Abstracts Presented at the Twenty-Ninth Annual Meeting of the Potomac Division of The American Phytopathological Society

Some effects of pH and nitrogen source on the hydrolysis of sucrose by Physalospora obtusa in culture. J. J. ALBERT & C. R. DRAKE (W. Va. Univ. Exp. Farm, Kearneysville, Va. Polytech. Inst. & State Univ., Blacksburg). *Physalospora obtusa* was cultured in liquid synthetic media containing sucrose as the carbon source and KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, and DL-aspartic acid as the nitrogen sources at initial pH values of 5.5 and 9.0. The medium also contained KH_2PO_4, 1.0 mg; $MgSO_4 \cdot 7H_2O$, 0.5 mg; $Fe(NO_3)_3 \cdot 9H_2O$, 0.2 mg; $MnSO_4 \cdot 4H_2O$, 0.1 mg; thiamine, 0.1 mg; and biotin, 0.005 mg. Chromatographic analyses of the cultural filtrates were made at 4-day intervals for 24 days. Hydrolysis of sucrose by *P. obtusa* was observed after 1 day of incubation at an initial value of pH 9.0 when aspartic acid served as the nitrogen source. All sugars were utilized between the 12th and 15th day at an initial pH value of 5.5, and between the 18th and 20th day at an initial pH value of 9.0. Partial hydrolysis was observed 1 day after incubation of the media at an initial pH value of 5.5, but not until the 4th day at pH 9.0 when KNO_3 was the nitrogen source. Complete utilization of the sugars by *P. obtusa* at both initial pH values was noted between the 15th and 18th days of incubation. The sugars were completely utilized at an initial value of pH 9,0, but not at pH 5.5 when NH_4NO_3 and $(NH_4)_2SO_4$ served as the sources of nitrogen. The sugars were not completely utilized at either pH value in the control without nitrogen.

A new strain of common bean mosaic in Puerto Rico. R. ALCONERO, J. P. MEINERS, & A. SANTIAGO (ARS, USDA, Mayaguez, P. R., Beltsville, Md.). Common bean mosaic virus was isolated from plant introductions of beans growing in field plantings in Puerto Rico. Inoculation of differential bean varieties produced systemic infection only on Bountiful, Columbia Pinto, Stringless Green Refugee, Red Kidney, and Puregold. Local lesions on primary leaves were observed with the Puerto Rican isolates as well as the type and Florida strains on Columbia Pinto, Great Northern U.I. 31, Great Northern U.I. 123, Red Mexican U.I. 35, Monroe, and Sanilac beans. The Puerto Rican strain produced symptoms on nonbean hosts as follows: *Vigna sinensis* 'No. 5 Blackeye', systemic mottle; *Dolichos lablab,* local lesions; *Phaseolus lathyroides*, local lesions, mild mottle; and *P. lunatus* 'Henderson Bush', mottle. The physical properties, seed transmission, morphology, and symptomatology were similar to those of known strains. Host reactions differed in some cases when isolates were tested in Puerto Rico and Maryland, possibly due to differing environmental conditions. Standardization of environmental conditions for tests and a standard set of differential bean varieties are needed to facilitate identification of common bean mosaic in different countries.

Isolation methods and per cent recovery of Fomes annosus and incidence on various soil hazards types. S. A. ALEXANDER & J. M. SKELLY (Va. Polytech. Inst. & State Univ., Blacksburg). The per cent of trees infected with *Fomes annosus* in selected 0.02 hectare plots on 5 "high hazard" sites (>70% sand content 8 to 12 inches deep) was obtained by two methods. Increment borer samples removed from each of two roots/tree indicated that 5, 6, 0, 26, and 16% of the trees were infected in each plot. When these same two roots were excised (46-cm sections) and isolations were made from several sound and decayed points, 29, 40, 13, 47, and 30% of the sampled trees were found to be infected. Seventy-nine trees were tested. In a second study using the two-root excision method, the average infection was 30% (41 of 136 trees; range 0-47%) on 9 high hazard sites and 10% (14 of 137 trees; range 0-50%) on 10 "low hazard" sites (<70% sand content 8 to 12 inches deep). All isolations were made on ortho phenylphenol medium. These results indicate that *F. annosus* may be much more important than was formerly reported. The "hazard" system of predicting the prevalence of *F. annosus* is reliable, but there is wide variability in incidence at specific sites.

Antifungal polyacetylene compounds associated with Phytophthora resistance in safflower. E. H. ALLEN & C. A. THOMAS (ARS, USDA, Beltsville, Md.). Six-week-old safflower plants of the breeding line Biggs and the variety Nebraska-10 were wound-inoculated in the first internode with *Phytophthora drechsleri* (virulent to Nebraska-10, avirulent to Biggs) and *P. megasperma* var. *sojae* (avirulent to both). Plants were held at 30 C with 2,200 ft-c of continuous light. Two antifungal polyacetylenes, safynol (*trans-trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol) and dehydrosafynol (*trans*-11-tridecene-3,5,7,9-tetrayne-1,2-diol), were extracted from infected stems and quantitated. When inoculated with *P. drechsleri*, Biggs stems (resistant) contained 956 μg (12 hr) and 1,472 μg (24 hr) safynol, and 47 μg (12 hr) and 297 μg (24 hr) dehydrosafynol/100 g fresh wt. Nebraska-10 (susceptible), inoculated with *P. drechsleri*, contained 943 μg (12 hr) and 1,590 μg (24 hr) safynol, and 27 μg (12 hr) and 200 μg (24 hr) dehydrosafynol/100 g fresh wt. When inoculated with *P. megasperma* var. *sojae*, the cultivars had similar safynol contents. Biggs and Nebraska-10 stems, inoculated with *P. megasperma* var. *sojae*, contained, respectively, 128 μg (12 hr) and 382 μg (24 hr), and 57 μg (12 hr) and 228 μg (24 hr) dehydrosafynol/100 g fresh wt. The rate of accumulation of dehydrosafynol was statistically correlated with the high disease resistance of Biggs.

