CELLULAR AND MOLECULAR BIOLOGY OF PLANT STRESS

Joe L. Key and Tsune Kosuge, Organizers

April 15 — 21, 1984

Plenary Sessions

Poster Sessions

Plant Responses To Environmental Stimuli I

1401 MOLECULAR ASPECTS OF PHOTOSYNTHESIS AT LOW LEAF WATER POTENTIALS, J.S. Boyer, USDA/ARS, Department of Botany and Department of Agronomy, 289 Morrill Hall, University of Illinois, 505 S.Goodwin Avenue, Urbana, Illinois 61801, U.S.A.

Chloroplasts lose activity when leaf photosynthesis is inhibited at low water potentials caused by withholding water from the soil in which the plants are growing. In sunflower, the losses often limit the rate of photosythesis even though the stomata also close at the same time. The chloroplasts show no structural degradation but the thylakoid lamellae are thinner than in leaves having high water potentials (1). Photophosphorylation is inhibited under these conditions and the activity of chloroplast coupling factor, a subunit of the membranes, is inhibited when the protein is prepared from leaves having low water potentials. The inhibition is associated with altered conformation of the protein and decreased binding affinity for ADP (2). The decreases in chloroplast activity are not caused by decreases in the free energy of water but are correlated with increases in the concentration of cellular constituents. The cells lose over half of their water content under our conditions, and the intrathylakoid spaces and stroma shrink. The changes in photophosphorylating activity and in coupling factor activity can be simulated by exposure of the chloroplasts or protein to Mg^{2+} concentrations 2 to 3 fold higher than normally present in the stroma (1 - 3 mM). These Mg^{2+} concentrations also cause conformational changes in coupling factor similar to those at low water potentials (3). Since high Mg^{2+} concentrations could readily occur in the stroma during dehydration of leaves having these shrinkage characteristics, high ion concentrations may be involved in the activity losses by the chloroplasts.

References

1. Fellows, R.J. and J.S. Boyer. 1976. Planta 132 : 229.
2. Younis, H.M., J.S. Boyer, and Govindjee. 1979. Biochim. Biophys. Acta 548 : 328.
3 Younis, H.M., G. Weber, and J.S. Boyer. 1983. Biochemistry 22 : 2505.

1402 **INTERACTIVE EFFECTS OF WATER, TEMPERATURE AND LIGHT ON PHOTO SYNTHETIC FUNCTION,** Olle Bjorkman, Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305

High light levels are generally beneficial to photosynthesis but under certain conditions may become excessive, resulting in damage to the photosynthetic system. Such photoinhibitory damage is manifest as a reduced photosynthetic activity both at the whole leaf and the chloroplast level with the photon yield being especially sensitive. Photoinhibition is also reflected in altered chlorophyll fluorescence characteristics at 77K and involves an inactiviation of the photochemistry associated with Photosystem II (cf. Abstract by C. Arntzen).

Recent work provides evidence that environmental stress predisposes the leaves to photoinhibitory damage. The photosynthetic system of several species, including the sclerophylls Nerium oleander and Schefflera actinophylla and the more mesic Macroptilium atropurpureum, suffers severe photoinhibitory damage when subjected to water stress in the presence of full daylight, whereas little or no such damage occurs in well-watered plants of the same species. Considerable photoihibition is also evident in exposed leaves of a number of mangrove species growing in sea water on the tropical coast of northeastern Australia while shade leaves of the same plants have normal photon yield for oxygen evolution and otherwise unimpaired photosynthetic function. These results provide evidence, that in addition to any direct effect of water status on the photosynthetic system, low leaf water potential (resulting from restricted water supply or high salinity) greatly increases the susceptibility to photoinhibition. Results with M. atropurpureum further show that high leaf temperature exacerbates this damage even in the temperature range where no direct heat injury occurs.

Photoinhibition may thus be an important component of the damage to the photosynthetic system that occurs in the field when plants are subjected to environmental stress. Any mechanism that reduces the accumulation of excess excitation energy should therefore be important in increasing the tolerance of the photosynthetic system to environmental stress. Reduction of the interception of radiant energy is an obvious and highly effective mechanism.

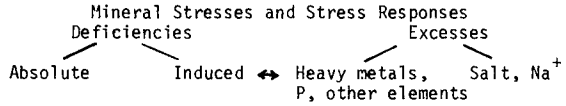
1403 MINERAL STRESSES: DEFICIENCIES AND EXCESSES, Emanuel Epstein, Department of Land, Air and Water Resources, University of California, Davis, CA 95616.

It is necessary, first, to define the concept of stress in general and mineral stresses in particular. Stress implies a departure from some norm or condition that represents no stress. This condition of non-stress is, however, itself elusive. For present purposes non-stress is any combination of external conditions allowing the maximal expression of the genetic potential of the plant in terms of growth, development, and reproduction, collectively called here performance. Stress-induced impairment of performance is called the stress response. Any component or combination of components of performance may be chosen, depending on the plant and the purpose of the investigation. Stress response then, is defined as follows:

$$\text{Stress response} = \frac{\text{potential performance - actual performance}}{\text{potential performance}}$$

Because all components of performance are a function of time, time must be included in discussions of stress. Finally, the genotype must be considered; a given condition may represent a stress for one genotype but not another, even if the two are closely related, like lines within a species.

Aspects of the mineral medium may represent stresses and elicit stress responses as defined above. They may be deficiencies of essential, or nutrient, elements, or excesses of either nutrient or non-nutrient elements:



A deficiency is absolute if the nutrient is in low supply (concentration, amount); it is induced if elicited by another element present in the medium, including H+ or OH- ions. Excesses are of two kinds. Some elements may be in excess if present at concentrations of some µmoles/L; salinity (most commonly Na+ salts) is only stressful to even salt-sensitive plants at concentrations higher by some orders of magnitude.

Examples of specific stresses and stress responses will be discussed, with emphasis on excesses and responses to them at the cellular level.

