

Abstracts of the Thirtieth Annual Meeting of the Potomac Division
of The American Phytopathological Society

Root depth and percentage infection by Fomes annosus in a loblolly pine plantation on two soil hazard types. S. A. ALEXANDER. (Va. Polytech. Inst. and State Univ., Blacksburg). The percentage infection of loblolly pine roots by *Fomes annosus* was found to increase with depth in a high hazard soil type (> 70% sand, 20- to 30-cm deep) and decrease with depth in a low hazard soil type (< 70% sand, 20- to 30-cm deep). Root systems of 10 trees were excavated, five from high- and five from low-hazard soil types. Length, depth, and direction from the stem were recorded for each root. The bark was removed, and chips were taken every 10 cm from the top and bottom sides of each root. Isolations were made on ortho-phenyl-phenol media and incubated at 24 C for 14 days. A total of 110 roots (2,625 chips) from depths of 0 to 95 cm was excavated, with 54 (1,552 chips) from the high hazard site and 56 (1,073 chips) from the low hazard site; the number of positive chips was 89 (6.1%) and 12 (1.1%), respectively, and the depths from which *F. annosus* was isolated were 5 to 95 cm and 10 to 45 cm, respectively. The percentage infection at depths of 20 to 30 cm was 10.3 (23 of 224 chips) and 1.2 (6 of 515 chips), respectively, for high- and low-hazard soil types and at depths of 35 to 95 cm, the average percentage infection was 20.7 (45 of 217 chips) and 4.5 (5 of 111 chips), respectively. These data further support the possibility of infection by percolated spores.

A comparison of properties of peanut stunt virus and cucumber mosaic virus. S. BOATMAN, J. M. KAPER, & S. A. TOLIN. (Hollins College, Va., ARS, USDA, Beltsville, Md., and Va. Polytech. Inst. & State Univ., Blacksburg). The comparison of a number of characteristics of peanut stunt virus (PSV) (ATCC PV-164) and cucumber mosaic virus (CMV-S) supports the classification of PSV as a strain of CMV. The same infectivity was observed for PSV at pH 6.9 and 8.6; decreased infectivity at pH 4.5 was apparently due to virus aggregation. Ribonuclease at 10^{-4} mg/ml inactivated PSV completely at all three pH's and at 10^{-8} mg/ml did not reduce infectivity. The sedimentation rate of PSV was unaffected by changes in pH. These properties of PSV are consistent with those observed for CMV. Purified PSV was degraded completely by 0.6 M KCl and NaCl and seemed more sensitive to LiCl, degrading between 0.24 M and 0.5 M. CMV-S was unaffected by 0.5 M LiCl and was completely degraded at 1.5 M. In both cases, the degradation products sedimented much like the respective phenol-extracted RNAs. PSV (0.2 mg/ml in 0.02 M NaPO_4 , pH 7.0 and 20 C) was extremely sensitive to sodium dodecyl sulfate (SDS), degrading between 0.005 and 0.015% SDS; CMV-S degraded between 0.005 and 0.02%. Phenol-extracted PSV RNA, analyzed by polyacrylamide gel electrophoresis, displayed five principal components which co-migrated with CMV RNA components and had molecular weights of 1.07, 0.95, 0.68, 0.33, and 0.11×10^6 Daltons.

A study of the relationship of late blight resistance to glycoalkaloid content in 15 potato clones. K. L. DEAHL, R. J. YOUNG, & S. L. SINDEN. (ARS, USDA, Beltsville, Md., W. Va. Univ., Morgantown). The possible role of steroid glycoalkaloids as resistance factors to late blight development in leaves of potato clones with high levels of multigenic resistance to late blight was investigated. Fifteen potato clones with resistance levels ranging from highly susceptible to highly resistant were inoculated with a zoospore-sporangial suspension of race 1, 2, 3, 4 of *Phytophthora infestans*. Resistance was evaluated 14 days after inoculation, at the same time that leaf samples from fungicide-protected plants were collected for glycoalkaloid analysis. No association was

found between leaf glycoalkaloid or tuber glycoalkaloid contents and the levels of foliar resistance in this sample of potato clones, suggesting that steroid glycoalkaloids are not closely associated with multigenic resistance to late blight in potato leaves. Steroid glycoalkaloid contents of tubers from blight-infected plants were not significantly higher than the contents of tubers from healthy (fungicide-protected) plants. These results indicate that plant pathologists and potato breeders should be able to develop lines with multigenic late blight resistance without increasing tuber glycoalkaloid levels.

Differentiation of Endothia gyrosa and E. parasitica by disk gel electrophoresis. G. H. EMERT, R. J. STIPES, R. D. BROWN, JR., & J. E. TALMADGE. (Va. Polytech. Inst. & State Univ., Blacksburg). Certain similar morphological and cultural attributes exhibited by the pin oak and chestnut blight pathogens, *Endothia gyrosa* and *E. parasitica*, respectively, have made precise taxonomic differentiation uncertain. Further clarification was attempted by disk gel electrophoresis of water-soluble intramycelial proteins as outlined in Mycologia 62:987-995, employing about 200 μg protein per gel. Seven isolates of *E. gyrosa* from species of *Quercus*, and five isolates of *E. parasitica* from species of *Castanea* and *Quercus* were studied. Gels were stained with Coomassie Blue for detecting general proteins, after which several different observers correctly separated the uncoded gels of both species by simple visual inspection, even though distinct intra-specific differences and inter-specific similarities existed. *E. parasitica* isolates yielded fewer and heavier bands than *E. gyrosa*. Recent, and 20-yr-old, isolates exhibited similar protein profiles. This technique has proven dependable in differentiating our isolates of the two species, and additional enzyme assays are in progress to further clarify and expand current findings.