Control of sweet potato scurf. R. E. BALDWIN (Va. Truck & Ornamentals Res. Sta., Painter). Effective chemical control of scurf (*Monilochaetes infuscans*) has been extremely difficult since the withdrawal of approved usage of mercury and other compounds as sweet potato slip treatments. Seed and sprout treatments in 1970 field trials indicated that benomyl and maneb gave excellent control of scurf when used as a 1-min dip. On a 0-100 scale, indices were 3.9, 0.4, and 14.9 for maneb, benomyl, and the untreated control, respectively, when used as seed treatments. Sprout treatment indices were 0.5, 0.3, and 27.9, respectively. In these trials, both maneb and benomyl were used at 2 lb. of the formulated product in 5 gal of water. The results of the 1971 trials indicate that the rate of maneb could be reduced, but benomyl could be reduced to 0.25 lb. of the product with excellent results as a sprout dip. Indices in 1971 were 13.4 and 22.7 for maneb at 2 and 1 lb., respectively. Indices for benomyl at 2.0, 1.0, 0.5, and 0.25 and the untreated control were 0.5, 0.6, 5.5, 6.0, and 51.4, respectively. The 1971 seed dip treatments were not as encouraging as the previous year.

Evidence that Xiphinema americanum transmits the causal agent of Prunus stem pitting. J. R. BLOOM, S. H. SMITH, & R. F. STOUFFER (Pa. State Univ., State College, Biglerville). Soil containing *Xiphinema americanum*, from an area of yew (*Taxus* sp.), was mixed with sterile and 1:1 (v/v). The soil-sand mixture (about 25 nemas/pint) was placed in 8-inch pots and transplanted with National Pickling cucumber and Tenn. Natural peach. Cucumbers were planted in a row through the center of the pot, and one peach seedling was planted on each side of the row near the edge of the pot. The same planting system was used in pots containing steamed greenhouse mix. Three days later, the cucumbers were rubbed with strains of tobacco ringspot or tomato ringspot virus or distilled water. Peach seedlings growing alone in steamed soil were similarly inoculated.

Peaches were maintained in the greenhouse for 8 months, then transplanted outdoors in Nemagon (1,2-dibromo-3-chloropropane)-treated soil and rated for stem pitting symptoms 12 months later. Symptoms developed only on peaches grown in nematode-infested soil. Eleven of 40 peaches grown with tobacco ringspot-inoculated cucumbers and 28 or 40 grown with tomato ringspot-inoculated cucumbers showed symptoms. Distilled water checks in infested soil showed 32.5% infection, indicating virus-contaminated nemas. Peach seedlings mechanically inoculated with tomato ringspot virus were stunted, but did not show typical stem pitting symptoms.

Inhibition of Aspergillus flavus aflatoxin production by Aspergillus niger. CHARLOTTE BURNETT, G. W. RAMBO, & G. A. BEAN (Univ. Md., College Park). *Aspergillus niger* (ATCC 10581) grown on peanut seed (*Arachis hypogaea* 'Early Runner') produced a metabolite that inhibited the production of aflatoxins by *A. flavus* (ATCC 2221). A conidial suspension of *A. niger* was added to autoclaved flasks of peanut seed and incubated at room temperature for 3, 6, 9, and 14 days, at which time the flasks were again autoclaved and inoculated with a conidial suspension of *A. flavus*. The cultures were incubated at 24 C for 14 days and extracted for aflatoxins. Aflatoxin concentration decreased with increasing length of incubation with *A. niger*. Peanuts incubated with *A. niger* for 14 days contained only 12% as much aflatoxins as controls that had not been inoculated with *A. niger*. Soaking peanuts in culture filtrates of *A. niger* before inoculation with *A. flavus* also reduced the aflatoxin concentration. When *A. flavus* was grown on a 9-day-old culture filtrate of *A. niger*, no aflatoxins could be detected and some reduction in mycelial growth was noted. Conidia of *A. flavus* germinated more slowly in *A. niger* culture filtrate than in the control, but percentages of germinated spores were similar in both.

Electron microscopy of viruslike particles in Penicillium stoloniferum. M. K. CORBETT (Univ. Md., College Park). Viruslike particles ca. 34 nm in diam have been isolated and purified by others from cultures of *Penicillium stoloniferum* ATCC No. 14586. Isolates of *P. stoloniferum* ATCC No. 14586 and ATCC No. 10111 were obtained from R. F. Bozarth, Boyce Thompson Institute for Plant Research. Materials for electron microscopy were obtained by placing a few drops of warm agar on 2-week-old fungal isolates growing on Czapek Dox agar slants. After the agar solidified, cubes ca. 2 mm containing mycelium and conidia were excised, fixed in Millonig's-buffered glutaraldehyde, and postfixed in 1% buffered osmium. The materials were dehydrated in a graded series of ethyl alcohol, embedded in Maraglas-Cardolite, and sectioned with a diamond knife in a Porter-Blum MT-1 microtome. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Hitachi HU-11C-1 electron microscope. Viruslike particles ca. 32 nm in diam were packed hexagonally in crystals within the cytoplasm of conidia of *P. stoloniferum* isolate No. 14586. Similar crystalline-packed particles were not found in sections of mycelium of isolate No. 14586, nor were they found in conidia or mycelium of isolate No. 10111.

Polyacrylamide disc-gel electrophoresis and isoelectric focusing of proteins released from soybean hypocotyl cell walls by culture filtrate of Sclerotium rolfsii. C. R. CURTIS & N. M. BARNETT (Univ. Md., College Park). Many proteins were released from cell walls of soybean hypocotyls (*Glycine max* 'Hawkeye 63') incubated with culture filtrate from *Sclerotium rolfsii*. Fungal culture filtrate was obtained from 4-week-old fungus cultures growing on an autoclaved mixture 1:1 (w/v) of fresh soybean hypocotyls and water. Incubation of 10 ml of hypocotyl cell wall suspension (50 mg dry wt of cell walls) with 1.0 ml of culture filtrate in pH 4.0 acetate buffer 0.1 M for 30 min at 30 C released many bound cell wall proteins. The mixture of cell wall proteins and filtrate was dialyzed, and the proteins were then subjected to either disc electrophoresis or isoelectric focusing in polyacrylamide gels in a pH 3-10 gradient. The gels were photopolymerized with riboflavin rather than persulfate-polymerized. Tests for peroxidase and esterase activity were made. The best separation into discrete bands of peroxidase or esterase activity occurred using isoelectric focusing techniques. Fungal culture filtrate contained no detectable peroxidase activity before or after disc electrophoresis, but the filtrate did have esterase activity.

