Abstracts of the 1972 Annual Meeting of the North Central Division of The American Phytopathological Society

Tobacco mosaic virus synthesis in a "hypersensitive" tobacco tissue culture. R. N. BEACHY & H. H. MURAKISHI (Michigan State Univ., East Lansing). Cultured cells of *Nicotiana tabacum Xanthi-nc* were inoculated with tobacco mosaic virus (TMV) and incubated on agar medium. At 12-hr intervals, cell samples were transferred to liquid medium containing ^{3}H-uridine and/or ^{14}C-leucine and incubated for 12 hr. Samples were then homogenized, and the virus was purified by differential and sucrose density-gradient centrifugations. Radioactivity in 1-ml fractions was determined by acid precipitation and liquid scintillation. Increases in extractable TMV, ^{3}H-uridine, and ^{14}C-leucine incorporation into complete virus were evident 24 to 36 hr after inoculation; rates of syntheses increased until 60-72 hr after inoculation. A decreasing rate of radioactivity incorporated into complete virus at 84-96 hr was followed by a second large increase in virus syntheses. Thereafter, the rate of virus syntheses again decreased to a low level, which was maintained. Symptoms comparable to local lesions were evident 40 hr after inoculation; more than 90% of the extractable virus was synthesized after this time.

Soil fungistasis: a possible mechanism for the inhibition of nutrient-independent propagules. P. R. BRISTOW & J. L. LOCKWOOD (Mich. State Univ., East Lansing). Germination of nutrient-independent propagules of several fungi is suppressed in soil or in a model system designed to imitate the soil energy source sink. The model system imposes a nutrient stress by leaching sand or glass beads upon which membrane filters bearing the propagules are incubated. Conidia of *Curvularia lunata* and sclerotia of *Sclerotium cepivorum* were collected from potato-dextrose agar amended with ^{14}C-glucose. Approximately twice the amount of radioactivity was lost from washed propagules of these two fungi incubated under leaching as under nonleaching conditions. Exudate collected from conidia of *C. lunata* contained anthrone- and ninhydrin-positive materials. Conidia of *Helminthosporium sativum, H. victoriae*, and *C. lunata* germinated to a greater extent when leached with exudate from *C. lunata* or a synthetic exudate composed of glucose and casein hydrolysate than with buffer solution. Nutrient-independent propagules contain nonspecific germination stimulants in their exudates which may be rendered unavailable to the propagules because of microbial competition in soil, resulting in fungistasis.

Effect of different concentrations of carbohydrates, amino acids, and growth substances on spore germination of Botrytis cinerea. L. G. CHOU (Ohio Agr. Res. Dev. Center, Ohio State Univ., Wooster). Standard conditions for germination of *Botrytis cinerea* were developed and used to evaluate the effect of leaf leachates from various tomato cultivars on *B. cinerea* spore germination in relation to pathogenicity. Spores of *B. cinerea* germinated in solutions containing 100 ppm, or more, of carbohydrates (glucose, fructose, or sucrose), but failed to germinate in distilled water or in distilled water containing amino acids (DL-aspartic acid or DL-glutamic acid) or growth substances (gibberellic acid, indole-3-acetic acid, or kinetin) alone or in combination. The maximum percentage germination (about 70%) occurred after 20-hr incubation at 23 C in a sucrose solution. Neither amino acid, any growth substance, nor any amino acid-growth substance combination appreciably altered the percentage germination when added to the sucrose solution.

Characteristics of a host-specific toxin produced by Phyllosticta maydis. J. C. COMSTOCK, C. A. MARTINSON, & B. G. GENGENBACH (Iowa State Univ., Ames, Univ. Ill., Urbana). A toxin with activity similar to *Helminthosporium maydis* race T toxin was produced by *Phyllosticta maydis* grown on modified Fries medium plus 0.1% yeast extract. Culture filtrates were concentrated in vacuo, methanol was added, and the precipitate and methanol were then removed to give a stock preparation one-twentyfifth the original filtrate volume. Toxin injected into leaf whorl tissues of corn inbreds induced chlorosis in Texas male sterile plants (Tcms) at a 1:4,000 dilution of the stock preparation, and in normal (N) cytoplasm plants at a 1:25 dilution. Inbred WF9 sometimes showed a greater sensitivity than other N inbreds with certain preparations. Chlorosis was induced in other plant species, but only at a toxin concentration that affected N corn. Toxin treatment inhibited root growth by 50% in Tcms and N seedlings at 1:1,000 and 1:25 dilutions, respectively. Toxin treatment of isolated mitochondria from Tcms corn stimulated or inhibited O_2 uptake depending on substrate being oxidized, caused swelling, and uncoupled oxidative phosphorylation. The effects were detected in W64A Tcms mitochondria at a toxin dilution 1:40,000; W64A (N) mitochondria were unaffected at a dilution of 1:40. A 4-hr toxin treatment caused a selective increased loss of electrolytes from Tcms corn leaves.