1404 HORMONES IN PLANT WATER STRESS, Hans Kende, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

During the past 20 years, it has been realized that phytohormones are involved in mediating some of the stress responses in plants. The role of three plant hormones - abscisic acid (ABA), cytokinins and ethylene - has been particularly well investigated with regard to water stress. By water stress we mean either conditions of drought or flooding. I shall discuss situations where stress translates into altered levels of hormones in the plant or redistribution of hormones within the plant, and where this changed availability of the hormone leads to a physiological response.

Roots synthesize cytokinins which are translocated to the shoot via the xylem. In this instance, cytokinins seem to fulfill the role of a real hormone, namely of a messenger substance between different organs of the plant. Under water stress, cytokinin production in the root decreases, and symptoms of senescence, indicative for cytokinin deficiency, appear in the leaves. These effects of drought could, at least in part, be overcome by treating the leaves with a cytokinin (for a review see ref. 1).

Leaves under drought conditions produce high levels of ABA, and ABA has been shown to cause rapid closure of stomata. It has been suggested that stomatal closure in plants under water deficit is mediated by ABA. Proving the correctness of this hypothesis has been complicated by the fact that stomatal closure usually precedes the first measurable increase in ABA content of the leaf. However, redistribution of existing ABA within the leaf and within compartments of the cell may trigger the initial closure of stomates, followed by the sharp increase in total ABA levels which may mediate the sustained response (for a review see ref. 2).

Flooding promotes internodal growth of deep-water rice plants. It has been shown that reduced levels of O_2 in the submerged parts of rice plants stimulate ethylene synthesis in the internodal tissue. This is a special adaptation found in rice as ethylene synthesis is usually reduced at lowered O_2 tensions. Ethylene accumulates in the submerged tissue because diffusion of the gas into water is about 10,000 times slower than its diffusion into air. Ethylene applied to non-submerged plants elicits a growth response which is very similar to the one observed under conditions of flood. Therefore, the response to flooding stress in rice and other semi-aquatic plants is mediated by ethylene (3).

References
1. Kende, H. Int. Rev. Cytol. 31:301-338 (1971)
2. Raschke, K. In: Plant Growth Substances 1982, P.F. Wareing, ed. pp. 581-598, Academic Press, 1982
3. Raskin, I. and H. Kende. Planta (in press)

Plant Responses To Environmental Stimuli II

1405 THE MOLECULAR RESPONSE OF CADMIUM RESISTANT PLANT CELLS TO HEAVY METAL STRESS, Paul J. Jackson, Genetics Group, Los Alamos National Laboratory, Los Alamos, NM 87545

Cadmium, a group IIB heavy metal, is a by-product of many energy and industrial processes. It is found in potentially harmful concentrations associated with mine tailings and a large number of other industrial processes. It is a major contaminant of oil shale processing waters and of the sewage sludge of most major industrial cities.

Datura innoxia cells have been selected for their ability to grow and divide rapidly in normally toxic concentrations of this metal ion. These resistant cells synthesize one or more heat stable, small molecular weight, cysteine-rich, metal binding proteins when grown in the presence of cadmium or zinc. Such proteins are not detectible in cadmium sensitive cells or within resistant cells grown in the absence of these metal ions. Upon addition of cadmium to the cells, rapid de novo protein synthesis allows detection of metal binding proteins within 45-60 minutes. This synthesis results in an accumulation of the metal binding proteins which reaches a maximum of 2 to 4% of the total, newly synthesized, soluble proteins within the cells, 8 to 12 hours following induction of protein synthesis.

De novo metal binding protein synthesis is directed by newly synthesized poly-A containing mRNA sequences. This suggests the presence in resistant cells of rapidly induced genes responsible of metal binding protein synthesis.

1046 BETAINE ACCUMULATION: METABOLIC PATHWAYS AND GENETICS, Andrew D. Hanson and Rebecca Grumet, MSU-DOE Plant Research Lab, Michigan State Univ. East Lansing, MI 48824

In certain groups of plants, water and salt stress provoke accumulation of betaine (glycine betaine). Betaine-accumulating taxa include the Chenopodiaceae and the grass tribe Hordeae (1,2). Circumstantial evidence indicates that stress-induced betaine accumulation is adaptive; betaine accumulated by stressed cells may function either as a non-toxic cytoplasmic osmoticum, as a stabilizing compound, or both (1).

Betaine accumulation is a potential metabolic component of stress tolerance that is simple enough to manipulate in genetic engineering of higher plants. To know whether this is either desirable or feasible, we need answers to many questions. Two of these are: I. *Is betaine accumulation really a heritable and adaptive trait?* No direct evidence yet shows that, within a species, naturally occurring levels of betaine are beneficial during stress, or that raising these levels will improve stress tolerance. With barley, we are using natural variation for betaine level (3) to investigate the inheritance of betaine, and to develop isopopulations differing in betaine level, in order to compare their performance under stress. This approach is possible because betaine level in barley is a highly heritable, predominantly additive trait; it is under the control of nuclear genes. II. *What are the enzymes..and..genes..unique to the betaine synthesis pathway?* Betaine is known to be synthesized in chenopods and barley from the primary metabolite choline by a two-step oxidation, of which the first may be regulated by stress (2). The supply of free choline for oxidation to betaine is also enhanced by stress. However, the pathway of free choline synthesis is poorly known in plants, and may be different in the chenopods and grasses (2). Most importantly, the steps in betaine synthesis are known only from radiotracer studies; essentially none of the corresponding enzymes have been detected in vitro, still less identified with protein bands on gels. Because choline-oxidizing enzyme(s) are probably of key significance, these are the first betaine pathway enzymes we are seeking.

More generally, we believe that advances in molecular genetics of higher plants make it timely to dissect simple metabolic traits that may confer stress-tolerance, of which betaine accumulation is only one example. This is because other types of traits conferring adaptation to stresses involve development, morphology or whole-plant physiology; they are therefore probably multigenic and beyond the scope of genetic engineering.

