MICROBIOLOGY AND MOLECULAR BIOLOGY

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Abstracts

Development of *Erysiphe polygoni* on susceptible and resistant races of *Oenothera biennis*. JEAN DICKEY and MORRIS LEVY, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.——*Oenothera biennis* (evening primrose) consists of numerous isogenic races. Each race, when grown in a greenhouse or garden, is characteristically either susceptible or resistant to the powdery mildew fungus *Erysiphe polygoni*. Both mildewed and non-mildewed plants are also found in nature. As the initial step in determining the basis of resistance, the time course of fungal development on susceptible and resistant *Oe. biennis* was studied. Leaves were artificially inoculated with *E. polygoni* conidiospores. At two hour intervals epidermal peels were taken and stained with aniline blue in lactophenol for examination by light microscope. In addition, some specimens of leaf tissue were fixed in formaldehyde-alcohol-acetic acid, dehydrated in acetone, critical-point dried and gold-coated for observation by scanning electron microscope.

Under conditions of 95% relative humidity, 20 C, conidiospores germinated within 5 hours, appressoria were formed from 5-12 hours, penetration had been effected and haustoria initiated by 20 hours. On resistant plants, there was no further growth of the fungus. Secondary hyphae were present but poorly developed. On susceptible plants, by 26 hours after inoculation secondary penetration occurred and secondary haustoria appeared. Sporulating colonies could be seen in 4-5 days.

Pathogenic Soil Amebas, CLYDE G. CULBERTSON, M. D. Lilly Laboratory for Clinical Research Indianapolis, Indiana 46202.——We have published a review of the literature regarding pathogenic soil amebas and have developed staining methods for identifying Naegleria and Acanthamoeba utilizing the indirect immunoperoxidase technique. Recently we have been able to improve this by utilizing Gill's modification of Mayer's hematoxylin, to first stain the formalin-fixed tissue sections or smears also fixed in formalin, thereafter applying the indirect immunoperoxidase procedure.

With the advent of the vertical illuminator for epifluorescence, immunofluorescence identification can be similarly used, but this gives only a temporary preparation. Using hyperimmune serum made against Entamoeba histolytica it is also possible to identify this parasite in autopsy or biopsy tissues.

The Effect of the *Colletotrichum graminicola* Conidial Matrix on Anthracnose Development in Maize. G. C. BERGSTROM and R. L. NICHOLSON, Department of

Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——*Colletotrichum graminicola* isolate 104 was grown on oatmeal agar under constant fluorescent light (3200 lux) at 24 C. Conidia were borne in an orange, mucilagenous matrix both in cluture and on anthracnose infected corn leaves. The water soluble spore matrix was washed from conidia by centrifugation (2,000*g*) of aqueous spore suspensions and was removed in the supernatant. Two week old susceptible corn plants (inbred Mo940) inoculated with unwashed spores exhibited more rapid development of anthracnose symptoms than did plants inoculated with washed spores. Removal of the matrix did not affect the viability of spores, since germination percentages (twelve hours after inoculation onto 2% water agar containing 1% sucrose) were the same for washed and unwashed spores. Addition of the macromolecule fraction (non-dialyzable) of the spore wash restored the ability of washed spores to cause rapid symptom development. Neither the spore wash dialyzate nor a leachate from oatmeal agar stimulated anthracnose development. Autoclaved spore wash also gave no stimulation. Thus, a heat-labile component of the macromolecule fraction of the spore matrix was associated with the stimulation of anthracnose seedling blight.

Acid invertase activity (pH optimum of 4.7) was found in the spore wash and exhibited a maximum specific activity of 4,000 g glucose equivalents liberated/hr/mg protein at 30 C. the presence of an extracellular fungal invertase is consistent with the organism's preference for sucrose as a carbon source and may afford the pathogen an advantage in colonizing corn tissue. Maximum anthracnose severity is observed in the field at two stages of plant development characterized by the presence of high levels of sucrose in the host tissue. These stages correspond to seedlings up to the six-leaf stage and mature plants immediately following pollination.

Role of the Cecum in Bild Acid Metabolism in Germfree Rats. D. MADSEN, M. BEAVER, E. BRUCKNER, and B. WOSTMANN. Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——High tissue levels of cholesterol in the germfree (GF) rodent has been suggested to be a function of the increased pools of bile acids. Increased cholesterol absorption from the GF rat gut has been demonstrated directly; increased bile acid absorption has been estimated from other data. The enlarged cecum of GF rodents is linked to several effects on metabolism. We have investigated the role of the cecum on bile acid metabolism and excretion in the GF and conventional (CV) rat. Indwelling cecal fistulas were established in GF and CV rats. 1.0 Ci of 14 -Na-deoxycholate (DOC) (2.0 mg) was injected and feces subsequently analyzed over 9 days for excretion and distribution of label. Total excretion of label by the GF rat was roughly half that in the CV rat. The percent of label found in the cholic acid fraction of GF feces was more than twice that in the CV rats. (In the rat, absorbed DOC is rehydroxylated by the liver to cholic acid.) This indicates greater absorption and retention of DOC in the enterohepatic circulation, since 12_{alpha}-hydroxylation in GF rat liver has been shown to be not greater than in CV rats.

