

BOTANY

Chairman: JOHN F. SCHAFER, Purdue University

C. FRANKLIN BISHOP, Goshen College, was elected chairman for 1966

ABSTRACTS

Nuclear changes and mitotic anomalies in tissue-cultured cells treated with extracts from marine algae. THEODORE J. STARR, MASHIRO KAJIMA and M. DIAZ-PIFERRER. University of Notre Dame, Department of Biology, Lobund Laboratory, Notre Dame, Indiana, and Instituto de Biologia Marina, Departamento de Biologia, C.A.A.M., Universidad de Puerto Rico, Mayaguez, Puerto Rico.—In a previous report, the antibacterial and antiviral activities of algal extracts were described (Starr *et al.*, *Tex. Rep. Biol. Med.*, 20:271, 1962). Results of continued efforts to characterize and concentrate the active factor directed our attention to specific nuclear changes and mitotic anomalies associated with treatment of tissue-cultured cells of animal origin. Algal extracts were added to cells on cover slips in Leighton tubes. Preparations were fixed, stained with acridine orange, and examined by fluorescence microscopy. Observed effects on normal development were multiple: 1) amitosis was widespread, large numbers of irregularly shaped nuclei were evident, multi-nuclear cells were apparently formed by budding and fragmentation of the nucleus; 2) after a short exposure to active algal fractions, metaphase configurations increased from a normal of 2 to 4 percent to more than 20 percent, 3) apparently some micronucleated cells were formed in a manner similar to that obtained with colchicine (Starr *et al.*, *Tex. Rep. Biol. Med.*, 21:412, 1963); and 4) in some instances miniature cells in clusters were observed and such anomalies may have resulted from a multiple cytokinesis.

Tissue Observations and Comparisons in the *Knotted Leaf (Kn)* Mutant of *Zea Mays L.* HARVEY D. TELINDE and S. N. POSTLETHWAIT, Purdue University.—The leaf of *Zea Mays L.* is characterized by having a flat blade delimited from the sheath by a so-called intercalary meristem.

The *Knotted (Kn)* gene conditions the production of localized protuberances or "knots" of tissue in the leaf blade. The nature and distribution of these protuberances suggest a relationship to a displacement of intercalary meristem tissue into the blade. These localized areas of meristematic activity surrounded by non-dividing differentiating cells result in folds or invaginations of leaf tissue.

Young leaf tissue, intercalary meristem tissue, knotted leaf tissue, and normal mature leaf tissue were histologically compared revealing certain similarities and differences between the four tissue types. The distal end of a "knotted area" retains normal leaf characteristics. The immediate proximal end of a "knotted area" develops a ligule-like structure delimiting an immature area resembling young leaf tissue undergoing intercalary growth and a simulated leaf sheath.

These observations suggest that certain cells associated with lateral bundles may retain intercalary meristem characteristics and thus form islands of meristematic activity surrounded by maturing tissue. The resulting structure is interpreted as a displaced intercalary meristem.

Some Effects of Dimethyl Sulfoxide upon Seed Germination, Plant Growth, and Herbicide Activity. H. W. MUSSELL, B. BUCKO, D. JAMES MORRÉ and R. J. GREEN, Purdue University.—Dimethyl sulfoxide (DMSO) may have applications in the plant sciences as a solvent for defoliant and herbicides. The effects of DMSO upon seed germination and plant growth were investigated using seeds of ten species soaked in various concentrations of DMSO. Results indicated no effect upon germination with concentrations up to and including 2.5 M DMSO. Concentrations of 5 M and 10 M DMSO delayed germination and in some cases caused a reduction in germination rate. DMSO concentrations that did not affect germination did not affect early growth. Daily foliar sprays of DMSO solutions did not affect the growth of cotton plants over a five week period. The only visible effect of the treatments was a slight leaf tip burn at the higher concentrations.

A preliminary field study using DMSO and Atrazine indicated that Atrazine is highly soluble in DMSO and the mixture can be applied to corn without affecting growth. Comparison of treatments indicated that the DMSO enhanced the effect of the Atrazine on dicots but not on monocots.

Effects of DMSO upon 2,4-D penetration and activity will be discussed and attempts to define the mode of DMSO enhancement of 2,4-D action will be presented.

