Physiological analysis of maize stunting caused by Pythium ultimum and involvement of Pythium sp. in maize culture

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Physiological analysis of maize stunting
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involvement of Pythium sp. in maize culture

by

Won Mok Park

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INTRODUCTION

Root rots are common diseases of plants, and many species of *Pythium* have been described as root rot pathogens on various plants. Root rot affects many established plants. The fibrous root system is attacked, and rootlets are softened and killed, the above-ground effects consist of stunting (34, 44), reduction of yield (34, 44), sudden wilting (110), or death (110).

Four hypotheses are proposed to explain the stunting phenomenon associated with *Pythium* infected seedlings. The first is deficiency of exogenous nutrients, minerals, and water due to a reduction in root absorption surface (116).

The second hypothesis is that stunting is due to a lower solubility or mobility of reserve nutrients, such as starch, in the endosperm in diseased than in healthy seedlings resulting in a deficiency at the growing point. Most nutrients required for shoot growth of seedlings are stored in the endosperms.

The third hypothesis is that stunting results from a phytotoxin produced by *Pythium*. Brandenberg (1950), as cited by Wilhelm (116), reporting on *Pythium* induced stunting and chlorosis on sugar beets, indicated that rootlets must be invaded to bring about the effect. He detected a proteinaceous toxin produced by *Pythium*.

The fourth hypothesis is that *Pythium* infection results in a hormonal imbalance in diseased plants. Most theories that have been proposed to explain growth abnormalities of diseased plants usually
include hormonal imbalances. Affected tissues frequently contain higher or lower than normal amounts of growth regulators in plants (95). These growth regulators are auxins, gibberellins, cytokinin, ethylene, and abscisic acid. Roots may function in the synthesis, distribution and activation of these hormones (105).

Since pathogenicity of *Pythium* is affected by soil antagonistic microorganisms, the effects of soil sterilization and inoculation methods on root rot development were tested.

Susceptibility of 17 maize inbred lines to *P. ultimum* Trow was also tested.
LITERATURE REVIEW

Pythium spp. are soil-borne fungi. They live saprophytically on plant debris in soil and parasitically on roots, stems, and foliage of seed plants (32). Not all Pythium species are pathogenic, but most are root pathogens, and some species incite stem and foliage blights (32).

Treub, as cited by Summers (100), referred to a Pythium as a possible cause of sugar cane root rot in 1885. It was not until 1921 that Carpenter (13) presented satisfactory evidence that the filamentous sporangial Pythium spp. were of major importance as root rot producing agents of monocotyledonous crops. After Carpenter's publication, several reports from widely separated localities indicated that Pythium spp. caused root rots in many crop plants (100, 110). Pythium spp. attack the fibrous roots, primarily near the root tip. Rootlets become necrotic and are killed, and the apical root meristem is destroyed. The above ground symptoms are stunting, sudden wilting, decline in plant vigor, and eventually death (110).

Environmental Effects on Nonmaize Pythium Diseases

The severity of Pythium injury depends upon environmental conditions. Leach (57) investigated the effect of temperature on the incidence of damping-off caused by P. ultimum, Rhizoctonia solani Kuehn, and Phoma betae Frank tested individually with spinach, watermelon, garden pea, and sugar beet. In all combinations of hosts and pathogens, damping-off was most severe at temperatures that were less favorable to the host than to the pathogen as measured by the ratio, host growth rate: pathogen growth.
rate.

Biesbrock and Hendrix (7) stated that saturated soils were important in infection and symptom production in peach trees grown in Pythium infested soils. Root necrosis, growth reduction, and mortality were closely associated with the length of time the soil was saturated. Those species readily producing zoospores were more dependent on high soil moisture levels for disease development than species that rarely produce zoospores (32). Soil moisture and soil temperature are the dominant factors affecting disease development by Pythium spp. Other factors are also important. For examples, the severity of peanut pod rot caused by Pythium spp. is affected greatly by crop sequence (26). Klisiewiez (53) noted that safflower root rot incidence was highest among plants grown in a low-light environment.

Environmental Effects on Maize Pythium Diseases

*D. ardianidermatum* (Edson) Fitzp., *P. arrhenomanes* Drechs., *P. debaryanum* Hesse, *P. graminicolum* Subr., *P. irregulare* Buis., *P. paroecandrum* Drechs., *P. rostratum* Butl., *P. splendens* Braun., *P. ultimum* Trow, and *P. vexans* d By. are pathogens causing maize root-rot and seedling blight problems (36, 38). Johann et al. (44) demonstrated that maize preemergence damping-off by Pythium was enhanced by low temperature, 12 to 16 C, and high levels of soil moisture. Van der Zwet (106) reported that maize planted in flooded soil 2 days after infestation by Pythium showed 38.5% root infection compared with 2.5% in the nonflooded control. Flor (25) demonstrated that injury to germinating corn and corn seedlings
was greatest at moisture levels above 50% of soil water holding capacity and at temperatures below 20°C.

Plant Hormones and Plant Disease

According to Weaver (112), it was believed, approximately 200 years ago, that sap produced in one part of the plant is moved to another part to control the growth in some manner. Duhamel du Monceau (1758) concluded that there was one sap that moved downward and one that moved upward. The downward-moving sap was said to originate in the leaves and then to move down and control the nutrition of the roots. Sachs (1882) revised Duhamel du Monceau's theory. He postulated that root-forming substances originated in the leaves. The substances which induce organ-formation are now known as hormones. The primary plant hormones are auxins, gibberellins (GA), cytokinins, ethylene and abscisic acid (ABA). There is evidence that plant hormonal imbalance can cause the abnormal growth of diseased plants (95).

Auxins

The usual sites for auxin formation in vascular plants are meristematic and enlarging tissues (59). Growth stimulation by auxin involves cell enlargement by nonpolar swelling. The stimulatory effects of auxin on growth vary considerably among tissues. In general, the most marked stimulations are obtained in seedling stems and coleoptiles. Green stems are often less responsive than etiolated stems, and in nearly all growing tissues high concentrations of auxin bring about growth inhibition rather than stimulation (102). Auxin generally inhibits stem
growth at concentrations above $10^{-3} M$, bud growth at concentrations above $10^{-5} M$, and root growth at concentrations above $10^{-3} M$. In addition to the effects on growth, auxins participate widely in regulation of growth differentiation and plant processes, e.g., the tropisms, apical dominance, and the regulation of organ differentiation phenomena (59).

Epinasty caused by excessive growth of the morphologically upper side, results two hours or less after auxins in sufficient concentrations are applied to plant. Twisting of stems, stunting, cupping, and development of abnormal venation, on newly developing leaves are other symptoms of auxin treatment (112).

The excessive amounts of auxins have been detected in diseased tissues for many diseases, e.g., crown gall caused by Agrobacterium tumefaciens (52), bacterial wilt caused by Pseudomonas solanacearum (94) and olive knot caused by Pseudomonas savastanoi (64).

Cytokinins

Relatively high levels of the cytokinins have been found in tissues in which there is active cell division, such as germinating seeds and young fruits. Cytokinins probably are synthesized in the root tips and move through the xylem to the leaves (112). Cytokinins are potent inducers of nutrient mobilization (76). Their roles in plant cell division (58), cell enlargement (60), and antisenescence (69) are well-known.

Some diseased tissues show increased cytokinin activity compared with healthy tissues, e.g., rust infected bean leaves (51), Corynebacterium fascians infected pea (103), and cabbage clubroot caused by
Plasmodiophora brassicae Wor. (49).

Gibberellins

The regulatory roles of gibberellins are cell division (92), cell enlargement (30), breaking of dormancy in some seeds (48) and unrolling of young leaves in response to light (62).

The exact site of gibberellic acid synthesis is not known for many plant species. Shoot apices (61), apical buds (47), roots (12, 14) and leaves (88) are sites of GA synthesis in some plants. GA's were detected in sieve tube sap (33), indicating shoot synthesized GA transport to the root via the phloem. Crozier and Reid (18) observed that removal of root apices resulted in the disappearance of $GA_1$, and accumulation of $GA_{19}$ in the leaves and apical buds, and in the subapical root remnants. They (18) suggested that $GA_{19}$ was synthesized in the shoot, and then moved to the root where it was converted to $GA_1$, which in turn was exported to the shoot. An important point in their scheme was that the shoot was the primary site of GA biosynthesis and the root was merely the site of a GA conversion to an active form. Abbott (1) stated that the young leaf laminal meristem provided a major source of gibberellins to the stem. GA biosynthesis may be also inferred to occur in embryo (81), cotyledons (Sebanek, 1965, cited by Leopold and Kriedemann (59)), fruits (6) and seeds (112). Wounding can stimulate GA biosynthesis in tubers (86). Gibberellic acid originating in seed embryos greatly enhances the synthesis of amylolytic enzymes in aleurone cells of barley (108, 81), wild oats (15, 79), and rice (80).
Bowen et al. (10) reported that light inhibited the rate of stem elongation of *Phaseolus coccineus* L. seedlings. GA₄, an endogenous component of the seedlings, promoted stem growth in the light but not in darkness. Dark-grown seedlings contained larger GA pools than light-grown plants. The capacity of seedlings to metabolize [³H] GA₄ was greater in the light than in darkness. This indicated that the availability of GA is a limiting factor only in the growth of light grown plants. However, the effect of light alone did not reduce the amount of GA available for growth because its inhibitory effect on stem elongation cannot be counteracted completely by application of exogenous GA.

Nadeau et al. (77) reported that uptake and metabolism of the [³H] GA₁ were markedly enhanced by simultaneous treatment with abscisic acid.

*Gibberella fujikuroi* (Saw.) Wr. which causes "Bakanae disease of rice" produces large quantities of GA (55, 118). The endogenous gibberellin content of thistle plants systemically infected diseased by *Puccinia punctiformis* (Str.) Rohl. is consistently higher than that of healthy plants during the earlier part of the season when the plants are growing rapidly (4). The estimation of cell number in the third leaf of barley infected with barley yellow dwarf virus (BYDV) showed a marked decrease in the mitotic activity of the infected plants. Endogenous gibberellins decrease the level of a substance corresponding to GA₃ in BYDV-infected plants (76). Early infection of cucumber by cucumber mosaic virus caused reduction in the net assimilation rate, pronounced stunting of the roots and reduced stem and leaf growth. Such stunting was associated with a reduction in the concentration of endogenous...
gibberellins (3). 'White Burley' tobacco with severe etch virus displayed a reduction of plant height which was overcome to a limited degree by spraying with GA (99). Spraying with a solution of GA$_3$ (1 mg/ml) thrice at weekly intervals to tobacco leaf-curl affected plants caused a reversal of stunting and suppression of leaf curl symptoms (78). The symptoms of cherry ring spot (CRS), and cherry yellows virus (CYV) on sour cherry trees were stunting and a reduction in yield. Foliar application of GA (0.1 mg/ml) was made on young Montmorency cherry shoots that were virus-free, infected with CYV and infected with CRSV. The GA treatments resulted in increased shoot length and diameter of the virus infected trees (39). The treatment of CYV-infected trees with GA$_3$ at a concentrations of 0.01 to 0.015 mg/ml (depending on the severity of the disease as indicated by the presence of long, barren twigs), reduced the percentage of blossom buds and increased the number of vegetative buds on terminal and lateral shoots. The year following treatment most of the vegetative buds produced spurs that eventually produced fruit the next year (24, 82).

