-benzoxazolinone (MBOA). Loomis et al. (62) detected an antifungal substance in corn extracts that inhibited corn borer larvae and D. maydis. Smissman et al. (93, 94) purified the compound responsible for the inhibitor noted by Loomis et al. (62) and concluded it was MBOA. Klun and Brindley (50) found a high correlation between MBOA content

of the whorl and field resistance ratings of 11 inbreds to European corn borer. Bioassays of MBOA showed it to be an inhibitor of borer pupation when incorporated into artificial diet. Klun and Robinson (52) conducted feeding bioassays with borers which suggested that the active agent might be 2,4-dihydroxy-7-methoxy-1,4 (2H)-benzoaxozin-3-one (DIMBOA) a precursor to MBOA. Subsequent investigation (51) into the genetic nature of the concentration of DIMBOA in a diallel set of eleven maize inbreds indicated that DIMBOA was responsible for resistance to the first brood of European corn borer. DIMBOA is not fungistatic at concentrations found in the corn plant (9, 20); however, a significant correlation has been demonstrated between DIMBOA concentration and resistance of corn to stalk rot (9), wheat to stem rust (29), corn to certain 2-chloro-s-triazine herbides (34), maize to northern corn leaf blight (5, 18), and corn to southern corn leaf blight (20).

In 1945 Johann and Dickson (45) furnished the first report of a fungal inhibitor in corn stalks. Ether extracts inhibited D. maydis, G. zeae and Nigrospora sphaerica Sacc. but had little effect on Gibberella fugikoroi Saw. Whitney and Mortimor (113, 114) extracted MBOA from corn and found it inhibited growth of Pyrenochaeta terrestris, D. maydis, G. zeae, F. moniliforme and Erwinia stewartii. Barnes (8) identified MBOA as the antifungal substance found in ether extracts of corn tissue that inhibited the growth of G. zeae.

Wahlroos and Virtanen (108) isolated a glucoside from 10-day-old corn seedlings which, when hydrolyzed by an enzyme

in the plant, yielded glucose and the aglucon, DIMBOA, which when heated yielded MBOA and formic acid. Whitney and Mortimore (115) found no correlation between MBOA content in the host tissue, expressed on a dry weight basis, and the stalk or root rot response. However, BeMiller and Pappelis (9) found that DIMBOA content, measured on a weight/volume basis (mg/cc of tissue sampled), was related to stalk rot response. They calculated that high density, resistant pith tissue composed of living cells contained between 0.6 and 1.6 mg of DIMBOA glucoside/cc of tissue, whereas low density, susceptible tissue containing great numbers of dead cells had less than 0.6 mg/cc of tissue. They suggested that when living cells died, the glucoside was lost and tissue became susceptible.

Dawe (20) tested pure 1,4 benzoxazolinone compounds extracted from corn leaves for inhibition of mycelial growth of Helminthosporium maydis Nisikado & Miyake race T. He reported that DIBOA and BOA significantly reduced mycelial growth compared to DIMBOA and MBOA, but that DIBOA and BOA were present in plant tissue at lower concentration than were DIMBOA and MBOA. Klun and Robinson (52) reported the concentration of DIBOA in corn was 5-10% of that of DIMBOA; thus DIBOA would not be expected to be a significant factor in the resistance of corn to the stalk rot complex.

The purpose of this experiment was to test the relationship between changes in benzoxazolinone concentration in stalks during a season and the succession of fungi in stalks.

Materials and Methods

Stalk tissue from four commercial field corn hybrids was tested for MBOA content. These hybrids were selected because in variety trials for stalk rot evaluation (Section IV) 'Pioneer 515' was susceptible, 'Pennington 7C11A' was resistant, 'Pennington Florida 200A' was more resistant to H. rostratum than to F. moniliforme, and 'McNair 508' was more resistant to F. moniliforme than to H. rostratum. Stalk samples of these hybrids, grown in Gainesville, Florida in 1974, were collected at stages of maturity ranging from early tassle to the full dent stage.

The MBOA extraction procedure was a modification of Barnes' (8) system. The first four internodes above ground were collected from each of 10 plants within each variety and macerated for two minutes in a heavy-duty blender with twice the tissue volume of distilled, demineralized water. The aqueous suspension was incubated at room temperature for 0.5 to 3.5 hours then filtered through cheesecloth and the marc was discarded. In certain experiments the filtrate was heated to 60-80 C, or boiled, for one hour. In other experiments the filtrate was not heated. The filtrate was then extracted two times with distilled, anhydrous diethyl ether in a separatory funnel. The water fraction was discarded and the ether fraction was centrifuged in order to separate any water remaining in the extract. The ether fraction was reduced in volume from ca. 100 ml to two or five ml in a vacuum desiccator. The resulting light brown concentrate constituted the crude ether extract.

The *in vitro* inhibitory activity of the extracts on spore germination and mycelial growth was bioassayed by the paper-disc plate method (60). Conidia or sclerotia of the test fungi were washed from wheat kernel cultures or PDA plates with sterile distilled water. The spore masses were gently fragmented in a tissue grinder and 10 ml of the suspension was pipetted into 150 ml of cooled, molten PDA which was immediately poured into petri plates. The assay discs, measuring six mm in diameter, were punched from Whatmann Number 1 filter paper, autoclaved, and infiltrated with 20 to 100 µl of the extract to be tested. The ether was allowed to evaporate from the discs before they were placed on the petri plate cultures. Control discs were treated with 60 µl of ether concentrate which was prepared by concentrating 200 ml of distilled ether down to five ml by means of a vacuum desiccator. Inhibitory activity was measured by the diameter of the zones of inhibition surrounding the assay discs.

The nature of the inhibitor(s) present in the crude ether extract was investigated by paper chromatography and spectrophotometric analysis. Portions of the ether extract were spotted on Whatmann Number 3 filter paper and chromatographed in ascending fashion in a methanol:water solvent system (1:2) for three hours. The paper was dried and exposed to UV light. Spectroanalysis of the crude extract dissolved in water was conducted on a spectrophotometer.

Results

The initial attempt to extract an inhibitor was successful. Stalks from the susceptible cultivar, 'Pioneer

515', in the tassle stage of development, were macerated, incubated for three hours, then filtered. In an effort to reduce the volume of the filtrate from two liters to ca. 0.5 liter the aqueous aliquot was placed in a rotary flash evaporator (water bath, 60 C) for one hour. This treatment did not reduce the volume of water, therefore it was discontinued and never repeated. The aqueous aliquot was then extracted with ether, concentrated, and bioassayed for activity against H. rostratum and F. moniliforme. Two replications of each of the following extract concentrations were allowed to evaporate from the sterile bioassay discs: 20 µl, 40 µl, 60 µl, 80 µl, 100 µl, and the ether control. When bioassayed against H. rostratum, the average width of the inhibition zones were: 2.5 mm at 20 µl, 2.0 mm at 40 µl, 3.5 mm at 60 µl, 4.5 mm at 80 µl, 6.5 mm at 100 µl, and 0 mm in the ether control. Chromatograms of the extract exhibited a dark spot at RF 0.61 when exposed to UV light. This observation agrees with the report of Barnes (8) which stated that pure MBOA was located at RF 0.61 when chromatographed as described above.

When the extraction procedure was repeated on all four test cultivars, the bioassays revealed no inhibitor activity and no UV light absorption could be detected in chromatograms of the extracts. In subsequent extractions, numerous variations of the procedure were tested in an effort to repeat the initial success. Over a period of six months the following variations were tested:

1. aqueous extract incubated for 30, 60, 90, 120, or 210 minutes after maceration;

2. bioassay plates incubated at 25, 15, and 4 C;

3. extraction from pith tissue versus rind tissue;

4. extraction from plants of different maturity;

5. boiling the aqueous aliquot after it had been incubated; and

6. heating the incubated, aqueous aliquot to 60-80 C for 60 minutes.

The final variation (heating) restored inhibitory activity to the extract. The hot water bath of the flash evaporator, which was used in the initial extraction only, had apparently provided the necessary heat in the first experiment.

To confirm the necessity of heating the incubated, aqueous aliquot the following experiment was conducted. Ten stalks of 'Pennington Florida 200A' in the hard dough stage of kernel maturity were macerated and incubated for 60 minutes. One half of the incubated fraction was heated while the other half was being filtered and extracted with ether. The results of the H. rostratum bioassay (Table 7) indicate that heating the aqueous fraction doubled its inhibitory activity. Furthermore, the zones of inhibition due to the non-heated extract, were not clear cut and free of growth as were those of the heated extract. The non-heated extract depressed, but did not stop growth of the fungus as did the heated extract.