Comparison of gall and egg-mass indices of two races of Meloidogyne incognita on ten differential hosts. J. A. FOX & L. I. MILLER. (Va. Polytech. Inst. & State Univ., Blacksburg). Experiments were initiated with two races of *Meloidogyne incognita*, provisionally designated as A from 'Coker 319' tobacco in Virginia and B from 'NC 95' tobacco in South Carolina, to determine the relationship of the number of galls to the number of egg masses formed on the roots of the following plants: 'NC 2' peanut (PN), 'California Wonder' pepper (PP), 'Dixie 82' corn (CN), 'Albritton' strawberry (SB), 'McNair 1032' cotton (CT), 'Allgold' (AG) and 'Puerto Rico' (PR) sweet potato, 'Rutgers' tomato (TM), 'NC 95' tobacco (TB), and 'Garrison' watermelon (WM). Ratings of gall and egg-mass production were made two months after plants were inoculated with chopped infected tomato roots. The two races were differentiated by their egg mass ratings on five hosts (CN, CT, PR, TB, WM). The number of galls was not related to the number of egg masses on five hosts (PN, CN, SB, CT, WM) of race A and three hosts (SB, CT, PR) of race B but the number of galls exceeded irregularly the number of egg masses formed. It is concluded that the number of galls formed is not a reliable index of the reproduction of root-knot nematodes.

Free amino acids in healthy and wilt-infected oak trees. D. W. FUNK & W. L. MAC DONALD. (W. Va. Univ., Morgantown). Differences in the free amino acids of four differentially susceptible oak species (*Quercus rubra*, *Q. velutina*, *Q. montana*, and *Q. alba*) were determined during a one-month period following inoculation with the oak wilt fungus, *Ceratocystis fagacearum*. Branches approximately four years old and 8 mm in diam were inoculated through razor-blade wounds; water-injected and non-treated branches

served as controls. Samples for analysis were collected 4, 16, and 30 days after inoculation. A two-gram portion of woody tissue adjacent to each inoculation point was ground in a Wiley Mill, extracted with ethanol, and analyzed for amino acids. Amino acids were determined both qualitatively and quantitatively by gas chromatography. Column packing was 80/100-mesh acid washed Chromosorb G coated with 0.325 (w/w)% ethylene glycol adipate (EGA). Predominant amino acids observed in the woody tissue of the four oak species were alanine, asparagine, aspartic acid, glutamic acid and serine; identification was confirmed by two-dimensional paper chromatography. Qualitative amino acid changes were not found among the three treatments.

Relationship of relative humidity to disease development of a seedborne bacterial wilt of watermelon. R. W. GOTH & R. E. WEBB. (ARS, USDA, Beltsville, Md.). Seeds of 40 watermelon (*Citrullus vulgaris*) cultivars were vacuum-infiltrated with broth suspensions containing 10^6 cells per ml of a non-fluorescent *Pseudomonad* originally isolated from seeds of a watermelon plant introduction. The seeds were planted in steam-sterilized soil held at 26 C (day) and 23 C (night) throughout the experiment. Treatments consisted of: greenhouse bench without additional moisture, 100% relative humidity (RH) plus free water for 16 hr for 10 days, and 100% RH plus free water for 24 hr for 10 days. In order of appearance symptoms included necrotic water-soaked lesions on the cotyledons, cotyledon collapse, and stem wilt. Occasionally, hypocotyl water soaking and immediate stem collapse were the initial symptoms. Seedlings grown in the greenhouse without additional moisture, were not infected. Both 100% RH plus free moisture for 16 hr and 24 hr produced rapid disease development in susceptible cultivars, and disease incidence was greatest in the 24 hr regime. Under these conditions, all plants of some varieties were killed, whereas plants of other varieties were symptomless. These results indicate the presence of watermelon germplasm resistance to this bacterium, and the feasibility of using vacuum infiltration and proper humidity as an assay method for this resistance.

Modification of the exogenous carbon and nitrogen requirements for chlamyospore germination of Fusarium solani by direct contact with soil. G. J. GRIFFIN (Va. Polytech. Inst. & State Univ., Blacksburg). Following exogenous carbon (C)-independent macroconidium germination by *Fusarium solani* at 1×10^4 conidia/ml in axenic culture, chlamyospores were formed terminally on germ tubes. Low chlamyospore germination was supported under axenic conditions by 0.004 ng C/spore, supplied as ethanol, or by 0.004 ng C/spore supplied as glucose or several other sugars. Chlamyospores in direct contact with a nonsterile loamy fine sand soil ($\text{NO}_3^- + \text{NH}_4^+ = 13.3 \mu\text{g N/g soil}$) had a greater exogenous carbon requirement and did not germinate until 2.0 ng glucose-C/spore was supplied. Supplemental exogenous nitrogen, added as NH_4Cl , had little or no influence on percentage germination at the threshold levels of exogenous carbon, both in soil and in axenic culture; however, increasing the amounts of glucose plus NH_4Cl supplied per spore, increased percentage chlamyospore germination more than increasing the amount of glucose alone for both soil and axenic systems. In axenic culture, complete germination was observed at 2.0 ng C plus 0.13 ng N/spore. Greater than 90% germination occurred for chlamyospores in contact with soil at the highest glucose-plus- NH_4Cl level examined (200 ng C plus 13 ng/spore), but not in glucose alone.

Influence of soil porosity and soil moisture on black shank of tobacco. S. B. HANOUNIK & W. H. WILLIS. (Va.