Abscisic acid

Abscisic acid (ABA) is a growth inhibitory hormone that is widespread in the plant kingdom. It interacts with growth promoters and thus has an important regulatory effect on growth phenomena. An important site for ABA synthesis is the plastids, especially the chloroplasts (67) and light is required (85, 101). Weinbaum and Powell (113) reported that ABA and its water soluble glucoside diffused from leaves through
petioles and along the axes of seedlings of *Malus hupehesis*, and the concentrations were greatest in growing shoot tips. In contrast, Pilet (85) reported ABA, which accumulated in the root after illumination, was synthesized mainly in the roots. ABA was assumed to the sole compound in the purified sample that absorbed ultraviolet light in the range 220 to 290 nm, and ABA moved to $R_f$ 0.7 to 0.8 on paper chromatograms developed in isopropanol: ammonia: water.

In bean axes continuously incubated in $10^{-5}$ M ABA, respiration was inhibited at all stages of germination, and in axes pretreated for 2 hours in $10^{-5}$ M ABA respiration was inhibited about 8% at all stages of germination (109). Chrispeels and Varner (16, 17) working on barley seeds, reported that ABA did not significantly alter the rate of respiration or the pattern of $^{32}P$ labelling in phosphorylated metabolic intermediates.

ABA significantly reduced maize root growth (84), the expansive growth of leaf sections of corn, and the GA-enhanced elongation of normal and dwarf peas (104). ABA inhibits the production of alpha-amylase which is triggered by GA in isolated barley aleurone layers (16).

The regulatory role for ABA in plants is best documented for responses to stress. There have been several reports of an increase in ABA in water-stressed tissues (68, 72, 75, 96, 117). Tobacco plants pre-exposed to leaf dehydration, mineral deprivation, salination, or borate toxicity exhibited increased resistance to subzero temperature and to reduced $O_2$ in the root medium. The stressed plants contained increased contents of leaf ABA. Treatment with ABA by direct application
to the leaves or by addition to root medium improved leaf resistance to subzero temperatures and to deprivation of root O$_2$ (9, 71). ABA treated alfalfa seedlings acquired an improved tolerance to subzero temperatures (90), but GA treated alfalfa did not acquire cold resistance. GA acted as an inhibitor of the cold hardening process in Acer negundo (43), while ABA increased the cold hardening level (42). The killing point was lowered from -16.7°C to -21.4°C with ABA. It is concluded that cold hardiness development appeared to be related more closely to a build-up of abscisic acid levels than to a reduction of gibberellin levels. Prechilled leaves of Xanthium strumarium contained more ABA than leaves of plants pretreated in a warm chamber (87).

ABA ($10^{-6}$M) treated woody angiosperms have reduced transpiration under water stress conditions (19). When the water potential of the leaf was -14 bars, stomata were closed and ABA levels were elevated (28). Free ABA concentration in water stress tolerant maize varieties is higher than that in susceptible varieties. Such high levels would be expected to have a profound effect in closing leaf stomata thereby reducing transpiration and protecting the plant against water loss (56).

Tobacco plants in NaCl and mannitol stress conditions contained higher concentration of ABA-like materials (73). Tobacco plants growing in half-strength Hoagland solution were deprived of nutrients by being transferred to distilled water and the ABA content of leaves in mineral-deprived plants increased. The mineral-deprived plants exhibited resistance to lack of aeration in the root medium, similar to that shown in salt-stressed plants or plants that were pretreated with ABA
The foregoing data support the hypothesis of Mizrahi et al. (73) that an increase in ABA is induced by conditions unfavorable to growth, and ABA controls metabolic patterns which facilitate plant adaptation to unfavorable conditions.

Steadman and Sequeira (98) reported that, in tobacco plants inoculated with Pseudomonas solanacearum E.F. Sm., there was a correlation between decreased internode elongation, maximum multiplication of the bacterium, and an increase in the concentration of a growth inhibitor (ABA) in stems, 4 to 12 days after inoculation. The inhibitor was not found in P. solanacearum culture medium. The pods of plants infected with pea-mosaic virus contains 2.5X more inhibitor than pods of healthy plants (107). The bioassays on ether-soluble acid extracts from healthy and Verticillium infected tomato plants, showed the presence of substances inhibiting growth of wheat coleoptiles in both healthy and infected leaves and stems, but the amounts were greater in the infected plants (83). The cotton leaves infected by the defoliating strain of Verticillium albo-atrum Reinke & Berth. contained about 2X more ABA than healthy leaves or the leaves of plants infected with a nondefoliating strain of the pathogen (114).

Gibberellin and kinetin had no effect on the percentage germination of Botrytis cinerea Pers. conidia, IAA stimulated germination slightly, but ABA (0.05 mg/ml) significantly decreased germination (8). ABA was slightly inhibitory to Aspergillus nidulans (Eidam) Wint. growth (97).
Maize Seed Metabolism

The enzymes hydrolyzing the starchy endosperm of maize seeds are alpha- and beta-amylases (20, 29). Dure (20) reported that alpha-amylase originated exclusively in the scutellum and was secreted into the endosperm during germination. Alpha-amylase accounted for 9/10 of the amylolytic activity found in the endosperm at the peak of amylolytic activity. Beta-amylase was the only amylase in the endosperm of the resting seed and was formed exclusively in situ in the endosperm during germination. Beta-amylase accounted for only 1/10 of the total amylolytic activity in the endosperm. Goldstein and Jennings (29) reported the development of alpha-amylase activity in de-embryonated maize kernels was independent of embryo activity and did not require any exogenous source of GA. Ingle and Hageman (40) determined that the catabolism of carbohydrates and proteins in excised endosperms of maize was stimulated by exogenous GA. The initiation of sugar production was completely dependent, whereas amino acid production was only partially dependent on added gibberellic acid. Glucose, a product of starch hydrolysis in the endosperm, was absorbed from the endosperm by the scutellum in germinating grain, was simultaneously converted to sucrose, and was transported in this form to the seedling (2). The scutellum contains all the enzymes required for sucrose synthesis (21). The maize scutellum absorbed sucrose actively without inversion and absorbed hexoses by two processes, diffusion and active transport, which operated simultaneously (115). In the early stages of maize seedling development (within 5 days after planting), dry weight, insoluble protein and fat in the endosperm decrease rapidly and
the soluble carbohydrate, soluble protein and soluble nucleotides in­
crease in the whole seedlings (41).

Soil Antagonists

Isolates of many different genera of soil microorganisms were
found to produce antibiotics which inhibited *Pythium arrhenomanes* (46).
These antagonistic microorganisms, especially the actinomycetes, were
important factors affecting the severity of *Pythium* root rot. Of 4,000
fungal isolates obtained from 46 sugar-cane soil samples in 13 locali­
ties of Lousiana, 15.4% were shown to be antagonistic to *P. arrhenomanes*
in pure culture. The antagonistic fungi included *Penicillium* sp.,
*Aspergillus* sp., and *Spicaria* sp. (63). Seven *Actinomycetes* and five
isolates of *Spicaria*, *Penicillium* and *Aspergillus* significantly reduced
the severity of root rot caused by *P. arrhenomanes* (45). Damping-off of
snapdragon seedlings from *P. ultimum* could be reduced by antagonistic
*Bacillus* sp. (11). These *Bacillus* sp. are favored by treatment of soil
with aerated steam at 60 C for 30 minutes (5). *Aphelenchus avenae* Bas­
tian could reduce the severity of root rot on maize by *P. arrhenomanes*
(89).

Genetic Resistance to *Pythium*

Erwin and Cameron (22) tested the susceptibility of five sweet corn
varieties to *Pythium graminicola* and found that Purdue 39A, Purdue 51B,
and Iowa 2000 appeared to be more tolerant to the fungus in the field
than Luther Hill and Wisconsin 23. Hooker (35) reported that corn
seedling resistance to *Pythium* did not appear to be specific for one
fungus genotype but was effective against genotypes of several *Pythium* species. Resistance is a function of embryo genotype. Hooker (36) showed that corn strains A73, 82, 225 and 829 were resistant and B6, B10, B36 and WF9 were susceptible to *P. graminicolum* and *P. debaryanum*. 
MATERIALS AND METHODS

Preparation of Plant Material and Pythium

Plant material

Seed  Maize inbred line W64A which was used in all general experiments, was kindly provided by Clyde Black and Son, Ames, Iowa. Seed of the various maize inbred lines were supplied by Dr. W. R. Russell.

Growth of maize  Maize seeds were surface sterilized in 1.0% NaOCl solution for 5 minutes and then washed five times with distilled water. Eight hundred gram of washed white silica sand was placed in a plastic cup (11 cm diameter and capacity of 1000 ml). Ten maize kernels were planted on top of the sand with the pedicel pointed downward. The seeds were carefully covered with an additional 200 g of sand and watered with 150 ml of Knop's solution. The 150 ml of solution was the field capacity of the sand. The pots were covered with transparent polyethylene bags to retain moisture in the pots. They were placed in a growth chamber at 25 C and a 14 hour photocycle.

Knop's solution is composed of 0.1017 g FeSO$_4$$\cdot$7H$_2$O chelated with 0.0745 g Na-EDTA, 0.285 g KH$_2$PO$_4$, 0.285 g CaCl$_2$$\cdot$2H$_2$O, 0.285 g KCl, 0.322 g NH$_4$NO$_3$, 0.427 g MgSO$_4$$\cdot$7H$_2$O, 1.57 mg MnCl$_2$$\cdot$4H$_2$O, 1 mg H$_3$BO$_3$, 0.1 mg ZnSO$_4$$\cdot$7H$_2$O, 0.02 mg CuSO$_4$$\cdot$5H$_2$O, 0.06 mg NH$_4$MoO$_4$ and 1000 ml of distilled water.

Isolation of Pythium

A collection of Pythium species was isolated from corn field soils
and from corn roots exhibiting fibrous root necrosis. The procedures of isolation were as follows;

**Water agar method**    Necrotic maize root tips (5 mm long) were dissected from the plant and washed with running distilled water. Solidified 1% water agar in a petri dish was lifted on the edge with a sterile spatula and a washed root sample was placed between the agar and the bottom of the petri dish. After 2 days incubation at 25°C, Pythium-like hyphae growing on the surface of agar (as observed with the aid of a stereoscopic microscope at 70X) were transferred to Difco potato-dextrose-agar containing streptomycin, 0.1 mg/ml (SPDA).

