BOTANY

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ABSTRACTS

Metabolism of Arbutin by Selected Fungi. PHYLLIS J. CONRAD, Purdue University.—Arbutin, a common glycoside of pears, consists of glucose linked to hydroquinone. This compound was found to be metabolized through different pathways by two different organisms. *Alternaria*, a weak pathogen of pears, breaks the linkage between glucose and hydroquinone. The hydroquinone can be detected by chromatography. *Helminthosporium carbonum*, a non-pathogen of pears, completely breaks down arbutin, leaving no trace of hydroquinone.

It was hpothesized that pathogens and non-pathogens of pears follow different pathways in the breakdown of arbutin. The following organisms were tested: *Fabreae maculata*, a pathogen; and *Fusarium moniliforme*, *Nigrospora* sp., *Septoria glycines*, *Gibberella zeae*, *Diaporthe sojae*, *Cercospora sojina* I, *Cercospora sojina* II, and *Venturia inaequalis* all of which are non-pathogenic to pears.

The pathogen, *F. maculata*, as well as the non-pathogens, *F. moniliforme*, *Nigrospora*, and *G. zeae*, metablolized arbutin, leaving traces of hydroquinone. The other non-pathogens, *S. glycines*, *C. sojina* I, *C. sojina* II, and *V. inaequalis*, left no trace of hydroquinone when arbutin was broken down.

It was concluded that there is no correlation between pathogens and non-pathogens as to their metabolism of arbutin.

Inheritance of Resistance of Barley to Covered Smut. JOHN F. SCHAFER and H. L. SHANDS, Purdue University and University of Wisconsin. —Varieties of spring barley, *Hordeum* spp., differ in their response to inoculation with *Ustilago hordei* (Pers.) Lagerh., the fungus causing covered smut. These responses vary from susceptibility through an intermediate reaction to high resistance. The high resistance of Brachytic, Jet, and Pillsbury as studied in hybrids of Hannchen x Brachytic, Valentine x Jet, Odessa x Jet, Trebi x Pillsbury, and reciprocal of the latter appeared to be conditioned by two independent, dominant genes each. The high resistance of Kitchin differed by one dominant gene from intermediate responding Chevron whereas an additional gene conditioning an intermediate response was suggested for Kitchin from study of the hybrid with susceptible Odessa. F_3 data of Pillsbury x Jet and Jet x Kitchin hybrids indicated that the genes conditioning resistance of Jet were different from those of either Pillsbury or Kitchin. One gene conditioning the resistance of Brachytic appeared associated with the brachytic character conditioned by a gene on chromosome 7. The resistance of Kitchin was associated with the "deficient" head type conditioned by a gene on chromosome 1.

Ultrastructural Studies of *Puccinia graminis* Infection of Wheat Possessing Sr 11 Resistance. JOHN F. SCHAFER, MARY A. EHRLICH, and HOWARD

G. EHRLICH, Purdue University and Duquesne University.—Wheat nearly isogenic to the 'Chinese' variety of *Triticum aestivum* L. ssp. *vulgare* (Vill. Host) MacKey, except for possessing the Sr 11 stem-rust-resistance gene, produced a '1' type response to infection by an isolate of *Puccinia graminis* Pers. f. sp. *tritici* race 56 at 65° F. The 'Chinese'-type near-isogenic susceptible counterpart produced a '3c' response. The resistant selection was distinguished ultrastructurally, seven days after inoculation, by the presence of an electron-optically opaque deposit in the corners of those smaller intercellular spaces containing fungus hyphae. Host endoplasmic reticulum occurred excessively in infected regions, apparently in even greater concentration than in the 'Chinese'-type counterpart. Lomasomes were occasionally observed in host cells adjacent to hyphae, apparently much more frequently in the resistant than in the more susceptible wheat line. The location of lomasomes suggested a response at points of incipient penetration. However, lomasomes and haustoria were observed in the same cell. Lomasomes were not observed in comparable resistant and susceptible materials grown at 80° F, although gross symptoms were similar to those of the respective lines grown at 65° F.

A Developmental Study of the Maize Mutant Silkless (*sk*). T. F. WEEKS and S. N. POSTLETHWAIT, Purdue University.—The development of the maize mutant **silkless** has been investigated anatomically and morphologically. This recessive gene has no affect on tassel development. However, in ear development three diverse flower patterns were recognized—those with (I) anthers only; (II) silks only; and (III) no anthers or silks. These phenotypes results from degenerating cells of the pistil primordium and a concommitant development of staminate structures. A "gradient" system appears to be functioning in the maize plant. Moreover, in (*sk*) the complete gradient is exhibited within the ear.