Polytech. Inst. & State Univ., Blacksburg). Six artificial soil mixtures with a wide range of sand-clay content were infested with *Phytophthora parasitica* var. *nicotianae*. In field and greenhouse experiments, black shank of *Nicotiana tabacum* 'Coker 319' was correlated with soil porosity, moisture and clay content. In the field, lowest disease incidence (4%) occurred in soil with the highest noncapillary porosity and the lowest capillary porosity, whereas highest disease (48%) was in soil with high capillary porosity. Soils with high clay content supported high disease, whereas low disease occurred on plants in soil with low clay content. In the greenhouse under controlled sub-irrigation, disease development in the same soils was highest in the high sand soils; in the high clay soils no disease developed; high disease was correlated with excessive gravitational water. In laboratory experiments, vertical movement of spores of the fungus through sandy soils was detected and correlated with disease development; whereas, no such movement was detected in the high clay soils and was correlated with absence of disease. Porosity characteristics determine water-holding capacity of the soils, which seemed to affect disease development by governing movement of zoospores in gravitational water.

Effect of relative humidity and air speed on release of conidia of Helminthosporium maydis race T. R. A. HYRE. (USDA, Pa. State Univ., University Park). Sporulating leaf sections of Texas male sterile cytoplasm P-A-G 15029¹ corn plants were sealed in a 1.27-cm (0.5-inch) diam Lucite tube connected to a K-C spore trap. Humidities used were: constant high (H-H) 91-100%; constant low (L-L) 20-36%; varying high to low (H-L); and varying low to high (L-H). Air flow was regulated by flowmeters calibrated with Pitot tubes. Three air speeds used were 33, 45, and 90 cm sec^{-1} (0.75, 1.0, and 2.0 mph). Maximum number of spores were trapped with H-L humidity air at 90 cm sec^{-1} . To reduce variation spores trapped during a 15-min test period are expressed relative to the spores trapped, with the same leaf section, during a succeeding 15-min reference period of H-L humidity air at 90 cm sec^{-1} . Relative number of spores trapped were: about one-and-one-half times as many at H-L humidity air at 90 cm sec^{-1} ; one-third as many with either L-L humidity air at 90 cm sec^{-1} or H-L at 45 cm sec^{-1} ; less than one-tenth as many with the other combinations. Thus, relative humidity favoring spore release can compensate for a lower air speed. Number of spores released increased as the air speed was increased.

Is Melanospora zamiae a mycoparasite. E. G. JORDAN & H. L. BARNETT. (W.Va. Univ., Morgantown). Isolations of *Melanospora zamiae* usually are accompanied by another fungus, often *Fusarium* sp., with which it apparently is constantly associated in nature. In agar culture *M. zamiae* grows and sporulates better when in the presence of several other fungi suggesting a nutritional relationship. When cultured with *Fusarium* sp. or *Tritirachium* sp., *M. zamiae* produces short hook-like cells contacting hyphae of the associated fungus, that resemble the special branches produced by contact biotrophic mycoparasites with their hosts. However, *M. zamiae* does not require the growth factor for which the contact biotrophic mycoparasites are deficient and it makes good axenic growth on various common media. Growth and sporulation occur when spores of *M. zamiae* are placed on washed living mycelium of *Fusarium* sp. *M. zamiae* is deficient for thiamine and biotin. Several single amino acids serve as nitrogen sources. Mannose, fructose, and glucose are the best utilized carbon sources. The evidence indicates that *M. zamiae* is parasitic on certain other fungi during the early stages of its development but may become independent after growth is established.

Distribution of compatibility types of the oak wilt fungus in northeastern West Virginia. H. W. KAUFMAN & W. L. MAC DONALD. (W. Va. Univ., Morgantown). Two-hundred-forty-six isolates of *Ceratocystis fagacearum* were obtained from 378 aerially-spotted suspect oaks in the Keyser Quadrangle, a 13.5 by 17.5 mile area in the eastern panhandle of West Virginia. Isolates were typed using known A and B cultures, grown for 10 days at 25 C on maltose-Casamino acids media, as females. Conidia of the test cultures were used to spermatize both A and B cultures. Perithecia usually appeared within 4 days on compatible cultures. Of the 245 isolates, compatibility types A and B were present in approximately a 1:1 ratio. Both types were found in only 6 of 41 multiple-tree centers. These results support the theory that most multiple-tree centers develop from a single source of inoculum.

Resistance-breaking biotypes in the root knot nematode. A. J. KISH & R. E. ADAMS. (W.Va. Univ., Morgantown). Resistance-breaking biotypes of *Meloidogyne incognita* on tomato (*Lycopersicon esculentum*) have been found in West Virginia collections, confirming and expanding the work of other researchers on named varieties. Single galls produced on resistant lines were increased on a susceptible variety and inoculated to seedlings of twenty-nine resistant lines. Also, 152 susceptible tomato plants were inoculated with single galls from the resistant lines. Thirty-eight percent were galled and eight percent were severely galled. Five resistant lines were selected for further testing. On reinoculation, one line showed severe galling, two showed moderate galling and two remained resistant to the selected populations.