Comparative disease reactions of Nicotiana species and cultivars to four virus isolates. V. D. DAMSTEEGT (Epiphytology Res. Lab., USDA, Frederick, Md.). Eighty-four entries representing 58 species from the World Collection of *Nicotiana* species, 21 foreign cultivars from the World Tobacco Collection, and other selected tobacco cultivars were evaluated for their susceptibility to disease caused by three individual virus isolates and a virus mixture. The viruses were the common strains of potato virus Y (PVY), tobacco mosaic virus (TMV), Datura 437 virus (D-437), and a mixture of PVY + TMV. Tobacco seedlings were inoculated with infectious plant extracts using an airbrush. All entries exhibited systemic symptoms of PVY infection, although the percentage of plants infected and severity of reaction varied. All entries except *N. benavidesii* and *N. tomentosa* var. *leguiana* were very susceptible to D-437. All entries lacking the *N. glutinosa* resistance factor were systemically infected by TMV and PVY + TMV. Entries that exhibited necrotic local lesions (typical of TMV infection) when inoculated with the PVY + TMV mixture later showed typical PVY symptoms on new growth. Measurements of height and fresh weight were taken at harvest, 21-24 days after inoculation. There was only slight variation in disease reaction among plants of an entry within a treatment. There was considerable variation among entries and among treatments.

Residual effectiveness of preharvest benomyl and triarimol applications for pre- and postharvest sooty blotch control. C. R. DRAKE & K. D. HICKEY (Va. Polytech. Inst. & State Univ., Blacksburg, Winchester). During a 3-year study, benomyl and triarimol were compared for their effectiveness for pre- and postharvest sooty blotch control on Golden Delicious apples when the standard seasonal spray applications were cut off 4, 6, 8, and 10 weeks before harvest. No preharvest sooty blotch developed on fruit when the last benomyl spray application was 4, 6, or 8 weeks before harvest. Only 1% sooty blotch occurred on a 3-year average when the last spray application was 10 weeks before harvest. Triarimol failed to provide commercially acceptable control of sooty blotch. Fruits that had the last triarimol spray applications 4, 6, 8, and 10 weeks before harvest during 1971 were 10, 16, 23, and 91% affected, respectively. The unsprayed fruits were 100% affected. The severity of postharvest sooty blotch development was correlated with the time interval from last spray application to harvest. Three months after harvest, fruits that had the last triarimol spray application 4, 6, 8, and 10 weeks before harvest were 6, 8, 16, and 24% affected, respectively, whereas benomyl-treated fruit on the same spray dates were 0, 0.6, 2, and 6% affected. Benomyl provided superior pre- and postharvest control, but was not significantly better than folpet.

Epiphytology of stripe rust of wheat caused by Puccinia striiformis in northeastern Oregon during 1971. R. G. EMGE

& D. R. JOHNSON (Epiphytology Res. Lab., USDA, Frederick, Md.). Factors affecting the increase of stripe rust, caused by *Puccinia striiformis*, and the resulting yield reduction were compared under field conditions. One-fifth hectare plots were established at Rew, Pendleton, and Weston, Ore. A 2.3 m^2 infection focus was established at tillering in the center of each plot in March 1971. Yield losses greater than 30% occurred over 12% of the plot at Rew, 32% at Pendleton, and 16% at Weston. Within 60 days from initial sporulation in the inoculated foci, the severity of rust at Rew, Pendleton, and Weston was 15, 42.5, and 20.4%, respectively. After 71, 62, and 69 days, respectively, 50% severities were observed. During this period, the frequency of incubation periods was 4.5, 1.75, and 3.5 days. Fifty per cent severities were attained during the heading stage at all locations. At Rew and Weston, 12 days elapsed from the attainment of the 50% level to the soft dough stage, whereas at Pendleton, 24 days elapsed. The time from milk to soft dough stage was 11, 18, and 5 days at Rew, Pendleton, and Weston, respectively. The frequency of incubation periods prior to, but not after, the time of the 50% severity level and the time the host remains in the flower, milk, and soft dough stages appear to be major factors affecting the amount of yield reduction.

Tobacco ringspot virus acquired but not transmitted by Tetranychus urticae. C. R. GRANILLO, S. H. SMITH, & W. M. BODE (Pa. State Univ., University Park, Biglerville). Tobacco ringspot virus (TRSV) was not transmitted by 4,110 two-spotted spider mites, *Tetranychus urticae*, reared on *Cucumis sativus* 'National Pickling' and *Phaseolus lunatus* 'Henderson Bush'. The virus-acquisition hosts included cucumber, lima bean, and *Vigna sinensis* 'Black Crowder'. The mites were placed on the acquisition hosts 5 days after inoculation with TRSV for periods of from 1 to 7 days. Mites were transferred from the acquisition hosts to the test plants (10 mites/plant) for transmission periods from 1 to 8 days. No symptoms were observed in any of the test plants over a 30-day period, and no virus could be recovered by mechanical inoculations. In five trials, virus was detected in the mites after a 24-hr acquisition period when homogenates (400 mites/ml of 0.05 M phosphate, pH 7.1) were rubbed on Carborundum-dusted cucumber cotyledons. An average of 5 local lesions/cotyledon was obtained when each 1 ml of homogenate was rubbed on 10 cotyledons. Exposing mites for 10 and 15 min to 2% formaldehyde prior to homogenizing did not reduce the number of local lesions. The virus recovered from the cotyledons inoculated with the mite homogenates was serologically identified as TRSV.