Epidemiology of white mold in western Nebraska. G. E. COOK, J. R. STEADMAN, & E. D. KERR (Univ. Nebraska, Lincoln). White mold of field beans caused by *Sclerotinia sclerotiorum* has become a serious problem in western Nebraska in recent years. Conservative estimates of yield loss averaged 9% in 1970 and 1971. Results from recent studies indicate that sclerotia are a primary source of inoculum in western Nebraska. Sclerotia overwintered for more than 1 year in the soil. In the greenhouse, white mold developed on leaves and stems in contact with sclerotia on the soil surface. Field observations indicate that initial infections of leaves and stems may result from contact of these organs with sclerotial mycelium on the soil surface. Ascospores of *S. sclerotiorum* may initiate infection in the plant canopy. Senescent leaves, flowers, and cotyledons of potted plants placed in the greenhouse on soil containing mature apothecia became infected with white mold within 2 weeks. Colonization of senescent tissue by the fungus provides a source of inoculum for secondary spread.

Virulence of biochemical mutants of Pseudomonas solanacearum. D. L. COPLIN, L. SEQUEIRA, & R. S. HANSON (Univ. Wis., Madison). The virulence of tryptophan auxotrophs of *Pseudomonas solanacearum* K60 was significantly lower than that of the wild type when inoculated on Bottom Special tobacco and Bonny Best tomato. These auxotrophs persisted at the site of inoculation for several weeks, but did not cause systemic infection. Auxotrophs for methionine and leucine biosynthesis, however, were virulent on tobacco and weakly virulent on tomato. The particular amino acid requirement of each auxotroph was determined in vitro and compared with levels of these amino acids in xylem sap collected from decapitated plants. Tobacco sap contained sufficient concentrations of required amino acids to support 0, 53, and 54% maximum growth in vitro of the try^{-}, met^{-}, and leu^{-} mutants, respectively. Tryptophan, methionine, and leucine levels in tomato sap supported 0, 3, and 13% maximum growth of the respective mutants in vitro. The virulence of tryptophan auxotrophs was restored by genetic transformation to

prototrophy and by supplying tryptophan to inoculated tobacco seedlings. The differences in virulence of the mutants to tobacco and tomato can be explained on the basis of growth limitations imposed by concentrations of required nutrients in vivo.

Variation among isolates of Macrophomina phaseoli (Rhizoctonia bataticola) from the same soybean plant. O. D. DHINGRA & J. B. SINCLAIR (Univ. Ill., Urbana). Single hyphal-tip isolates were made from 15 cultures of *Macrophomina phaseoli* isolated separately from roots, stem, petiole, pod, and seed of three field-grown soybean plants. They differed in colony type, in vitro growth rate, sclerotial size, and ability to cause soybean seedling blight. Root isolates from the three plants caused 80-100% death of wound-inoculated seedlings within 10 days, or severe rosetting on surviving seedlings; pod isolates caused 10-20% mortality; and remaining isolates were intermediate. After 4 days, sclerotia produced by root isolates averaged 112-188 × 77-127 μ; and stem isolates, 84-100 × 60-71 μ. Growth rate and colony type varied among isolates from the same plant and between isolates from different plants on different media (commercial potato-dextrose agar [PDA], nutrient agar, and soybean-seed-extract agar) and within different incubation temperatures (15, 20, 25, 30, 35 C). At 30 C on PDA, stem isolates produced fluffy growth, root isolates were partially fluffy, and all other isolates were appressed. The most growth was at 35 C for pod and seed isolates and two isolates from root, stem, and petiole; and 30 C was optimum for the remaining three. Each isolate performed consistently through three trials.

Increased snow mold of winter wheat after fall clipping. D. M. HUBER, B. J. HANKINS, & G. E. SHANER (Purdue Univ., Lafayette, Ind.). Early seeding and a prolonged autumn resulted in extensive growth of winter wheat (30-45 cm). Since heavy foliage favors snow mold, plots were established to determine the effect of late fall clipping on disease severity. Wheat at several locations in Indiana was clipped with a rotary mower to within 10 cm of the ground during late November 1971. Clippings (ca. 1,000 kg/hectare with the most growth) were either left on the plots or removed. Snow mold, caused by *Typhula* sp. and *Fusarium nivale*, was more severe on clipped wheat (49% of the plants killed), especially where clippings remained on the plot (62% of the plants killed), than on nonclipped wheat (7% of the plants killed). Spring vigor was greatest for nonclipped wheat (index of 4 on a 0-5 scale). Clipped plots were less vigorous (2.5), especially where clippings were not removed from the plots (2.0). Snow mold was severe on commercial wheat sown before 25 September in northern Indiana, especially in fields that were mowed. Although considerable regrowth of molded plants occurred, plants were chlorotic and stunted in comparison with later seedings. Later seedings (15-20 cm of fall growth) failed to develop snow mold even when clipped.