Effect of volatiles from decomposing organic amendments on Rhizoctonia solani. J. A. LEWIS & G. C. PAPAIVIZAS. (ARS, USDA, Beltsville, Md.). Hyphae of *R. solani* were darkened or "melanized" in PDA cultures by volatiles emanating from several plant tissues decomposing in soil. Isolates of the fungus differed in their response to the volatiles. The lower the carbon:nitrogen (C:N) ratio of the decomposing amendment, the greater was the tendency for the volatiles to cause melanization. Materials that induced the darkening, included crucifer residues and immature grain crops. Although tissues had different minimum threshold concentrations which initiated hyphal darkening, approximately 2% amendment in soil was necessary in most cases. The volatiles responsible for the hyphal darkening were trapped when the headspace vapors over decomposing tissues were passed through a solution of H_3BO_3 or H_2SO_4 . This suggests that NH_3 may be one of the implicated volatiles. Addition of inorganic N salts to amendments of high C:N ratio to reduce the C:N ratio of the system did not cause formation of vapors responsible for melanization. Hyphae, melanized on perlite and PDB as a result of amendment volatiles, failed to grow when perlite pieces were placed on water agar. The melanin or melanin-like material was not extracted at 100 C with 1 N KOH from darkened hyphae treated in an ultrasonic disintegrator.

Differential isolation of Pythium spp. from soil by means of selective media, temperatures, and pH. R. D. LUMSDEN. (ARS, USDA, Beltsville, Md.). *Pythium* propagules were estimated by a most probable number technique for high populations or by a smear plate technique for low populations in soil dilutions suspended in 0.3% water agar. Species with optimum growth at ca. 25 C were readily isolated on gallic acid medium (GAM) when incubated for 24-48 hr at 20 C. This medium (pH 4.5) allows germination and growth of the following species: *P. ultimum*, *P. paroeccandrum*, *P. irregulare*, *P. mammatum*, and an unidentified *P. sp.* *P. aphanidermatum*, *P. myriotylum*, and *P.*

spinosum grew well on GAM but could not be isolated from soil on this medium. *P. aphanidermatum* was isolated if GAM was adjusted to pH 6.0, but was more effectively isolated on a pimaricin-vancomycin-rose bengal medium (PVRB) (pH 6.5) incubated 24 hr at 38 C. Although all *Pythium* spp. encountered were isolated on PVRB, *P. aphanidermatum* was favored since it grew rapidly at 38 C, with colonies measuring 1-5 cm in diam. *P. myriotylum* grew similarly to *P. aphanidermatum*, but was only rarely isolated, even from soils with known high *P. myriotylum* disease potential. This procedure is, therefore, especially useful for selective isolation of *P. aphanidermatum* from soils with mixed *Pythium* populations.

Lesion development and sporulation in the brown spot disease of tobacco caused by Alternaria alternata. C. E. MAIN, C. J. NUSBAUM & G. B. LUCAS. (N.C. State Univ., Raleigh). The dynamics of lesion development on leaves of naturally infected, susceptible, and resistant tobacco cultivars were studied in the field. Increase in invaded, necrotic, lesion area was linearly regressed on time over a nine-day period. Leaf-age and level of cultivar resistance affected the regression values. Area of the non-invaded chlorotic halo zones associated with the lesions increased in a curvilinear manner. Application of a log area (mm^2): response (sporulation probability) transformation to the sporulation data showed conidia were present on 50 percent ($P = .5$) of the lesions when necrotic area reached $23 mm^2$. This suggests that necrotic area is related to fungal biomass and/or quantity of some host substrate required for initiation of sporulation. Number of conidia produced per unit necrotic area, was least for resistant cultivar PD121 and greater for moderately resistant breeding line NC80383-5-9 and susceptible cultivar 'Coker 298'. The quantification of lesion development and sporulation based upon lesion populations is considered in terms of computing latent periods and predicting brown spot epidemics.

Serological studies of corn stunt diseases. J. H. MC BEATH & T. A. CHEN. (Rutgers Univ., New Brunswick, N.J.). Antisera were produced in rabbits against stunt-infected and healthy corn plants. Five g of the atactosteles of diseased or healthy corn stems were cut into thin slices, to which 3 ml of phosphate buffered physiological saline (0.1 M) was added. Slices were then ground and filtered through a layer of glass wool. The filtrates were centrifuged at 1,500 rpm for 5 min and supernatants filtered through a 1.2- μ Millipore filter. Rabbits were injected intravenously with 0.2-ml portions of these partially cleared extracts. When no anaphylactic reactions were observed in the rabbits, 3 more injections (volumes of 0.8, 1.0, and 1.0 ml) were made in the same manner at 7-day intervals. Two weeks after the last injection, 5 boosters, 2 intravenous of 1.0 ml each and 3 subcutaneous of 4.0 ml each, were made over a one-month period. Blood was then collected from the marginal vein of the rabbit ears, and sera were prepared. Two antigens were found in the healthy corn extract. The results of absorption and immunodiffusion tests indicated that at least six antigens were present in the diseased corn extracts, of these four were serologically unrelated to the antigens of the healthy corn extract. The antigenicity of the corn in early stages of infection was similar to the antigenicity of the plant with fully developed symptoms.

Mechanical transmission of a whitefly-borne disease agent of beans in El Salvador. J. P. MEINERS, R. H. LAWSON, F. F. SMITH, & A. J. DIAZ. (ARS, USDA, Beltsville, Md.; Ministry of Agr., El Salvador). A disease agent producing symptoms in bean (*Phaseolus vulgaris*) similar to mottled dwarf and golden mosaic diseases in Brazil was transmitted

with the sweet potato whitefly (*Bemisia tabaci*) from a wild legume (*Calopogonium mucunoides*). Whitefly-infected beans from El Salvador were used as inoculum sources in mechanical transmission tests at Beltsville. The agent was sap-transmitted in the greenhouse at temperatures varying from 20 to 30 C. A higher percentage of the inoculated plants showed conspicuous disease symptoms with increasing temperature under 26,900 lx (2,500 ft-c) on a 12-hr photoperiod. At 21, 24, 27, 32, and 35 C the percentage of plants with golden mosaic and mottled dwarf symptoms was 24, 33, 52, 66, and 66%, respectively. Symptoms often appeared about 1 week after inoculation when plants were held at 27 C or above, and about 2-3 weeks after inoculation when plants were incubated at 21 and 24 C. The golden mosaic disease described in Brazil is transmitted only by *B. tabaci*, and mottled dwarf is mechanically transmitted only with difficulty. The whitefly-borne agent we report may differ from the incitants of golden mosaic and mottled dwarf diseases in Brazil.