Propagule densities of Aspergillus flavus and A. niger group in Virginia field soils. G. J. GRIFFIN & K. H. GARREN (Va. Polytech. Inst. & State Univ., Blacksburg; ARS, USDA, Holland). The propagule density of *Aspergillus flavus* was determined by the dilution plate method in field soils to form a basis for spore germination and survival experiments in soil. Ten-g soil samples, collected in August 1971 at six locations for each of five fields planted with peanuts in the vicinity of Holland, were assayed with a peptone-glucose-chlorotetracycline-streptomycin-mineral salts medium supplemented with 3% NaCl plus 1 μg/ml Botran (M3SB) (2,6-dichloro-4-nitroaniline) or 3% NaCl plus 33 μg/ml rose bengal (M3SR). Recovery of *A. flavus* from field soil was similar on both media. The mean populations in the peanut fruiting zone for the five fields assayed with M3SB were 12.8, 4.3, 0.8, 3.7, and 11.8 propagules/g soil. Individual assays ranged from 0 to 102.7 propagules/g soil, and varied considerably among subsamples from a given location and among locations in a field. The mean *A. niger* group populations were 20.2, 14.0, 7.9, 58.3, and 4.0 propagules/g soil, respectively, for the same fields. With M3SR, the mean *A. flavus* propagule densities in 1970 in two fields sampled were also less than 15 propagules/g soil, but the population in soil containing decomposing rye debris was much greater. In 1971, which was characterized by higher rainfall, a higher *A. flavus* population associated with rye was not observed.

Effects of Penicillium simplicissimum on growth, chemical composition, and root exudation of axenically grown marigolds. K. M. HAMEED & H. B. COUCH (Va. Polytech. Inst. & State Univ., Blacksburg). Axenically grown marigolds (*Tagetes erecta*) were inoculated by the introduction of washed conidia of *Penicillium simplicissimum* in the rooting medium at concentrations of 7-8 × 10^6 conidia/plant. In two experiments, root exudates were collected and plants harvested 20 and 34 days after inoculation. In a third experiment, exudates were obtained in five samplings at weekly intervals. In all experiments, shoots of inoculated plants were taller, greater in fresh and dry weights, and flowering was earlier than in the noninoculated plants. After 20 days, the foliage of inoculated plants was higher in reducing sugars and lower in P and K. Root exudates from the inoculated plants were lower in total organic matter and protein. After 34 days, root colonization and degradation were extensive. The foliage of the inoculated plants was higher in Ca and total carbohydrates, and the root exudates were higher in organic compounds, than in the noninoculated plants. The exudates obtained at weekly intervals showed a decrease in amounts of organic compounds released at flowering from noninoculated plants only.

Differential action of benomyl and methyl-2-benzimidazolecarbamate in Saccharomyces pastorianus. R. S. HAMMERSCHLAG & H. D. SISLER (Univ. Md., College Park). Benomyl and its hydrolysis product, methyl-2-benzimidazolecarbamate (MBC), usually are equally toxic to most fungi but differ in toxicity to *Saccharomyces pastorianus*. The two fungicides appear to act by different mechanisms in this organism, at least in short-term experiments. Concentrations of benomyl (1-10 μg/ml) which inhibited growth also inhibited glucose or acetate oxidation 50-75%, and severely inhibited DNA, RNA, and protein syntheses. No morphological changes occurred in the cells after addition of the toxicant. MBC (10-15 μg/ml), on the other hand, did not inhibit glucose or acetate oxidation. DNA, RNA, and protein syntheses were not inhibited initially, but DNA synthesis was sharply curtailed after 1 hr. RNA and protein synthesis continued for several hours with only moderate inhibition. The cells became greatly enlarged and somewhat distorted. They reproduced slowly but did not separate,and there was a marked loss of viability. The data suggest that, in *S. pastorianus*, benomyl acts on a primary phase of metabolism, whereas MBC inhibits cytokinesis or mitosis.

Effect of spray interval on performance of triarimol for control of apple rot. K. D. HICKEY & C. R. DRAKE (Va. Polytech. Inst. & State Univ., Winchester, Blacksburg). Triarimol failed to give adequate control of late season diseases caused by *Physalospora obtusa* and *Glomerella cingulata* on Golden Delicious apple when used during a 3-year study in standard seasonal dilute spray programs under epiphytotic conditions. Under less severe conditions, the level of control approached commercial acceptance but was not equal to folpet. The data showed that the interval between the last triarimol spray and harvest had a direct effect on the per cent of fruit infected. In one test, fruits sprayed 15 and 30 days before harvest were 2.1 and 4.5% infected,

respectively, and were not significantly different from fruits sprayed with folpet 45 days before harvest. Data obtained in three other tests where the interval was 30, 34, and 42 days showed triarimol to be significantly inferior to folpet in fruit rot control. Fruits that were sprayed with triarimol 45, 60, and 75 days before harvest were 20.3, 15.0, and 22.0% infected, respectively, under conditions that resulted in 100% infection on the unsprayed fruits. Triarimol has been shown to be effective for control of apple scab, rust, and mildew, but these data indicate that it must be supplemented for control of late-season rots.

An association of the black turpentine beetle, Dendroctonus terebrans, and Fomes annosus in loblolly pine. W. E. HIMES & J. M. SKELLY (Va. Polytech. Inst. & State Univ., Blacksburg). A 32-year-old loblolly pine stand that had a high incidence of *Fomes annosus* and abundant black turpentine beetle (*Dendroctonus terebrans*) activity was located. Nine trees that exhibited this association were selected for study. A total of 1,438 insects in various stages of development were removed from the roots and lower boles and placed on pine discs. The discs were incubated at 23 C for 14 days. The per cent isolation of *F. annosus* from adults, larvae, and pupae was 16.2 (92 of 566), 8.1 (66 of 819), and 13.2 (7 of 53), respectively. The average per cent isolation from all stages was 11.5. At the end of the 14-day incubation period, 130 of the 819 larvae tested had bored through the discs. The *Oedocephalum* stage was found within nine of the canals. None of the 13 canals produced by adults on the discs was positive. Because 11.5% of the insects tested were positive, it is likely that *D. terebrans* functions as a vector of *F. annosus*.