**Selective medium**    Washed Pythium infected maize roots were placed on modified SA-PCNB agar (93) and incubated at 25°C. SA-PCNB agar is composed of 2.4 g sucrose, 0.27 g asparagine, 0.15 g KH₂PO₄, 0.15 g K₂HPO₄, 0.1 g MgSO₄·7H₂O, 0.0044 g ZnSO₄·7H₂O, 0.001 g FeSO₄·7H₂O, 0.07 mg MnCl₂·4H₂O, 2 mg Thiamine·HCl, 0.01 g ascorbic acid, 0.01 g Cholesterol (2 ml N,N-Dimethylformamide sol.), 0.01 g benomyl, 0.021 g pentachloronitrobenzene, 0.1 g neomycin sulfate, 0.01 g chloroamphenicol and 20 g Bacto agar in 1000 ml of distilled water. Pythium isolates were transferred to SPDA.

**Bait method**    Maize field soil was brought to the laboratory, moistened, and baited with autoclaved maize kernels. After 2 days, the kernels were removed from the soil, serially washed with sterilized water and placed on the SA-PCNB agar. The kernels were incubated at 25°C for 2 days. Hyphae growing from the kernels were transferred to
Each Pythium isolate was numbered and its pathogenicity was tested on young maize seedlings by the following procedure. All isolates were cultured separately on PDA for 5 days at 25 C. An agar disc (1.7 cm diameter) was cut in the culture with a #10 cork borer and the disc was placed in the center of a pot which contained 800 g white silica sand. Ten maize seeds were placed around the disk, 200 g more sand was poured over seed, and the sand was watered with 150 ml of Knop's solution. The seeds were grown in a growth chamber at 25 C for 6 days. Shoot emergence and root damage was rated. Root damage rating is from 1 (healthy) to 5 (severe root rot). The isolate selected for intensive study was #61, Pythium ultimum Trow. Identity of this isolate was kindly confirmed by Dr. A. F. Schmitthenner, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Preparation of inoculum

White proso millet seeds (Panicum milaceum L.) were washed with tap water to remove dust and loose contaminants, and boiled in excess tap water for 30 minutes. The seeds were drained and placed in 250 ml Erlenmeyer flasks to near 1/5 of flask capacity. The flasks were plugged with cotton and autoclaved at 1 atmosphere pressure for 90 minutes. Pieces of P. ultimum culture on PDA were seeded into the flasks and incubated at 25 C for 5 days with daily shaking of the culture. The inoculum was then stored at 4 C. The millet inoculum (0.5 g/pot) was mixed with the sand before planting seed.
Relation between Shoot Growth and Root Damage

Stunting due to Pythium ultimum and surgical removal of roots

Maize seeds were sown in P. ultimum infested sand and grown in the growth chamber for 6 days. Total of 85 seedlings were harvested and the root damage rated into 5 classes.

Thirty pots were filled with white sand. Fifteen pots were inoculated with Pythium ultimum and the other 15 pots were uninoculated. Ten maize seeds were sown into each pot. They were watered with Knop's solution and placed in the growth chamber. After 4 days, 60 seedlings grown in uninoculated pots were dug out and their roots were completely pruned by knife. Then they were replanted in the same pots where they were grown. This surgical operation was to mimic the damage from Pythium. Pruning was done every other day, to keep seedlings from rooting. The shoot growth was monitored every 3 days until the seedlings were 10 days old. A comparison was made in shoot growth among healthy, diseased and root pruned seedlings.

The shoots of 6 days old healthy and diseased seedlings were harvested. The fresh weight of them was measured, and then they were dried in a mechanical convection oven at 85°C for 3 days before determinations of the dry weight and moisture content.

Effects of Pythium ultimum on seedlings grown in the light and dark

After the seeds were sown in P. ultimum infested sand (6 pots) and uninoculated sand (6 pots); 3 pots of each treatment (10 seeds per pot) were placed in a dark box. They were kept in a growth chamber at 25°C for
7 days and then the shoot growth was measured.

Utilization of Reserved Nutrients in Endosperm

**Analysis of alpha-amylase activity**

Maize seeds were planted in *Pythium* infested and noninfested sand. When seedlings were 4 days old, the roots of half of the healthy seedlings were surgically pruned and the pruned seedlings were replanted. Alpha-amylase activity in scutellum and endosperm tissues was monitored for healthy, diseased, and root-pruned seedlings.

The endosperm and scutellum tissues were dissected separately from maize seeds (1 day old) and seedlings (4, 7, and 10 days old). The tissue from each seed and seedlings was ground individually with 1 ml of ice cold deionized distilled water in a mortar and pestle, and transferred to a centrifuge tube. Another 4 ml of ice cold deionized distilled water was used to rinse the residue from the mortar and pestle into the centrifuge tube. The homogenate was centrifuged at 12,000 x g for 15 minutes at 2 C and the supernatant was collected. The pellet was resuspended in 5 ml of water and centrifuged again. The supernatants were pooled and the volume adjusted to 10 ml with deionized distilled water. This crude extract was assayed for amylase activity.

The assay mixture consisted of 0.5 ml of enzyme extract, 1.5 ml of 0.001 M acetate buffer (pH 4.5) and 1 ml of a starch solution. The reaction was stopped at 2 minutes by adding 1 ml of iodine reagent (iodine stock solution containing 6 mg of potassium iodine and 600 mg of iodine in 100 ml water was used after a 1/10 dilution with 0.05 N HCl).
The mixture was diluted with 4 ml of distilled water and the O.D. measured in a Spectronic 20 at 620 nm. Starch solution was obtained by boiling a starch suspension containing 75 mg of starch, 600 mg of KH₂PO₄, and 200 umoles of CaCl₂ in a final volume of 100 ml. A zero time control (1 ml of starch solution, 1 ml of iodine reagent, 1.5 ml of buffer, and 4.5 ml of distilled water) was prepared to obtain the reading for the unhydrolyzed starch. The enzyme activity was calculated by the formula (23),

\[
\frac{(A)(V)}{(T)(V')} = \text{unit of alpha-amylase}
\]

\[ A = \text{absorbance of a zero time control minus the absorbance after reaction time (T) minutes,} \]
\[ V = \text{total volume of enzyme solution,} \]
\[ V' = \text{volume of enzyme used in assay.} \]

The cold supernatant was saved for sugar analyses.

**Analysis of reducing sugars**

The 6-day-old healthy and diseased seedlings were harvested. They were dissected into endosperm and scutellum and then analyzed to determine concentrations of reducing sugar and sucrose in them. Reducing sugars in the supernatant were quantitatively assayed by an adaptation of the dinitrosalicylic acid test (DNS) of Miller (70). A sample of 0.5 ml of supernatant (1/10 of total volume) was added to a 16 mm (diameter) test tube containing 2.5 ml of distilled water and 3 ml of fresh DNS reagent (1 g dinitrosalicylic acid, 0.2 g phenol, 1 g NaOH, and 0.05 g Na₂SO₃/100 ml distilled water). The mixture was heated in a boiling
water bath for 15 minutes. One ml of a 40% solution of Rochelle salt (potassium-sodium tartrate) was added to stabilize the color of the reaction mixture. The mixture was cooled in cold water and the volume was adjusted to 25 ml with distilled water. The O.D. was measured in a Spectronic 20 at 575 nm. Reducing sugar was calculated from a standard curve for glucose. The data presented represents the average of 10 replications. The supernatant was saved for sucrose analysis.

**Analysis of sucrose**

A 0.5 ml aliquot of the supernatant (above) and 0.5 ml of invertase (10 units to 2 ml of distilled water) were incubated for 30 minutes at 23 C to hydrolyze sucrose into reducing sugars. Total reducing sugars were measured by DNS test and then the amount of sucrose was calculated by the formula,

\[ S = \frac{(A-B)}{2} \]

- **A** = mg of reducing sugars after hydrolysis
- **B** = mg of reducing sugars before hydrolysis
- **S** = mg of sucrose

The data presented represents the average of 10 replications. The amount of invertase used in this assay was more than necessary for total hydrolysis of the sucrose found in any of the samples.

**Analysis of total soluble carbohydrate**

Maize seeds were planted in *Pythium* infested and noninfested sand, and half of the healthy seedlings were root-pruned 3 days later. Endosperm and scutellum tissues were dissected at 3, 5, and 7 days. The tis-
sue from each seed was ground separately with 2 ml of distilled water in a mortar and pestle. After the homogenate was transferred to a centrifuge tube, 4 ml of distilled water was used to rinse the mortar and pestle. The rinse was pooled with the homogenate and centrifuged at 3,000 x g for 5 minutes. The supernatant was diluted 5 times with distilled water. A 0.1 ml sample was added to 2.9 ml of distilled water and then this was added to 6 ml of anthrone reagent (4 g of anthrone in 1000 ml of conc. H₂SO₄) in the test tube and mixed by shaking. The tubes were placed in a boiling water bath for 3 minutes and then cooled in running tap water adjusted to ambient temperature. The O.D. of the reaction mixture was measured immediately in a Spectronic 20 at 600 nm. Total soluble carbohydrate was calculated from a standard curve for glucose.

**Determination of dry weight of insoluble material in endosperm**

Maize seeds were planted in P. ultimum infested and noninfested sand. Eight seedlings were harvested at 4, 7, and 10 days after planting. The endosperm tissue was removed and individually ground in 5 ml of distilled water by mortar and pestle. The homogenates were centrifuged at 12,000 x g for 15 minutes to separate the soluble portion and insoluble portion (pellet). The pellets were collected and dried at 105 C for 5 days. The amount of insoluble material in the endosperm tissue was measured.

**Measurement of respiration in shoot**

The shoots were harvested from healthy and diseased seedlings, when they were 3 and 6 days old. For respiration measurement of 3-day-old
seedlings, the whole shoots were used, and for 6-day-old seedling, 2 cm shoot fragments were cut from the first node. Four shoots were placed in each respirometer flask and each treatment had 3 replications. The respiration was measured in a Gilson respirometer at a temperature of 28.3 °C. Measurements were taken for 30 minutes after stabilization. After the measurements, the shoots were dried in an oven at 85 °C for 2 days to determine the dry weight of the shoots.

O₂ uptake was calculated by the formula,

\[
\frac{(273)(BP)}{(T+273)(762)} = F
\]

\[
\text{O₂ uptake} = \frac{C \times F}{t \times DM}
\]

BP = atmosphere pressure in Bar,
T = temperature (°C)
C = change volume in ul
t = time
DM = dry weight.

Effects of Growth Regulators on Shoot Stunting

**Exogenous auxin treatment**

Maize seeds were planted in Pythium infested sand and noninfested sand. When the seedlings were 4 days old, the seedlings were gently washed from the sand and the roots were washed with distilled water. The shoots were pruned 2 cm above the first node. The seedlings were placed in auxin solutions at concentrations of 0, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M IAA and incubated at 27 °C growth chamber with lights on (24 hour/day).
The solutions were replaced daily. Six days after the treatments, the growth of the seedlings was measured. Each treatment had 3 replications.