The presence of MBOA in the heated ether extract was indicated by chromatograms on which a dark spot appeared at RF 0.60. Ultraviolet spectral analysis of the extract indicated absorption maxima at 230 and 287 mu. Tipton et al. (98) and Loomis et al. (62) reported nearly identical values for the absorption spectrum of synthesized and extracted MBOA. Attempts to crystallize the inhibitor were unsuccessful.

The activity of the inhibitor was tested in two experiments. The first experiment tested the change in concentration of the inhibitor in stalks as a function of plant maturity. The activity of the inhibitor was assayed using F. moniliforme and H. rostratum as test organisms. The concentration of the inhibitor in stalks did decrease as plants matured from the flowering to the dent stages (Table 8). In this experiment equal volumes of tissue from 10 plants were used for each extraction rather than equal weights of tissue. The results reported here are in accord with those of BeMiller and Pappelis (10) who found that death of pith cells was accompanied by a decrease in the glucoside fraction content of tissue.

In the second experiment, the sensitivity of certain fungi to the ether extract was tested as a function of plant maturity and resistance to stalk rotting fungi. The extracts from the susceptible variety ('Pioneer 515') were no less inhibitory to certain fungi than were those from a more resistant variety ('Florida 200A') (Table 9). Nor does there appear to be a relationship between changes in MBOA concentrations in stalks and the succession of fungi in stalks under

Table 7. Influence of ether extracts from the stalks of corn cultivar 'Pennington Florida 200A' on the in vitro growth of Helminthosporium rostratum using the paper disc-plate technique. The aqueous aliquot of one extract was heated to 60-80 C for one hour prior to ether extraction

Treatment	Amount of extract (µl)	Mean width of inhibition zone in mm[a]
Heated	20	3.0
"	40	4.5
"	60	6.5
Nonheated	20	0
"	40	2.0
"	60	3.0
Ether control	60	0

[a]Measurements were made from the edge of the assay discs to the edge of the inhibition zone. Average of two replications.

Table 8. In vitro inhibitory activity of 60 µl aliquots of the ether extracts of corn hybrid 'Pennington Florida 200A' against Fusarium moniliforme and Helminthosporium rostratum as a function of stage of maturity of the hybrid

Test Fungus	Isolate	Mean width of zone of inhibition (mm) at indicated stage of kernel maturity		
		Silk	Dough	Dent
Fusarium moniliforme	A 14	1.5[a]	1.5	1.5
Fusarium moniliforme	A 11	2.0	1.5	0.5
Helminthosporium rostratum	S 3	1.0	1.0	0.5
Helminthosporium rostratum	A 2	1.0	1.0	1.0
Ether control	---	0	0	0
Average of all isolates		1.4	1.3	0.6

[a]Measurements made from the edge of the assay discs to the edge of the inhibition zone. Average of two replications.

Table 9. Inhibitory activity of 20 µl aliquots of the ether extracts of a stalk rot-resistant corn hybrid ('Pennington Florida 200A') and a stalk rot-susceptible hybrid ('Pioneer 515') on fungi in vitro as a function of stage of kernel maturity of the hybrids

Fungus	Isolate	Ether check	Resistant		Susceptible	
			Silk	Dent	Silk	Dent
Diplodia maydis	JM 3	-[a]	+	-	++	+
Macrophomina phaseolina	C 8	-	+	-	+	+
Mortierella sp.	A 6	-	-	+	+	-
Curvularia sp.	JM 9	-	-	+	+	-
Curvularia sp.	JL 1	-	-	+	+	+
Fusarium moniliforme	A 11	-	+	+	+	-
Fusarium moniliforme	A 14	-	+	+	+	+
Trichoderma viride	JM 4	-	+	-	+	-
Trichoderma viride	A 1	-	-	-	-	-
Trichoderma viride	C 2	-	-	-	-	-
Helminthosporium rostratum	JM 11	-	-	-	+	-
Helminthosporium rostratum	S 3	-	+	+	++	+
Helminthosporium rostratum	A 2	-	+	+	+	+

[a] + = majority of the four replications/treatment had zones of inhibition up to 1 mm in width.

++ = majority of the four replications/treatment had zones of inhibition greater than 1 mm in width.

- = two or less of the four replications/treatment were inhibitory.

the test conditions described above. The response of T. viride is an interesting exception. Trichoderma is one of the few fungi found in the stalks of seedlings and mature plants and it was generally the only fungus whose isolates were not inhibited by extracts from plants of any maturity. An exception was T. viride isolate JM4 which was sensitive to the extracted inhibitor.

Discussion

An antifungal compound was isolated from corn stalks and tentatively identified as MBOA. The decline of inhibitor concentration in stalks, as measured by the bioassay system, was correlated with declining resistance of maturing corn plants to stalk rot, but the presence of this substance could not be related to fungal succession in corn stalks nor could inhibitor concentration be correlated with susceptibility or resistance of certain cultivars. Several things might be proposed to explain resistance. There is potentially enough benzoxazolinone in both cultivars tested to totally inhibit growth of the test fungi. However, the inhibitory compounds may not be available in the susceptible variety while they are readily available in the resistant variety. Food sources or stimulators may mask the inhibitory compound. There may be intermediates in MBOA synthesis or breakdown products of MBOA which are fungitoxic. Phenolic acids of stalk tissue, which exist in the form of glucosides (11) may be involved in host resistance. Molot (68) studied the sensitivity of Fusarium spp. to five phenolic compounds and

MBOA. He postulated that fungi may penetrate stalks without complex reactions taking place; there are then large amounts of phenolic glucosides but small amounts of free phenols. Later, phenolic glucosides may be hydrolyzed by pathogen β-glucosidases and the release of phenolic resistance factors takes place. Before the specific relationships among suscepts, pathogens, and benzoxazolinone compounds can be fully understood, the complete metabolic pathways of benzoxazolinone biosynthesis and breakdown will have to be elucidated. Background information of this nature would surely be necessary before bioassay of ether extracts could be utilized as a means of screening corn inbreds for resistance to stalk rot.

The ether-soluble moiety extracted during this study evidently does not exist in the living corn plant as an uncombined, free compound. The conversion of DIMBOA to MBOA is presumably catalyzed by an enzyme, although such an enzyme has not been demonstrated (BeMiller, personal communication). Apparently the conversion is controlled in some way by the attack of a pathogen, although heat, as demonstrated in this study, will also cause the conversion. Wahlroos and Virtanen (108) reported that, upon heating, DIMBOA yields MBOA and formic acid. Heating is, therefore, an essential step in the extraction process, but this fact is seldom mentioned in the literature. The objectives of this study could have been answered more completely if the bioassays had been conducted with synthesized rather than extracted MBOA since the purity and concentration of the extracted material was doubtful.

SECTION III
THE INCIDENCE OF STALK ROT AND ITS EFFECT ON CORN YIELDS IN FLORIDA

Introduction

The economic impact of corn stalk rot is considerable. Stalk rots are responsible for direct losses in yield due to premature dying of infected plants and a softening of the rind resulting in much of the lodging in corn (40, 118). Lodged plants are difficult to harvest because many ears are missed and those in contact with moist soil are soon destroyed by fungi.

In Minnesota, in 1956 the losses caused by stalk rots were estimated to be 10% of the value of the crop (21). Michaelson and Christensen (66) showed that D. maydis and G. zeae reduced yields of inoculated plants by 9.7% and 6.8% respectively. Wilcoxson (116) found that yields were suppressed 8.5% in corn hybrids inoculated with Fusarium graminearum. Koehler (53), following his extensive study of corn stalk rot, considered an average annual loss from stalk rot of 7 to 10% to be valid for Illinois. Hooker and Britton (40) determined an average annual loss of 8.6% for Illinois. In Nebraska, Wysong and Kerr (118) estimated loss amounting to 11% annually.

The following experiments and observations were made in order to assess the extent and severity of stalk rot in Florida.

Materials and Methods

Incidence of Stalk Rot in Commercial Fields

Fourteen commercial fields in northcentral and northwest Florida were surveyed in September, 1974 in order to assess the percentage of plants which were lodged. In each field the incidence of lodged plants was determined in replications of 50 plants. The total number of plants surveyed in each field ranged from 100 to 400.