The Photographing of Serial Microscope Sections on 16 mm Movie Film. S. N. POSTLETHWAIT and ROY MILLS, Purdue University.—Through alignment of a microscope, a microtome and a 16 mm movie camera, it has been possible to photograph in perfect registration serial microscope sections. The authors have collaborated to produce an automated machine which in effect permits one to insert a specimen embedded in paraffin into the machine and to harvest a movie film at a later time on which has been photographed each individual section of a specimen in sequence and appropriately aligned. Photographs of the machine in operation and several loop film products will be presented.

An Analysis of Calcium-induced Inhibition of Cell Expansion. JAMES S. COARTNEY, WILLIAM R. EISINGER and D. JAMES MORRÉ, Department of Botany and Plant Pathology, Purdue University.—According to classical concepts of plant growth regulation, calcium ions were considered to inhibit cell expansion by crosslinking anionic wall polymers. When etiolated pea internode sections were incubated in varying concentrations of calcium chloride, expansion was inhibited by high concentrations but stimulated by low concentrations both in the presence and absence of growth stimulatory concentrations of auxin

(IAA). With calcium-treated sections, cell wall extensibility (the ability of the walls to yield under externally imposed load) closely paralleled growth both in the presence and absence of auxin. The change in extensibility and growth due to auxin was not reduced until the calcium concentration exceeded 10^{-3} M. However, auxin-induced changes were eliminated at 10^{-1} M calcium chloride. When the response of living pea tissues was compared with that of pea tissues killed by freezing and thawing, the extensibility of the frozen and thawed sections was unaffected by either calcium or IAA.

If the effect of calcium were purely physical, the effect should increase as a function of concentration rather than having an optimum with a rapid decrease as growth is inhibited. In addition, if the effect of calcium were simply to cross-link anionic wall polymers, an effect of calcium on the dead walls would have been predicted. At least with peas, the results suggest that calcium-induced wall stiffening is a metabolic event as is auxin-induced wall loosening. Furthermore, the possibility that calcium inhibition may result from changes in the cation balance of the cell or more specifically of the cell membrane should be considered, in addition to a possible effects on the cell wall. We are now faced with the complicated problem of understanding how calcium might affect the regions of the protoplast which establish wall organization or provide the biodynamic driving force of cell expansion. It is hoped that further exploration of interactions between calcium, auxin and inhibitors of metabolism will provide clues as to the nature of the active metabolic events governing cell expansion rates.[1]

Preliminary Evidence for Secretion of Cell Dispersing Enzymes during Bean Petiole Abscission. D. JAMES MORRÉ, SUSAN KAMPMEYER and DAWN HALL, Department of Botany and Plant Pathology, Purdue University. —Previous studies of abscission events have focused attention on cell wall breakdown as a key structural change. It has been generally assumed that the enzymes are produced by cells in or adjacent to the separation layer. The cell membrane would normally act as a barrier restricting enzyme movement into this region of the cell wall. Results with neutral red staining coupled with plasmolytic studies have demonstrated that protoplasts remain intact during cell separation. Thus we conclude that the enzymes responsible for dissolution of the intercellular cementing substances are secreted through the plasma membrane and into the cell wall region. Chemical analysis of abscission zones revealed that compositional changes are largely restricted to hot water extractable constituents of the wall which would include the classical pectic fraction. That enzymes are produced during abscission is evidenced by the fact that inhibitors of RNA and protein synthesis block abscission and that ethylene-induced abscission is preceded by sequential rises in rates of both RNA and protein synthetic activities (Abeles, F. B. and R. E. Holm, Plant Physiol. Abs. liii, 1966). However, the nature of the proteins synthesized or of the enzymes secreted is unknown.

1. Work supported in part by a contract with the U. S. Army Biological Center, Fort Detrick, Mayland.

Assays for wall dispersing enzymes were based on a method measuring cell separation of blocks of cucumber pericarp described previously. Chloroamphenicol (100 μg/ml) was added to enzyme preparations and dispersion assays to prevent buildup of microorganisms. Explants were prepared from the monofoliate leaf of 15 day old bean plants and contained the abscission zone nearest the leaf base. Enzymes active in cell dispersion were secreted from the petiole-derived portion of the explant in linearly increasing amounts beginning 24 to 36 hours before the time of 50% abscission. Activity in the petiole-derived portion of the explant did not appear until after abscission and was due primarily to secondary invasion by microorganisms.

With intact plants grown in the greenhouse, the monofoliate leaves abscise when the plants were about 30 days old. Analysis of petioles from such plants of different ages revealed that cell dispersing activity reached a maximum between 26 and 30 days after planting and then plateaued or declined slightly, the maximum level of cell dispersing activity coinciding with the time of natural abscission.