Cellulolytic and pectolytic enzymes produced by Helminthosporium maydis Nisikado & Miyake (Race T). P. O. LARSEN & R. M. RIEDEL (Ohio State Univ., Columbus). Filtrates from *Helminthosporium maydis* cultures and extracts from healthy and diseased Texas male sterile (Tms) and normal (N) cytoplasm corn were assayed for cellulolytic and pectolytic enzymes. Culture filtrates for enzyme assays were obtained from *H. maydis* (race T) shake cultures grown for 10 days on decoction of potato supplemented with 1.0% glucose and 0.1% sodium carboxymethyl cellulose (CMC) or 0.1% pectin N.F. Plant extracts were obtained from distilled water triturates of healthy and diseased corn leaf tissue. Cellulase, polygalacturonase, and polymethylgalacturonase activities were detected in culture filtrates and extracts from diseased plants by reduction in viscosity on substrates of CMC, sodium polypectate, and pectin N.F., respectively. Slow release of reducing groups and rapid reduction in viscosity of reaction mixture substrates indicated that all three enzymes were of the endotype. More cellulase, polygalacturonase, and polymethylgalacturonase activities were detected in diseased plant extracts with Tms cytoplasm than N cytoplasm. Cellobiase activity was detected in culture filtrates and diseased plant extracts when incubated on cellobiose substrate and analyzed for the presence of glucose as an endproduct using paper chromatography.

Evidence for chloroplast involvement in maize dwarf mosaic virus replication. D. MAYHEW, JOANNE KLEINWORT, & R. E. FORD (Iowa State Univ., Ames). Healthy and maize dwarf mosaic virus (MDMV) infected corn leaf tissues were fixed in Formalin-acetic acid, embedded in Tissuemat, sectioned, and treated with either 0.1% pancreatic ribonuclease (in H_2O, pH 6.8) or distilled H_2O (pH 6.8) at 37 C for 4 hr. The sections were stained with 0.1% acridine orange and viewed in ultraviolet light with a fluorescence microscope. All RNA fluoresced orange-red. In healthy tissue, treatment with ribonuclease removed all fluorescent RNA from chloroplasts and cytoplasm. Infected tissue, however, even 1 day after inoculation, showed the presence of ribonuclease-resistant RNA in the chloroplasts, primarily in the bundle sheath parenchyma cells. Infectivity was associated with chloroplast-rich fractions of MDMV-infected corn prepared by discontinuous sucrose density-gradient centrifugation. The acridine orange technique was repeated with an albino mutant of corn that supports MDMV replication. The ribonuclease-resistant RNA appeared to be associated with the plastids. Thus, the presence of presumed double-stranded, ribonuclease-resistant RNA in treated infected tissue, correlated with infectivity of the chloroplast-rich fractions of infected corn, suggests that the RNA of MDMV replicates in corn chloroplasts.

Differential effect of protein and nucleic acid inhibitors on the multiplication of two closely related plant viruses. G. D. MC LEAN & J. S. SEMANCIK (Univ. Nebraska, Lincoln). Actinomycin-D (AMD) inhibited the multiplication of cowpea mosaic virus (CPMV) in excised etiolated hypocotyls of *Phaseolus vulgaris* Pencil Pod Wax by 90% at 2 hr, with a progressive decrease to 10% by 12 to 15 hr postinoculation. In contrast, bean pod mottle virus (BPMV) was not inhibited when AMD was added during the same time intervals, but was stimulated by as much as 200%. Both viruses are in the "Comovirus" group, and are beetle-transmitted. Cycloheximide, and inhibitor of protein synthesis, was added to each of the virus-host systems for 4-hr intervals. When applied 2 hr after inoculation, both viruses were inhibited by 80 to 95%. However, at intervals begun 24 hr postinoculation, CPMV was inhibited by as much as 40% more than BPMV. The AMD results indicate that CPMV replication may require the synthesis of a host messenger RNA during early infection, whereas BPMV does not. This suggestion was supported by the observation that BPMV replication was less sensitive to protein synthesis inhibitors than CPMV replication at early times postinoculation.

Quantitative isolation of Macrophomina phaseoli (Rhizoctonia bataticola) from soils using a selective medium. W. A. MEYER, J. B. SINCLAIR, & M. N. KHARE (Univ. of Ill., Urbana, & J. Nehru Agr. Univ., Jabalpur, India). A selective medium was developed for assaying populations of *Macrophomina phaseoli* in artifically and naturally infested field soils. The medium, designated "CMR", was prepared

by: boiling 10 g of polished rice in 1 liter distilled water for 5 min; filtering through cheesecloth; adding 20 g Difco agar to the filtrate and autoclaving; then adding the following: 300 mg active ingredient chloroneb as Demosan 65 WP; 7 mg $HgCl_2$; 90 mg rose bengal; 40 mg streptomycin sulfate; 60 mg penicillin; and lactic acid to give pH 6. Autoclaved and nonautoclaved field soil was infested with either 25, 50, or 75 mg sclerotia/40 g of soil of "Jabalpur 1" isolate of *M. phaseoli*. Ten-mg samples from these infested soils and noninfested soil were finely ground, spread on the surface of CMR plates, and incubated at 30 C. The number of colonies recovered was directly proportional to the amount of sclerotia added for both soils. *M. phaseoli* colonies were recognized after 4 days; sclerotia developed at 6 days. Compared to control plates, contaminating colonies were restricted to a few of very limited growth. A soil sample from an Illinois field with a history of soybean cropping and charcoal rot showed populations of *M. phaseoli* of over 100 propagules/g of air-dried soil.