1. Wyn Jones, R.A. and R. Storey. 1981. In Physiology and Biochemistry of Drought Resistance, L.G. Paleg and D. Aspinall, eds. Academic Press, Sydney.
2. Hanson, A.D. and W.D. Hitz. 1982. Annu. Rev. Plant Physiol. 33: 163-203.
3. Ladyman, J.A.R., K.M. Ditz, R. Grumet and A.D. Hanson. 1983. Crop Sci. 23: 465-468.

1407 POLYAMINE METABOLISM AND PLANT STRESS. Arthur W. Galston, Hector E. Flores and Nevin D. Young. Department of Biology, Yale University, New Haven, CT. 06511

Cereal plants exposed to various stresses accumulate large quantities of putrescine (Put; 1,4-diaminobutane) but not the higher polyamines spermidine and spermine (1,2). Peeled oat leaf segments exposed to osmotic stress (0.4 to 0.6 M sorbitol or other osmoticum) or to pH values < 5.0 increase their Put level manyfold within 6 hr. of exposure, a significant increase being shown after only 2 hr (3,4). This increase in Put is paralleled by a rise in the activity of arginine decarboxylase (ADC), while the analogous enzyme ornithine decarboxylase (ODC) is unaffected. DL-α-difluoromethylarginine, a specific enzyme-activated irreversible inhibitor of ADC, prevents the stress-induced rise in both ADC activity and Put levels, implicating ADC in the stress response. L-canavanine and D-arginine are also effective inhibitors, and leaves of other cereals, such as wild oat, barley, corn and wheat respond similarly. Rises in ADC and Put are also prevented by cycloheximide (10-50 μg/ml) suggesting that protein synthesis is involved in the response.

K^+ deficiency (< 6 mM K^+) also resulted in a marked rise in ADC activity. Thus, oat seedlings grown on washed quartz sand for 18 days at 6 μM K^+ showed a sixfold elevation in ADC activity; in the first leaf, the activation was thirtyfold. Replacing K^+ with Na^+ or Li^+ partially inhibited the increase in ADC activity, while Rb^+ depressed activity below that in normal plants.

ADC was purified from 18 day old K^+-deficient oats by ammonium sulfate and acetone fractionation, gel filtration and ion exchange chromatography. This resulted in a 650 fold enrichment in activity with a 5% recovery. The final preparation was homogeneous, migrating as a single band with M_r 39,000 during SDS-polyacrylamide gel electrophoresis. Leaf segments were stressed and incubated with ^{35}S-methionine and labelled proteins extracted, separated by electrophoresis and identified by fluorography. Six bands were enhanced by osmotic stress, and 2 bands by acid stress. One protein band enhanced in both treatments comigrated with purified ADC. The labeling of this band was increased 28% by osmotic stress. The significance of this stress-induced rise in ADC and Put is not yet known.

(1) Smith, T.A. (1970) Ann. N.Y. Acad. Sci. 71, 988-1001. (2) Galston, A.W. (1983) BioScience 33, 382-388. (3) Flores, H.E. & A.W. Galston (1982) Science 217, 1259-1261. (4) Young, N.D. & A.W. Galston (1983) Pl. Physiol. 71, 767-771.

Plant Responses To Environmental Stimuli III

1408 PHYSIOLOGICAL AND MOLECULAR ANALYSES OF THE HEAT SHOCK RESPONSE OF PLANTS, Joe L. Key, Janice Kimpel, Chu-Yung Lin, Ronald Nagao, Ewa Czarnecka and Fritz Schöffl, University of Georgia, Athens, GA 30602

We have studied the heat shock (hs) response in soybeans and a number of crop plants using a variety of physiological and molecular approaches. The hs condition (i.e. accumulation of hs mRNAs and hs proteins, decrease in normal protein synthesis, and the development of thermotolerance) may be achieved under a number of hs or hs-like conditions in soybean seedlings: 1) incubation at 40°C (the "breakpoint" temperature for soybean) for about 2 hrs, 2) incubation for 10 min. at 45°C (a lethal temperature if treatment continues) followed by return to the normal growth temperature for about 2 hrs, 3) a gradual increase in temperature to 47.5°C, and 4) treatment with 50 μM arsenite for 3 hr (the latter closely but not completely mimics hs at 40°C). Genomic clones to a number of genes have been isolated and are being characterized. The hs system of soybean (and other plants) is highly complex relative to animal systems; there are at least 40 to 50 hs proteins in plants compared to 8 or so major hs proteins in Drosophila. The genes for these hs proteins reflect over-lapping families of related sequences based on hybrid selection/translation analyses, Southern hybridization analyses, and finally DNA sequence analyses. The complex low molecular weight hs proteins are much more abundant in soybean than are the high molecular weight hs proteins. Yet, the high evolutionary conservation of the hs response is suggestive of an essential function for the system, presumably the development of thermo-tolerance to otherwise non-permissive temperatures.

Relevant references to our work include:

Key, J.L., Lin, C.Y., and Chen, Y.M. (1981). Proc. Natl. Acad. Sci. 78, 3526.
Schöffl, F., and Key, J.L. (1982). J. Mol. Appl. Genet. 1, 301.
Key, J.L., Lin, C.Y., Ceglarz, E., and Schöffl, F. (1982). In "Heat Shock: From Bacteria to Man" (M. Schlesinger, M. Ashburner and A. Tissières, eds.) p. 329. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Key, J.L., Czarnecka, E., Lin, C.Y., Kimpel, J., Mothershed, C., and Schöffl, F. (1983A). In "Current Topics in Plant Biochemistry and Physiology Symposium" (D.D. Randall, D.G. Blevins, R.L. Larson, B.J. Rapp, eds.) p. 107. Univ. of Missouri, Columbia, Missouri.
Czarnecka, E., Edelman, L., Schöffl, F., and Key, J.L. (1983). Plant Mol. Biol. (in press).
Lin, C.Y., Roberts, J.K., and Key, J.L. (1984). Plant Physiology (in press).

This work is supported by contract DE-8509-80ER10678 from DOE and a contract from Agrigenetics Research Corporation.

1409 POTATO COLD HARDINESS AND FREEZING STRESS, Paul H. Li, Laboratory of Plant Hardiness, Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108.