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The metabolism of Arbutin by *Alternaria mali* and *Helminthosporium carbonum*. PHYLLIS CONRAD, Purdue University.—This study was conducted to compare the breakdown of Arbutin (the most commonly occurring glycoside in pear) by *Helminthosporium carbonum* and *Alternaria mali*. *A. mali* is weakly pathogenic, while *H. carbonum* is not pathogenic to pear. Unseeded and seeded broth extracts containing 10^{-2} M/1 arbutin were incubated at 28°C for one week and were then analyzed for arbutin, hydroquinone and quinone. After the solutions were centrifuged and filtered they were spotted on chromatograms. These were developed in 4:1:5 butanol, acetic acid, water and sprayed with diazotized sulfanilic acid. Results indicate that the two organisms do not metabolize arbutin in the same manner. *A. mali* apparently breaks arbutin down to hydroquinone and glucose. *H. carbonum* does not stop with hydroquinone. Solutions of arbutin with the organism showed no arbutin nor hydroquinone after one week's incubation. However, when sodium metabisulfite was added to filtrates of these and more arbutin was added, hydroquinone was detected after 4 days. Thus, it seems that *H. carbonum* breaks down arbutin to hydroquinone and then further breaks down hydroquinone to an unidentified compound or compounds.

Mature Plant Resistance of Oats to Crown Rust. BRIAN C. CLIFFORD and JOHN F. SCHAFER, Purdue University.—The development of mature plant resistance of *Avena sativa* L. to *Puccinia coronata*. Cda. var *avenae* Fras. & Led. was studied under growth chamber, greenhouse and field conditions. Resistance related to the age of the leaf at time of infection, older leaves being generally more resistant. The host: parasite interaction was influenced by temperature, resistance developing earlier at low temperatures in a range of 15-30°C. Neither infection type nor rate of symptom development was significantly affected by preinoculation temperature. Post-inoculation temperature was important in this respect. Comparisons between growth chamber, greenhouse and field experiments indicated the importance of low night temperatures in conditioning resistance. Day length in a range of 12-20 hours had no appreciable effect on the outcome of the host: parasite encounter. Resistance to the two rust races tested (264 & 294) was race specific and apparently governed by fairly complex genetic mechanisms. The temperature and ontogenetic components of resistance suggest that the activity of a resistance gene is modified by inhibitory mechanisms. It appears that the resistance gene is in a state of suppression in young leaves. At lower temperatures the degree of inhibition decreases with leaf development. At higher temperatures a higher level of inhibition is maintained.

Tordon, A New Synthetic Growth Regulator. WILLIAM EISINGER and D. JAMES MORRÉ, Purdue University.—Tordon (4-amino-3,5,6-trichloropicolinic acid) is a new herbicide which is potentially useful for vegetation control along highway right-of-ways and in other non-crop areas. Several sources have indicated that Tordon has growth regulatory properties. A series of biological tests was set up to investigate this possibility. In straight growth tests using etiolated oat coleoptiles, etiolated pea epicotyls and soybean hypocotyls, and green pea epicotyls, Tordon was shown to promote growth at low concentrations and inhibit growth at high concentrations. Translocation studies using leaf application of Tordon and 2,4-D on whole bean plants indicate that the two herbicides seem to translocate at approximately the same rate. In cell wall deformability tests, only weight application experiments have been completed. Tests using the Instron (a mechanical stress analyzer) are planned. Tordon has shown considerable activity in the corn root inhibition tests. Inhibition kinetics have been compared to relate the modes of action of 2,4-D and Tordon. Studies using tissue culture have indicated differences in the type of callus induced by the two materials.

The mode of action of Tordon will be discussed in comparison with that of IAA, 2,4-D and other growth regulations.

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Determination of Auxin-induced Cell Wall Deformability in the Presence and Absence of Actinomycin D. COARTNEY, JAMES S. and D. J. MORRÉ, Purdue University.—The Instron (a mechanical stress-strain analyzer) was used to mechanically assess the changes in cell wall de-

formability accompanying auxin (IAA or 2,4-D) induced growth. Sections 1.0 cm in length were taken from 27-32 mm *Avena* coleoptiles, the 3d internode of light-grown peas or from dark-grown maize mesocotyls. These sections were pretreated in the presence or absence of actinomycin D (an inhibitor of DNA-dependent RNA synthesis) and then transferred to auxin to evaluate the effect of the actinomycin D pre-treatment. Growth measurements were made throughout the incubation period using a binocular scope fitted with a scale calibrated to the nearest 0.1 mm. Sections were then boiled in methanol for 4-5 minutes and stored in methanol until mechanical analyses were performed.

The results with light-grown peas and maize mesocotyls revealed that actinomycin D pretreatment severely inhibited auxin-induced growth and cell wall deformability. With *Avena* a small increment of auxin-induced growth and deformability remained even after a 6 hour actinomycin D pre-treatment. RNA determinations performed on similarly treated *Avena* sections suggested that RNA synthesis was only partially inhibited by such a pretreatment.

These results will be discussed in terms of the requirement for RNA synthesis for auxin-induced growth and cell wall deformability.