Effect on Yield in Controlled Experiments

Yield reductions caused by stalk rot are the result of the debilitating effects of certain fungi on plant growth and development. Indirect yield losses can occur in the form of unharvested ears, ear rot, and harvesting problems. Direct yield losses attributable to soilborne stalk rotting pathogens were measured in the course of the 1973 ecology study. Field plot design and preparation are described in Section I. The yield of 'PAG 751' grown in soil fumigated with methyl bromide was compared to its yield when grown in non-fumigated plots. Yield data was collected from the two center rows of each replication by measuring the cob length. Grain weight could not be determined because of a severe infestation of weevils. The cobs of every plant in a yield row were measured, including those of lodged plants. Lodging data was also collected at the end of the season.

Indirect yield and monetary losses were not measured, but their importance is discussed on the basis of personal observations.

Results

Incidence of Stalk Rot

The survey of commercial corn fields revealed that lodging is a common but variable occurrence. The results indicate that lodging varied from 1 to 54% with an average of 10.6% of the plants in each field being downed (Table 10).

Effect on Yield

The yield of 'PAG 751' was increased 17% by preplant soil fumigation. Analysis of variance of this data indicated that the difference is not statistically significant but, there appears to be crop improvements caused by fumigation. The beneficial effect of fumigation is also evident in the stalk lodging data collected on the plants in the yield test (Table 11). Soil fumigation had the effect of increasing yield and decreasing lodging.

Broken stalks may result in large losses because many ears are missed by mechanical pickers and consequently not harvested. However, if a harvesting combine is properly adjusted, ear losses average about one third the percentage of lodged stalks (6). In order to reclaim this percentage of downed ears, it is necessary to practice good weed control, which many Florida growers fail to do (Fig. 23). When operating a combine in a weed infested field, it is necessary to raise the snouts and increase the opening between the roller bars to reduce the chance of pulling weeds into the machinery. Unfortunately, these adjustments make it more difficult to successfully lift lodged stalks off of the ground and harvest their ears.

Table 10. Percentage of lodged corn plants in 15 fields in northcentral and northwest Florida in September, 1974

Field #	Total number of plants counted/number lodged	% Lodged
1	3/150	2
2	1/100	1
3	8/200	4
4	9/150	6
5	25/200	12
6	44/200	22
7	18/400	5
8	6/200	3
9	7/200	4
10	1/100	1
11	4/200	2
12	26/200	13
13	20/200	10
14	41/200	20
15	135/250	54
		$\bar{X}$ = 10.6%

Table 11. The effect of soil fumigation with methyl bromide on cob length of 'PAG 751'

Treatment	Replication	Cob length (cm)	Percentage lodged
Fumigated	1	15.6[a]	10
	2	15.5	10
	3	15.2	12
		$\bar{X}$ = 15.4	$\bar{X}$ = 11
Nonfumigated	1	11.2	38
	2	13.5	40
	3	14.9	28
		$\bar{X}$ = 13.2	$\bar{X}$ = 35

[a]Average based on 40 plants.

Fig. 23. A corn field moderately infested with weeds.

When determining the losses caused by stalk rot it is important to consider expenditures in time, labor, and capital in harvesting the crop. Stalk rot may increase harvesting expense in a field with lodged stalks. Although it is possible for a combine to pick up downed plants, it may be necessary to operate the combine at a slow ground speed or the stalks will snap and be lost when the snouts lift them off the ground. Increased harvesting time and fuel consumption are the result.

Discussion

The incidence of lodging averaged 10% in the commercial fields surveyed in this study. However, lodging is an

unreliable index of stalk rot because of variations in loading (wind velocity, ear height and weight) and stalk strength (31). Upright stalks may or may not lodge with a slight increase in loading. The incidence of infected plants at the end of the season, as shown in the ecology study (Section I), may reach 50-100% for certain fungi in the stalk rot complex. However, plants infected late in the season may not suffer from direct yield loss due to premature dying and may retain sufficient residual stalk strength after decay has run its course to resist lodging. Therefore, incidence of infection by itself is not a reliable index of stalk rot. Additional experimental information is needed in order to accurately evaluate disease incidence; however, based on data in Section I a disease incidence of 50% is a conservative estimate.

Measuring yield loss due to stalk rot is difficult because healthy plants are not always available for comparison, and the effects of stalk rot are difficult to isolate from other factors affecting yield such as foliar disease, or moisture stress. Yield reduction in the fumigation study was 17%. Part of this reduction might be attributed to weed, soilborne insect, and nematode infestations, all of which are reduced by soil fumigation. However, nematode assays, as discussed in Section I, indicated that nematode infestations were of no consequence in the fumigated and nonfumigated treatments, and the field was hand cultivated to remove weeds. On the other hand, additional yield losses not reflected in this statistic would occur in the form of unharvested ears,

lodged plants, ear rots and harvesting problems. Thus, it is believed that 17% is a reasonable estimate of yield reduction attributable to stalk rot.

The multiplication of disease incidence (50%) by average yield reduction of diseased plants (17%) indicates that field losses may average at least 8.5%. Thus, approximately 1,360,000 bushels are lost per year in Florida based on the most recent four year average of corn production in the state. Based on the average price per bushel for the same four year period, this loss would be valued at $2,900,000/year. Stalk rot losses are not fully appreciated because they occur more or less continually year after year, with little fluctuation in severity, whereas other plant diseases may develop in epiphytotic proportions in certain years and thereby receive widespread attention.

SECTION IV
EVALUATION OF STALK ROT RESISTANCE IN CORN VARIETIES BY SEVERAL METHODS

Introduction

The most feasible control for corn stalk rot is use of resistant hybrids (16). The present corn breeding method involves the isolation of inbred lines, or their equivalents, and the recombination of these lines into hybrids. Hybrids are usually resistant in proportion to the number of resistant inbred lines used in their synthesis (38). The breeding objective, therefore is the isolation of suitably resistant inbred lines.

Many corn breeders, including those in Florida, depend on natural lodging as an index of stalk rot resistance. However, selection in the field is a slow process, and reliable selection can only be made after several years of testing in many localities because natural infection is variable and undependable (4). The objective of this study was to find a simple laboratory or greenhouse test that could be used to eliminate the vast majority of breeding material which has poor stalk rot resistance. To date, the only method to determine resistance is by the examination of mature plants. Studies with corn indicate that certain morphological characteristics and resistance to various stalk rot pathogens are closely correlated with resistance to stalk lodging (16, 91). Mechanical devices have been

developed to test crushing strength, rind thickness, weight of 5.1 cm sections, and breaking load. Considerable effort has also been devoted to artificial inoculation and rating procedures. Semeniuk (90) suggested that seedling reactions to artificial inoculation might be useful for selecting resistant plants. However, Blaak (12) states that the results of numerous workers are inconclusive in respect to correlations between seedling blight and stalk rot caused by G. zeae. Methods for inoculating stalks, which have been reviewed by Kappelman and Thompson (47), include: spore suspension inoculator; infested toothpicks, threads, or oat kernels; spore-soaked pipe cleaner sections; and pieces of agar medium cultures placed in holes made by a cork borer. Positive screening techniques by which plants can be evaluated for resistance prior to physiological maturity are desirable. The evaluation of a bioassay of ether extracts as a means of screening for resistance is discussed in Section II and because such a technique is not yet dependable other techniques need to be considered. In the following studies certain inbreds and hybrids were evaluated for resistance to stalk rotting fungi by inoculation of seedlings and mature plants.

Materials and Methods

Seedling Test

The reaction of seedlings of commercial hybrids to stalk rotting fungi was tested in three experiments. In the first experiment, seedlings of 'PAG 751' and 'McNair 508' were

tested for resistance to seedling blight. Lots of soil, which had been autoclaved twice at 24 hour intervals, were infested with conidia of F. moniliforme isolates, F. solani isolates, or F. lateritium isolates. Twelve, four and three isolates of F. moniliforme, F. lateritium, and F. solani, respectively, were individually tested. The soil was stored at room temperature for one week, and then assayed for Fusarium populations by soil dilution using pentachloronitrobenzene agar medium (73). Infested soil was mixed with steamed, greenhouse potting mixture in proportions necessary to yield 10,000 propagules per gram of soil. Two one-week-old corn seedlings were transplanted from vermiculite to each of three four inch clay pots filled with infested soil for each of the 19 isolates. The seedlings were examined for symptoms three weeks after they had been transplanted.