The potato here is referring to the tuber-bearing Solanum species in addition to the commonly cultivated S. tuberosum potato. According to leaf cold hardiness and ability to acclimate to cold, the potato can be grouped into five groups (1): a) frost hardy and able to acclimate, b) frost hardy but unable to acclimate, c) frost sensitive but able to acclimate, d) frost sensitive and unable to acclimate, and e) chilling sensitive. The commonly cultivated potato belongs to d). Using NMR, the amount of unfrozen water present at sub-zero temperature was measured from six potato species with hardiness ranged from -3 to -6°C (killing temperature). At the killing temperature, a significant correlation between hardiness and % of unfrozen water was observed among them (2). Results confirmed that in the potato cold hardiness is due to the tolerance of freeze-induced dehydration. Additional evidence indicates that the thermal stability of proteins plays a significant role in freezing tolerance of the potato (3). During cold acclimation, an elevation of ABA concentration was observed in the potato which belongs to frost hardy and able to acclimate group (4). It is believed that the elevation of ABA may induce specific-protein synthesis which in turn is responsible for the development of cold hardiness in the potato.

1. Chen, H. H. and P. H. Li. Characteristics of cold acclimation and deacclimation in tuber-bearing Solanum species. Plant Physiol. 65:1146-1148, 1980.
2. Chen, P. M., M. J. Burke and P. H. Li. The frost hardiness of several Solanum species in relation to the freezing of water, melting point depression, and tissue water content. Bot. Gaz. 137:313-317, 1976.
3. Huner, N. P. A., J. P. Palta, P. H. Li and J. V. Carter. Comparison of the structure and function of ribulosebisphosphate carboxylase-oxygenase from a cold-hardy and non-hardy potato species. Can. J. Biochem. 59:280-289, 1981.
4. Chen, H. H., P. H. Li and M. L. Brenner. Involvement of abscisic acid in potato cold acclimation. Plant Physiol. 71:362-365, 1983.

1410 MEMBRANE DAMAGE AND REPAIR IN STRESSED CHLOROPLASTS, Charles Arntzen, David Kyle, Bruce Runk, and Herb Nakatani, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Two strategies for coping with stress-induced damage to the chloroplast have been characterized. The first involves a potentiation for membrane repair by elevated temperature acclimation; this allows the chloroplast to recover from a "heat shock". The second involves a rapid, selective thylakoid protein turnover process that remains active throughout the life of the plant and allows plants to constantly restore activity of electron transport chains damaged as a result of high light stress (photoinhibition).

We have shown that the primary site of photoinhibition damage results in inactivation of the secondary quinone (Q_B) electron acceptor of photosystem II (PS II). This renders PS II inactive due to the damage and subsequent removal of the Q_B apoprotein. Reactivation (repair) of damaged PS II centers occurs in vivo and requires synthesis of a "32" kilodalton polypeptide and its insertion and integration into the PS II reaction center. This damage and repair process explains the previously observed rapid turnover of the 32 kDa polypeptide and is consistent with the constant high levels of mRNA coding for this polypeptide in mature plant chloroplasts.

Severe heat stress (48°C, 5 min) results in damage to the PS II complex of chloroplasts in intact maize leaves. Recovery from this damage also requires protein synthesis. Unlike high light stress, however, the heat stress itself severely limits the amount of protein synthesis that can occur in a treated chloroplast. A preadaptation of the seedlings (39°C, 3 hr) acclimated the system allowing treated seedlings to fully recover from the stress. Recovery was found to correlate directly with rates of synthesis of new thylakoid polypeptides.

In summary, stress-induced damage to chloroplast thylakoids can be studied both with respect to the site of the primary lesion as well as in regard to the repair processes involved in recovery from the damage. The latter allow a better understanding of developmental strategies which have evolved to allow plants to tolerate environmental stress.

This research was supported by DOE contract # DE-AC02-76ERO-1338 to Michigan State University

Plant Responses To Environmental Stimuli IV

1411 ANAEROBIC GENE EXPRESSION IN MAIZE

M.M. Sachs*, W.L. Gerlach, E.S. Dennis, A. Elizur and W.J. Peacock
CSIRO, Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T., 2601 Australia

Anaerobic stress drastically alters the pattern of gene expression in maize seedlings. Pre-existing mRNA translation is repressed, while approximately 20 novel anaerobic polypeptides are selectively synthesized (1). Among the anaerobic polypeptides are the alcohol dehydrogenase isozymes encoded by Adh1 and Adh2. cDNA clones were made to mRNA from anaerobically treated maize seedlings (2). These clones were screened with cDNAs from anaerobic and aerobic mRNA and the anaerobic specific cDNA clones were further analyzed. The anaerobic specific cDNA clones were grouped into families by hybridization and each family was analyzed by hybrid selected translation and RNA blot hybridization. Among the several cDNA clone families are those which encode ADH1 and ADH2 and one family which hybridizes to two mRNA classes which allow translation of two of the previously identified anaerobic polypeptides. All of these cDNA clones hybrize to anaerobic specific mRNAs.

(1) Sachs, M.M., M. Freeling and R. Okimoto, 1980 Cell 20 761-767
(2) Gerlach, W.L., A.J. Pryor, E.S. Dennis, R.J. Frel, M.M. Sachs and W.J. Peacock 1982 Proc. Natl. Acad. Sci. USA 79 2981-2985

*Present address: Department of Biology, Washington University, St. Louis, MO 63130.

1412 THE Adh GENES OF MAIZE: COMPONENTS OF THE ANAEROBIC RESPONSE, E.S. Dennis, J. Ellis, E.J. Finnegan, W.L. Gerlach, D. Llewellyn, W.J. Peacock and M.M. Sachs, CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

In maize, both alcohol dehydrogenase genes (Adh1 and Adh2) are induced by anaerobic conditions. The two genes are unlinked, Adh1 is on chromosome 1, Adh2 on chromosome 4. The gene products, the enzymes ADH1 and ADH2, are similar; both enzymes are dimers of 40,000 dalton subunits and both homodimers and heterodimers are enzymatically active. Antibodies to ADH1 partially cross-react with ADH2.