The influence of a geographically isolated source of oxides of nitrogen on growth of flue-cured tobacco (Nicotiana tabacum). L. D. MOORE. (Va. Polytech. Inst. & State Univ., Blacksburg). The tobacco cultivars 'Coker 319', 'Hicks', and 'McNair 14' were grown in charcoal filtered and non-filtered chambers, and in open plots 0.457 km (site I) and 6.4 km (site II) downwind from a source of air pollution. The high 24-hour average of total oxidants monitored at sites I and II were 22 pphm and 7.3 pphm, respectively. The tobacco was grown in a uniform soil mixture in 15-liter cans and was harvested either 10-14 days following flowering in August or just prior to maturity in October. Flowering of the three cultivars was delayed up to 14 days at site I as compared to site II. In the open plots, leaves of each cultivar exhibited 50-90% oxidant fleck with the level of fleck increasing as the plants matured. With an increase in the percentage fleck of each of the cultivars, there were decreases in fresh weight and dry weight of the deveined leaves, stem diameter and number of attached leaves. Leaf abscission increased with an increase in the percentage fleck. At the final harvest, leaves of plants with 50-90% fleck weighed 55-70% less than plants with 10% or less fleck; there were about 35% fewer leaves, and stems were 0-25% smaller. Tobacco grown in filtered chambers was taller, had thicker stems, less leaf loss, and leaves weighed more than tobacco grown in non-filtered chambers.

Helminthosporium triseptatum, a major pathogen of some cultivars of Kentucky bluegrass (Poa pratensis). S. A. OSTAZESKI & J. J. MURRAY (ARS, USDA, Beltsville, Md.). In recent years, plots of 'Belturf' Kentucky bluegrass at Beltsville, Md., have declined in quality and vigor. Symptoms during mid-summer include prominent light-brown leaf lesions, so numerous that plots may show an overall brownish-gray color. Isolates of *Helminthosporium triseptatum* and *Curvularia* spp. have been easily obtained from Belturf, but only *H. triseptatum* proved pathogenic. Forty-four Kentucky bluegrass cultivars and selections were seeded in rows in flats, in July 1972, and inoculated with *H. triseptatum* 10 and 14 weeks later. Cultivars P-142, 'Baron', KL-138, and 'Princess' were the most resistant entries; 'Belturf', 'Campina', 'Adelphi', and 'Merion' were highly susceptible. The remaining entries had intermediate reactions.

Artificial reproduction of Fusarium wilt of the mimosa tree under greenhouse and field conditions. P. M. PHIPPS & R. J. STIPES. (Va. Polytech. Inst. & State Univ., Blacksburg). Mimosa trees (*Albizia julibrissin*) were subjected to various inoculation techniques in the greenhouse and to trunk inoculations in the field to determine the

location of infection courts and the conditions favoring disease development. Root injury, inoculum dosage, and soil temperature were important in determining the rate and severity of disease development in greenhouse seedlings. More disease occurred in soils artificially infested with milled, oat-grain cultures of the pathogen, *Fusarium oxysporum* f. sp. *perniciusum* than in soils infested with milled, washed mycelium. Disease development was more rapid at 28 C soil temperature than at 23 C, 33 C, and 17 C, in descending order of severity. The root dip-spore suspension method of seedling inoculation produced the most uniform and predictable levels of disease. Trunk inoculations at biweekly intervals with washed conidia in suspension failed to produce disease. Reisolation of the pathogen above the inoculation point revealed that the fungus had not spread significantly beyond distances of initial conidia up-take. These findings indicate that (i) an active defense mechanism was operating in stem tissues, and/or (ii) a threshold level of root infection may be required before successful colonization of the shoot can occur.

Characterization of a NEPO virus isolated from Euonymus. C. W. PUFFINBERGER & M. K. CORBETT. (Univ. Md., College Park). A virus which incited chlorotic rings and oak-leaf patterns on young leaves of *Euonymus kiautschovica* or *E. fortunei* var. *vegeta* was mechanically transmitted to a wide range of hosts. The virus was also transmitted by dodder (*Cuscuta campestris*), grafting, and nematodes (*Xiphinema* spp.), but it was not transmitted by seed. In crude sap, the thermal inactivation point of the virus was 59-60 C for 10 min; the dilution end point was between 1:100-1:1,000 in 0.01 M phosphate buffer at pH 7; and sap diluted 1:3 in buffer had a longevity of 28-32 hr at 25 C. Tobacco plants systemically infected with tomato ringspot virus or tobacco ringspot virus were protected from infection by *Euonymus* ringspot virus (EuRSV) but tobacco plants infected with EuRSV were not protected against the other two viruses. Preparations of purified virus were infectious and gave an ultraviolet absorption spectrum characteristic of a nucleoprotein with a maximum (260 nm): minimum (240 nm) ratio of 1.35. Density-gradient centrifugation of purified preparations yielded one visible zone containing infectious 28-30 nm icosahedral virus particles with a sedimentation coefficient of 131 S. EuRSV is a member of the NEPO virus group but preliminary serological tests indicate no relationship to other members of the group so far tested.