In the second experiment the above test was repeated with the following changes. Inoculum was prepared from petri plate cultures of six Fusarium isolates (three, two and one isolate of F. moniliforme, F. lateritium, and F. solani, respectively) by fragmenting one culture of each isolate in a blender with 150 ml sterile tap water. Seedlings were inoculated by dipping roots into the culture suspension, transplanted to sterile soil, and observed for 21 days. Roots were cultured on APDA to ascertain percentage infection.

In the third variation of the seedling test, three-day-old seedlings of corn cultivars 'Pennington 7C11A' (resistant) and 'Pioneer 3009' (susceptible), were inoculated with isolate A14 of F. moniliforme or isolate JM12 of H. rostratum,

or both fungi by the root dip technique described above. The seedlings were then transplanted to paper cups containing pasteurized soil using one plant per cup and 25 cups per fungus per cultivar. After 14 days, the plants were rated for shoot length, root wet weight, percentage infection, and symptom development. A variation of this experiment conducted at a later date, rated seedlings inoculated with isolate JM12 of H. rostratum on the basis of root dry weight.

Stalk Inoculation

In August 1973, seed of 40 commercial hybrids were planted in a randomized block design replicated twice at the Agronomy Farm of the University of Florida, Gainesville. Fifty seeds of each variety were planted 12 inches apart in the row using 36 inch row centers. Corn plants were maintained according to standard field procedures for this area.

At the mid-silk stage of ear development, 25 plants of each variety within each block were artificially inoculated with a virulent culture of F. moniliforme and the remaining 25 plants were inoculated with a virulent culture of H. rostratum using the toothpick method (119). A single toothpick, infested with the fungus, was inserted into a puncture in the first expanded internode above the brace roots.

Ratings on the severity of stalk rot were taken four weeks after inoculation. Twenty of the 25 plants in each block inoculated with each fungus were cut longitudinally at right angles to the long axis of the toothpick and scored for resistance according to the following scheme suggested by

Hooker (38): 1 = 0-25 per cent, 2 = 26-50 per cent, 3 = 51-75 per cent, 4 = 76-100 per cent of the inoculated internode discolored, and 5 = discoloration extending into adjacent internodes. Tissue from 5 stalks of each variety inoculated with each fungus per block was assayed for the test fungus via the culture plate technique previously described.

In April 1974, seed was planted in a randomized design, replicated four times with 15 plants/replication. Five plants/fungus/replication of each variety were inoculated as in the 1973 experiment using H. rostratum and F. moniliforme recovered from inoculated stalks in 1973. The cultures of these fungi were stored in a lyophylized state from 1973 to 1974. Stalk rot severity was rated as it was in 1973.

Also, in 1974, 240 breeding lines (inbreds) were screened for resistance to H. rostratum according to the procedures described above.

Results

Seedling Test

Seedlings grown in infested soil developed no foliar symptoms, nor was seedling growth retarded compared to that of the check plants of any cultivar. The seminal roots of some individuals showed damping-off symptoms, but, in general, root systems appeared healthy.

Seedlings of all cultivars inoculated by the root dip technique exhibited no visible symptoms on roots or aerial parts. Crown and seminal root tissue, incubated on APDA,

predominantly yielded T. viride, Rhizopus sp., and bacteria. Only three of the Fusarium isolates could be recovered, and these were at low levels (less than 20%). Root inoculation of susceptible and resistant cultivars with Fusarium spp. did not result in disease.

The third experiment measured the effect of seedling inoculation on growth, and symptom development. It was found that neither F. moniliforme nor H. rostratum, alone or in combination, significantly reduced shoot height or root wet weight of either variety. As in the previous experiment, few of the plants inoculated with F. moniliforme became diseased (23% recovery). H. rostratum, however, was recovered from 86% of the plants inoculated with the fungus. Although the fungus caused no statistically significant weight loss, roots of plants inoculated with H. rostratum were darker and frequently exhibited small brown lesions and burned root tips. The combination of both fungi was no more virulent than either fungus by itself.

The root dry weight of seedlings of resistant and susceptible cultivars was not reduced by inoculation with H. rostratum. The average dry weight of 'Pennington 7C11A' roots was 400 mg in healthy plants, and 440 mg in inoculated plants. Cultivar 'Pioneer 3009' roots weighed an average of 390 mg, whether from healthy or inoculated plants. Helminthosporium rostratum could be recovered from the roots, mesocotyl and seed of both varieties, but root inoculation with the fungus failed to differentiate between the susceptible and resistant cultivars.

Stalk Inoculations

Means for the stalk rot reaction for cultivars tested in 1973 and 1974 and overall means for both years with both fungi, appear in Table 12. The statistical comparisons of stalk rot ratings made in each year with each fungus appear in the appendix. Differences in resistance to both F. moniliforme and H. rostratum do exist among commercial hybrids. None of the cultivars tested were immune to stalk rot, nor did their reactions place them into discreet groups. Rather, the resistance exhibited was that of degree as would characterize a quantitatively inherited characteristic.

The inbreds rated for stalk rot resistance in this study were the breeding lines of Dr. Earl Horner, University of Florida Agronomy Department. Stalk rot reactions were consistent within inbreds but differential reactions occurred between inbreds. Approximately 12% of the 238 lines gave highly resistant reactions (1 on the rating scale). Resistance has, therefore, been demonstrated to exist in both commercial hybrids and inbred breeding lines.

Discussion

Seedling blight in the greenhouse could not be correlated with mature plant stalk rot in the field because of resistance within corn seedlings to certain stalk rot pathogens. The resistance of corn seedlings to certain fungi was also reflected in the low isolation frequencies of particular fungi in the ecology study (Section I). Similarly, Kingsland (48) was unable to induce blights or reduce the dry weight of

Table 12. Mean stalk rot ratings and overall stalk rot ratings of commercial corn hybrids artificially inoculated[a] in 1973 and 1974 in Gainesville, Florida with Fusarium moniliforme and Helminthosporium rostratum

Cultivar	Stalk Rot Rating[b]				Average of all tests
	Fusarium moniliforme		Helminthosporium rostratum		
	1973	1974	1973	1974	
Greenwood 471	1.15[c]	1.55[d]	1.25[c]	1.65[d]	1.40
Asgrow RX450A	1.47	1.35	1.80	1.75	1.59
Coker 71	1.57	1.35	1.57	1.95	1.61
Pennington 9P3A	1.30	1.65	1.22	2.50	1.67
Pennington Fla 200A	2.00	1.15	1.32	2.20	1.67
Pennington 7C11A	1.07	2.00	1.20	2.40	1.67
McCurdy 67-14	1.55	2.10	1.20	1.85	1.67
Greenwood 228	1.42	1.80	1.20	2.40	1.70
Asgrow RX132	1.52	1.70	1.42	2.30	1.73
Coker 814	1.42	1.80	1.55	2.33	1.77
Dekalb 1214	1.50	1.75	1.75	2.15	1.78
Greenwood 95	1.75	1.40	1.72	2.25	1.78
PAG 653W	1.62	1.95	1.35	2.20	1.78
Dekalb XL80	1.82	2.00	1.37	2.40	1.90
Dekalb SL85A	1.25	2.15	1.38	2.90	1.92
Pioneer 3030	1.65	1.55	2.27	2.55	2.00
McNair X300	1.47	2.40	1.62	2.64	2.03
PAG 748	1.47	1.95	2.17	2.53	2.03
Coker 54	2.00	1.80	2.07	2.25	2.03
Pioneer 3161	1.60	1.90	2.02	2.65	2.04
Pioneer 3369A	1.53	2.00	2.02	2.60	2.04
Dekalb XL380	1.62	2.70	1.37	2.50	2.05
PAG 751	1.32	2.35	1.72	2.80	2.05
McCurdy 72-227	1.32	2.15	2.37	2.40	2.06
ACCO AR-02801	1.80	2.12	1.75	2.67	2.08
McNair 73011	1.65	2.10	2.60	2.05	2.10
McNair S-338	1.40	2.20	1.87	2.95	2.10
McNair 508	1.35	1.95	2.67	2.55	2.13
Dekalb XL99	1.62	2.10	2.32	2.55	2.15
Funks G4949A	1.87	1.95	2.42	2.50	2.18
Funks G5945	2.12	2.35	2.17	2.10	2.18
Greenwood 45	1.50	1.85	2.75	2.65	2.18
Funks G4864	1.97	2.15	2.22	2.50	2.21
Coker 56	1.37	2.13	2.12	3.40	2.25
Dekalb XL-89	1.77	2.15	1.92	3.50	2.34
McNair X210	1.65	2.85	2.12	2.80	2.35
Greenwood 801	2.25	2.25	2.50	2.60	2.40
Asgrow RX115	1.70	3.20	2.00	3.10	2.50
Pioneer 3009	2.25	2.75	2.55	2.85	2.60
Pioneer 515	2.40	2.80	2.90	3.28	2.84

Table 12 - Continued

---

[a]Plants inoculated by the toothpick technique.