We have cloned and sequenced the two Adh genes of maize. A comparison of the nucleotide sequences of the two genes indicates that they arose from a single gene which was duplicated. The coding regions of Adh1 and Adh2 have identical lengths and are about 80% homologous at both the amino acid and nucleotide sequence level. Both genes are interrupted by 9 introns in identical positions in the gene; for any intron the nucleotide sequence and length is not conserved.

The transcription start has been mapped in both genes and a presumptive TATA box identified about 35 bp upstream in each case. In the Adh1 gene a CCAAT box-like sequence is present at position -88 bp; this sequence is not seen in Adh2. The promoters are being analysed in order to identify the sequences responsible for anaerobic induction.

Anaerobic conditions induce the specific Adh1 and Adh2 mRNAs about fifty-fold. The kinetics of appearance of these two mRNAs are similar but the Adh1 mRNA level is maintained for a much longer time than the Adh2 mRNA.

Analysis of these two related genes should enable the identification of the sequences responsible for the anaerobic response as well as those responsible for the tissue specific expression of the two genes.

1413 MOLECULAR ANALYSIS OF ANAEROBIC GENES IN MAIZE, Sarah Hake, Phillip M. Kelley, William C. Taylor and Michael Freeling, Department of Genetics, University of California, Berkeley, California, 94720.

Anaerobiosis results in the selective synthesis of a particular set of polypeptides in the maize root including the two alcohol dehydrogenases (Sachs, et al., 1980) and glucose phosphate isomerase (Kelley and Freeling, 1984). We have identified two additional major anaerobic proteins, ANP33 and ANP35.5, as corresponding to fructose-1, 6-diphosphate aldolase. cDNA clones to five anaerobic mRNAs have been identified and characterized. We identify the cDNA clones to polypeptides using hybrid select translation and show that one is to fructose-1, 6-diphosphate aldolase mRNA. Using the cDNA clones to measure the kinetics of mRNA accumulation, we show that the increase in concentration of the different mRNAs is not simultaneous. Each message displays different kinetics. The kinetics of accumulation of aldolase mRNA differs from the kinetics of the aldolase polypeptide during anaerobic induction. Genomic DNA blotting experiments demonstrate that the genes encoding the anaerobic polypeptides are for the most part single copy. Adh1 and glucose phophate isomerase are located on the long arm of chromosome 1 (1L) (Kelley and Freeling, 1984). A translocation line that generates progeny that contain 1, 2 and 3 doses of 1L allowed us to test for anaerobic gene clustering: 3 of the anaerobic genes tested do not reside on chromosome 1L.

Kelley, P.M., M. Freeling. The anaerobic expression of maize glucose phosphate isomerase 1. J. Bio. Chem. accepted for publication.

Sachs, M.M., M. Freeling, R. Okimoto. The anaerobic proteins of maize. Cell 20: 761-768 1980.

1414 METABOLIC ACTIVITY AND SYNTHESIS OF MACROMOLECULES UNDER ANOXIA, Alain Pradet, Bernard Mocquot and Michel Delseny*, INRA Bordeaux, France and *CNRS Perpignan, France.

The ability of plants to synthesize macromolecules in anoxia was recently pointed out. DNA, RNA and protein can be synthesized without oxygen. It is variable depending on the plants. Fermentative metabolism and ATP production are also very different. The energy charge can reach "normal" values (about 0.8) under anoxia. The intensity of protein and RNA synthesis are modified in a parallel manner with the energy charge changes.

The modified patterns of polypeptides synthesized during the anoxic treatment were analyzed by two-dimensional polyacrylamide gel electrophoresis. Analysis of RNA shows that rRNA and mRNA are synthesized and that the processing of rRNA precursor is altered. Polyribosomes are present throughout the adaptation period although their amount is reduced during the first hour of anoxia. Changes in poly(A) content were noticed, showing that some mRNA are rapidly degraded.

Our data indicate that high metabolic activity occurs in rice embryo under anoxia, which can be correlated with a high energy charge value. The metabolic adaptation of rice embryos allows a survival of the rice seedlings for several days in the absence of oxygen. Such an important synthesis ofmacromolecules and survival under anoxia are not observed in most plant materials. The correlation between these phenomena and the ability of plant tissues to synthesize ATP under anoxia will be discussed.

Plant Responses To Biological Stimuli I

1415 GENETIC CONTROL OF PATHOTOXIN INDUCED STRESS IN PLANTS, David G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616

The scope of mechanisms used by pathogens to effect physiological stress include macromolecules involved in vascular plugging, enzymes involved in the digestion of polymeric host components, and metabolites toxic to host cells. In particular, host-specific toxins of fungal origin have been accorded great promise for yielding details of chemical mechanisms of pathogen induced stress because of their inherent host-specificity and because their action in symptom production is often under simple genetic control by the host. A host-specific toxin is defined as a metabolic product of a pathogen which is toxic only to hosts which are susceptible to that pathogen. Such toxins, if they induce the symptoms associated with the disease, are direct tools to study the molecular development of disease and the molecular basis of resistance to disease development. Molecular modes of action have been proposed for few pathotoxins and fewer still for host-specific toxins. However, recent evidence obtained with AAL-toxins produced by Alternaria alternata f. sp. lycopersici, causal agent of Alternaria stem canker of tomato, suggests a mechanism for genetic control of toxin induced stress. In this system genetic control of toxin sensitivity and disease resistance is determined by a single locus which expresses complete dominance for pathogen resistance and incomplete dominance for toxin sensitivity. The presence of an aspartate-like moiety in the toxin prompted metabolite competition studies which focused attention on aspartate carbamoyl transferase (ACTase) as a possible site of action. Partial purification and detailed kinetic characterization of the enzyme from green tomato leaf tissue revealed sigmoid substrate saturation kinetics for both substrates, aspartate and carbamyl phosphate, and sensitivity to inhibition by UMP and the AAL-toxins. ACTase from both resistant and sensitive (susceptible) genotypes was indistinguishable in kinetic response to the substrates and UMP but exhibited a two-fold higher toxin-enzyme dissociation constant (Kiapp) from the resistant genotype. The combination of UMP and AAL-toxins, when assayed at variable aspartate concentration, appear to act synergistically to form a double dead-end inhibitor complex which drove the ACTase reaction to zero at concentrations of UMP that were not inhibitory in the absence of AAL-toxins. The UMP:AAL-toxin synergism was forty-fold greater with ACTase from the susceptible compared to the resistant genotype. Feedback loops regulating intermediary metabolism of arginine, glutamine and pyrimidine biosynthesis provide the basis for a testable hypothesis of events leading from toxin induced stress to cell death. Application of this scenario to other host-toxin interactions will be discussed.