Mycoplasmalike bodies in yellows-diseased Scoparia dulcis. HIROYUKI HIRUMI & KARL MARAMOROSCH (Boyce Thompson Inst., Yonkers, N.Y.). After recent observations indicated the possible mycoplasma etiology of the lethal yellowing disease of coconut palms, a search was made for yellows-diseased weeds in the proximity of dying coconut palms in Togo, West Africa. Patches and isolated plants of a common weed, identified as *Scoparia dulcis* (*Scrophulariaceae*), were observed affected by a witches'-broom disease in coconut groves along the coastal area between Lome and Kaincope. An electron-microscopic study revealed the presence of typical mycoplasmalike bodies in phloem elements of the diseased *Scoparia*. The working hypothesis that the yellows disease of the *Scoparia* and lethal yellowing are due to the same etiologic agent, carried by an insect vector from weeds to palms, could explain the observed pattern of spread of the coconut disease in West Africa, but confirmation awaits transmission studies.

Electron microscopy of Phytophthora megasperma var. sojae in resistant and susceptible soybean hypocotyls. W. L. KLARMAN & M. K. CORBETT (Univ. Md., College Park). *Phytophthora* species are generally intercellular pathogens. When soybean hypocotyls infected with *P. megasperma* var. *sojae* (*Pms*) were examined by electron microscopy, however, the fungus was found to be both inter- and intracellular. Hypocotyls of 1-week-old plants, Harosoy (susceptible) and Harosoy 63 (resistant), were wound-inoculated with *Pms*. Within 48 hr, typical hypersensitive reactions had occurred on resistant plants, whereas susceptible plants were water-soaked and flaccid. Pieces of inoculated hypocotyls 2-3 mm long were fixed in Millonig's-buffered glutaraldehyde, postfixed in 1% buffered osmium, dehydrated with alcohol, embedded in Maraglas-Cardolite, and sectioned with a diamond knife in a Porter-Blum MT-1 microtome. Sections were stained with lead citrate and uranyl acetate, and examined in a Hitachi HU-11C-1 electron microscope. Sections from resistant plants 24 hr after inoculation did not appear infected; after 72 hr, *Pms* was inter- and intracellular in all tissues adjacent to the point of inoculation, but integrity of the tissues was maintained. Sections from susceptible plants 24 hr after inoculation contained abundant inter- and intracellular mycelium, and infected tissues were disorganized. In some sections, haustorialike bodies were observed.

Disease and yield reduction in greenhouse-grown rice inoculated with Xanthomonas oryzae, the cause of bacterial leaf blight of rice. J. S. MELCHING (Epiphytology Res. Lab., USDA, Frederick, Md.). Seedlings of rice, cultivars Taichung Native 1 and Nato, in 4-inch pots died within 8 days when the stems at the crown were injected by hypodermic needle with 0.5 ml of bacterial suspension (ca. 10^7 cells) 17 days after emergence. Potted plants 26 days old inoculated by the pricking of the stems and leaves with a bacteria-laden needle or by atomizing bacteria on wounded (sterile needle-pricked) or nonwounded plants showed yield decreases in weights of dry grain produced of 47, 30, and 22%, respectively, as compared with the appropriate controls. Rice grown in simulated "paddy" conditions showed much less yield reduction than pot-grown plants when both were similarly inoculated. Addition of bacteria to paddy water resulted in disease and in yield reductions of 21, 26, and 32% when 0.25, 2, and 4 liters, respectively, of inoculum (ca. 10^8 bacteria/ml) were added to 0.91 × 1.22 m (3 × 4 ft) paddies flooded with 10 cm (4 inches) of water. Plants were 6 weeks old at the time of inoculation. The disease decreased yields by causing a reduction in numbers of panicles formed, grains formed per panicle, and in the average weight of grain produced.

Deleterious effects of tobacco smoke upon spore germination in Puccinia graminis tritici, P. striiformis, Piricularia oryzae, and an Alternaria sp. J. S. MELCHING, J. R. STANTON, & D. L. KOOGLE (Epiphytology Res. Lab., USDA, Frederick, Md.). Uredospores of *Puccinia graminis tritici* and *P. striiformis* and conidia of *Piricularia oryzae* and an *Alternaria* sp. were unable to germinate on 1.25% water agar when 50 cc of cigarette smoke were injected into the incubation chamber (volume 8,000 cc), whereas in a representative experiment, the germination percentages for the respective organisms on the control plates (50 cc of laboratory air injected) were 81, 91, 42, and 48. Decreased concentrations of smoke down to about 5 cc smoke/8,000 cc air caused inhibition; the magnitude of this effect varied with spore lot and with species. An additional incubation period in smoke-free air, following the smoke exposure, resulted in some increase in germination on the plates showing inhibition providing that the original exposure was not greater than about 30 cc/8,000 cc air. Exposure of agar to smoke prior to seeding with spores and incubation in a smoke-free atmosphere also inhibited or prevented germination, depending on the concentration and duration of exposure to smoke. Smoke from cigar and pipe tobacco had essentially the same effect as that described above.

The influence of soil **texture** *on the survival of Belonolaimus longicaudatus.* L. I. MILLER (Va. Polytech. Inst. & State Univ., Blacksburg). A survey was made of cultivated fields on 431 farms (25 soil types in an 87,000-hectare area) in Southampton County, Va., to determine the occurrence of the sting nematode (*Belonolaimus longicaudatus*). The nematode was found in 68 of the fields, and the soils of these fields (four soil types) were classified as sands or loamy sands with a deep, nonaggregated A horizon. The sand content of the nematode-infested soils ranged from 84 to 94%, and the

available water values ranged from 1.75-2.75. Several plots, in fields with soils lighter and heavier than those in which the nematode occurred in the surveyed area, were experimentally infested with the nematode occurred in the surveyed area, were experimentally infested with the nematode and planted to a host crop. The dematode reproduced and developed on the lighter soils when irrigation water was supplied during dry periods, but the nematode did not survive on the heavier soils with or without supplementary water during periods of water stress. It was concluded that the nematode is limited to soils of a certain texture, and that sand content and available water values, two important factors related to texture, can be used as an index of whether or not the sting nematode will survive in a soil.