**Gibberellin and kinetin treatment**

Maize seeds were planted into Pythium-infested and noninfested sand. When the seedlings were 4-days-old, the roots of some of the healthy seedlings were pruned, and then each of the diseased and root-pruned seedlings was placed in $10^{-6}$M solutions of $\text{GA}_3$, kinetin (zeatin), and $\text{GA}_3 + $ zeatin. Solutions were replaced daily. The shoot growth was measured 3 days after the treatments started.

**Abscisic acid treatment**

Maize seeds were planted in Pythium infested and noninfested sand. When the seedlings were 5-days-old, the seedlings were trimmed to a uniform shoot length (2 cm) and the healthy seedlings were placed into solutions of 0, 100 and 1000 units of ABA. (1 unit = 5.28 mg ABA/1000 ml water). Some healthy seedlings were root pruned at the same time. The Pythium-infected seedlings and root pruned seedlings were placed in distilled water. The seedlings were grown at 25 C for 4 days. There were 5 replications. The ABA and distilled water was changed daily. Shoot growth was measured daily.

**Analysis of Gibberellin Concentration**

**Purification**

**Extraction** Healthy and diseased 6-day-old seedlings were
harvested from the sand and 110 g (fresh weight) were homogenized in 500 ml of 80% methanol in a Waring blender and the homogenate was placed at 4 C for 48 hours. The methanol extract was filtered through Whatman #1 filter paper in a Buchner funnel and the filtrate was evaporated to the water phase in a reduced pressure flash evaporator at 40 C. The pH was adjusted to pH 7.5 with 5% NaHCO3. The water phase was extracted once with the same volume of alcohol-free ethyl acetate. The aqueous layer, containing most of the active acidic gibberellins, was adjusted to pH 3.0 with sulfuric acid and again extracted twice with ethyl acetate. The water phase was discarded. The ethyl acetate layer was dried with Na2SO4 and evaporated to dryness (31).

**Paper chromatography** The gummy residue was purified further by descending paper chromatography on Whatman 3MM paper using as solvent isopropanol : ammonium hydroxide (specific gravity 0.897) : water, 10 : 1 : 1 (vol : vol : vol) (Jones and Phillips, 47). The chromatograms were dried when the solvent front line reached about 25 cm from the origin. The chromatogram was divided into 10 equal strips corresponding to Rf values. A 6 cm wide portion of each strip was eluted and bioassayed with lettuce seed germination to find the position containing inhibitors. Strong inhibitors were found at Rf zones of 0 to 0.1, 0.1 to 0.2, and 0.6 to 0.7 (Fig. 9). Those 3 strips were discarded. The other strips were eluted with 80% ethanol. The eluates were combined and dried under reduced pressure for further thin layer chromatography (TLC).

**Thin layer chromatography** The dried material was dissolved with
3 ml of 80% ethanol and strip loaded onto a Brinkman precoated TLC plate of silica gel 60 F-254 (thickness of 0.25 mm). Reference spots of authentic GA3 and abscisic acid were chromatographed at the same time as extracts. Chromatograms were developed with n-butanol : ammonium hydroxide (specific gravity 0.897), 3 : 1 by volume, and the region between Rf 0.0 and 0.3 was scraped from the plate and eluted with 80% ethanol (3). The silica gel was pelleted by centrifugation at 12,000 x g for 15 minutes. The ethanol supernatant was concentrated to a small volume by evaporation with moving air and rechromatographed on a TLC plate using benzene : n-butanol : acetic acid, 70 : 25 : 5 by volume (31). The development was stopped when the solvent front reached about 15 cm from the origin. The TLC plates were divided into ten equal zones corresponding to Rf values and each zone was eluated with 80% ethanol. The eluates were dried and dissolved in 2 ml of distilled water. They were stored in a refrigerator at about 5 C.

Bioassay for gibberellin

Lettuce seeds, Lactuca sativa L. (variety Grand Rapid), were sown in a 60 x 15 mm petri dish on one layer of Whatman #1 filter paper (5.5 cm diameter) which was moistened with 0.5 ml of the test solution. The petri dishes were placed in the dark at 20 C for 2 days, and then under light at 23 C for 4 days when the hypocotyl lengths were recorded. The mean length of the ten hypocotyls in each dish was expressed as a % of the water controls (i.e. water control = 100%). Stimulation of hypocotyl elongation resulted in values > 100% and inhibition was < 100%. (27).
Analysis of Abscisic Acid Concentration

**Purification**

**Extraction**  The shoots (30 g of fresh weight) were excised at the first node on 6-day-old seedlings from each treatment, (healthy, diseased and root-pruned) grown under light and grown under dark. The shoots were cut into about 5 mm pieces and extracted in 500 ml of 80% methanol for 48 hours at -20°C under dark. The original extract was decanted and replaced with 500 ml of fresh 80% methanol. After 48 hours, the solvent was again decanted and the solvents were pooled. After the removal of methanol under reduced pressure at 35°C, the extract was partitioned twice between 250 ml of 2% sodium bicarbonate at pH 8 and 250 ml of diethyl ether. The aqueous fraction was acidified to pH 2.8 with 3 N HCl and partitioned twice with equal volumes of diethyl ether. The ether extract was dried over anhydrous sodium sulphate. The solvent (ether) was completely removed by evaporation. The dried material was dissolved in 2 ml of 80% ethanol for thin layer chromatography (56).

**Thin layer chromatography**  Further separation was carried out on 20 x 20 cm fluorescent silica-gel coated TLC plates (Merck Silica gel 60 F-254, 0.25 mm thick), which were developed in n-propanol : n-butanol : ammonium hydroxide (specific gravity 0.897) : water, 6 : 2 : 1 : 2 by volume (56) for 4 hours. The solvent front line ran 14.5 cm from origin. The zone on each plate corresponding to an ABA standard marker spot (observed with ultraviolet light) was scraped from the plate and the
Silica gel was eluted with 80% ethanol for final purification.

**Paper chromatography** Final purification was carried out by ascending paper chromatography on Whatman #1 paper using isopropanol : ammonium hydroxide (specific gravity 0.897) : water, 10 : 1 : 1 by volume, for 12 hours. The developed chromatograms were cut into 10 sections corresponding to $R_f$, and eluted with 80% methanol. The methanol fractions were evaporated to dryness (117). These were assessed for inhibitory activity in the wheat coleoptile straight growth bioassay and by UV absorbance at 285 nm. The standard solution of abscisic acid (mixed isomers) was purchased from Sigma Chemical Co. St Louis, MO.

**Bioassay for abscisic acid**

Undamaged wheat grain, *Triticum aestivum* L. (variety Era), which was surface sterilized with 1.05% NaOCl for 5 minutes, were sown with embryo oriented upwards, on moist blotter paper in a polystyrene box. The box was covered with aluminum foil and kept in the dark at 22 C for 3 days. Ten coleoptile tips (10 mm in length) were cut from uniform 15 mm etiolated coleoptiles and were incubated in petri dish (60 x 15 mm) containing Whatman #1 filter paper moistened with 1 ml of the sample (eluate from chromatogram) or distilled water. All procedures with the coleoptiles were done with only green safe-light illumination. Coleoptiles were incubated at 25 C under dark for 24 hours, then the growth was measured. The mean length of the ten sections in each dish was expressed as a % of the water controls (i.e. water control = 100%) and plotted against the position of the sample from the chromatogram.
gram (101).

Saprophytic Survival and Pathogenicity of *Pythium ultimum*

**Effects of soil sterilization and soil additives on saprophytic survival of Pythium ultimum**

The soil was taken from a corn field near Ames, Iowa. The soil moisture was adjusted to near field capacity which was determined to be 9.35%. The soil was divided into 3 lots: one was amended with sucrose (1% of soil weight), the second was amended with dried corn debris (2% of soil weight) and the third was left as the nonamended control. Each soil was placed into 6 petri dishes (100 x 15 mm). Three petri dishes of each treatment were autoclaved (1 Atmos. - 90 minutes). The following day, *P. ultimum* inoculum (millet culture) was placed into all the petri dishes. Three colonized millet grains were placed on top of the soil in each petri dish and pressed lightly into the soil. The petri dishes were wrapped with polyethylene bags to retain moisture and all were incubated at 23 C. After 20 days, the **Pythium** colonies were observed under a dissecting microscope (70X). A 0.1% solution of acid fuchsin in lactophenol was added to aid in the detection of living **Pythium** mycelium. Random isolations to SPDA were made to confirm the identity of **Pythium**. If the colony could not be observed by 70X magnification, a small piece of the inoculum (a fragment of millet) was taken from the soil and observed under a compound microscope to ascertain the presence of **Pythium** mycelium in the soil around the millet grain. A subjective rating of the mycelium was made as follows: - (no mycelium),
+ (sparse mycelium), to ++++ (extensive mycelial development). The results presented are an average of 10 samples.

**Effects of soil sterilization and amounts of inoculum on Pythium ultimum pathogenicity**

Soil was taken from a corn field near Ames, Iowa. Half of the soil (about 50 kg) was autoclaved at 1 Atmos. for 90 minutes. One-liter plastic pots were filled with 1000 g (oven dry weight) of either the sterilized soil or the unsterilized soil. *P. ultimum* inoculum (millet culture) was mixed into the soil in each pot at rates of 0, 0.4, 2.0 and 10.0 g/pot. Ten maize seeds were planted in each pot and moisture was adjusted to field capacity. Three replications (randomized complete block), were placed in the growth chamber for 8 days. The plants were rated for percentage emergence, shoot height, and root damage.

Another method was tried for infestation of *P. ultimum* inoculum into unsterilized soil. Soil was taken from a corn field near Ames, and the moisture was adjusted to near field capacity. About 50 kg of the soil was autoclaved at 1 Atmos. for 90 minutes. The balance of the soil was stored in a plastic covered container at 25 C. The day following soil sterilization, *Pythium* inoculum (50 g of millet culture) was mixed uniformly with the sterilized soil (50 kg). The soil was covered with polyethylene film to maintain moisture and incubated at about 23 C for 2 weeks. The mycelium grew throughout the soil and this was labeled infested soil. The infested soil was mixed with the nonsterilized soil at the following ratios; (infested soil : nonsterilized soil) 1 : 0, 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32, and 0 : 1. The latter was only
unsterilized noninfested soil. The mixed soils were placed into the 1 liter plastic pots and 10 maize seeds were planted/pot. Three replications of each treatment were placed in the growth chamber for 7 days at 25 °C and 14 hour photoperiod. The pots were watered with tap water daily until termination of the experiment.