Vacuum storage of plant pathogens. C. W. ROANE. (Va. Polytech. Inst. & State Univ., Blacksburg). Lyophilization techniques for storage of microorganisms have been used for many years, but the methods have not been generally employed by plant pathologists. In a modified method, the key lies in the use of a suitable vehicle such as sterile glass beads, small seeds, or short wooden sticks for introducing the culture into a vacuum storage tube and for transferring it after storage from the tube to a culture medium. The vehicle of choice is implanted into a nutrient agar in plates and the agar is sowed with an organism. After the organism grows upon or into it, the vehicle with adherent culture is transferred to a sterile tube for evacuation at ambient temperature for 4 hr at <100 μ Hg. The tube is sealed and stored at 2-5 C. With this method, species of *Diaporthe*, *Fusarium*, *Helminthosporium*, *Pseudomonas*, and *Rhynchosporium* have been stored successfully up to 15 years, and species of *Phytophthora* and *Pythium* for 2 years. Organic vehicles are favored because the organisms may form therein propagules resistant to the treatment. The method precludes freezing before drying and the cultures remain genetically unchanged and contaminant-free.

The effect of air and methane gas on root development of Pinus virginiana and on mycorrhiza formation with Amanita rubescens. V. M. RUSSO & W. L. KLARMAN. (Univ. Md., College Park). Various flow rates of air and air plus methane were passed through sterile, nutrient-saturated, vermiculite-peat moss in one-liter flasks. After treatment of the medium for six days, 16-day-old axenic seedlings of *Pinus virginiana* were planted in these flasks. Some flasks were also inoculated with *Amanita rubescens*, a fungus commonly mycorrhizal with *P. virginiana*. Seedlings were maintained under continuous illumination for thirty days at 24 C and roots were then examined to determine development and/or mycorrhizal association. Development of root systems of trees planted in flasks inoculated with *A. rubescens* increased with increase of air flow to 0.5 liters per hour. Air flow-rates above 0.75 liters per hour caused drastically reduced root development. Root development of trees alone in flasks receiving air reached maximal development at 0.5 liters per hour of air, but showed a sharp decline at higher flow rates. When 1% methane gas was passed through flasks containing no fungus, almost universally poor development occurred at all flow rates. When *A. rubescens* was present, maximal root development occurred at air flow-rate of 0.5 liters per hour and decreased sharply at higher flow rates. Mycorrhizae development in all inoculated flasks generally paralleled root development.

Peroxidase activity and heat-induced susceptibility of flue-cured tobacco to Phytophthora parasitica var. nicotianae. G. E. SANDEN & L. D. MOORE. (Va. Polytech. Inst. & State Univ., Blacksburg). Intact plants of 'Coker 187' (resistant) and 'Virginia Gold' (susceptible) tobacco were treated as follows: (i) roots immersed in a water bath at 50 C for one minute and inoculated with an aqueous mycelial suspension of *Phytophthora parasitica* var. *nicotianae*; (ii) roots immersed only; (iii) roots inoculated only; and (iv) untreated control. Root tissue and basal stem tissue were analyzed for peroxidase activity, both quantitatively (colorimetrically) and qualitatively (disc gel electrophoresis), immediately (0 days) and 10 days following a treatment. Disease severity of both cultivars was increased up to 100% by heating. This increase in severity, however, was not correlated with levels of peroxidase activity, and the peroxidase patterns of extracts of Virginia Gold roots and basal stem tissues were not altered by any treatment. The heat treatment induced four distinct peroxidase bands in the acrylamide gels of noninoculated and inoculated Coker 187 roots at 0 and 10 days that were not present in gels of unheated Coker 187 root extracts. It is possible that the unaltered peroxidases were active in black shank resistance, and the heat induced peroxidases were not.

The effect of ambient and filtered air and distance from pollutant source on two forest tree species. J. M. SKELLY & L. L. STONE. (Va. Polytech. Inst. & State Univ., Blacksburg). Charcoal filtered or noncharcoal filtered air was passed through 2 m x 4 m x 2 m chambers located at three distances from a source of air pollution, an industry manufacturing nitrated products and nitric acid. These sites, I, II, III, were established at 0.457 km downwind, 6.4 km downwind and 19.3 km upwind, respectively. An open air plot was also established at each site. Within each chamber and the open air plot, five seedlings of sweetgum and European larch were planted in containers with a uniform soil mix. The high 24-hour average of total oxidants monitored at the three sites, I, II, III were 22 pphm, 7.3 pphm, 3.4 pphm, respectively. The average height increase of all sweetgum seedlings grown in filtered or non-filtered chambers or open plots was 6.3, 5.7, and 3.3 cm, respectively, and purple coloration of foliage was more

prominent on all open and non-filtered chamber-grown trees. Similar results occur on larch with 2.4, 1.7, and 2.0 cm growth recorded, respectively. Open, and non-filtered, chamber-grown trees of both species exhibited premature leaf coloration and abscission several weeks before filter chamber-grown trees. There was a direct correlation of all data with respect to distance and direction from the source.

Effect of etch virus infection on Southern Maryland tobacco cultivars under field conditions. H. A. SKOOG (ARS, USDA, Beltsville, Md.). The etch virus disease of tobacco increased rapidly in Maryland during the 1971 and 1972 growing seasons. In 1972, eight Maryland cultivars and 'Hav. 307', a Wisconsin cultivar, were grown in a field split-plot design of three replications with 17 plants per replicate/cultivar. Hav. 307 was included for comparison because previous observations indicated it was consistently highly tolerant to etch. Late in the season plants within each plot were scored for virus symptoms using a 0 to 5 rating for no symptoms to severe symptoms, respectively. The disease index for the artificially inoculated material ranged from an average of 0.50 for Hav. 307 to 4.67 for 'Md. 609'. 'Wilson' and 'Md. 64' had the lowest ratings (1.50) of the Maryland cultivars. The ratings of the naturally infected material averaged from 0.33 for Hav. 307 to 3.17 for Md. 609 and 'Md. 59'. Of the Maryland cultivars, Md. 64 had the lowest score (1.00) followed closely by Wilson (1.17). Relative susceptibility of the cultivars was similar in the inoculated and naturally infected plots.