[b]Rating scheme: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of the inoculated internode discolored.

[c]Based on two replications/variety, 20 plants/replication.

[d]Based on four replications/variety, 5 plants/replication.

seedlings planted in F. moniliforme infested soil. Resistance of corn in the seedling stage may have a morphological or physiological basis. Embryonic plants and seedlings contain high concentrations of fungitoxic benzoxazolinones which decrease as plants mature (52). Voorhees (106) reported that F. moniliforme is retarded in the seedling by the endodermis of the radicle. Whatever the basis, resistance of corn seedlings to certain stalk rot pathogens means that seedling tests could not be utilized as a screening technique.

The toothpick method of stalk inoculation was the most effective screening procedure tested, although it was time consuming. Other methods of stalk inoculation, such as syringe or inoculator, are more rapid, but the toothpick technique affords the least rating variability due to a more positive infusion of inoculum into the plant (47). There is evidence that stalk rot reactions are influenced by environmental conditions during the growing season (47, 53, 65, 88) and this may account for the variation in stalk rot reaction between years within cultivars, especially since one test was conducted in the fall and the other in the spring. The variation between years exhibited by certain varieties underscores the caution necessary when selecting hybrids; the decision should not be based on a single year's data.

The development of corn hybrids with improved standability has been a major objective in corn improvement for many years. According to Christensen and Wilcoxson (15), "Standability of a corn plant refers to its ability to remain upright in the field when the plant has reached full maturity and tissues

have died. Poor standability includes root breakage, stalk lodging, and stalk breakage, caused by disease and insect damage. There may, of course, be inherent structural weaknesses in plants. A secondary factor is wind strength, especially late in the season."

Both the rind and the pith contribute to stalk strength and stalk rot resistance may not reflect any morphological resistance to lodging that the host may possess (15, 72). The present study revealed that certain commercial cultivars may be resistant to stalk rot, but resistance was not correlated with their standability. Certain types of standability may be due to morphological rather than physiological characteristics and the standability of a variety is not necessarily an indication of resistance to rot within the stalk. Wilcoxson (116) reported that certain sources of corn with good standability may actually be susceptible to stalk rot and thus sustain considerable reduction in yield. Gibberella stalk rot, for example, can cause premature death of infected plants due to pectin occlusions within ground parenchyma, xylem and phloem (59). Vascular occlusions can also be formed by F. moniliforme (57) and H. rostratum (Section I). Therefore, stalk quality and stalk rot resistance are both important characteristics to consider when evaluating corn. Stalk rot ratings per se are useful criteria for developing resistant hybrids (38, 47), but they should be used in combination with stalk quality criteria rather than in their stead.

SUMMARY

1. It is proposed that five fungal communities exist in stalks and roots of hybrid field corn grown in Florida. These communities constitute a serule.
2. The fungal complex of stalk rot pathogens includes: F. moniliforme, H. rostratum, and D. maydis. In addition, T. viride and M. phaseolina, which were weak parasites in living plants, were capable of extensive saprophytic decay activity in dead tissue.
3. The succession of fungi in corn stalks cannot be directly attributed to seasonal fluctuations in temperature or moisture.
4. The mode of invasion of corn stalks by H. rostratum may be similar to that reported for D. maydis; that is, airborne spores lodge between stalks and sheaths, germinate and penetrate the stalks.
5. Helminthosporium rostratum and D. maydis were unable to survive in stalk residue buried in the field, whereas F. moniliforme and T. viride were able to survive.
6. The succession of fungi in corn stalks could not be directly attributed to the effects of changing sucrose concentrations on growth of fungi in vitro.
7. Fungal succession in corn stalks cannot be solely attributed to differences in the relative saprophytic

ability of the fungi involved, although this may be one of the contributing factors.

8. An antifungal compound was isolated from corn stalks and identified as MBOA. The decline of inhibitor concentration in stalks was correlated with the declining resistance of maturing plants to stalk rot, but could not be related to fungal succession.
9. In Florida, the losses caused by stalk rots were estimated to be 8.5% of the value of the crop, or nearly three million dollars annually.
10. Resistance to F. moniliforme and H. rostratum was demonstrated in some commercial hybrids and in some inbred breeding lines.

LITERATURE CITED

1. Abney, T. S. and D. C. Foley. 1971. Influence of nutrition on stalk rot development in Zea mays. Phytopathology 61:1125-1129.

2. American Phytopathological Society. 1973. A compendium of corn diseases. Amer. Phytopath. Soc., Inc., St. Paul. 64 p.

3. Amosu, J. O. and A. L. Hooker. 1970. Weight loss of mature corn stalk tissue induced by twelve fungi. Phytopathology 60:1790-1793.

4. Andrew, R. H. 1954. Breeding for stalk-rot resistance in maize. Euphytica 3:43-48.

5. Anglade, P. and P. M. Molot. 1967. Mise en evidence d' une relation entre les sensibilites de lignees de mais a la pyrale (Ostrina nubilalis HBN.) et a l'helminthosporiose (Helminthosporium turcicum pass.). Ann. Epiphyt. 18:279-284.

6. Anonymous. 1973. Check corn lodging, then adjust your combine. Crops and Soils Mag. 26(1):20-21.

7. Ark, P. A. 1940. Bacterial stalk rot of field corn caused by Phytomonas lapsa. Phytopathology 30:1 (Abstr.).

8. Barnes, J. M. 1960. Investigations on stalk rot of corn caused by Gibberella zeae: Part I. A comparison of two methods of evaluation the severity of stalk rot in several corn varieties. Part II. Aspects of the biochemical nature of stalk rot resistance. Ph.D. Thesis. Cornell University. 44 p.

9. BeMiller, J. M. and A. J. Pappelis. 1965. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, 1-butanol glycoside fraction content of pith cores and stalk rot resistance. Phytopathology 55:1237-1240.

10. BeMiller, J. M. and A. J. Pappelis. 1965. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. II. Isolation of 6-methoxy-2(3)-benzoxazolinone fraction as a measure of glucoside content and tissue differences in glucoside content. Phytopathology 55:1241-1243.

11. BeMiller, J. M., R. L. Rimsay, and A. J. Pappelis. 1967. Extraction and purification of phenolic acids from corn stalk tissue. Illinois Acad. Sci. Trans. 60:68-71.

12. Blaak, G. 1957. Histological studies of seedlings and older plants of several varieties of corn infected by Gibberella. M. S. Thesis. Cornell University. 39 p.

13. Burrill, T. J. and J. T. Barrett. 1909. Ear rots of corn. Illinois Agr. Exp. Sta. Bull. 133:63-109.

14. Campbell, C. M. 1964. Influence of seed formation of corn on accumulation of vegetative dry matter and stalk strength. Crop. Sci. 4:31-34.

15. Christensen, J. J. and R. D. Wilcoxson. 1966. Stalk rot of corn. Amer. Phytopathol. Soc. Monogr. No. 3. 59 p.

16. Cloninger, F. D., M. S. Zuber, O. H. Calvert, and P. J. Loesch, Jr. 1970. Methods of evaluating stalk quality in corn. Phytopathology 60:295-300.

17. Cook, G. E., M. G. Boosalis, L. D. Dunkle, and G. N. Odvody. 1973. Survival of Macrophomina phaseolia in corn and sorghum stalk residue. Plant Dis. Reptr. 57:873-875.

18. Couture, R. M., D. G. Routley, and G. M. Dunn. 1971. Role of cyclic hydroxamic acids in monogenic resistance of maize to Helminthosporium turcicum. Physiol. Plant Pathol. 1:515-521.

19. Craig, J. and A. L. Hooker. 1961. Relation of sugar trends and pith density to Diplodia stalk rot in dent corn. Phytopathology 51:376-382.