The Response of 17 Maize Inbred Lines to Infection by P. ultimum

Seventeen maize inbred lines, kindly provided by Dr. W. R. Russell, were tested for their response to infection by P. ultimum. The lines were A239, A619, B14A, B57, B73, B75, B76, B77, B79, B81, C123, H98, H99, PRC517, Mol7, W17, and W64A. Ten seeds of each line were planted into each pot of white-silica sand. There were 4 pots of each line. Two pots were Pythium infested and two pots were noninfested for control. They were grown in a 25 °C growth chamber with a 14 hour photocycle. After 6 days, the seedlings were washed from the sand and their germination percentage, shoot height measurements and root damage ratings were taken.
RESULTS

Relation of Root Damage to Shoot Growth

Stunting due to Pythium ultimum and surgical removal of roots

The root damage was rated into 5 classes (Fig. 1). As root damage by the fungus increased, there was less shoot growth by the plant (Fig. 2). There was no significant difference (0.05 level) in shoot growth among classes 1, 2, and 3. But there were significant differences between class 3 and class 4; and between class 4 and class 5. A slight damage by Pythium did not decrease shoot growth.

A comparison was made in shoot growth among healthy, diseased, and root pruned seedlings (Fig. 3). At 4 days, the average shoot lengths of healthy and diseased seedlings were not significantly different (0.05 level). At 7 and 10 days after planting, the shoots of healthy seedlings were significantly longer than the shoots of diseased and root pruned seedlings. At 7 days, the shoots of root pruned seedlings were significantly longer than the shoots of diseased seedlings. At 10 days, pruning the roots of seedlings caused a stunting of the shoot similar to severe Pythium damage.

Pythium infection significantly decreased both fresh weight and dry weight of the shoots when compared to healthy seedlings (Table 1).

Effects of Pythium ultimum on seedlings grown in the light and dark

The shoots of diseased seedlings grown in the light were considerably stunted when compared to the healthy seedlings. However, the
Figure 1. Root damage rating system. (broken lines indicate the damaged portion).
Figure 2. Shoot growth of 6-day-old maize seedlings grouped into 5 classes of root damage by *Pythium ultimum*. 1 = no damage, 5 = roots rotted completely. Brackets indicate confidence interval (0.05 level).
Figure 3. Shoot growth of healthy, diseased, and root pruned seedlings. Average of 10 seedlings per date for each treatment.
Table 1. Fresh weight, dry weight, and moisture content of the shoots from healthy and *Pythium*-infected maize seedlings after 6 days growth

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th><em>Pythium</em>-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight (mg)</td>
<td>629.5</td>
<td>465.5**</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>56.1</td>
<td>45.0**</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>91.07</td>
<td>90.27*</td>
</tr>
</tbody>
</table>

**Significantly less than the healthy controls (0.01 level).
*Significantly less than the healthy controls (0.05 level).

^Averages of 8 samples.
shoots of diseased seedlings grown under continuous darkness was as tall as the shoots of healthy etiolated seedlings (Fig. 4). The light and dark treatments had no effect on root rot development by *Pythium*. The stunting was restricted to the shoot growth above the first node (Table 2).

Utilization of Reserved Nutrients in Endosperm

*Alpha-amylase activity in endosperm tissue and scutellum tissue of healthy, diseased, and root pruned seedlings*

*Endosperm* The alpha-amylase activity increased similarly in both healthy and diseased seedlings until 4 days old. At 7 days, the amylase activity had decreased greatly in diseased seedlings, while the activity in healthy and root pruned seedlings decreased only slightly. At 7 days, there was a significant difference (0.05 level) between diseased and healthy seedlings, and between diseased and root pruned seedlings, but no significant difference was shown between healthy and root pruned seedlings. At 10-day-old, the amylase activity in healthy seedlings was much lower than at 7-day-old. The decrease in amylase activity of root pruned seedlings seemed to be greater than the healthy seedlings but the difference was not statistically significant. The amylase activity of *Pythium* infected seedlings remained low, significantly (0.01 level) less than healthy seedlings, but not significantly less than root-pruned seedlings (Fig. 5).
Figure 4. Healthy and diseased seedlings grown in 14 hours light daily or continuous darkness for 7 days. Dark grown seedlings are the three on right in each group.
Table 2. Shoot growth of maize seedlings after 7 days growth in infested or noninfested sand with 14 hours light daily or continuous darkness

<table>
<thead>
<tr>
<th></th>
<th>14 hours light daily</th>
<th>Continuous darkness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (mm)(^{a})</td>
<td>Diseased (mm)(^{a})</td>
</tr>
<tr>
<td>Shoot above 1st node</td>
<td>90.7</td>
<td>62.8**</td>
</tr>
<tr>
<td>Coleoptile</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mesocotyl</td>
<td>13.0</td>
<td>13.8(^{n.s.})</td>
</tr>
<tr>
<td>Whole shoot</td>
<td>103.7</td>
<td>76.6**</td>
</tr>
</tbody>
</table>

**Significantly shorter than healthy control (0.01 level).

\(^{n.s.}\)No significant difference from the healthy control.

\(^{a}\)Averages of 13 samples.
Figure 5. Alpha-amylase activity in endosperm tissue of healthy, diseased and root pruned seedlings at various seedling ages.
**Scutellum**  The amylase activity in scutellum increased similarly in both healthy and diseased seedlings until 7 days old. At 10 days, the amylase activity had decreased in both healthy and diseased seedlings (Fig. 6) The enzyme activity in scutellum tissues was not significantly affected by *Pythium* infection.

**Analysis of reducing sugar and sucrose concentration in endosperm and scutellum tissues**

Six-day-old healthy and diseased seedlings were harvested and the seeds were dissected into endosperm and scutellum tissues. Reducing sugar and sucrose analysis were made on 10 single plant replications of each tissue. The amount of reducing sugar in the endosperm tissues of healthy and diseased seedlings was essentially the same (Table 3). However, the amount of the sucrose and reducing sugar concentrations in the scutellum tissues were significantly greater from diseased than from healthy seedlings.

**Total soluble carbohydrate in endosperm and scutellum tissues of healthy, root pruned, and diseased seedlings**

The seedlings were harvested at 3, 5, and 7 days after sowing, and dissected into scutellum and endosperm tissues. The amount of soluble carbohydrate was determined by the anthrone test. Three replications of one plant each were harvested for each treatment on every sampling date. The mean total soluble carbohydrates in both tissues was lower from healthy seedlings than from either root pruned and diseased seedlings at the 5 and 7 day sampling times (Fig. 7).
Figure 6. Alpha-amylase activity in scutellum tissue of healthy and diseased seedlings at various seedling ages.
Table 3. Concentration of reducing sugars in endosperm tissues and reducing sugars and sucrose in scutellum tissues of 6-day-old healthy and diseased maize seedlings

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/seed)^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reducing sugars in endosperm</td>
<td>7.89</td>
<td>8.62^ns</td>
</tr>
<tr>
<td>Reducing sugars in scutellum</td>
<td>0.341</td>
<td>1.175**</td>
</tr>
<tr>
<td>Sucrose in scutellum</td>
<td>1.102</td>
<td>1.582*</td>
</tr>
</tbody>
</table>

**Significantly more than the healthy control (0.01 level).

*Significantly more than the healthy control (0.05 level).

^ns. No significant difference from the healthy control.

^Averages of 10 samples.
Figure 7. Concentration of soluble carbohydrate in healthy, diseased, and root pruned seedlings at various seedling ages.
Dry weight of insoluble material in the endosperm tissues of healthy and diseased seedlings

The homogenates of endosperms from healthy and diseased seedlings were centrifuged to separate the soluble portion and insoluble portion (pellet). The pellets were collected and dried at 105°C for 5 days. The amount of insoluble material in the endosperm tissue decreased more rapidly in healthy than in diseased seedlings (Table 4).

Respiration in shoots of healthy and diseased seedlings

The O₂ consumption by 3-day-old healthy and diseased seedlings was 1.36 μl/min/mg and 1.08 μl/min/mg, respectively. The consumption by 6-day-old healthy and diseased seedlings was the same for each tissue (0.63 μl/min/mg). None of the differences was significant.

Effects of Growth Regulators on Shoot Stunting

Effect of exogenous auxin on growth of stunted plants

Diseased seedlings were significantly shorter than healthy seedlings in all concentrations of IAA and in the distilled water control (Table 5). Exogenously applied IAA did not recover shoot stunting due to Pythium root rot, and auxin significantly inhibited shoot growth of both healthy and diseased seedlings at high concentration (10⁻⁵M).

Effects of exogenous gibberellin and kinetin on shoot growth of diseased and root pruned seedlings

Shoot growth of diseased seedlings with the GA₃ treatment was nearly as tall as healthy seedlings. Shoot growth of diseased and root
Table 4. Dry weight of insoluble material in endosperm tissues of healthy and diseased maize seedlings at various ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Diseased (mg)(^a)</th>
<th>Healthy (mg)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day (dry seed)</td>
<td>141.36</td>
<td>141.36</td>
</tr>
<tr>
<td>4 days</td>
<td>87.75</td>
<td>88.09(^n.s.)</td>
</tr>
<tr>
<td>7 days</td>
<td>66.90</td>
<td>47.11*</td>
</tr>
<tr>
<td>10 days</td>
<td>43.69</td>
<td>26.67**</td>
</tr>
</tbody>
</table>

\(^{**}\) Differences between healthy and diseased are significant at 0.01 level.

\(^*\) Differences between healthy and diseased are significant at 0.05 level.

\(^{n.s.}\) No significant difference from the healthy control.

\(^a\) Average of 8 samples.
Table 5. Effect of exogenous auxin on shoot growth of 4-day-old healthy and diseased maize seedlings grown in the various concentrations of auxin solutions at 27 °C for 6 days

<table>
<thead>
<tr>
<th>Auxin conc.</th>
<th>Healthy (mm)</th>
<th>Diseased (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (distilled water)</td>
<td>83.7 a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>54.0 b</td>
</tr>
<tr>
<td>10&lt;sup&gt;-7&lt;/sup&gt;M</td>
<td>76.7 a</td>
<td>55.0 b</td>
</tr>
<tr>
<td>10&lt;sup&gt;-6&lt;/sup&gt;M</td>
<td>77.0 a</td>
<td>54.7 b</td>
</tr>
<tr>
<td>10&lt;sup&gt;-5&lt;/sup&gt;M</td>
<td>62.0 b</td>
<td>40.7 c</td>
</tr>
</tbody>
</table>

<sup>1</sup>Averages of 3 samples.

<sup>2</sup>Values followed by the same letter are not significantly different (0.05 level).
pruned seedlings could not be restored with zeatin treatment alone. With the GA$$_3$$ plus zeatin treatment, the shoot growth of diseased and root pruned seedlings was between that of GA$$_3$$ treatment alone and zeatin treatment alone (Table 6).

Production of stunting by abscisic acid

The seedlings treated with 100 units of ABA were slightly stunted (Fig. 8), but the differences between the healthy control seedlings and those treated with 100 units ABA were not significant (0.05 level). The seedlings treated with 1000 units of ABA showed severe stunting and had the same foliage symptoms of poor lateral leaf expansion as did Pythium infected seedlings.