Interactions of Meloidogyne incognita, Helicotylenchus nannus, and Pratylenchus penetrans with breeding lines of Lycopersicon esculentum in agar culture. W. R. SLABAUGH & R. E. ADAMS. (W.Va. Univ., Morgantown). Seedlings were inoculated alone and in all combinations with *M. incognita*, *H. nannus*, and *P. penetrans*. The variety 'Success' was severely galled when inoculated with *M. incognita*. Galling was reduced when *H. nannus* or *P. penetrans* was present. Lines 621 (F₁) and 455 were not galled when inoculated with *M. incognita* or in combination with *H. nannus*, whereas galling occurred when *P. penetrans* and *M. incognita* were combined. Line 607 was not galled when inoculated with *M. incognita*. This line was galled when *M. incognita* was in combination with *H. nannus* or *P. penetrans*. Line 601 was totally resistant to galling. All three reduced the growth of the variety Success when inoculated singly or in combination. Growth of 621 was stimulated when inoculated with *M. incognita* but was decreased by *P. penetrans* and *H. nannus* and all combinations. Growth of 601 was increased by *H. nannus* alone, but was decreased by *P. penetrans* or *M. incognita*. This reduction of 601 by *M. incognita* was reversed by the combination of *M. incognita* and *H. nannus*. Growth increased in lines 607, 455, 506, and 544 when inoculated with *H. nannus*, but was reduced when inoculated with *H. nannus* and *M. incognita*.

Effectiveness of fungicide treatments against Xylaria mali in vitro and in preventing root infections on potted apple (Malus pumila) seedlings. D. A. SMITH & K. D. HICKEY. (Va. Polytech. Inst. & State Univ., Blacksburg). The effectiveness of captan, benomyl, thiabendazole, and triarimol against *Xylaria mali*, the apple black root rot pathogen, was determined in vitro and in greenhouse studies. Sharvelle's modified paper-disk bioassay for fungitoxicity was used to determine the amount of fungal growth inhibition by the fungicides. The high degree of contact activity of captan at 30 µg per bioassay disk was shown, whereas benomyl and thiabendazole were ineffective. Triarimol at 100-200 µg prevented mycelial growth for up to 15 days. In another experiment, where the fungicides were suspended in a liquid

medium, thiabendazole and benomyl were very effective fungistats at concentrations as low as 0.5 ppm. The effect of fungicide drench or soil-incorporation treatments in preventing root infections on potted apple seedlings was determined. Fungicides in drenches were more effective than when incorporated into the potting medium at similar rates. Captan at 4,000 ppm applied as a drench was the most effective treatment in reducing disease severity. Benomyl (300 ppm) and captan (1,000 ppm) drenches gave similar results, but were significantly less effective than captan at 4,000 ppm. Thiabendazole and triarimol were relatively ineffective.

ATP in second-stage larvae of Anguina tritici activated from cryptobiosis. H. W. SPURR, JR. (ARS, USDA, N.C. State Univ., Oxford). Desiccated, second-stage larvae of *A. tritici* were extracted from "wheat galls" and revived by the addition of water. Movement was observed within 24 hr after wetting. Adenosine triphosphate (ATP) per nematode was assayed by measuring the luciferin-luciferase reaction with a Du Pont 760 Luminescence Biometer. Although precautions were taken to avoid contamination by other microorganisms, bacteria were present in the water with nematodes at a constant level of 5.1×10^5 bacteria per ml. Sonification was more efficient for obtaining ATP from nematodes than the butanol-octanol disruption procedure commonly used with bacteria. After activation, second-stage larvae averaged 1.84×10^{-4} μg ATP excluding bacterial ATP. The assay procedure was sensitive enough to measure the ATP in one nematode. ATP per nematode declined gradually over a period of days following activation and incubation in water at 26 C. This procedure for measuring ATP should facilitate studies of cryptobiosis, senescence, and quiescence in nematodes.

Resistance to Alternaria alternata in Nicotiana tabacum. J. R. STAVELY, T. W. GRAHAM, G. R. GWYNN, J. F. CHAPLIN, & C. E. MAIN. (ARS, USDA, Beltsville, Md., Florence, S.C., Oxford, N.C., & N.C. State Univ., Raleigh). In 1970, all 990 tobacco introductions (TIs) and in 1971 all 486 cultivars of *N. tabacum* maintained by the USDA were exposed to field infection by the brown spot pathogen, *A. alternata*, at Oxford, North Carolina. In 1971, 50 apparently resistant TIs and 15 resistant breeding lines from T. W. Graham; and in 1972, the 28 most-resistant TIs and breeding lines and 20 apparently resistant cultivars were planted in four replicate blocks at Beltsville, Florence, and Oxford for further field evaluation. Susceptible cultivars were included as checks. Inoculation was artificial at Beltsville, but natural at the other locations. None of the entries were immune. However, some were as resistant or nearly as resistant as 'Beinhart 1000-1' and PD121, the only entries previously identified as highly resistant in North America. The newly identified, highly resistant, cultivars were 'Beinhart 1000', 'Ambalema', and 'Cigar Wrapper' (Dominican Republic). The highly resistant TIs, in descending order of resistance, were 1467, 820, 995, 1043, 1138, 505, 835, 764, 822, and 804; the best breeding lines were G-34, 35, and 36. Some newly identified resistant entries may be more desirable sources of resistance because of certain agronomic characteristics.