Ultrastructure of roots of corn and Johnson grass infected with maize dwarf mosaic virus. H. E. MOLINE (Iowa State University, Ames). An electron microscopic study of roots of corn systemically infected with maize dwarf mosaic virus (MDMV) revealed that pinwheels characteristic of the potato virus Y group were present in young epidermal cells. Root tips of plants grown in vermiculite were fixed in 3% glutaraldehyde, postfixed in 2% OsO_4, and embedded in araldite-epon. Pinwheels in epidermal cells undergoing division were somewhat different from those found in leaves of MDMV-infected corn. In addition, only a small percentage of root sections contained inclusions. Bundle-type viral inclusions also were found, not in the same cells as pinwheels, but in parenchyma surrounding differentiating protoxylem in corn roots and in numerous meristematic cells of Johnson grass. This is the first report of pinwheel inclusions in roots of infected plants. No inclusions were found in healthy root tissue. Other ultrastructural changes included changes in size and number of dictyosomes, increased endoplasmic reticulum proliferation, morphological changes in mitochondria, and irregularities in the tonoplast of infected cells.

A virus isolated from Physalis sp. in Iowa serologically related to Belladonna mottle virus. H. E. MOLINE & R. E. FRIES (Iowa State Univ., Ames). A virus was recovered from *Physalis angulata* with symptoms of yellow mottle and leaf distortion. Host range is limited to the Solanaceae and *Chenopodium quinoa*. The dilution end point is 10^{-6}, and the longevity in vitro is 10-14 days in 0.01 M phosphate buffer, pH 7.0. Purification by a modification of Steere's chloroform-butanol method from *Nicotiana glutinosa, N. tabacum,* and *Datura stramonium* gave high yields of infectious virus. Cesium chloride gradients yielded a top component and bottom component with 260:280 ratios of 1.07 and 1.60, respectively. Analytical ultracentrifugation of the two components in distilled water gave sedimentation (S) rates of 54 S (top component) and 114 S (bottom component). Normal diameter of the polyhedron was 29 nm. Antiserum produced in rabbits had a homologous titer of 1:512 in microprecipitin tests. Immunodiffusion tests were negative against tobacco ringspot, bromegrass mosaic, cucumber mosaic, turnip yellow mosaic, wild cucumber mosaic, and tomato aspermy virus antisera. Homologous reactions were obtained with H. L. Paul's (Braunschweig, Germany) Belladonna mottle virus antiserum. This data provides evidence for a new member of the Andean potato latent virus group in the USA.

Homologous interference between the components of tobacco rattle virus. T. J. MORRIS & J. S. SEMANCIK (Univ. Nebraska, Lincoln). Tobacco rattle virus, Brazillian strain, has two nucleoprotein rod particles (520 Å and 1,970 Å) which interact on infection to produce progeny virus. Inoculation at multiplicities of short to long nucleoprotein rods of 100- to 1,000-fold, based on A_{260} units, resulted in a decrease of 70% or more in local lesion numbers, and 50% or more in progeny virus compared to inoculations at multiplicities of 1 to 10. Interference was also observed at high multiplicities of extracted RNA from short and long rods. However, when the short nucleoprotein rods (1,150 Å) from a serologically distinct California isolate were inoculated at high multiplicities with the Brazillian long nucleoprotein rods, no interference was observed with respect to local lesion production, and no progeny virus was recovered as these two components did not complement one another. These results suggest that although both long and short particles cooperate during infection, interference at high multiplicities of short rods may result from competition between the different-length RNA species for the viral replicase.

Detection and identification of wheat diseases by aerial photography C. L. NIBLETT, J. E. HUNTER, M. G. EVERSMEYER, & J. F. SCHAFER (Kansas State Univ., Manhattan). Aerial photography was used in preliminary studies to develop remote sensing techniques for the assessment of wheat diseases and crop losses. Kodak film/Wratten filter combinations were: color infrared (IR) No. 2443/15, IR No. 2424/89B, Plus-X Pan/25, and Plus-X Pan/58. Photographic observations were closely correlated with ground studies. IR films were more sensitive than Plus-X. In color IR photographs, fields infected with wheat streak mosaic virus (WSMV) were light red to off-white depending upon disease severity. Healthy fields were dark red and uniform in color and texture. Despite uniform infection incidence, in some fields severely diseased light-colored sections with distinct boundaries were noted. This was attributed to previous cropping practices; areas previously in wheat were more severely diseased than those planted to sorghum or left fallow. Fields infected with soil-borne wheat mosaic virus also photographed as a lighter color, but unlike fields uniformly infected with WSMV, these contained scattered foci of infection. Other crops could be identified by slight differences in color tone and texture. These studies indicate that remote sensing may be useful in assessing productivity of wheat in relation to diseases and other factors.