Interrelation of stem pitting in various Prunus spp. S. M. MIRCETICH & E. L. CIVEROLO (ARS, USDA, Beltsville, Md.). The causal agent(s) of stem pitting was readily graft-transmitted by cross inoculation of different *Prunus* spp. Pitting symptoms in indicator plants were similar to those in the same *Prunus* sp. naturally pitted regardless of the donor *Prunus* spp. Apparently, stem pitting in different *Prunus* spp. is caused by the same or closely related strains of the same causal virus or viruses. Spread of stem pitting occurred in seedling orchards from infected to adjacent healthy trees of the same or different *Prunus* sp. A virus serologically related to tomato ringspot virus and to peach yellow bud mosaic virus was recovered from soil around peach, nectarine, and apricot orchard trees. The same virus was also recovered from apricot, European plum, and Nanking cherry seedlings inoculated with root chips from naturally pitted peach and apricot trees. This virus and the stem pitting causal agent(s) were not uniformly distributed in infected trees. However, we found no correlation between the recovery of this virus from soil and stem pitting in orchard trees or consistent association of this virus with pitted indicator plants. Apricot seedlings planted to sites of naturally pitted peach orchard trees became infected and developed stem pitting within 5 months after planting.

Effects of temperature and duration of dew period, under controlled conditions, on infection of corn by Helminthosporium maydis. C. E. PEET & M. A. MARCHETTI (Epiphytology Res. Lab., USDA, Frederick, Md.). Corn plants, cultivar XL 45, at the 3- to 4-leaf stage were inoculated with dry conidia of *Helminthosporium maydis* in a turntable tower at spore release rates of 0.5 mg (ca. 5×10^4) spores/8 plants, then put into dew chambers controlled at air temperatures of 16, 18, 21, 24, 27, or 29 ± 0.5 C for periods of 4, 6, 8, 10, 12, 15, or 18 ± 0.5 hr. After dew exposure, plants were put in a greenhouse at 21 C (night) and 27 C (day) temperatures and 40-70% relative humidity. Lesions were counted on all leaves 2-3 days after inoculation. With a 4-hr dew period, no infection occurred at < 21 C, but at >24 C, 0.1-0.4 lesions/leaf appeared. With a 6-hr dew period, 0.1 lesion/leaf was found at 18 C but none at 16 C. At 16 C, >8 hr of dew were required for infection. Between 18 C and 29 C, each incremental increase in dew period from 6 through 12 hr gave a progressive increase in disease; longer periods did not result in any further increase. When dew periods were >12 hr, about 30 lesions/leaf occurred regardless of the dew period temperature over the range 18-29 C.

Electron microscopy of African maize streak. BILJANA PLAVSIC-BANJAC & KARL MARAMOROSCH (Univ. Sarajevo, Yugoslavia, Boyce Thompson Inst., Yonkers, N.Y.). Maize streak has been considered a virus disease since the classical work with its leafhopper vectors was reported by H. H. Storey in 1928. Recent findings that a number of plant diseases are probably caused by leafhopper-transmitted, mycoplasmalike organisms rather than viruses prompted a reinvestigation of the cause of maize streak. Portions of leaves from healthy and diseased corn plants, collected in Ibadan, Nigeria, were excised in the field and fixed immediately in 3% glutaraldehyde. Further fixation and electron microscopy studies were carried out in the laboratory during the following weeks. Thin-sections of chlorotic spots from diseased leaves revealed the presence of polyhedral viruslike particles in the nuclei of cells. No fungi, bacteria, or pleomorphic mycoplasmalike bodies were detected. Controls from healthy plants appeared normal and contained no viruslike particles. The results indicate that the original assumption of Storey was correct and that maize streak is indeed a virus disease.

Mode of action of triarimol in Ustilago maydis. N. N. RAGSDALE (Univ. Md., College Park). Triarimol (EL-273) is a fungitoxic, trisubstituted methanol derivative with a structure similar to triparanol, a known inhibitor of cholesterol biosynthesis in mammalian systems. An investigation of the mode of action of triarimol in sporidia of *Ustilago maydis* revealed that 2 μg/ml of the chemical terminated sporidial multiplication after the first division, which was completed by 3.5 hr. Oxidation of glucose or acetate in nongrowing sporidia was not affected. DNA, RNA, and protein syntheses were only slightly limited during a 9.5-hr period. Preliminary investigations indicated substantial changes in the lipid fraction with a marked effect on sterol synthesis. Increase in total digitonin-precipitable sterols was inhibited 60-90% in 5.5 hr. The quantity of ergosterol in treated sporidia after 5.5 hr was less than that present in the initial inoculum even though the dry wt had increased 4-fold. Ergosterol added to agar medium seeded with sporidia of *U. maydis* did not alleviate toxicity, but polyoxyethylene sorbitan monolaurate (Tween 20), oleic acid, and several other fatty acids gave a delayed, partial reversal of toxicity. The action of the toxicant is probably at several sites in lipid biosynthesis.

Antimycin A and azide-tolerant respiration in Ustilago maydis. J. L. SHERALD & H. D. SISLER (Univ. Md., College Park). Sporidia of *Ustilago maydis* possess an antimycin A and azide-tolerant electron transport pathway which apparently diverts electrons to O_2 at a site on the substrate side of cytochrome b. The alternate pathway (induced by 0.5 μg/ml antimycin A or 5×10^{-4} M azide) supports a respiratory rate 1.5 to 2 times that of the normal system, but has a terminal oxidase with a lower than normal affinity for O_2. An equally high respiratory rate is supported by the normal pathway when uncoupled by 4 μg/ml of 4,5-dichloro-2-trifluoromethylbenzimidazole, but a high affinity for O_2 in this case indicates that the normal terminal oxidase is utilized. Respiration by the normal pathway is only slightly or moderately inhibited by 5×10^{-4} M 8-hydroxyquinoline or 1.5×10^{-3} M benzohydroxamic acid. The alternate pathway, however, is inhibited 70 and 85%, respectively, by these two compounds. Both compounds apparently act at a site in the alternate pathway which is not part of the normal electron transport system.