20. Dawe, D. H. 1973. The relation of 1,4 benzoxazolinones and related compounds in Zea mays to Helminthosporium maydis resistance. Ph.D. Thesis. Iowa State University. 83 p.

21. DeVay, J. E., R. P. Covey, and P. N. Nair. 1957. Corn diseases and their importance in Minnesota in 1956. Plant Dis. Reptr. 41:505-507.

22. Durrell, L. W. 1923. Dry rot of corn. Iowa Agr. Exp. Sta. Res. Bull. 77.

23. Durrell, L. W. 1925. A preliminary study of fungus action as the cause of down corn. Phytopathology 15:146-154.

24. Eddins, A. H. 1930. Corn diseases in Florida. Florida Agr. Exp. Sta. Bull. 210.

25. Eddins, A. H. 1930. Dry rot of corn caused by Diplodia macrospora Earle. Phytopathology 20:439-448.

26. Eddins, A. H. and R. K. Voorhees. 1933. Physalospora zeicola on corn and its taxonomic and host relationships. Phytopathology 23:65-72.

27. Edmunds, J. E. 1963. The relationship of soil fumigation with D-D for the control of Pratylenchys penetrans (Cobb 1917) Chitwood and Oteifa 1952 to subsequent development of root and stalk rot of corn. M. S. Thesis. Cornell University.

28. Edmunds, L. K. 1964. Combined relation of plant maturity, temperature and soil moisture to charcoal rot development in grain sorghum. Phytopathology 54: 514-517.

29. Elnaghy, M. A. and P. Linko. 1962. The role of 4-0-glucosyl-2-4-dihydroxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiol. Plantarum 15:764-771.

30. Foley, D. C. 1962. Systemic infection of corn by Fusarium moniliforme. Phytopathology 52:870-872.

31. Foley, D. C. 1969. Stalk deterioration of plants susceptible to corn stalk rot. Phytopathology 59:620-627.

32. Garren, K. H. 1959. The stem rot of peanuts and its control. Virginia Ag. Exp. Sta. Bull. 144. 29 p.

33. Garren, K. H. 1966. Peanut (groundnut) microfloras and pathogenesis in peanut pod rot. Phytopath. Z. 55:359-367.

34. Hamilton, R. H. 1964. Tolerance of several grass species to 2-chloro-5-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. J. Agr. Food Chem. 12:14-17.

35. Ho, Wen-Chun and I. E. Melhus. 1940. Succession of soil-inhabiting fungi attacking roots of maize. Phytopathology 30:10 (Abstr.).

36. Holbert, J. R., W. L. Burlison, B. Koehler, C. M. Woodworth, and G. H. Dungan. 1924. Corn root, stalk, and ear rot diseases, and their control through seed selection and breeding. Illinois Agr. Exp. Sta. Bull. 255.

37. Holbert, J. R. and P. E. Hoppe. 1935. Some factors affecting infection with and spread of Diplodia zeae in the host tissue. Phytopathology 25:1113-1114.

38. Hooker, A. L. 1956. Association of resistance to several seedling, root, stalk, and ear diseases in corn. Phytopathology 46:379-384.

39. Hooker, A. L. 1972. The worst diseases of corn. Crops and Soils Mag. 24(4):15-18.

40. Hooker, A. L. and M. P. Britton. 1962. The effects of stalk rot on corn yields in Illinois. Plant Dis. Reptr. 46:9-13.

41. Hornby, D. and A. J. Ullstrup. 1967. Fungal populations associated with maize roots. Quantitative rhizosphere data for genotypes differing in root rot resistance. Phytopathology 57:76-82.

42. Huber, D. M. and D. W. Nelson. 1974. Control of corn stalk rot through stabilization of ammonium nitrogen with nitrpyrin. Down to Earth Mag. 30(1): 1-3.

43. Hunter, J. W. and N. E. Dalbey. 1937. A histological study of stalk-breaking in maize. Am. J. Bot. 24:492-494.

44. Jackson, C. R. 1968. A field study of fungal associations on peanut fruit. Georgia Agr. Exp. Sta. Bull. 26.

45. Johann, H. and A. Dickson. 1945. A soluble substance in corn stalks that retards growth of Diplodia zeae in culture. J. Agr. Res. 71:89-110.

46. Josephson, L. M. 1962. Effect of potash on premature stalk dying and lodging of corn. Agron. J. 54: 178-179.

47. Kappelman, A. J. and D. L. Thompson. 1966. Inoculation and rating procedures for corn stalk rot in the south. Plant Dis. Reptr. 50:655-659.

48. Kingsland, G. C. 1958. Etiology and epiphytology of stalk rot of corn in Pennsylvania. Ph.D. Thesis. Pennsylvania State University.

49. Kingsland, G. C. and C. C. Wernham. 1962. Etiology of stalk rots in Pennsylvania. Phytopathology 52: 519-523.

50. Klun, J. A. and T. A. Brindley. 1966. Role of 6-methoxy-benzoxazolinone in inbred resistance of host plant (maize) to first brood larvae of European corn borer. J. Econ. Entomol. 59:712-718.

51. Klun, J. A., W. D. Gutherie, A. R. Hallauer, and W. A. Russel. 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy 2H-1,4 denzoxazin-3-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. Crop Sci. 10: 87-90.

52. Klun, J. A. and J. F. Robinson. 1969. Concentration of two 1,4-benzoxazolinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. J. Econ. Entomol. 62:214-220.

53. Koehler, B. 1960. Cornstalk rots in Illinois. Illinois Agr. Exp. Sta. Bull. 658.

54. Koehler, B., G. H. Dungan, and J. R. Holbert. 1925. Factors influencing lodging in corn. Illinois Agr. Exp. Sta. Bull. 266.

55. Koehler, B. and J. R. Holbert. 1930. Corn diseases in Illinois: their extent, nature, and control. Illinois Agr. Exp. Sta. Bull. 354.

56. Kucharek, T. A. 1973. Stalk rot of corn caused by Helminthosporium rostratum. Phytopathology 63:1336-1338.

57. Kucharek, T. A. and T. Kommedahl. 1964. Histological changes in corn stalks infected with Fusarium moniliforme. Phytopathology 54:898 (Abstr.).

58. Kucharek, T. A. and T. Kommedahl. 1966. Kernel infection and corn stalk rot caused by Fusarium moniliforme. Phytopathology 56:983-984.

59. Littlefield, L. J. and R. D. Wilcoxson. 1962. Studies on necrotic lesions in cornstalks. Amer. J. Bot. 49:1072-1078.

60. Loo, Y. H., P. S. Skell, H. H. Thornberry, John Erlich, J. M. McGuire, G. M. Savage, and J. C. Sylvester. 1945. Assay of streptomycin by the paper-disc plate method. J. Bact. 50:701-709.

61. Loomis, W. E. 1934. The translocation of carbohydrates in maize. Iowa State J. Sci. 9:509-520.

62. Loomis, R. S., S. D. Beck, and J. F. Stauffer. 1957. The European corn borer, Pyrausta nubilalis (Hubn), and its principal host plant. V. A chemical study of host plant resistance. Plant Physiol. 32: 379-385.

63. McNew, G. L. 1937. Crown infection of corn caused by Diplodia zeae. Iowa Exp. Sta. Res. Bull. 216.

64. Melhus, I. E. and L. W. Durrell. 1922. Dry rot of corn. Iowa Agr. Exp. Sta. Circ. 78.

65. Michaelson, M. E. 1957. Factors affecting development of stalk rot of corn caused by Diplodia zeae and Gibberella zeae. Phytopathology 47:499-503.

66. Michaelson, M. E. and J. J. Christensen. 1953. Reduction in yield of corn due to stalk rot. Phytopathology 43:479 (Abstr.)

67. Miller, R. E. 1963. A root rot complex of field corn: symptomatology, sequence of fungi involved and the effect of corn root exudate on these fungi. Ph.D. Thesis. Cornell University. 58 p.

68. Molot, P. M. 1969. Studies on the resistance of maize to Trichometasphaeria turcica and Fusarium spp. III. Behavior of phenolic compounds. Annals Phytopath. 1:367-383.

69. Morris, V. H. 1931. Effect of barrenness and reduction of leaf area on the sugar content of corn stems. Ohio Agr. Exp. Sta. Bull. 470:23-24.