1416 EXPRESSION OF LATENT GENETIC INFORMATION FOR DISEASE RESISTANCE IN PLANTS, Joseph A. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

Plants developed effective mechanisms for resistance to all infectious agents in their environment to survive the selection pressure of evolution. Work in our laboratory provides biological and chemical evidence to support the hypothesis that all plants contain the genetic potential for resistance mechanisms to fungal, bacterial and viral diseases. The determinant of resistance would then be the speed and magnitude with which this potential is expressed.

Working with green bean, tobacco, cucumber, watermelon and muskmelon, we demonstrated that plants are systemically immunized against diseases caused by fungi, bacteria and viruses by restricted infection with fungi, bacteria, or viruses. Immunization protects cucumber, watermelon, muskmelon and tobacco throughout the season, and a single immunization protects cucumber against at least 12 unrelated diseases.

Multiple mechanisms for the containment of infectious agents are rapidly activated after infection in immunized plants. Plants are sensitized to respond rapidly as a result of immunization, but responses are most apparent after challenge with the infectious agent.

Immunization against blue mold also increased the growth of Burley tobacco. Immunized unchallenged plants at the start of flowering were 40% taller, had a 30% increase in fresh weight and 5-7 more leaves than control plants.

In some interactions general defense responses of the host, including phytoalexin accumulation, are elicited or suppressed. In the interaction of Phytophthora infestans and potato tuber, arachidonic and eicosapentaenoic acids produced by the fungus elicit phytoalexin accumulation in potato, whereas glucans produced by the fungus enhance or suppress the response.

1417 PLANT DEFENSE RESPONSES TO VIRAL INFECTIONS. G. Loebenstein and Adina Stein, Virus Laboratory, Agricultural Research Organization, Bet Dagan, Israel.

A defense response can be defined as a mechanism that after introduction of the virus into the plant prevents or restricts virus multiplication or spreading, and requires transcription of cellular DNA. The subliminal infection and the local lesion response, where virus after inoculation invades a few or several hundred cells respectively, but does not spread to other tissues are two of the most notable resistance mechanisms.

Various observations and hypotheses concerning localization, as ultrastructural changes in advance of the infection, barrier substances and inactivation of a "translocation protein" will be discussed. Evidence will be presented that localization is an active process, requiring translation of the host genome, and associated in Samsun NN tobacco infected with TMV, with the production of an inhibitor(s) of virus replication (IVR).

IVR inhibited virus replication in protoplasts from both local lesion-responding resistant, Samsun NN and systemic-responding susceptible, Samsun plants, when applied up to 18 hr after inoculation. It was not produced in protoplasts from susceptible plants nor from noninoculated protoplasts of the resistant cultivar. IVR was partially purified using $ZnAc_2$ precipitation, and yielded two biologically active principles with molecular weights of about 26,000 and 57,000, as determined by gel filtration. Further purification of IVR with HPLC is in progress. Actinomycin D and chloramphenicol, which enhanced TMV replication when applied to Samsun NN protoplasts up to 24 hr after inoculation, concurrently inhibited IVR production.

IVR also inhibited replication of several plant viruses when applied to infected leaf tissue disks, and replication of TMV in intact tobacco plants, when applied by spraying indicating that IVR is neither virus nor host specific. An antiserum to IVR was obtained, beeing a prerequisite for future isolation of the IVR specific m-RNA.

Activation of the localizing mechanism by polyanions and induction of systemic resistance in non-invaded tissues, and their association with "pathogenesis-related proteins", hormones and other elicitors will be presented.

Active defense responses are also known in several systemic infections, as in certain cucumber cultivars and in tobacco infected with CMV. In the later, an uneven distribution of virus is evident, producing striking "green islands" that are resistant to reinfection. A substance similar to IVR was released from protoplasts obtained from "green island" tissue, giving a partial cross reaction with IVR antiserum.

These results may open new approaches for the control of plant viruses by substances associated with natural resistance phenomena.

1418 MOLECULAR BASIS OF IMMUNITY OF COWPEAS TO COWPEA MOSAIC VIRUS, George Bruening and Janet L. Sanderson, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

Approximately 60 lines of cowpea (Vigna unguiculata) are designated as being operationally immune to cowpea mosaic virus strain SB (CPMV-SB) according to specific criteria (1). CPMV-SB replication in seedlings of these lines is restricted so effectively that no virus can be detected by sensitive infectivity and immunological assays performed several days after inoculation. The cowpea line Arlington is the only immune line from which resistant protoplasts have been isolated. Compared to inoculated protoplasts of the susceptible line Blackeye 5, Arlington protoplasts accumulate only approximately one tenth the amount of CPMV-SB, whereas protoplasts of other cowpea lines yield as much or nearly as much virus. The immunity of seedlings and the resistance of protoplasts of Arlington appear to be inherited in parallel as a simple dominant character, implying that both phenomena reflect a restriction of virus replication at the cellular level. Several aspects of the CPMV-SB replication cycle in Blackeye 5 and Arlington protoplasts have been compared. Two observations seem most significant. The level of (-)RNA (i.e., RNA that is complementary to the (+) or messenger polarity RNA of the virus particles) is greatly reduced in Arlington protoplasts, and capsid antigen accumulates to a lesser extent, per unit of (+)RNA (M.C. Kiefer, G. Bruening and M.L. Russell, Virology, in press). The genetic and biochemical results are most easily explained by the production in Arlington cells of an inhibitor of CPMV-SB replication. The two genomic RNAs of CPMV-SB are translated into polyproteins that are cleaved to give functional proteins. Our working hypothesis is that the postulated inhibitor acts on a virus specified proteinase to reduce formation of coat proteins and of one or more subunits of a virus specified RNA dependent RNA polymerase. We have demonstrated in extracts of Arlington protoplasts an activity that interferes with the specific cleavage of a precursor of the CPMV-SB capsid proteins. The degree of inhibition was dependent upon the amount of Arlington protoplast extract added. The inhibitor action was heat sensitive and was enhanced by partial fractionation of the extracts.