Fusarium oxysporum f. sp. perniciosum from lenticellar sporodochia on Fusarium-wilted mimosa trees in Virginia. R. J. STIPES & P. M. PHIPPS. (Va. Polytech. Inst. & State Univ., Blacksburg). Cultural and pathogenicity tests were conducted to confirm the identity of *Fusarium oxysporum f. sp. perniciosum* (Fop) in lenticellar sporodochia on the bark of Fop-wilted mimosa trees at Blacksburg, Va. Biopsy tissues from 1,440 lenticels on 20 diseased and four healthy mimosa trees were plated on glucose-yeast extract and modified Nash

& Snyder's PCNB media, each containing 200 $\mu\text{g}/\text{ml}$ chloramphenicol. About 38, 83, and 79% of lenticels from trees which exhibited initial foliar symptoms in 1972, 1971, and 1970, respectively, yielded cultures identified morphologically and culturally as *F. oxysporum*; 90, 85, and 96% of these cultures, respectively, produced foliar and vascular discoloration symptoms of mimosa wilt on greenhouse seedlings inoculated by the root dip-spore suspension technique. Vascular discoloration was closely correlated with foliar symptoms. Only one lenticel from one asymptomatic tree yielded Fop. Since aerobiological studies have documented the airborne nature of *Fusarium* conidia, these findings suggest that lenticellar sporodochia may function as major sources of inocula by which rapid overland spread of the disease might occur.

Growth fluctuations of two forest tree species proximal to a periodic source of air pollution. L. L. STONE & J. M. SKELLY. (Va. Polytech. Inst. & State Univ., Blacksburg). A geographically isolated industrial complex has been manufacturing nitrated products and nitric acid periodically since 1941. High relative air pollution emissions have been associated with the following production levels: 1941-1946 (89% of capacity); 1950-1957 (47%); 1966-1971 (73%). The interval periods were of zero or low production levels. This industrial complex is surrounded (within 0.2 miles) by natural stands of eastern white pine and yellow poplar. To measure growth of these species, increment cores were taken at d.b.h., dated, and measured with a dendrochronograph at a magnification of $\times 2.5$. The annual growth rate from 1935 to 1971 of 45 white pines was compared with the annual industrial production levels. An inverse correlation, significant at the 99.5% level, was found between tree growth and production levels ($r = .50$). A positive correlation between seasonal (Apr.-Sept.) rainfall and tree growth was significant at the 97.5% level ($r = .37$). Similar measurements were recorded for 50 even-aged, yellow poplar trees for the period of 1941-1971. An inverse relationship was found between growth and production levels and was significant at the 99.5% level ($r = .52$), whereas correlation with annual rainfall was significant at the 97.5% level ($r = .43$).

The occurrence of peanut stunt virus in crown vetch (Coronilla spp.) and its effect on various cultivars. S. A. TOLIN. (Va. Polytech. Inst. & State Univ., Blacksburg). A virus isolated from crownvetch, *Coronilla varia*, has been identified as a strain of cucumber mosaic virus that is closely related to peanut stunt virus (PSV). On the basis of host range, symptomatology, physical properties, serological reactions, and aphid transmissibility, crownvetch virus (CVV) and PSV were nearly identical. CVV was transferred from crown vetch to peanut and back to crownvetch by the leaf-rub technique. Foliar symptoms on 'Penngift', 'Emerald', and 'Chemwing' cultivars of *C. varia* were mild mottle, stunting, and distortion of the leaflets. Of 38 plant introductions inoculated with CVV in the greenhouse, the following *C. varia* entries appeared tolerant: P.I. 228373, 228411, 229968, 274041, and 278698. A mild mottle was evident on 11 *C. varia* entries, whereas 15 entries of this species showed a more striking mottle and a reduction in vigor. *C. coronata* leaflets showed a chlorotic mottle and were stunted. *C. globosa* was yellowed and stunted. Five entries of *C. scorpioides* were severely mottled, stunted, or killed by the virus. Susceptible cultivars in plantings near commercial peanut, soybean, or tobacco fields may provide a source of virus having the potential of causing severe damage to these crops.

Interactions of wood-inhabiting fungi as influenced by

substrate. R. P. TRUE & W. L. MAC DONALD. (W. Va. Univ., Morgantown). The competitive abilities of three lignicolous imperfect fungi and three hymenomycete wood rotters were compared on two media. Fungi were grown in paired competition on a glucose-yeast extract agar and on autoclaved half-inch disks cut from red-oak branches two inches in diam and lacking heartwood. The ability of each fungus to overgrow or be overgrown by each of the other five fungi was evaluated by a + or - rating from 1 to 3. Total

dominance ratings for each fungus when grown in competition with five others on nutrient agar and on wood, respectively, were: *Gliocladium roseum* +10, -2; *Paecilomyces (Spicaria) sp.* -5, -12; *Trichoderma viride* +9, -6; *Polyporus adustus* +1, +15; *Stereum purpureum* -7, -1; *Polyporus versicolor* -8, +6. The relative dominance of the three lignicolous imperfects was greater on agar than on wood. The reverse was true of the hymenomycete wood rot fungi.

Abstracts presented at the 1973 (30th) Annual Meeting of the Potomac Division of The American Phytopathological Society held 22-23 March 1973 at the Lakeview Inn, Morgantown, West Virginia.