70. Mortimore, C. G. and C. M. Ward. 1964. Root and stalk rot of corn in southwestern Ontario III. Sugar levels as a measure of plant vigor and resistance. Can. J. Plant Sci. 44:451-457.

71. Murdy, W. H. 1960. The strengthening system in the stem of maize. Ann. Mo. Bot. Gard. 47:205-226.

72. Nagel, C. M. 1973. Techniques and methods useful in the selection of root and stalk rot resistance in corn. Proc. Ann. Corn and Sorghum Res. Conf. 28:51-56.

73. Nash, S. M. and W. C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology 52:567-572.

74. Nelson, D. W. 1963. The relationship between soil fertility and the incidence of Diplodia stalk rot and northern leaf blight in Zea mays. M. S. Thesis. University of Illinois, Urbana. 56 p.

75. Nyvall, R. F. and T. Kommedahl. 1968. Individual thickened hyphae as survival structures of Fusarium moniliforme in corn. Phytopathology 58:1704-1707.

76. Nyvall, R. F. and T. Kommedahl. 1970. Saprophytism and survival of Fusarium moniliforme in corn stalks. Phytopathology 60:1233-1235.

77. Otto, H. J. and H. L. Everett. 1956. Influence of nitrogen and potassium fertilization on incidence of stalk rot of corn. Agron. J. 48:301-305.

78. Pappelis, A. J. 1965. Relationship of seasonal changes in pith condition ratings and density to Gibberella stalk rot or corn. Phytopathology 55:623-626.

79. Pappelis, A. J. 1970. Effect of root and leaf injury on cell death and stalk rot susceptibility in corn. Phytopathology 60:355-357.

80. Pappelis, A. J. 1971. Stalk rot of corn. Proc. Ann. Corn and Sorghum Res. Conf. 26:148-164.

81. Pappelis, A. J. and L. V. Boone. 1966. Effect of planting date on stalk rot susceptibility and cell death in corn. Phytopathology 56:829-831.

82. Pappelis, A. J. and L. V. Boone. 1966. Effect of soil fertility on cell death in corn stalk tissue. Phytopathology 56:850-852.

83. Pappelis, A. J., S. Mayama, M. Mayama, J. N. BeMiller, J. A. Murphy, P. Mumford, G. A. Pappelis, and M. S. Kang. 1973. Parenchyma cell death and Diplodia maydis susceptibility in stalks and ears of corn. Plant Dis. Reptr. 57:308-310.

84. Pappelis, A. J. and F. G. Smith. 1963. Relationship of water content and living cells to spread of Diplodia zeae in corn stalks. Phytopathology 53:1100-1105.

85. Pepper, E. H. 1967. Stewart's bacterial wilt of corn. Amer. Phytopathol. Soc. Monogr. No. 4. 36 p.

86. Peterson, J. L. 1961. Studies on the prevalence and comparative pathogenicity of fungi associated with corn stalk rot. Plant Dis. Reptr. 45:208-210.

87. Roelfs, A. P., V. A. Dirks, and R. W. Romig. 1968. A comparison of rod and slide samplers used in cereal rust epidemiology. Phytopathology 58:1150-1154.

88. Russell, W. A. 1961. A comparison of five types of testers in evaluating the relationship of stalk rot resistance in corn inbred lines and stalk strength of the lines in hybrid combinations. Crop Sci. 1:393-397.

89. Sayre, J. D., V. H. Morris, and F. D. Richey. 1931. The effect of preventing fruiting and of reducing the leaf area on the accumulation of sugars in the corn stem. Agron. J. 23:751-753.

90. Semeniuk, G. 1944. Seedling infection of dent maize by Sclerotium batiticola Taub. Phytopathology 34: 838-843.

91. Sleper, D. A. and W. A. Russell. 1970. Interrelationships among several stalk characteristics in maize and their significance in resistance to natural stalk breakage. Iowa State J. Sci. 45:197-209.

92. Smith, A. L. and J. R. Holbert. 1931. Cornstalk rot and ear rot. Phytopathology 21:129 (Abstr.)

93. Smissman, E. E., O. Kristiansen, and S. D. Beck. 1962. Presence of 6-methoxy-benzoxazolinone in uninjured corn tissue. J. Pharm. Sci. 51:292.

94. Smissman, E. E., J. B. LaPidus, and S. D. Beck. 1957. Corn plant resistance factor. J. Org. Chem. 22:220.

95. Sumner, D. R. 1968. Ecology of corn stalk rot in Nebraska. Phytopathology 58:755-760.

96. Sumner, D. R. 1968. The effect of soil moisture on corn stalk rot. Phytopathology 58:761-765.

97. Thayer, Paul and L. E. Williams. 1960. Effect of nitrogen, phosphorus, and potassium concentrations on the development of Gibberella stalk and root rot of corn. Phytopathology 50:212-214.

98. Tipton, C. L., J. A. Klun, R. R. Husted, M. D. Pierson. 1967. Cyclic hydroxamic acid and related compounds from maize. Isolation and characterization. Biochemistry 6:2866-2870.

99. Tuite, J. 1969. Plant pathological methods. Burgess Publishing Co., Minneapolis. 239 p.

100. Turner, E. M. 1956. The nature of the resistance of oats to the take-all fungus. II. Inhibition of growth and respiration of Ophiobolus graminis and other fungi by a constituent of oat sap. J. Exp. Bot. 7:80-92.

101. Virtanen, A. I. and P. K. Heitala. 1955. 2(3) Benzoxazolinone, an anti-fusarium factor in rye seedlings. Act. Chem. Scand. 9:1543-1544.

102. Virtanen, A. I. and P. K. Heitala. 1959. On the structure of the precursors of benzoxazolinone in rye plants. Suomen Kemistilehti B32:252.

103. Virtanen, A. I. and O. Wahlroos. 1958. On the anti-fungal activity of wheat and rye seedlings. Suomen Kemistilehti B31:402.

104. Voorhees, R. K. 1933. Effect of certain environmental factors on the germination of the sporangia of Physoderma zeae-maydis. J. Agr. Res. 47:609-615.

105. Voorhees, R. K. 1933. Gibberella moniliformis on corn. Phytopathology 23:368-378.

106. Voorhees, R. K. 1934. Histological studies of a seedling disease of corn caused by Gibberella moniliformis. J. Agr. Res. 49:1009-1015.

107. Voorhees, R. K. 1934. Sclerotial rot of corn caused by Rhizoctonia zeae. N. sp. Phytopathology 24:1290-1303.

108. Wahlroos, O. and A. I. Virtanen. 1959. The precursors of 6-methoxy-benzoxazolinone in maize and wheat plants: their isolation and some of their properties. Acta Chem. Scand. 13:1906-1908.

109. Wall, R. E. and C. G. Mortimore. 1965. The growth pattern of corn in relation to resistance to root and stalk rot. Can. J. Bot. 43:1277-1283.

110. Weaver, J. E. and F. E. Clements. 1938. Plant Ecology. McGraw-Hill Book Co., Inc., New York. 601 p.

111. Whitehead, M. D. and O. H. Calvert. 1959. Helminthosporium rostratum inciting ear rot of corn and leaf-spot of thirteen grass hosts. Phytopathology 49: 817-820.

112. Whitney, N. J. and C. G. Mortimore. 1959. An anti-fungal substance in the corn plant and its effect on growth of two stalk-rotting fungi. Nature 183: 341.

113. Whitney, N. J. and C. G. Mortimore. 1959. Isolation of the antifungal substance, 6-methoxybenzoxazolinone, from field corn (Zea mays L.) in Canada. Nature 184:1320.

114. Whitney, N. J. and C. G. Mortimore. 1961. Effect of 6-methoxybenzoxazolinone on the growth of Xanthomonas stewartii Dowson and its presence in sweet corn (Zea mays var. saccharata). Nature 189:596-597.

115. Whitney, N. J. and C. G. Mortimore. 1962. Evaluation of a bioassay of ether extracts from corn (Zea mays L.) as a means of screening inbreds for resistance to root and stalk rot. Can. J. Plant Sci. 43:302-307.

116. Wilcoxson, R. D. 1962. Stalk rot in relation to yield of corn. Phytopathology 52:416-418.

117. Wysong, D. S. and A. L. Hooker. 1966. Relation of soluble solids content and pith condition to Diplodia stalk rot in corn hybrids. Phytopathology 56:26-35.

118. Wysong, D. S. and E. Kerr. 1969. Hybrid corn yield reduction by stalk and root rot in Nebraska. Plant Dis. Reptr. 53:326-329.