For purposes of enzyme isolation, 40 g samples of petioles from 28 to 30 day old bean plants were harvested, homogenized in buffer and the proteins fractionated using ammonium sulfate. After exhaustive dialysis, cell dispersing activity was recovered in the fraction precipitated between 0 to 60% of saturation of ammonium sulfate with an active concentration of the enzyme in the fraction between 20 and 40% of saturation. Ammonium sulfate fractionation has provided no more than a 7-fold purification of the activity on a total protein basis, however.

"Aseptically" prepared explants abscise normally and contain the dispersing enzyme. However, microorganisms are an ever present source of concern in research related to cell dispersing enzymes. Regardless of the source of the enzyme, the results do demonstrate a rise in a pectinase-like cell dispersing activity coinciding with the onset of bean petiole abscission.[1]

Ultrastructural Changes during Secretion of a Polygalacturonase by the Fungus *Fusarium moniliforme*. DONALD TRUMBULL, STANLEY GROVE, D. JAMES MORRÉ and SUSAN KAMPMEYER, Depatment of Botany and Plant Pathology, Purdue University.—The metabolic systems involved in secretion are localized in several cell components including sites of synthesis (endoplasmic reticulum), sites of concentration and modification (Golgi apparatus) and vehicles for transport through the plasma membrance to the extracytoplasmic environment (secretion vesicles). Not all secretory pathways involve all these cell components as conspicuous or obligate participants. The actual secretory pathway may depend upon the nature of the secreted materials as well as the kinds of transitions necessary to facilitate transfer of product (Mollenhauer, H. H. and D. J. Morré, Ann. Rev. Plant Physiol. 17: 27, 1966).

1. Results supported in part by NSF GB 1084, a contract with the U. S. Army Biological Center, Fort Detrick, Maryland and NSF Undergraduate Research Participation Program Gy-67.

In the present study, a fungus, *Fusarium moniliforme*, was chosen for study because it lacks a morphologically recognizable Golgi apparatus and can be induced to secrete substantial quantities of at least one enzyme, polygalacturonase, into the extracellular environment. Enzyme secretion was induced by culturing the fungus on a liquid mineral salts medium containing sodium pectate as the sole carbon source. Hyphae grown on a glucose containing medium served as controls. Hyphae were examined after 2 and 3 days of growth for ultrastructural modifications related to enzyme secretion. Polygalacturonase activity of extracts was estimated by their activity in dispersing cucumber pericarp tissues into single cells and by the number of reducing groups released from a standard solution of sodium pectate.

Between 2 and 3 days after inoculation, the fungus was secreting significant amounts of polygalacturonase into the pectin-containing medium. Hyphae were collected and prepared for electron microscopy by fixation in potassium permanganate and were embedded in an epon-araldite resin mixture for sectioning. Hyphae grown on the pectin-containing medium were generally smaller and with fewer vacuoles than those grown on glucose. These differences, however, are probably not related to enzyme secretion. A difference which might be related to secretion, is the presence in pectin-grown cells of conspicuous, complex masses of electron luscent material, concentrated near the cell surface, resembling and often continuous with the cell wall. The results will be discussed in relation to known secretion pathways.[1]

Physiology of Resistance of *Glycine* and *Phaseolus* Species to Fungi. W. L. BIEHN, Purdue University.—The youngest trifoliate leaf of *Glycine max* reacts hypersensitively to *Helminthosporium carbonum*. Numerous hypersensitive reactions are also produced on etiolated hypocotyl tissue after penetration by *H. carbonum*. Polyamide thin layer chromatography of ethyl acetate extracts showed that three major diazotized sulfanilic acid (DSA) reacting compounds appeared or increased after the hypocotly tissue had just started to react hypersensitively to *H. carbonum*. A much higher yield of these compounds resulted when most of the epidermis was removed from the etiolated seedlings prior to inoculation. Ethyl acetate extracts of such injured inoculated soybean tissue inhibited the growth of *H. carbonum* to a much greater extent than the uninoculated injured tissue. Chromatographic data and ultraviolet spectra revealed that the same substances were produced with *Monilinia fructicola*, an *Alternaria* species and several other fungi.

Inoculation of injured hypocotyl tissue of *Phaseolus limensis* and *P. vulgeris* with *H. carbonum* resulted in the appearance and/or accumulation of DSA reacting compounds which were distinct from those produced in the *G. max-H. carbonum* interaction. It appears that the specific chemical compounds produced in a resistant interaction are dependent on the host and largely independent of the fungus. The DSA reacting compounds may be involved in a general mechanism of resistance to fungi.

1. Work supported in part by NSF GB 1084, NSF GB 03044, and a contract with the U. S. Army Biological Center, Fort Detrick, Maryland.