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Characterization *Zea Mays* Root Cap Slime Secretion. D. D. JONES, D. J. MORRÉ, Purdue University and H. H. MOLLENHAUER, C. F. Kettering Research Laboratory, Yellow Springs, Ohio.—Dictyosomes of the plant Golgi apparatus are important organelles in concentration and modification of secreted substances. In the maize root tip, secretion is accompanied by hypertrophy of dictyosome cisterna to form large vesicles. These vesicle contents are released from the cytoplasm by fusion of the vesicle membrane with the plasma membrane (Mollenhauer, H. H., Whaley, W. G. and Leech, J. H., *J. Ultrastructure Res.* 5, 193-200, 1961). This system was selected for study of the physiology and biochemistry of the secretion process. During secretion, a slime droplet forms on the maize root tip, the size of which has been related by these studies to secretory activity. By utilizing an index rating of 0 to 10 mgs. of slime per root tip, steady state secretion kinetics (4 hrs.) were established with a Q_{10} of 1.57-2.3. The high Q_{10} suggests an active process. A temperature independent periodicity (period of 3 hrs.) was also indicated. Secretion amounts increased by supplying sucrose, lactose, and other carbohydrates. A 2-fold slime increase was obtained with 0.04 M sucrose. High sucrose concentrations inhibited slime secretion. Boric acid (20 mg/l) occasionally promoted secretion with variable results. No correlation was found between slime amount and growth rate.

A positive periodic acid-Schiff reaction (PAS positive) given by the secreted slime provided a means of localizing the secretion intercellularly. By observing the pathway light microscopically, secretion is tentatively established as outward, following the free space of the cell wall region after discharge from the symplast. Optimum movement rates were maintained only in saturated water atmospheres. A larger accumulation of Golgi product was found in cells of roots grown under

water stress (conditions where external secretion was prevented). Upon transfer to water, external slime accumulation appeared. Under these conditions, electron microscopy verifies that secretion vesicles seldom accumulate in large amounts at the interior wall surface. This process exhibited a Q_{10} smaller than two, suggesting that extrusion of slime is not an active process but influenced by the increased turgor.

A tentative method for slime isolation has been developed, and chemical characterization is under way.

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Evidence for Secretion of Cell Dispersing Enzymes from Maize Root Cap and Epidermis. DAWN HALL, H. H. MOLLENHAUER and D. J. MORRÉ, Purdue University and C. F. Kettering Research Laboratory, Yellow Springs, Ohio.—Normal sloughing of root cap cells has been assumed to occur primarily through a dissolution of the intercellular cementing materials. Except for hypertrophy of the Golgi apparatus and associated secretory activity, the separating cells show normal ultrastructural patterns. They have turgid protoplasts surrounded by a continuous wall layer. Voeller (The Cell, Brachet and Mirsky, eds. VI, p. 274, 1964) suggested that the Golgi apparatus secretion vesicles of the outer root cap cells of maize might contain a cell wall hydrolyzing enzyme which would aid in separation and eventual breakdown of the cap cells. Secretion of digestive enzymes has not yet been shown to be a Golgi apparatus function in plants as it has in animal cells.

An assay based on dispersion of cucumber pericarp was used to measure cell dispersing enzyme activity along the root axis. Uniform tissue blocks prepared from mature cucumber fruits were incubated either with intact root pieces or with enzyme preparations. Dispersion activity was based on the amount of cell separation during standard test conditions. Cell separation was proportional to concentration of pectinase and other commercial cell wall hydrolyzing enzymes. Pectinase is also active in separation of root cap cells.

Preliminary results using the cucumber assay method indicate cell dispersing enzymes as components of maize root cap and root hair secretions. The root hair zone showed the greatest activity. Results will be discussed in relation to normal development of the root cap and root hair formation.

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Cytogenetics of Radiation-induced Chromosomal Aberrations in *Collinsia heterophylla* Buist. M. AKHTARUZZAMAN and K. S. RAI, University of Notre Dame.—Seedlings of *Collinsia heterophylla* at the cotyledon stage were gamma-irradiated at doses ranging from 1050-1600r. A tetraploid, a triploid, three trisomics and 13 reciprocal translocations were detected in the parental generation (X_1). Of these aberrations, the behavior of reciprocal translocations was followed in details in sequential generations.

In a few cases, chromosomal chimeras were observed in X_1 generation. One of the two branches of a plant, for example, was tetraploid and the other was normal, disomic. Chimeras in plants heterozygous for reciprocal translocations were also observed in a few cases.

Twelve of the reciprocal translocations involved two chromosome pairs and the remaining one, three pairs. Chromosome orientation at metaphase-1 in majority of the translocation heterozygotes was directional and occurred more frequently as rings than chains in most cases. Chiasma-frequency was higher for chromosomes involved in the reciprocal translocations. The pollen-stainability and seed-set in translocation heterozygotes varied from 50-95% and 22-95% respectively. Approximately 10-25% of the pollen-mother-cells in some translocation heterozygotes disclosed seven bivalents only.

Studies of chromosome association at metaphase-1 in the triploid and the tetraploid plants indicated that disjunction in these, too, was directional.