Analysis of Gibberellin Concentration

The paper chromatogram which was developed by the solvent isopropanol : ammonium hydroxide : water, was divided into 10 equal strips corresponding to R$$^f$$ values. A portion of each strip was eluted and bioassayed with lettuce seed germination to find the position containing inhibitors. Strong inhibitors were found at R$$^f$$ zones of 0 to 0.2, and 0.6 to 0.7 (Fig. 9). The quantity of GA was determined by lettuce hypocotyl bioassay. Almost the same amount of gibberellins were found in both healthy and diseased seedlings (Fig. 10). Total gibberellin concentration appearing in R$$^f$$ 0.3 to R$$^f$$ 0.6 of healthy seedling was 8.0 x 10$$^{-8}$$M in 2 ml of solution and that of diseased seedling was 8.1 x 10$$^{-8}$$M in 2 ml of solution. A gibberellin-like substance was found in diseased seedlings between R$$^f$$ 0.9 and R$$^f$$ 1.0; these could possibly be GA$_4$ and
Table 6. Effects of gibberellic acid (GA3) and zeatin treatment of 4-day-old Pythium-diseased and root-pruned maize seedlings on the subsequent shoot growth after 3 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Additional shoot growth (mm)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diseased</td>
</tr>
<tr>
<td>GA₃</td>
<td>57.25 a¹</td>
</tr>
<tr>
<td>Zeatin</td>
<td>44.0 bc</td>
</tr>
<tr>
<td>GA₃ + zeatin</td>
<td>50.75 ab</td>
</tr>
<tr>
<td>Distilled water</td>
<td>42.25 bc</td>
</tr>
</tbody>
</table>

¹Values followed by the same letter are not significantly different (0.05 level).

²Average of 4 samples.
Figure 8. Induction of stunting in healthy maize seedlings by exogenous application of abscisic acid at 100 and 1000 units.
Healthy control, Pythium-diseased and root-pruned seedlings were placed only in distilled water.
Figure 9. Lettuce seed germination assays of the first chromatogram of extracts of healthy and diseased seedlings grown with a 14 hour photocycle.
Figure 10. Lettuce hypocotyl assays of the final chromatogram of extracts of healthy and diseased seedlings grown with a 14 hour photocycle. (100% = 5.16 growth in distilled water).
GA\textsubscript{7} which move at this speed in this solvent system. Between \( R_f \) 0.7 and 0.8, the diseased seedlings showed very high concentration of a growth inhibitory material; this could be abscisic acid which normally appears at \( R_f \) 0.8 in this solvent system.

Analysis of Abscisic Acid Concentration

**ABA concentrations in seedlings grown in light**

The quantity of ABA was determined by wheat coleoptile bioassay and UV spectrophotometry. The ABA appeared at \( R_f \) 0.7 and \( R_f \) 0.8 in the final solvent system. Both diseased seedlings and root pruned seedlings contained higher concentration of ABA than healthy seedlings (Fig. 11 and Fig. 12) as determined by the wheat coleoptile bioassay. Diseased seedlings contained 2.5 times more ABA than healthy seedlings (Fig. 11) and root pruned seedlings contained about 2 times more ABA than healthy seedlings (Fig. 12). Analysis of the ABA by UV spectrophotometry showed that diseased tissues contained 11 mg ABA/g tissue and healthy tissues contained 7 mg/g tissue. The root-pruned tissues contained 16.9 mg/g tissue and the healthy tissues contained 11.8 mg/g tissue in a separate experiment.

**ABA concentrations in the seedlings grown in dark**

The quantity of ABA was determined by wheat coleoptile bioassay. There was a very small amount of inhibitor (ABA) at \( R_f \) 0.7 and \( R_f \) 0.8 from all treatments and the differences among treatments were insignificant (Fig. 13).
Figure 11. Wheat coleoptile assays of the final chromatogram of extracts of healthy and diseased seedlings grown with a 14 hour photocycle. (100% = 15.8 mm growth in distilled water).
Figure 12. Wheat coleoptile assays of the final chromatograms of extracts of healthy and root pruned seedlings grown with a 14 hour photocycle.
Figure 13. Wheat coleoptile assays of the final chromatograms of healthy, diseased, and root pruned seedlings grown under continuous darkness for 6 days.
ABA quantity in *Pythium ultimum* culture

A SA-PCNB broth medium was prepared and *P. ultimum* was cultured in 100 ml of the medium in 250 ml Erlenmeyer flasks. Two flasks in which *P. ultimum* was not added, remained as controls. The flasks were placed in a 25 C incubator for 2 weeks and then abscisic acid extraction procedure was used on these media. No abscisic acid was detectable from either the *Pythium* culture or the uninoculated control broth.

**Saprophytic Survival and Pathogenicity of *Pythium* Isolates**

**Comparision of pathogenicity among the isolates of *Pythium* spp.**

Twenty three isolations of *Pythium* spp. were made from necrotic fine roots of maize and maize field soils near Ames, Iowa. There was a broad spectrum of pathogenicity among the isolates (Table 7). The highest root damage rating and the lowest shoot emergence was due to isolate #61. The identity of isolate #61 was *Pythium ultimum*.

**Effects of soil sterilization and soil additives on saprophytic survival of *Pythium ultimum***

The results presented are an average of 10 samples (Table 8). The saprophytic growth of *P. ultimum* in unsterilized soil was very poor and survival of any mycelium in the unsterilized soil surrounding the the millet (inoculum) was not apparent except where 2% corn debris was added to the soil. The fungus grew very well in the sterilized soil.

**Effects of soil sterilization and amounts of inoculum on *Pythium ultimum* pathogenicity**
Table 7. Comparison of the pathogenicity among collected isolates of *Pythium* spp. on maize seedlings.

<table>
<thead>
<tr>
<th>Isolate Nr.</th>
<th>Root damage ratinga</th>
<th>Shoot emergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary root</td>
<td>Seminal roots</td>
</tr>
<tr>
<td></td>
<td>Root tip</td>
<td>Fine roots</td>
</tr>
<tr>
<td>check</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>25</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>26</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>27</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>29</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>37</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>44</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>45</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>46</td>
<td>1.7</td>
<td>2.6</td>
</tr>
<tr>
<td>47</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>48</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>49</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>50</td>
<td>1.5</td>
<td>2.3</td>
</tr>
<tr>
<td>52</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>54</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>60</td>
<td>1.8</td>
<td>2.6</td>
</tr>
</tbody>
</table>

aRoot damage rating: 1=healthy, 5=severe root rot.
Table 7. (continued)

<table>
<thead>
<tr>
<th>Isolate Nr.</th>
<th>Root damage rating&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Shoot emergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary root</td>
<td>Seminal roots</td>
</tr>
<tr>
<td></td>
<td>Root tip</td>
<td>Fine root</td>
</tr>
<tr>
<td>61</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>64</td>
<td>2.3</td>
<td>3.3</td>
</tr>
<tr>
<td>66</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>68</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>79</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table 8. Effects of soil sterilization and soil amendments on saprophytic growth and survival of *P. ultimum*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Density of <em>Pythium</em> hyphae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Survival (%) of <em>Pythium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unsterilized soil</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no amendment</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>1% sucrose</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>2% debris</td>
<td>+</td>
<td>55</td>
</tr>
<tr>
<td><strong>Sterilized soil</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no amendment</td>
<td>++</td>
<td>91</td>
</tr>
<tr>
<td>1% sucrose</td>
<td>+++</td>
<td>100</td>
</tr>
<tr>
<td>2% debris</td>
<td>+++</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> - no mycelium present, + mycelium sparse, to ++++ extensive mycelial development.
There was a significant difference in shoot growth, root damage and shoot emergence, between sterilized soils and unsterilized soils, and among the levels of inoculum (Table 9). In the sterilized soil, minute amounts of inoculum caused severe damage to the seedlings. In unsterilized soil large amount of inoculum were required to induce any significant damage to the seedlings. Root damage and reduced shoot growth was very obvious in sterilized soil, when small amounts of *Pythium* inoculum were added to the soil. When 0.4 g of inoculum was mixed into sterilized soil, only 21% of seedlings were healthy and 38% of the seeds rotted. But in unsterilized soil, at the 0.4 g level of inoculum, 67% of the seedlings were healthy and only 8% of the seeds rotted. When 2 g of inoculum was added into sterilized soil, no healthy seedlings were found and 58% of the seeds rotted. However, in unsterilized soil at the 2 g level of inoculum, 27% of seedlings were healthy and no seed rot was noticed. Under heavy inoculum dosages (10 g of inoculum/pot), all seedlings in both sterilized and unsterilized soils were severely damaged and 50% and 53% seed rot, respectively, were found.

In a separate experiment, sterilized soil was infested with *Pythium ultimum* for 2 weeks and then this was mixed with unsterilized soil at various ratios. The infested soil, even at the lowest level of infestation, reduced shoot growth and induced severe root damage (Table 10). Unfortunately, damage in the control (unsterilized) soil was high. This appeared to be an excellent procedure for infesting natural soil and demonstrated that the disease observed in sand could be reproduced under more natural conditions.
Table 9. Effect of soil sterilization and amount of *Pythium* inoculum on maize damage

<table>
<thead>
<tr>
<th>Inoculum dosage (g inoculum/1 kg soil)</th>
<th>% shoot emergence</th>
<th>shoot length (mm)</th>
<th>Root damage rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilized soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>76.7 n.s.</td>
<td>96.7 a</td>
<td>1.9 a</td>
</tr>
<tr>
<td>0.4</td>
<td>50.0</td>
<td>59.1 bc</td>
<td>3.5 bc</td>
</tr>
<tr>
<td>2.0</td>
<td>33.3</td>
<td>15.6 c</td>
<td>4.4 c</td>
</tr>
<tr>
<td>10.0</td>
<td>40.0</td>
<td>25.0 c</td>
<td>4.3 c</td>
</tr>
<tr>
<td>Unsterilized soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>80.0</td>
<td>86.7 ab</td>
<td>1.8 a</td>
</tr>
<tr>
<td>0.4</td>
<td>73.3</td>
<td>89.3 a</td>
<td>2.4 ab</td>
</tr>
<tr>
<td>2.0</td>
<td>86.7</td>
<td>95.7 a</td>
<td>2.3 a</td>
</tr>
<tr>
<td>10.0</td>
<td>43.3</td>
<td>47.7 b</td>
<td>3.7 b</td>
</tr>
</tbody>
</table>

n.s. No significant difference among treatments.

1 Values followed by the same letter are not statistically different (0.05 level).

2 Averages of 3 replications.
Table 10. Effect of *P. ultimum* inoculum density in soil on maize seedling growth and root rot.

<table>
<thead>
<tr>
<th>Ratio of infested soil : noninfested soil</th>
<th>Shoot length (mm)</th>
<th>Root damage rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 : 1</td>
<td>84.5 a(^1)</td>
<td>2.9 a(^1)</td>
</tr>
<tr>
<td>1 : 32</td>
<td>58.9 b</td>
<td>4.4 a; b</td>
</tr>
<tr>
<td>1 : 16</td>
<td>47.7 b</td>
<td>5.0 a, b, c</td>
</tr>
<tr>
<td>1 : 8</td>
<td>53.0 b</td>
<td>5.2 a, b, c</td>
</tr>
<tr>
<td>1 : 4</td>
<td>40.8 b</td>
<td>5.1 a, b, c</td>
</tr>
<tr>
<td>1 : 2</td>
<td>0.0 c</td>
<td>5.9 b, c</td>
</tr>
<tr>
<td>1 : 0</td>
<td>0.0 c</td>
<td>6.0 c</td>
</tr>
</tbody>
</table>

\(^1\)Values followed by the same letter in the column are not significantly different (0.05 level).