Aspartate transcarbamylase activity in etiolated cowpea hypocotyls infected with cowpea mosaic virus. C. L. NIBLETT & L. B. JOHNSON (Kansas State Univ., Manhattan). Two possible sources of nucleotides for synthesis of viral RNA are de novo synthesis and salvage from degraded host nucleic acids. De novo synthesis could be indicated by an increase in activity of aspartate transcarbamylase (ATC). ATC catalyzes the first reaction unique to de novo pyrimidine synthesis; i.e., condensation of aspartic acid (ASP) and carbamyl phosphate to ureidosuccinate (US). ATC activity in buffer extracts of etiolated cowpea (*Vigna unguiculata*) hypocotyls was assayed by incorporation of ^{14}C ASP into US. Labeled substrate and product were separated on Dowex 50 columns (H^+ form). ATC activity in hypocotyls infected with cowpea mosaic virus (CPMV) first exceeded that in healthy rubbed hypocotyls 96-120 hr after inoculation, and was 3-4 times greater after 168 hr. Virus concentration increased rapidly, and total buffer-soluble protein nearly doubled during this

period. Both ATC and soluble protein decreased in healthy controls in the 168-hr period. Inhibition of ATC by uridine monophosphate did not indicate whether increased ATC activity was due to de novo enzyme synthesis or to release of pre-existing enzyme from a feedback-inhibited state. These data suggest that increased de novo biosynthesis of pyrimidines occurs during CPMV replication in etiolated cowpea hypocotyls.

Sooty stripe of sorghum in Nebraska. G. N. ODVODY, L. D. DUNKLE, & M. G. BOOSALIS (Univ. Nebraska, Lincoln). Sooty stripe is a foliar disease of *Sorghum spp.* which is common in some southern states. It was first discovered in Nebraska during the summer of 1971. The causal fungus, *Ramulispora sorghi*, survived the winter months primarily as sclerotia either dissociated from leaf tissue in the soil or attached to leaf material on and above the soil surface. A method was devised for quantitative isolation of sclerotia from soil. The procedure involves a combination of wet screening, ammonium sulfate flotation, and discontinuous sucrose density-gradient centrifugation. Earlier studies suggested that a sporogenic sclerotium of *R. sorghi* germinated by producing a large mass of branched conidia through the top portion of the sclerotial wall. Results from our studies indicate that the mass of conidia is extruded through that portion of the sclerotial wall previously attached to subepidermal sporodochial tissue. In greenhouse studies, international sorghum lines previously exhibiting some resistance to sooty stripe showed varying degrees of susceptibility to a particular Nebraska isolate of *R. sorghi.*

Wheat leaf necrosis incited by Pseudomonas syringae. J. D. OTTA (South Dakota State Univ., Brookings). A severe flag leaf necrosis of South Dakota winter wheat has been observed annually since 1968. Isolations from necrotic lesions in 1971 consistently yielded a bacterium which produced green-fluorescent water-soluble pigment on King's medium B. This bacterium produced yellowing and necrosis when atomized onto winter wheat in the greenhouse. The leaf symptoms observed were very similar to those described for basal glume rot which is incited by *Pseudomonas atrofaciens*. However, preliminary tests with the isolated organism determined that it is oxidase-negative, inhibits *Geotrichum candidum*, is serologically indistinguishable from *P. syringae*, and produces holcus leaf spot when inoculated onto sorghum. All of these tests indicate that the bacterium isolated from necrotic wheat leaves is *P. syringae*. The same pathogenic bacterium has been isolated from seed of several cultivars of winter wheat obtained from Saskatoon, Saskatchewan; Lethbridge, Alberta; Minot, Casselton, and Dickinson, No. Dak.; Brookings, So. Dak.; and Lincoln, Neb. The yield loss due to this disease is unknown. There appear to be some differences in cultivar susceptibility; with Froid, Hume, Lancer, and Winoka being particularly susceptible.

Concentration and infectivity of barley stripe mosaic virus. M. K. PALOMAR (Univ. Nebraska, Lincoln). Three zones were obtained when purified barley stripe mosaic virus (BSMV) was sedimented through sucrose gradient columns. The zones were identified as monomers (unaggregated virus), dimers, and a mixture of trimers and higher aggregates, because all had nucleic acid that sedimented at the same rate. A plot of infectivity at a constant ultraviolet absorbance against depth for fractions from the virus zone from a centrifuged density-gradient column showed a bell-shaped curve. The unaggregated virus gave little or no infection, whereas the more rapidly sedimenting dimers and higher aggregates were infectious. However, the ribonucleic acids of unaggregated virus and of "polymers" were similar in infectivity and sedimentation coefficient. This study revealed no longlasting immunity in the plant from the virus. Symptoms were more severe in plants grown at a lower temperature than at higher, but this was not directly correlated to virus concentration. The peak concentration and specific infectivity in the youngest leaves was similar at 13, 17, 21, and 25 C, and remained relatively constant throughout the life of the plant.