119. Young, H. C., Jr. 1943. The toothpick method of inoculating corn for ear and stalk rots. Phytopathology 33:16 (Abstr.).

120. Young, H. C., Jr., R. D. Wilcoxson, M. D. Whitehead, J. E. DeVay, C. O. Grogan, and M. S. Zuber. 1959. An ecological study of the pathogenicity of Diplodia maydis isolates inciting stalk rot of corn. Plant Dis. Reptr. 43:1124-1129.

121. Younts, S. E. and R. B. Musgrove. 1958. Chemical composition, nutrient absorption and stalk rot incidence of corn as affected by chloride in potassium fertilizer. Agron. J. 50:426-429.

APPENDIX

Table 13. Mean stalk rot ratings of commercial corn hybrids artificially inoculated[a] with Helminthosporium rostratum in 1973

Variety	Stalk Rot Rating[b]
Greenwood 228	1.20[c]
McCurdy 67-14	1.20
Pennington 7C11A	1.20
Pennington 9P3A	1.22
Greenwood 471	1.25
Pennington Fla 200A	1.32
PAG 655W	1.35
Dekalb XL-380	1.37
Dekalb XL-80	1.37
Dekalb XL-85A	1.38
Asgrow RX132	1.42
Coker 814	1.55
Coker 71	1.57
McNair X300	1.61
Greenwood 95	1.72
PAG 751	1.72
ACCO ARO2801	1.75
Dekalb 1214	1.75
Asgrow RX450A	1.80
McNair S-338	1.87
Dekalb XL-89	1.92
Asgrow RX 115	2.00
Pioneer 3161	2.02
Pioneer 3369A	2.02
Coker 54	2.07
McNair X210	2.12
Coker 56	2.12
Funks G5945	2.17
PAG 748	2.17
Funks G4864	2.22
Pioneer 3030	2.27
Dekalb XL99	2.32
McCurdy 72-227	2.37
Funks G4949A	2.42
Greenwood 801	2.50
Pioneer 3009	2.55
McNair 73011	2.60
McNair 508	2.67
Greenwood 45	2.75
Pioneer 515	2.90

Table 13 - Continued

---

[a]Plants inoculated by the toothpick technique.

[b]Rating scheme: 1) = 0-25%, 2) = 26-50%, 3) = 51-75%, 4) = 76-100% of the inoculated internode discolored. Bars indicate Duncan's multiple range groupings of treatment means which do not differ significantly at the 1% level.

[c]Based on two replications/variety, 20 plants/replication.

Table 14. Mean stalk rot ratings of commercial corn hybrids artificially inoculated[a] with Helminthosporium rostratum in 1974

Variety	Stalk Rot Rating[b]
Greenwood 471	1.65[c]
Asgrow RX450A	1.75
McCurdy 67-14	1.85
Coker 71	1.95
McNair 73011	2.05
Funks G5945	2.10
Dekalb 1214	2.15
Pennington Fla 200A	2.20
PAG 653W	2.20
Greenwood 95	2.25
Coker 814	2.33
Dekalb XL-80	2.40
Greenwood 228	2.40
Pennington 7C11A	2.40
McCurdy 72-227	2.40
Dekalb XL-380	2.50
Funks 64864	2.50
Funks G4949A	2.50
Pennington 9P3A	2.50
PAG 748	2.53
Dekalb XL99	2.55
McNair 508	2.55
Pioneer 3030	2.55
Greenwood 801	2.60
Pioneer 3369A	2.60
Greenwood 45	2.65
McNair X300	2.65
Pioneer 3161	2.65
ACCO AR02801	2.67
McNair X210	2.80
PAG 751	2.80
Pioneer 3009	2.85
Dekalb XL-85A	2.90
McNair S-338	2.95
Asgrow RX115	3.10
Pioneer 515	3.28
Coker 56	3.40
Dekalb XL-89	3.50

Table 14 - Continued

---

[a]Plants inoculated by the toothpick technique.

[b]Rating scheme: 1) = 0-25%, 2) = 26-50%, 3) = 51-75%, 4) = 76-100% of the inoculated internode discolored. Bars indicate Duncan's multiple range groupings of treatment means which do not differ significantly at the 1% level.

[c]Based on four replications/variety, five plants/replication.

Table 15. Mean stalk rot ratings of commercial corn hybrids artificially inoculated[a] with Fusarium moniliforme in 1973

Variety	Stalk Rot Rating[b]
Pennington 7C11A	1.07[c]
Greenwood 471	1.15
Dekalb XL-85A	1.25
Pennington 9P3A	1.30
McCurdy 72-227	1.32
PAG 751	1.32
McNair 508	1.35
Coker 56	1.37
McNair S338	1.40
Greenwood 228	1.42
Coker 814	1.42
McNair X300	1.47
Asgrow RX450A	1.47
PAG 748	1.47
Greenwood 45	1.50
Dekalb 1214	1.50
Asgrow RX132	1.52
Pioneer 3369A	1.53
McCurdy 6744	1.55
Coker 71	1.57
Pioneer 3161	1.60
Dekalb XL-380	1.62
PAG 653W	1.62
Dekalb XL99	1.62
McNair X210	1.65
McNair 73011	1.65
Pioneer 3030	1.65
Asgrow RX115	1.70
Greenwood 95	1.75
Dekalb XL-89	1.77
ACCO AR02801	1.80
Dekalb XL-80	1.82
Funks G4949A	1.87
Funks G4864	1.97
Coker 54	2.00
Pennington Fla 200A	1.97
Funks G5945	2.12
Greenwood 801	2.25
Pioneer 3009	2.25
Pioneer 515	2.40

Table 15 - Continued

---

[a]Plants inoculated by the toothpick technique.

[b]Rating scheme: 1) = 0-25%, 2) = 26-50%, 3) = 51-75%, 4) = 76-100% of the inoculated internode discolored. Bars indicate Duncan's multiple range groupings of treatment means which do not differ significantly at the 1% level.

[c]Based on two replications/variety, 20 plants/replication.

Table 16. Mean stalk rot ratings of commercial corn hybrids artificially inoculated[a] with Fusarium moniliforme in 1974

Variety	Stalk Rot Rating[b]
Pennington Fla 200A	1.15[c]
Coker 71	1.35
Asgrow RX450A	1.35
Greenwood 95	1.40
Pioneer 3030	1.55
Greenwood 471	1.55
Pennington 9P3A	1.65
Asgrow RX132	1.70
Dekalb 1214	1.75
Coker 84	1.80
Greenwood 228	1.80
Coker 54	1.80
Greenwood 45	1.85
Pioneer 3161	1.90
PAG 748	1.95
Funks G4949A	1.95
PAG 653W	1.95
McNair 508	1.95
Dekalb XL-80	2.00
Pioneer 3369A	2.00
Pennington 7C11A	2.00
Dekalb XL99	2.10
McNair 73011	2.10
McCurdy 67-14	2.10
ACCO AR02801	2.12
Coker 56	2.13
Dekalb XL-89	2.15
Dekalb XL-85A	2.15
Funks G4864	2.15
McCurdy 72-227	2.15
McNair S338	2.20
Greenwood 801	2.25
PAG 751	2.35
Funks G5945	2.35
McNair X300	2.40
Dekalb XL-380	2.70
Pioneer 3009	2.75
Pioneer 515	2.80
McNair X210	2.85
Asgrow RX115	3.20

Table 16 - Continued

---

[a]Plants inoculated by the toothpick technique.

[b]Rating scheme: 1) = 0-25%, 2) = 26-50%, 3) = 51-75%, 4) = 76-100% of the inoculated internode discolored. Bars indicate Duncan's multiple range groupings of treatment means which do not differ significantly at the 1% level.

[c]Based on four replications/variety, five plants/ replication.

BIOGRAPHICAL SKETCH

Thomas R. Young was born in San Francisco, California, on December 25, 1946. He received the degree of Bachelor of Arts in biology from Occidental College in 1969. In 1972, he received the Master of Science degree from California State Polytechnic University at Pomona. In the Fall of 1972, he began studies toward the degree of Doctor of Philosophy at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas A. Kucharek
Chairman
Assistant Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Daniel A. Roberts
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert E. Stall
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Kimbrough
Professor of Botony

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell
Assistant Professor of Plant Pathology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1975

Dean, College of Agriculture

Dean, Graduate School