\(^2\)Averages of 3 replications.
The Response of 17 Maize Inbred Lines to Infection by *P. ultimum*

All lines except B77 were stunted when their roots were infected by *P. ultimum* (Table 11). Lines B77, A239, H99, B73 and H98 were classified as resistant; B57, B75, W64A, A619, B79 and Mo17 were classified as moderately susceptible; Bl4A, B81, B76, W17, PRC517 and C123 were classified as susceptible. The inbred line W64A was used in all previous experiments.
Table 11. Response of 17 maize inbred lines to *Pythium ultimum*

<table>
<thead>
<tr>
<th>Line</th>
<th>Height (% of control)</th>
<th>Stand (%)</th>
<th>Stand (% of control)</th>
<th>Root damage&lt;sup&gt;a&lt;/sup&gt; &lt;br&gt;rating&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B77</td>
<td>107.0</td>
<td>100</td>
<td>100</td>
<td>1.8 (1.2)</td>
</tr>
<tr>
<td>A239</td>
<td>92.5</td>
<td>100</td>
<td>100</td>
<td>3.0 (1.8)</td>
</tr>
<tr>
<td>H99</td>
<td>88.9</td>
<td>85</td>
<td>100</td>
<td>2.5 (1.6)</td>
</tr>
<tr>
<td>B73</td>
<td>85.7</td>
<td>75</td>
<td>83.3</td>
<td>2.5 (1.1)</td>
</tr>
<tr>
<td>H98</td>
<td>85.3</td>
<td>95</td>
<td>100</td>
<td>3.4 (2.1)</td>
</tr>
<tr>
<td>B57</td>
<td>83.0</td>
<td>80</td>
<td>94.1</td>
<td>4.1 (1.7)</td>
</tr>
<tr>
<td>B75</td>
<td>81.8</td>
<td>65</td>
<td>68.4</td>
<td>4.0 (2.2)</td>
</tr>
<tr>
<td>W64A</td>
<td>79.2</td>
<td>80</td>
<td>89.5</td>
<td>2.6 (1.3)</td>
</tr>
<tr>
<td>A619</td>
<td>74.5</td>
<td>85</td>
<td>95.0</td>
<td>3.3 (1.4)</td>
</tr>
<tr>
<td>B79</td>
<td>73.4</td>
<td>65</td>
<td>77.8</td>
<td>4.1 (2.7)</td>
</tr>
<tr>
<td>Mo17</td>
<td>71.5</td>
<td>70</td>
<td>86.6</td>
<td>4.5 (3.4)</td>
</tr>
<tr>
<td>B14A</td>
<td>65.6</td>
<td>80</td>
<td>88.2</td>
<td>4.5 (3.1)</td>
</tr>
<tr>
<td>B81</td>
<td>64.8</td>
<td>30</td>
<td>75.0</td>
<td>5.4 (4.3)</td>
</tr>
<tr>
<td>B76</td>
<td>63.5</td>
<td>70</td>
<td>70.0</td>
<td>3.7 (1.2)</td>
</tr>
<tr>
<td>W17</td>
<td>63.0</td>
<td>80</td>
<td>84.2</td>
<td>3.6 (1.4)</td>
</tr>
<tr>
<td>PRC517</td>
<td>62.1</td>
<td>60</td>
<td>66.7</td>
<td>4.4 (2.3)</td>
</tr>
<tr>
<td>C123</td>
<td>46.8</td>
<td>50</td>
<td>52.6</td>
<td>4.7 (1.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Root damage rating - 1=healthy, 5=severe root rot, and 6=seed rot.

<sup>b</sup>() indicates root condition in noninfested sand (control).
DISCUSSION

Many workers have reported that seedlings damaged by *Pythium* sp. exhibit a stunting of top growth (7, 37, 44, 53, 65, 116). The present data indicate that the shoot stunting was associated with high root disease ratings (Fig. 2). The possibility exists that the estimates of root damage can be made by shoot growth measurement. But, inhibition of plant growth is affected by many factors, e.g. microclimate, nutrient and water availability and distribution, presence of toxic materials, and soil physical factors. Without effective controls, it would be difficult to estimate disease severity in the field.

The shoot stunting is apparently a general symptom of *Pythium* root infection. Four hypotheses were put forth to explain the stunting phenomenon. 1) Stunting is due to a lack of exogenous nutrient and water uptake, 2) stunting is due to insufficient solubilization of reserve nutrients, 3) stunting results from the production of toxic materials by *Pythium*, and 4) stunting is the result of a hormonal imbalance due to root damage.

If the stunting is due to a lack of nutrient uptake by the necrotic root system, the shoot growth of healthy and diseased seedlings grown in distilled water should be nearly equal. Distilled water cannot supply nutrients to the healthy or diseased seedlings. Nevertheless, the shoots of healthy seedlings grown in distilled water were significantly taller than those of diseased seedlings (Table 5). The stunting even became noticeable in 4-day-old seedlings. At that early stage of development, most nutrients required for shoot development are reserved
in the endosperm. These results do not support the hypothesis that the stunting is due to poor nutrient uptake by the necrotic root system.

The second hypothesis poses the question of whether or not the reserve materials in the endosperm can be transformed into soluble forms for equal mobility in healthy and diseased seedlings. The reserve carbohydrate in maize endosperm is immobile starch. The starch must be hydrolyzed by alpha- and/or beta-amylase to the simple sugar glucose (20). The glucose is absorbed by the scutellum (21, 115), and simultaneously is converted to sucrose in scutellum. Sucrose is the form of sugar transported to the growing tissues (21).

To test the second hypothesis, I monitored the activity of alpha-amylase and the concentration of total soluble carbohydrate, reducing sugar and sucrose in endosperm and scutellum tissues of healthy and diseased seedlings. With 4 day old seedlings, even when the root damage was definitely noticeable, there was no significant difference between healthy and diseased seedlings in shoot growth (Fig. 3), activity of alpha-amylase (Fig. 5), and amount of soluble carbohydrate (Fig. 7) in endosperm tissue. It is assumed that at this stage, the young seedlings did not respond to the Pythium ultimum infection, because most metabolic activities such as synthesis of enzymes, and hydrolysis of reserved materials, occurred before infection. After 7 days, the shoot growth and alpha-amylase activities in the endosperm of diseased and root pruned seedlings were significantly reduced in comparison with healthy seedlings (Fig. 5), but the enzyme activity in the scutellum was not reduced either in healthy seedlings or in diseased seedlings.
Dure (20) reported that alpha-amylase which originated exclusively in scutellum of maize accounted for 9/10 of the amylolytic activity found in the endosperm at the peak of amylolytic activity. But Goldstein and Jennings (29) reported that the development of alpha-amylase activity in de-embryonated maize kernels was independent of an embryo or an exogenous source of gibberellic acid. The present results indicate the alpha-amylase activity in endosperm is very sensitive to Pythium infection (Fig. 5), but the enzyme activity in scutellum is not (Fig. 6). It may be assumed that the nature of alpha-amylase from endosperm and that of scutellum differ. The enzyme in the endosperm may be very sensitive to ABA as described by Chrispeels and Varner (16, 17). The infected seedling produced excessive amount of ABA (Fig. 11) which affected enzyme activity in endosperm and decreased the activity. There is no literature describing the sensitivity of scutellum originated alpha-amylase to ABA. Thus, it is difficult to explain why the enzyme activity in scutellum did not decrease after infection. Possibly the enzyme in the scutellum may not be affected by ABA. Maize seed possibly has 2 isoenzymes of alpha-amylase, one ABA sensitive alpha-amylase which originates from endosperm, and another ABA insensitive one which originates from the scutellum. This hypothesis should be studied further.

The amounts of the total soluble carbohydrate, reducing sugar and sucrose in the scutellum and endosperm of diseased and root-pruned seedlings were significantly higher than those in the scutellum and endosperm of healthy seedlings (Fig. 7, Table 3). These results
indicate that the diseased and root-pruned seedlings have as much mobilized available nutrient as healthy seedlings. Also, the dry weight of insoluble materials in the endosperm of healthy seedlings decreased more rapidly than that in diseased seedlings (Table 4). It is assumed the higher accumulation of soluble sugars in the endosperm and scutellum of diseased seedlings and relatively slower decrease of reserve insoluble material in endosperm of diseased seedlings resulted from a slower consumption of sugars by slowly growing stunted shoots. The healthy shoot needed more sugars to build cell walls and protoplasm, than stunted diseased plants. Stunting is not likely due to a lack of solubilization and mobilization of reserve materials in endosperm of diseased seedlings. The diseased seedlings did not show nutrient deficient symptoms or water stress wilting. The results are unfavorable to support the second nutrient hypothesis.

The possibility of toxin production by *P. ultimum* was also tested. As described by Wilhelm (116), Brandenber (1950) described a stunting phenomenon in sugar-beet plant which was due to a proteinaceous toxin by *Pythium*. Because the process of root-pruning duplicated the foliar symptoms of *Pythium* injury, it is difficult to envision that a toxin is involved (Fig. 3). A toxin could be involved in the induction of root necrosis, but there is no evidence to support a hypothesis that stunting is due to a toxin. Also, no ABA-like growth inhibitors were detected from the *P. ultimum* cultural medium. In several unreported exploratory experiments, I failed to detect stunting materials from crude culture filtrates. These results are unfavorable to support the toxin hypothesis.
The diseased seedlings were treated with growth promotors which are kinetin (zeatin), IAA and GA$_3$. IAA did not stimulate shoot growth at all (Table 5). At a high concentration (above $10^{-6}$M), IAA inhibited shoot growth. This is same phenomenon as Thimann (102) described for IAA. Since auxins are synthesized in meristemic and enlarging tissues (59), root damage probably does not affect auxin synthesis and stunting is not due to a deficiency of free auxins in diseased seedlings. The possibility still exists that stunting is a result of hyperauxinity.

Zeatin ($10^{-6}$M) was supplied to diseased seedlings. This treatment increased shoot growth slightly in diseased and root pruned seedlings. But the differences in shoot growth were not significant between the zeatin treated seedlings and the control seedlings which were grown in the absence of exogenous zeatin (Table 6).

GA$_3$ ($10^{-6}$M) treatment restored shoot growth completely (Table 6). When GA$_3$ and zeatin ($10^{-6}$M) both were applied to either diseased or root pruned seedlings, the shoot growth of the seedlings was intermediate between the results obtained from separate GA$_3$ and zeatin treatments. No synergistic effects were found. By the above results, it is possible to conclude that the stunting is due to lack of GA, as described by Russell and Kimmins (91) in barley infected with BYDV, and Bailiss (3) in cucumber infected with cucumber mosaic virus.