Dothiostroma pini found infecting Scotch pine, Pinus sylvestris, a first report. J. M. SKELLY (Va. Polytech. Inst. & State Univ., Blacksburg). A new needlecast disease of Scotch pine was found to be caused by *Dothiostroma pini*. This is the first report of this particular pathogen infecting and causing moderate damage to Scotch pine in the United States. The affected Christmas tree plantation is located near Montvale, Va. Symptoms occurred predominantly on the lower branches, and were characterized by very thin,

light-brown foliage. Affected needles exhibited yellow-brown spots, with black pycnidia of the causal fungus breaking through the needle surface. The terminal portion of many needles was dead beyond the point of infection. Approximately 30 trees have died, and several dozen more exhibit advanced symptoms.

Induction of stem pitting in peaches by mechanical inoculation with tomato ringspot virus. S. H. SMITH, R. F. STOUFFER, & D. M. SOULEN (Pa. State Univ., Biglerville, Pa. Dept. Agr., Harrisburg). Isolates of tomato and tobacco ringspot viruses were obtained from soil around stem-pitted peach and cherry trees. These isolates and known strains of tomato ringspot, tobacco ringspot, and *Prunus* necrotic ringspot viruses were used to inoculate mechanically Halford and Tennessee Natural peach seedlings. Cucumbers infected with these viruses were ground in 0.05 M phosphate buffer at pH 7.1 and rubbed on Carborundum-dusted leaves of peach seedlings 13 to 16 cm in height. Inoculated peach seedlings were maintained in the greenhouse for 8 to 9 months before planting outside in soil previously fumigated with Nemagon [1,2-dibromo-3-chloropropane, 2 gal active (12.1 EC)/acre]. The trees were examined for stem pitting symptoms 13 months later. Five of the six isolates of tomato ringspot virus induced the development of stem pitting. Of the 270 trees inoculated with the tomato ringspot viruses, the percentage showing stem pitting varied between isolates from 2.5 to 50. Stem pitting did not appear in any of the 98 controls, the 174 peaches inoculated with tobacco ringspot viruses, or the 87 trees inoculated with the two isolates of *Prunus* necrotic ringspot virus.

Control of postharvest decay of peaches with combination of pre- and postharvest treatments. W. L. SMITH, Jr., & H. L. KEIL (ARS, USDA, Beltsville, Md.). Fruits from peach trees sprayed with 57 g or 113 g/378 liters (2 or 4 oz/100 gal) benomyl 1 and 2 weeks before harvest developed significantly less decay (*Monilinia fructicola*) during postharvest holding at 18 C for 4 and 8 days than those from unsprayed trees. Preharvest treatments of 113 g/378 liters benomyl resulted in less fruit decay than the controls, but the treatment of 57 g/378 liters was ineffective. Postharvest-treated fruit with benomyl (57 g and 113 g/378 liters) or fruit dipped in water at 52 C for 2.5 min developed significantly less decay than did control fruit which had neither preharvest nor postharvest treatment. Peach fruits treated with a combination of either of the preharvest benomyl sprays and any of the postharvest treatments usually developed significantly less decay than fruit receiving only postharvest treatments. However, none of the pre- and/or postharvest treatments resulted in significantly better fruit decay control than that obtained by the postharvest dip treatment in water at 52 C alone.

Root chip inoculation, a reliable indexing method for Prunus stem pitting. D. M. SOULEN, S. H. SMITH, & R. F. STOUFFER (Pa. Dep. Agr., Harrisburg, Pa. State Univ., Biglerville). Approximately 50% detection of stem pitting resulted when 1-year-old peach seedlings were inoculated with root chips from 20 stem pitted *Prunus* source trees. The seedlings developed typical symptoms of stem pitting during the first growing season after inoculation. No stem pitting developed with comparable inoculations from 10 source trees free of obvious symptoms. Inocula consisted of root pieces, ca. 0.5-1.0 cm in diam, collected from an area of the root within 30 cm of the trunk. The 30 inocula sources included trees of Montmorency sour cherry and peach. Each of four peach seedlings was inoculated with three root chips from each inoculum source within 24 hr after the inoculum was collected. The root tissue was inserted into T-shaped cuts in the peach seedling trunk ca. 17 cm above the soil line; the cut was immediately wrapped with a rubber budding strip. The field-grown Halford seedlings used as index plants in this study were grown in soil that had received a preplant treatment of Dowfume MC-33 (methyl bromide 67%, chloropicrin 33%).

Resistance to Meloidogyne javanica and Cercospora nicotianae transferred from Nicotiana repanda to N. tabacum breeding selections. J. R. STAVELY, G. W. PITTARELLI, & L. G. BURK (ARS, USDA, Beltsville; Md., Oxford, N.C.). *Nicotiana repanda* is resistant to nine pathogens of tobacco (*N. tabacum*), but these two species have never been successfully crossed. Attempts to transfer resistance by a bridge-cross procedure through *N. repanda* × *N. sylvestris*, backcrosses to *N. sylvestris*, and finally to *N. tabacum* have been hindered by loss of resistance or sterility barriers. We crossed an autotetraploid form of *N. tabacum* 'C139' with amphidiploid 4N (*N. repanda* × *N. sylvestris*). Many plants in the resultant progeny were immune to *Meloidogyne javanica* and *Cercospora nicotianae*, pathogens for which there are no known resistant tobacco cultivars. These hybrids were sterile. However, some fertile plants resulted from treating immune hybrid seedlings with 0.4% aqueous colchicine for 4 hr. They were used as male or female parents in crosses with tobacco. The first generation from backcrosses with *N. tabacum* (B_1), had approximately 3 and 12% plants immune to *M. javanica* and *C. nicotianae*, respectively. They retained 11-19 of the 72 chromosomes theoretically introduced from the two wild species. Resistance to the two diseases was not linked and the reactions to each disease varied over the entire range. Progeny from selfing immune B_1 plants were 3 and 7% immune, while about 4 and 1% of the B_2 plants were immune to *M. javanica* or *C. nicotianae*, respectively.