We conclude that bile acid absorption from the cecum of the GF rat is much greater than in the CV rat. This may be due to greater available cecal surface

area, greater absorptive capacity, or to variations in cecal emptying and intestinal transit time.

***In Vitro* Selection of Somatic Callus Sectors High in Regeneration Capacity.** N. P. MAXON, E. M. JONES, R. L. NICHOLSON, and C. L. RHYKERD. Departments of Agronomy and Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——Expression of totipotency in plant cells is dependent on genotype by environmental (*in vitro*) interactions. Alfalfa plants, *Medicago sativa* L., have been regenerated in our laboratory from primary callus. Examination of newly initiated callus revealed morphologically distinct callus sectors which were present in most callus that eventually regenerated. By selecting out and subculturing these sectors regeneration frequency was increased.

These callus sectors contained cells organized in such a way as to resemble pre-embryo structures. When cells were examined 14-17 days after callus initiation they contained large numbers of starch granules surrounding the nucleus. After 18-20 days callus contained xylem cells apparently dispersed at random through the tissue. Following an additional 3 to 4 days growth interconnected elements of vascular tissue were evident. When callus was initiated on a proper auxin: cytokinnin medium, embroyogenesis was detected within 28 days.

If callus sectors, such as the type found in alfalfa, occur in other plant genera and species, *in vitro* selection for these cell types may increase the regeneration capacity and reduce the time involved in the regneration of viable plants. This would be of particular importance for species which at present are difficult to regenerate from callus.

Enumeration and Identification of Bacterial Chitinoclasts in Selected Indiana Waters with Emphasis on the Actinomycetes. S. G. NEWMAN and C. E. WARNES. Department of Biology, Ball State University, Muncie, Indiana 47306.——Four borrow pits located in East Central Indiana were examined quantitatively and qualitatively for aerobic bacterial chitin decomposition from January through July, 1977. Speciation of chitinolytic actinomycetes was accomplished primarily by patterns of carbon compound utilization and sporulating qualities of the isolates. Numbers of chitinoclasts were lowest (50-150/ml) in winter with increasing counts through spring and summer months (60-4200/ml). Three major groups of chitinoclasts were isolated: gram-negative, nonfermentative rods (Group I); gram-negative fermentative rods (Group II); and the actinomycetes (Group III). Actinomycetes comprised 0-20% of the chitinolytic bacterial community, attaining the highest percentages in the winter samples. A possible seasonal selection for different groups of actinomycetes was noted, with members of the genus *Micromonospora* predominating in the cold winter waters and *Streptomyces* spp. in warmer spring and summer waters.

The Hypersensitive Response of Tomato to the Bacterial Wilt Pathogen, *Pseudomonas solanacerarum*. C. Y. LIN, W. R. STEVENSON, and R. L. NICHOLSON, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——Inoculation of stem sections of resistant and susceptible tomato lines with an avirulent isolate of *Pseudomonas*

solanacearum elicits a hypersensitive response characterized by intense browning of the tissue. A similar response is observed on resistant tomato stem sections inoculated with virulent isolates of the pathogen, but not on inoculated susceptible stem sections. The browning response is visible 48 hours after inoculation and increases in color intensity with time. By 96 hours after inoculation, susceptible stem pieces inoculated with virulent isolates are only slightly discolored whereas other tissue-isolate combinations are deep brown to black. Intact resistant plants inoculated with the virulent isolate respond with the development of brown coloration of internal and external tissues 48 hours after inoculation. Water-soaking near the point of inoculation is the only symptom apparent at this time on susceptible plants inoculated with the virulent isolate. A localized necrosis of tissues surrounding the site of inoculation with the virulent isolate appears on otherwise symptomless resistant plants 96 hours after inoculation. Wilting and internal tissue maceration are observed at this time on susceptible plants inoculated with the virulent isolate.

The intensity of browning of resistant stem sections is greater when the virulent isolate inoculum is adjusted to 5.2×10^7 cells/ml and less intense at higher and lower concentrations. Bacterial populations in inoculated resistant and susceptible plants increase at the same rate during the first 48 hours after inoculation. After 48 hours the bacterial population in susceptible plants continues to increase while the population in resistant plants rapidly decreases. Reduction of bacterial populations in resistant plants corresponds to the appearance of browning associated with the hypersensitive reaction. Bacterial populations in susceptible plants continue to increase until the time of plant wilt.