Incorporation of uridine-H^3 into cellular and viral nucleic acids in the presence of a high kinetin concentration. L. E. PELCHER & H. H. MURAKISHI (Mich. State Univ., East Lansing). Suspension culture cells of *Nicotiana tabacum* 'Havana-38' were inoculated with 250 μg/ml of TMV. The cells were washed with fresh medium and incubated on agar. Forty-eight hr after inoculation, the cells were transferred to 2 ml liquid medium containing uridine-H^3 (25 μc/ml). Kinetin was added to a final concentration of 2.5×10^{-4} M. After a 12-hr incorporation period, the nucleic acids were extracted and subjected to electrophoresis in 2.4% polyacrylamide gels. The gels were scanned at 260 nm, and sliced into 1-mm sections for radioactivity determination. Incorporation of uridine-H^3 into cellular ribosomal RNA species was inhibited by approximately 70% in the presence of 2.5×10^{-4} M kinetin, whereas uridine-H^3 incorporation into viral RNA was virtually unaffected. Studies using density-gradient analysis of complete virus synthesized during 2.5×10^{-4} M kinetin treatment indicate that accumulation of complete virus was inhibited by ca. 30%; also, uridine-H^3 incorporation into complete virus was inhibited 30%. However, the specific activity (cpm/μg complete virus) was approximately the same in kinetin treated and nontreated infected cells.

Interactions of Atrazine with soil microorganisms: population shifts and accumulation. J. A. PERCICH (Mich. State Univ., East Lansing). The herbicide, Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), at 30 and 100 μg/g, increased populations of actinomycetes, bacteria, and fungi in a loam soil. Populations of fungi tolerant to Atrazine increased in Atrazine-treated soil. *Fusarium* spp. accounted for 11% of the total fungi in untreated soil and 20% in soil treated with 30 μg/g Atrazine. Severity of pea root rot caused by *F. solani* f. sp. *pisi* was increased in soil treated with 2.5 μg/g Atrazine, a concentration nontoxic to pea. Several soil fungi and actinomycetes accumulated Atrazine several-fold above ambient levels when incubated in distilled water containing 5 μg/g. In a nutrient solution, accumulation was less but metabolic products of Atrazine were produced. In soil, organisms accumulated the herbicide only to ambient levels in 3 days without evidence of breakdown. Mycelia of *Sclerotium rolfsii* were grown in a nutrient solution containing 20 ug/ml Atrazine. Sclerotia formed after transfer of the mycelia to soil contained approximately half of the Atrazine accumulated by the mycelia. Concentrations in the sclerotia were ca. 20 times higher, on a fresh weight basis, than in the original medium.

Immunodiffusional and immunoelectrophoretic comparison of *Erwinia carotovora* and *E. atroseptica.* A. PHILIP & S. C. Y. LIU (Eastern Michigan Univ., Ypsilanti). Antisera were produced in rabbits against 12 isolates of *Erwinia carotovora* and *E. atroseptica*. In homologous and heterologous tests, these isolates were compared and analyzed, with ATCC 495, ATCC 4446 as "types", in Ouchterlony's plates and in immunoelectrophoresis (Ionagar 0.85%, barbiturate buffer 0.04 M, at pH 7.5). In Ouchterlony's plates, homologous *E. carotovora* gave four

precipitation bands whereas *E. atroseptica* gave three bands. Between the two "types", there was one band of identity considered as "group-specific". The other isolates, when plated, gave two-three bands varying from nonidentity to identity, including the group-specific. The varied number of bands, and the pattern of reactions, reflected the varied immunological relationships among these isolates. These were confirmed by cross absorption tests. Immunoelectrophoretic patterns and mobility tended to confirm the results of Ouchterlony's tests. Results thus far obtained in both immunodiffusion and immunoelectrophoresis, as shown by the number of bands, lines of reactions, and uncommon antigenic components in cross absorption tests, seemed to warrant the conclusion that *E. carotovora* and *E. atroseptica* were two distinct species.

Effect of various cations on the in vitro dissociation of tobacco mosaic virus. C. A. POWELL (Univ. Nebraska, Lincoln). Purified tobacco mosaic virus (TMV) was placed in 0.02 M Tris [tris (hydroxymethyl) amino methane] buffer, pH 9.0, containing bentonite. After 24 hr at 2 C, density-gradient centrifugation patterns showed that the virus had dissociated completely into protein and free nucleic acid. Varying concentrations of cesium, potassium, sodium, magnesium, or calcium added to equivalent virus solutions protected against complete stripping. Degradation proceeded to certain sites along the nucleic acid and stopped. There were at least five of these sites. Apparently the cations were bound at these sites and increased nucleic acid-protein interactions. The sites were the same for all the ions, but cation concentrations required for equivalent results were different for different ions. Low concentrations of divalent cations (10^{-6}-10^{-3} M) had the same effect as high concentrations of monovalent cations (10^{-3}-1.0 M). This indicates different affinities of the sites for different ions. When the magnesium was buffered with citrate to give constant concentrations of free magnesium ion, the results were significantly different from those obtained using unbuffered magnesium solutions with the same initial concentration of the free ion. The results were more reproducible, and protection was better with the buffered system.