Stereo electron microscopy of freeze-etched, corn stunt-diseased plant tissue. R. L. STEERE & R. E. DAVIS (Plant Virology Lab., USDA, Beltsville, Md.). Freeze-etched and freeze-fractured preparations of corn stunt(CS)-infected phloem cells reveal characteristic flattened bodies with attached helical filaments. The fracture face of the main body plasma membrane has a narrow, smooth band which contrasts sharply with the over-all particulate nature of this surface. Spiral filaments have a diameter approximating 250 nm, with the helical diameter approximating about 0.6 μ. These 3-dimensional structures are clearly visible in stereo electron micrograph pairs.

Soil transmission of Prunus stem pitting. R. F. STOUFFER, S. H. SMITH, F. H. LEWIS, & D. M. SOULEN (Pa. State Univ., Biglerville). *Prunus* stem pitting was shown to be transmitted through soil, but we obtained no evidence that it was transmitted by aerial vectors. Eighteen severely pitted peach trees were removed from an area in which most of the trees appeared to be diseased. At each tree site, a 55-gal steel barrel was sunk into the ground to within 6-8 inches of the rim. The barrels were filled to within 6 inches of the top with soil that was treated in one of the following wasy: (i) untreated field soil taken from the root zone of the pitted trees; (ii) comparable soil that was steam sterilized; (iii) comparable soil that was treated with Dowfume MC-2 (98% methyl bromide, 2% chloropicrin) at the rate of 454 g/barrel; (iv) steam-sterilized greenhouse soil mix; and (v) MC-2-treated greenhouse mix. One-year-old Sunhigh peach trees were planted into the barrels in May 1969. Six of the 18 trees were enclosed in insect-proof saran screen (32 × 32 mesh) cages. All trees were removed in October 1971. The bark was stripped and the trees were examined for pitting symptoms. Four of the six trees growing in untreated field soil showed the typical pitting syndrome. One of the four

trees which became infected had been enclosed in a cage. None of the trees growing in any of the treated soils showed pitting symptoms.

Frogs for production of antibodies to icosahedral plant viruses. SUE A. TOLIN, S. BOATMAN, & J. H. DEMOS (Va. Polytech. Inst. & State Univ., Blacksburg, Hollins College, Va.). Antibodies specific to peanut stunt virus (PSV), strain Y of cucumber mosaic virus (CMV), and brome mosaic virus (BMV) were produced in 4-inch grass frogs (*Rana pipiens*) after intraperitoneal injections with virus suspended in saline. Frogs were maintained at 28 ± 2 C in water in plastic pans and were sacrificed at intervals from the initial immunization date. From each frog, 1- to 2-ml blood was collected from the anterior vena cava with a hypodermic needle. Each frog was immunized with a total of 0.5 mg PSV in three injections 10 days apart. Serum from frogs after 25 or 35 days contained no antibodies detectable in gel diffusion tests. The titer was 1/4 from 48-day frogs and 1/16 from 55-day frogs. Similarly, 0.1 mg CMV induced a 1/8 titer in 55 days. Serum from frogs each given a total of 1 mg BMV in three weekly injections had a titer of 1/512 on day 54, whereas that from rabbits given 3 mg BMV had a 1/16 titer. Thus, frogs required a smaller total amount of virus to produce antisera with higher titer than did rabbits. Precipitin bands were sharper with frog sera than with rabbit sera. Additional antigen may induce greater antibody response in the frogs.

Interaction of genetic and environmental factors influencing sex determination of Meloidogyne graminis. A. J. WEBBER, Jr., & J. A. FOX (Va. Polytech. Inst. & State Univ., Blacksburg). Two populations of *Meloidogyne graminis*, characterized by low per cent males (L-7) and high per cent males (H-57), were used to study the interaction effect of genotype and different environmental regimes of temperature and host nutrition. Rooted sprigs of Tifgreen Bermudagrass (*Cynodon* sp.) were inoculated with 150 larvae and incubated at 26 C. The larvae had been conditioned at 20 or 26 C and at a low (0.01 Hoagland's) or high (Hoagland's) host nutrient level for 3 months. The sprigs had been conditioned at 26 C or 32 C and at a low or high nutrient level for 3 months. Males were counted at 21, 28, and 35 days after inoculation as they emerged from the roots. Females were counted at 35 days in cleared and stained roots. Both populations had a proportionately higher number of males when environmental conditioning was applied to the Bermudagrass rather than to the nematode, but the H-57 population consistently had a higher per cent of males than L-7 population, indicating a predominant genetic effect.

Ultrastructural investigation of microbodies in the Fusarium wilt organism. W. P. WERGIN. (ARS, USDA, Beltsville, Md.). The fine structure of parasitic fungal hyphae was examined in the vascular tissue of tomato plants inoculated with the Fusarium wilt organism, *Fusarium oxysporum* f. sp. *lycopersici*. Three to five days after inoculation, fungal hyphae were found localized in the metaxylem vessels of the tomato stems. These hyphae contained the normal complement of fungal organelles; in addition, the cytoplasm of the fungus also included numerous, well-developed microbodies. A negative staining reaction, which was obtained with the DAB (3,3-diaminobenzidine) method, indicated that these microbodies did not have peroxidase activity. The fungus was also cultured on potato-dextrose agar and processed for electron microscopic observation. Examination of this material disclosed that saprophytic hyphae did not contain the large microbodies which were commonly present in the parasitic hyphae.

The 1972 Annual Meeting of the Potomac Division was held 16-17 March 1972 at Beltsville, Maryland.