GA was extracted from healthy and diseased seedlings. Diseased seedlings had almost an equal amount of GA as healthy seedlings. Both healthy and diseased seedlings showed high GA activity at $R_f$ 0.4 and $R_f$ 0.5, but the diseased seedlings showed high GA activity at $R_f$ 1.0.
on the chromatogram (Fig. 10). The diseased seedling possessed very high growth inhibitor activity between $R_f$ 0.7 and $R_f$ 0.8 on the chromatogram developed for GA purification. The present data showed the total amounts of GA in shoots of diseased and healthy seedlings are about the same, but the quality of the GA's differ between diseased and healthy seedlings. Similar results were obtained by Crozier and Reid (18).

They found that removal of root apices from light-grown Phaseolus coccineus L. seedlings inhibited shoot growth. The major GA in control plants was $GA_1$ and traces of $GA_{19}$ were detected. Removal of root apices resulted in the disappearance of $GA_1$ and the accumulation of $GA_{19}$ in the leaves and apical buds and in the subapical root remnants. Based on the data they proposed a scheme that the shoot was the site of synthesis of $GA_{19}$, which moved to the root where it was converted to $GA_1$, an active form, which in turn was exported to the shoot. They did not consider the possibility that root-pruned plants may have produced excessive amounts of ABA. My present data indicate that root-pruned and diseased seedlings have more ABA than healthy seedlings (Fig. 11, Fig. 12). Bown et al. (10) showed that dark-grown Phaseolus coccineus seedlings contained larger GA pools than light-grown seedlings, and concluded that the capacity of seedlings to metabolize $GA_4$ was greater in the light than in dark. My present data (Fig. 13) indicate that the plants grown under dark did not produce ABA. Light stimulates or is necessary for the synthesis of ABA (67, 85, 101). It has been reported that ABA stimulates the uptake and metabolism of the $GA_1$. The $GA_1$-metabolites which are $GA_2$, $GA_3$-glucoside and $GA-X$ (not identified), are
unable to stimulate alpha-amylase activity (77). I think the disappearance of \( \text{GA}_1 \) and accumulation \( \text{GA}_{19} \) in the leaf and apical buds of root-pruned seedlings, which was the result obtained by Crozier and Reid (19), was not due to removal of the interconversion site (root), but due to the production of excessive amounts of ABA in root-pruned seedlings. The ABA stimulates the metabolic activity of GA, which results in the reduction of active \( \text{GA}_1 \) and the accumulation of inactive \( \text{GA}_{19} \) in shoot. Also, this could be the reason why diseased and healthy seedlings had different amounts of different GA species in my experiments.

It has not been determined with certainty whether the site of GA biosynthesis is located in the roots or in the stems and leaves. Reid et al. (88) reported barley leaves could synthesize gibberellins and Hoad and Bowen (33) also described shoot synthesized GA's. But Carr et al. (14) and Butcher (12) demonstrated that the roots were the sites of GA production. Since my present data showed no difference in total amounts of GA's between healthy and root diseased seedlings, the root of maize probably is not the sole site of GA biosynthesis.

ABA was extracted from healthy, diseased and root pruned seedlings. The diseased and root-pruned seedlings contained much more ABA than healthy seedlings (Fig. 11, Fig. 12). Seedlings were grown under complete darkness and even though the roots were severely damaged by \( \text{P. ultimum} \), the shoots of the diseased etiolated seedlings were not stunted (Fig. 4, Table 2). The healthy, diseased, and root-pruned, etiolated plants had minute ABA activity. This is in agreement with reports (67, 85) that light is required for ABA synthesis. ABA was
applied to healthy seedlings and at the 1000 unit concentration, stunting was noticed (Fig. 8). The treated healthy seedlings had the same foliar symptoms of poor leaf expansion as *P. ultimum* infected seedlings. These results support a hypothesis that the stunting is due to excessive amounts of a growth inhibitor, probably ABA. Root damaged seedlings produce excessive amounts of ABA in the light and the excessive ABA produces stunted plants.

Some articles reported that the stunted plants contained low amounts of endogenous GA (3, 91). The authors discarded and lost most growth inhibitors during purification of the gibberellins and disregarded the possible activity of growth inhibitors. Without consideration of growth inhibitors, it is hard to conclude that the reason for the stunting is the reduction of endogenous GA. The bioassay of lettuce seed germination showed that the crude extract of stunted seedlings showed high levels of growth inhibitors (Fig. 9). But the results of final chromatography for GA purification indicated that most of the inhibitors were removed during the processes of chromatography (Fig. 10).

GA application to stunted diseased plants has restored the plant growth (39, 78, 99). The same phenomenon was observed in this study. Diseased seedlings treated with GA₃ grew as tall as healthy seedlings (Table 6). However, healthy and diseased seedlings had the same total amount of GA's. The exogenous application of GA₃ probably stimulated shoot growth via antagonism or competition with ABA in the diseased plants. Another evidence of an involvement of ABA is the decreased alpha-amylase activity in the endosperm of diseased seedlings (Fig. 5).
Chrispeels and Varner (16) reported, that ABA inhibited the production of alpha-amylase in endosperm which was triggered by GA.

The teliological question is raised, "Why do diseased plants produce excessive amounts of ABA and what is the role of ABA?"

A few papers have described that ABA synthesis is stimulated by unfavorable conditions for plant growth such as water stress (96, 117), mineral deprivation (9), salination (9, 74), $\text{BO}_3^{--}$ toxicity (9), salt-stress (71), low temperature (42, 43, 87) and infection of pathogens (83, 98, 107). Some papers (8, 97) also described that ABA inhibited spore germination of some species of fungi. It could be assumed that ABA is a physiological trigger for protection of plants against the adverse conditions and against invasion of pathogens. The possible regulation of phenolics might be extended to ABA metabolism since Walton and Sondheimer (111) demonstrated that ABA stimulated the activity of phenylalanine-ammonia-lyase in bean plants. This is the key enzyme for the synthesis of trans-cinnamic acid, which eventually turns to various phenols like scopoletin and caffeic acid and various phytoalexins like pisatin, phaseollin, and glyceollin. The possibility that ABA might be involved in or regulate the synthesis of phytoalexins in infected plants should be studied further.

My present hypothesis is that the stunting of young maize seedlings following severe root infection by *Pythium ultimum* is primarily due to the production of excessive amounts of ABA by the plant and this hormonal imbalance induces seedling stunting.
Pythium ultimum showed very low pathogenicity in natural soil in many early tests. It seems that the pathogenicity or activity of the pathogen was greatly decreased by soil antagonism. Many papers (11, 46, 63) have showed the presence of antagonistic microorganisms in soil. Our results showed that the fungus had low ability of saprophytic survival in natural soil (Table 8) in the absence of a substrate for colonization. In natural soil, a large amount of inoculum was required to develop root disease (Table 9). It is a commonly held belief that when a pathogenic fungus is grown in artificial media for many successive generations, the fungus tends to lose pathogenicity. Throughout this study, isolate #61 of P. ultimum did not lose its ability to cause disease in natural soil systems like sand and sterile soil. The use of infested soil as inoculum was helpful to demonstrate that P. ultimum is virulent in a natural soil system. Pythium growth through soil is known to be affected by soil fungistasis (54). Thus, the infested soil inoculum provided for a more diffuse dispersal of Pythium inoculum than could be obtained with millet seed.

The susceptibility of maize inbred lines to Pythium ultimum was variable. Inbred lines B77, A239, M99, B73 and H98 were resistant, B57, B75, W64A, A619, B79 and Mo17 were intermediate, and B14A, B31, B76, PRC517 and C123 were susceptible (Table 11). Erwin and Cameron (22) showed the variance of susceptibility of sweet corn lines to P. graminicola, and Hooker (35, 36) showed the difference of susceptibility of field corn lines to P. graminicola, and P. debaryanum. The present data showed all maize lines, except B77, were stunted by Pythium infec-
tion, and the importance and possibility of producing lines to control *Pythium* root rot.
Maize seedlings with severe root rot incited by *Pythium ultimum*, become stunted. Four hypotheses were put forth to explain the stunting phenomenon: 1) a lack of exogenous nutrient and water due to a reduction in root absorption surface, 2) less solubilization or mobilization of reserve nutrient in endosperm tissues of diseased seedlings than that of healthy seedlings, 3) a toxin produced by *P. ultimum*, 4) a hormonal imbalance in the infected plants.

The shoots of healthy maize seedlings grown in distilled water were significantly taller than those of diseased seedlings. This result did not support the hypothesis that the stunting was due to poor nutrient uptake by the necrotic root system.

The amounts of the total soluble carbohydrate in the scutellum and endosperm of diseased and root-pruned seedlings were significantly higher than those in the scutellum and endosperm of healthy seedlings. The amount of reducing sugar in the endosperm of healthy and diseased seedlings was nearly the same. The sucrose and reducing sugar concentrations in the scutellum were lower in the healthy seedlings than diseased seedlings. These data support the conclusion that the activity in the growing tissues was such that it depleted the concentration of sugar in the healthy seed. Alpha-amylase activity in diseased tissues was less in endosperm than in the scutellum. These data do not support the second hypothesis.
Because the process of root-pruning duplicated the foliar symptoms of *P. ultimum* injury, it is difficult to envision that a toxin was involved. Also, no growth inhibitors were detected from *P. ultimum* cultural media.

Exogenously applied GA₃ at 10⁻⁶ M concentration restored shoot growth of diseased seedlings, but IAA and zeatin did not restore shoot growth. The total amounts of GA in shoots of diseased and healthy seedlings were about the same, but the quality of the GA's differed between diseased and healthy seedlings. The diseased and root-pruned seedlings contained about 2X more ABA than healthy seedlings. Seedlings were grown under complete darkness and even though the roots were severely damaged by *P. ultimum*, the shoots of the diseased etiolated seedlings were not stunted. The healthy, diseased, and root-pruned etiolated seedlings contained little ABA activity. ABA applied to healthy seedlings at a 1000 unit concentration caused stunting of the healthy seedlings and the same foliar symptoms of poor leaf expansion as *P. ultimum* infected seedlings. The results support a hypothesis that the stunting is due to a growth hormone imbalance and is most likely caused by excessive amounts of growth inhibitor, probably ABA.

*P. ultimum* had low saprophytic survival ability in natural soil in the absence of a substrate for colonization. In natural soil, a large amount of inoculum was required to develop root disease.

The susceptibility of maize inbred lines to stunting and root necrosis caused by *P. ultimum* was variable. Inbred lines B77, A239, M99, B73 and H98 were judged as resistant, B57, B75, W64A, A619, B79 and Mo17 were
intermediate, and B14A, B81, B76, PRC517 and C123 were susceptible to 
P. ultimum.
LITERATURE CITED


*de novo* synthesis of enzymes: Density labeling with H_{2}O^{18} of barley 


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