Detection of systemic movement and retention of Thiabendazole and benomyl in sugar beets. J. H. RIESSELMAN & J. L. WEIHING (Univ. Nebraska, Lincoln). Benomyl and Thiabendazole (TBZ) are known to be systemically distributed in plants when applied to the soil. Soil was amended with 10, 20, 40, 80, 160, and 320 ppm benomyl or TBZ. The methods of application were a preplant incorporation into the soil and a postplant soil drench. Standard bioassay procedures were used for detection of fungicides in leaf and soil extracts. Areas of zones of inhibition on V-8-agar plates were measured with a planimeter. *Cercospora beticola* was the bioassay organism. Zones of inhibition in the soil drench treatments ranged from an average of 0.71 sq. inches at 40 ppm to 4.85 sq. inches at 320 ppm, benomyl; and 0.62 sq. inches at 160 ppm to 1.25 sq. inches at 320 ppm, TBZ. Benomyl was active in both leaf tissue and treated soil 5 months after the initial preplant treatment at rates greater than 160 ppm. TBZ was not detected at this time. In both treatments, TBZ was phytotoxic at rates exceeding 20 ppm. Benomyl was not phototoxic at any rate.

Bacterial blight of carrot in Wisconsin. S. M. SAAD & E. K. WADE (Univ. Wisconsin Center-Washington County, Univ. Wisconsin, Madison). Bacterial blight of carrot caused by *Xanthomonas carotae* was found for the first time in Wisconsin during the summer of 1971. The disease was observed on the leaves and petioles of carrots, cultivar Spartan Sweet. The Nantes varieties showed only a trace of infection. Greenhouse studies revealed that a high level of relative humidity of 90-100% for 48-72 hr after inoculation is necessary for the bacteria to cause infection. The disease symptoms were as follows: At first there were small, irregular, water-soaked areas at the margins of the leaf segments. These soon turned dark brown in color and became dry and brittle. In many cases, a sharp halo surrounded each lesion. Typical symptoms appeared on the foliage within 12-15 days, with severe infection developing at the end of 20 days at 74 F. Repeated isolations from diseased leaves and petioles on a heart infusion medium constantly yielded a yellow bacterial organism. The organism was found to be rod-shaped, 1-1.5 μ long, and gram-negative. The electron microscope revealed that the bacteria had one polar flagellum. The Hugh-Leifson test was negative.

Direct observation of Fusarium solani f. pisi chlamydospore germination in the spermosphere of peas. G. E. SHORT & M. L. LACY (Michigan State Univ., East Lansing). Conover loam artificially infested with 1.6×10^6 chlamydospores/g oven-dry soil of *Fusarium solani* f. *pisi* was placed in 25-mm-diam Pyrex tubes, and a pea seed (*Pisum sativum*) planted 0.5 inch below the soil surface in each tube. Soil moisture levels of 20 and 50% (oven-dry basis) were established in the top inch of soil by placement of the lower ends of 81- and 330-mm soil columns in water, allowing water to move upward by capillary action. At 24, 48, and 72 hr after planting, columns were removed from the water source, air-dried by application of vacuum to the lower end, infiltrated with 2% molten agar, and cooled, and the agar was hardened by soaking in ethanol. Blocks of soil ($1 \times 2 \times 3$ mm) were removed at measured distances from the seeds. The blocks were placed in 5 N HCl to dissolve the agar, and chlamydospores were stained with 0.1% aniline blue in lactic acid. Spore germination was as high as 70% in the millimeter of soil adjacent to the seed, and declined to zero at distances of 208 mm, depending on pea cultivar and soil moisture. The spermosphere of Alaska pea, but not Miragreen, was larger in the region of radicle emergence than in other areas. Spermosphere radius was greater at 50% moisture than at 20% with both cultivars, and was directly related to amount of carbohydrate exuded.

Production of antifungal compounds in bean leaves infected with tobacco mosaic virus. J. L. STARR & M. O. GARRAWAY (Ohio State Univ.). Detached trifoliate leaves of *Phaseolus vulgaris* 'Pinto' were examined for local lesion development and content of phenolic compounds after inoculation with sap from tobacco mosaic virus (TMV) -infected tomato. Twenty-four hr after inoculation, local lesions developed on leaves incubated in continuous light or continuous dark. No lesions developed on controls. The content of phenols of control and inoculated leaves was 20 μg/mg dry wt at 0 hr, and increased to 43 μg/mg at 48 hr in the light-incubated diseased leaves. There was no significant change in the content of phenols of control and dark-incubated diseased leaves at 48 hr. Ethyl acetate extracts from light or dark-incubated diseased leaves separated on silica gel thin-layer plates with pentane-ether-acetic acid (75:25:1) revealed several components, detectable with ultraviolet light or phenol-detecting reagents, not observed in the controls. The component at R_F 0.65 gave a phenol reaction to $FeCl_3$-$K_3Fe(CN)_6$ and diazotized sulfanilic acid, and had

ultraviolet absorbance maxima at 281-277 nm. In water agar containing 1.3 μg/ml (phaseollin equivalents) of this compound, spore germination of *Fusarium solani* f. sp. *phaseoli* was < 1%, whereas germination of *F. solani* f. sp. *cucurbitae* was 58%. Phytoalexin produced in TMV-infected bean leaves appears to differ from phaseollin.