1. Beier, H., Siler, D.J., Russell, M.L., and Bruening, G. (1977). Phytopathology 67, 917-921.

Plant Responses To Biological Stimuli II

1419 SPONTANEOUS MUTATIONS IN MAIZE ASSOCIATED WITH VIRAL INFECTION, Stephen L. Dellaporta[1], John P. Mottinger[2], and James B. Hicks[1], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, [2]Dept. of Botany, University of Rhode Island, Kingston, RI 02881.

Genetic studies have indicated that a high incidence of spontaneous mutations is the basis for the virus-induced phenomenon termed Aberrant Ratio (AR) in maize. We have examined the molecular basis of spontaneous mutations at the shrunken locus recovered from AR lines previously infected with Barley Stripe Mosiac Virus and Wheat Streak Mosiac Virus. Our results indicate:

(1) Most mutations contain structural rearrangements of the Sh DNA which are genetically unstable.
(2) Detailed molecular analysis of two Sh mutants reveal insertions having characteristics of transposable elements.
(3) These elements lack homology with the inciting viral genome but are present as middle repetitive DNA sequences in all maize lines tested.

Our results suggest that viral infection in maize may induce genome reorganizational events. Although the interaction between the virus and maize genome may be indirect, the outcome of this stress process is the activation of transposable elements which contribute to the genetic instability observed in the AR phenomenon. These results may have general implications of how the plant genome responds to environmental and physical disturbances.

Plant Responses To Biological Stimuli III

1420 STUDIES OF L-CANAVANINE TOXICITY AND DETOXIFICATION IN INSECTS, Gerald A. Rosenthal, T.H. Morgan School of Biological Sciences and the Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky 40506

The nonprotein amino acid, L-canavanine is the guanidinooxy structural analogue of L-arginine. This higher plant allelochemical has proven to be an excellent compound for analyzing the chemical defenses of higher plants and the biochemical mechansims employed by insects to detoxify foreign allelochemicals.

Some of the biochemical bases for the marked insecticidal properties of this nonprotein amino acid will be discussed, particularly the relationship of L-canavanine to insectan protein synthesis and the formation and turnover of canavanine-containing proteins.

The Neotropical bruchid beetle, Caryedes brasiliensis provides an ideal mechanism for probing and analyzing the biochemical mechanisms evolved by this highly successful seed predator in adapting to this potentially poisonous allelochemical. The biochemical adaptations of this seed predator to its principal food and ovipositional resource, Dioclea megacarpa, a canavanine-laden legume, will be presented.

1421 CHEMICAL MODIFICATION OF PLANT STRESS RESPONSES, Terrence L. Graham, B. J. Castanho, S. J. Wratten, N. Le-Van, N. S. G. Kishore, C. C. Hodges, Monsanto Agricultural Products Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167

During their growth and development, plants "recognize" a complex array of endogenous and exogenous environmental and biological signals which direct the appropriate developmental event or the appropriate response to alleviate an applied stress. The plant response to a given stress usually involves a complicated matrix of metabolic shifts which work together to "modify" the plants physiological status toward that stress. Even the recognition and transmital of the stress signal for a given response involves a complicated "cascade" of molecular events in a number of cellular compartments including the cell wall, membrane, cytoplasm and nucleus. This molecular cascade of recognition events is moreover apparently under complex regulation by factors such as the magnitude (threshold) of the stress signal, the prioritization of other concomitant stress or environmental signals and the developmental/cell cycle "permissability" of responsiveness.

To control such remarkably complex biological phenomena one must be able to appropriately model its various components, chemically monitor the critical molecular components from the initial signal to the physiological effect and then finally develop chemical probes of the system which initiate or regulate the cascade in a highly specific manner. Feedback from such a "systems analysis" approach allows continued refinement of initial models and eventually may lead to the development of agricultural chemicals to control discrete physiological responses.

Such models and monitors have been developed in our laboratories largely based on our early work in soybean phytalexin elicitation. This work and its implications will be presented as well as a description of our analytical monitors, our cascade regulation models and our systems analysis approach to plant biochemical phenomena. Finally, specific examples of potential regulatory molecules will be described which may ultimately find use not only as probes or controls for discrete plant biochemical events but also in the discrete control of expression of blocks of genetic information in plants.

1422 ADAPTATION BY PLANT PATHOGENS TO INHIBITORY CHEMICALS IN DISEASED PLANTS, Hans D. VanEtten, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Plants often create a localized inhibitory environment in response to the stress caused by microbial infection. In some cases microbes promote decompartmentalization and mixing of preformed plant substrates and enzymes, which produce toxic compounds. In other cases microbes induce de novo synthesis of antimicrobial compounds (termed phytoalexins) by activating latent biosynthetic pathways in the plant.

Plant pathogens are often uniquely tolerant of phytoalexins and other inhibitory chemicals produced by their own hosts. In many cases the biochemical basis of the tolerance is unknown, but one common tolerance mechanism involves metabolic degradation of the toxic chemicals to less inhibitory products. To illustrate this phenomenon and the importance of such detoxification mechanisms for plant pathogenesis, the interaction of the fungal pathogen Nectria haematococca with one of its hosts, pea (Pisum sativum), will be discussed.