Leaf rust resistance of Waldron wheat: a genetic analysis. G. D. STATLER (North Dakota State Univ., Fargo). The inheritance of resistance to wheat leaf rust incited by *Puccinia recondita* Rob. ex Desm f. sp. *tritici* was investigated in Waldron, a leaf rust-resistant, hard red spring wheat. Waldron was crossed to the leaf rust-susceptible variety Little Club, for the genetic analysis. Culture 70-1 (race 1), a widely avirulent culture of wheat leaf rust, was used to test progeny of reciprocal crosses. The F_1 plants tested were all resistant to culture 70-1. The F_2 and backcross-F_1 plants satisfactorily fit a monogenic-dominant ratio. The F_2 plants segregated approximately three resistant to one susceptible, or 13 resistant to three susceptible ($P>.25$). The backcross-F_1 plants segregated ca. one resistant to one susceptible ($P>.25$). However, the backcross-F_2 families fit a 2:1:1 ratio ($P>.10$) and indicated a second recessive gene for resistance. Sixty-three backcross-F_2 families segregated three resistant: one susceptible or 13 resistant: three susceptible; 33 were homozygous-susceptible; and 23 segregated one resistant: three susceptible. Based on these data, the hypothesis was formulated that seedling resistance in Waldron to culture 70-1 *P. recondita* was conditioned by a single dominant gene and a single recessive gene.

Correlation coefficients between various criteria for evaluating brown stem rot resistance in soybean. H. TACHIBANA & L. C. CARD (ARS, USDA, Iowa State Univ., Ames). Two experiments were carried out in each of 2 years to obtain correlation coefficients (r) to determine the best disease criterion for selection of brown stem rot (BSR, caused by *Cephalosporium gregatum*) -resistant soybeans with the highest yield potential. Percentage of plants infected versus yield had the lowest r values, and percentage of stem infected versus yield had the highest r values. The values for percent stem infected versus yield ranged from -0.377 to -0.582 and were always significant at the .01 level of probability, whereas values for percent plants infected ranged from -0.013 to -0.243 and were never statistically significant. The values for cm of stem infected without consideration of plant height ranged from -0.113 to -0.486, and these were not always significant. Among disease criteria, all r values were positive and significant and ranged from 0.560 to 0.967. Significant positive correlations of 0.278, 0.412, and 0.459 were calculated between lodging and percent plants infected, cm of BSR, and percent stem infected, respectively. The correlation between yield and lodging of late maturity soybeans was -0.507 and significant. Percentages of mottled seeds from two experiments were highly correlated, -0.509 and -0.423, with cm of BSR and percent stem infected, respectively, in the case of early but not late maturity soybeans.

Degradation and synthesis of chloroneb by soil microorganisms. M. V. WIESE & J. M. VARGAS (Mich. State Univ., East Lansing). The fate of chloroneb was studied in soil, in grass plants (*Poa pratensis* 'Merion'), and in cultures of soil microorganisms. The fungicide and two of its analogs, 2,5-dichloro-4-methoxyphenol (MP) and 2,5-dichlorohydroquinone (HQ) were assayed by a new gas-liquid chromatographic procedure sensitive to 0.1 ng of these residues. Chloroneb was the only residue recovered from grass plants (1.2 and 21.1 μg/g fresh wt of leaves and roots, respectively) 7 days after the fungicide was drenched in soil. The fungicide was unaltered after 30 days in sterile soil regardless of moisture status, and was also unchanged in natural soil and in liquid shake cultures where growth of organisms was minimal or static. Of 23 organisms grown in the presence of 5 μg/ml chloroneb in liquid culture, 13 degraded the fungicide to MP. *Fusarium solani* and *Cephalosporium gramineum* were most active in this respect, converting more than 50% of the chloroneb to MP in 5 days. When grown in cultures containing 5 μg/ml MP, 8 of the 23 organisms, especially a *Mucor* sp., converted up to 20% of the MP to chloroneb. *C. gramineum* converted MP to chloroneb and to HQ. Chloroneb may present unique residue problems, since it can be degraded and/or resynthesized from its breakdown products by certain soil microorganisms.

Clover club-leaf: a possible rickettsial disease of plants. I. M. WINDSOR & L. M. BLACK (Univ. Ill., Urbana). Bodies resembling rickettsias were found in transverse sections of the phloem of *Vinca rosea alba* and *Trifolium incarnatum* showing symptoms of clover club-leaf, a yellows-type disease transmitted by the leafhopper, *Agalliopsis novella*. No such bodies were found in unaffected plants. Some bodies appeared roughly spherical, with a diameter of ca. 200 mμ, whereas others were elongated measuring 200 mμ diam by 2 μ long. It was concluded that the spherical bodies probably represented transverse sections of the elongated form. They contained ribosomes smaller than those of the host, faint strands of DNA-like material, and, in addition to having a limiting unit membrane, were bounded by a second trilaminar structure considered analogous to a wall. Each of these structures measured approximately 80 Å thick and were separated by a space of 70-160 Å. Probability studies showed that the chance that these bodies were associated with the diseased plants purely fortuitously was not greater than 1/924. Remission of symptoms, but not cure, of the disease was obtained by root treatment with solutions of both penicillin and achromycin at concentrations of 200-1,000 μg/ml. The evidence suggests that clover club-leaf is caused by a rickettsialike organism.

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