Pisum sativum produces the pterocarpanoid phytoalexin pisatin in response to infection by N. haematococca. Naturally occurring, highly virulent isolates of the pathogen detoxify pisatin by an O-demethylation reaction catalyzed by a cytochrome P-450 monooxygenase. A number of isolates of N. haematococca which lack this detoxification mechanism have also been identified. All of these isolates are of low virulence on pea. Genetic analysis of naturally occurring isolates that do or do not demethylate pisatin indicates that high virulence on pea is always linked to pisatin demethylation. More detailed studies have revealed that N. haematococca isolates can possess more than one gene conferring pisatin demethylation, and that not all of these genes are related to virulence. Some of these genes confer a phenotype characterized by rapid demethylation of pisatin, especially after induction by the substrate. The other phenotype is characterized by a slow rate of demethylation. Only the rapidly demethylating phenotype is associated with high virulence. N. haematococca is also capable of performing at least three other monooxygenase-catalyzed detoxification reactions on pterocarpanoid phytoalexins produced by plants of which it is a pathogen. These observations suggest the existence in this fungus of a family of genes coding for various cytochrome P-450s. We hypothesize that one factor leading to the evolution of a pathogenic relationship between N. haematococca and P. sativum is the natural selection of a specific cytochrome P-450 system that is especially adapted to detoxifying pisatin.

1423 CHEMICAL CONJUGATION AND COMPARTMENTALIZATION: PLANT ADAPTATIONS TO TOXIC NATURAL PRODUCTS, Eric E. Conn, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616.

Higher plants produce a large variety of chemically reactive, natural (secondary) products (1,2). These include compounds such as phenols, quinones, amines, carboxylic acids, cyanohydrins, hydroxamic acids, aldehydes, ketones, diazo compounds, terpenes and alkaloids. In some instances concentrations in the millimolar range represent a potentially toxic environment in which the primary metabolic processes of respiration, photosynthesis and the assimilation of NH_3 and H_2S must occur. Plants appear to have at least two major mechanisms for providing protection against these reactive species. The first is structural compartmentation whereby natural products are physically sequestered in specific organs (e.g., seeds, roots, leaves), tissues (e.g., epidermal layers), cells (e.g., resin cells, laticifers, glandular cells), organelles (e.g., vacuoles, chloroplasts) and extra cytoplasmic spaces (e.g., cell wall, subcuticular space). Whether the storage compartment is also the site of synthesis, or whether it only serves as a receptacle for storage of a natural product produced elsewheres, is largely unknown. There is a need for research in this area.

The second protective mechanism may be termed chemical compartmentation to describe the fact that numerous natural products occur in the plant as derived forms (e.g., glycosides, esters, O-methyl ethers) in which their physiologically active functional group is masked. Many other natural products also occur as chemical conjugates (e.g., glycosides, esters, dipeptides) for which a physiological significance is less apparent. Of prime importance to chemical compartmentation are the existance and localization of enzyme(s) which catalyze the synthesis and catabolism of such chemical conjugates. Studies of the substrates utilized by these enzymes has often disclosed a high degree of specificity and therefore supports the concept of a role for the chemically conjugated, natural product.

1. Secondary Plant Products, E.A. Bell and B.V. Charlwood, eds. Vol. 8 of Encyclopedia of Plant Physiology, Springer-Verlag, Berlin (1980).

2. Secondary Plant Products, E.E. Conn, ed. Vol. 7 of The Biochemistry of Plants, a Comprehensive Treatise, Academic Press, New York (1981).

Plant Genes and Their Expression in the Transformed State

1424 ORGANIZATION AND EXPRESSION OF DEVELOPMENTALLY REGULATED GENES IN SOYBEAN, Robert B. Goldberg, Department of Biology, University of California, Los Angeles, CA 90024.

Soybean seed protein genes represent an excellent model for the study of gene regulation during plant development. Mutant lines exist which are unable to synthesize specific seed proteins. Experiments will be presented which provide insight into the molecular basis of several seed protein gene mutations. In addition, comparative DNA sequencing studies will be discussed which indicate that there is a short consensus sequence in the 5 upstream region of several different seed protein genes.

Plant Responses To Biological Stimuli IV

1425 INDUCTION OF RESISTANCE-RELATED mRNAs BY UV LIGHT OR FUNGAL ELICITOR IN CULTURED PLANT CELLS, Klaus Hahlbrock, Joseph Chappell, David N. Kuhn, Michael Walter and Elmon Schmelzer, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, and Biologisches Institut II der Universität, D-7800 Freiburg, FRG

We have studied the biochemical responses of cultured parsley cells (*Petroselinum hortense*) to various forms of environmental stress, including pathogens and pathogen-derived elicitors, UV irradiation, heat shock, changes in culture conditions, and various combinations of these treatments. Although our major aim is to elucidate the plant's defense reactions against fungal and bacterial pathogens, some similarities between these responses, particularly to irradiation with UV light and treatment with elicitor, have led us to investigating these reactions in parallel.

The enzymes of phenylpropanoid pathways are rapidly induced when cell suspension cultures of parsley are treated either with UV light (1) or with a cell-wall fraction (elicitor) of the fungus *Phytophthora megasperma* f. sp. glycinea (2). Biosynthetic end products are either flavonoids (1), which are probably involved in protecting the plant from excess UV irradiation, or furanocoumarins (3), which might act as antimicrobial agents (phytoalexins) in the plant's defense against potential pathogens. We have used cloned cDNAs to demonstrate that the induction in both cases is due to transiently increased rates of transcription of the genes encoding the respective enzymes (4,5 and unpublished results). Different elicitor preparations have been compared, and the responses to simultaneous treatments with different biological or physical stimuli have been studied.

1. Hahlbrock, K., Knobloch, K.-H., Kreuzaler, F., Potts, J. R. M. & Wellmann, E. (1976) Eur. J. Biochem. 61, 199-206.
2. Hahlbrock, K., Lamb, C. J., Purwin, C., Ebel, J., Fautz, E. & Schäfer, E. (1981) Plant Physiol. 67, 768-773.
3. Tietjen, K. G., Hunkler, D. & Matern, U. (1983) Eur. J. Biochem. 131, 401-407.
4. Kreuzaler, F., Ragg, H., Fautz, E., Kuhn, D. N. & Hahlbrock, K. (1983) Proc. Natl. Acad. Sci. USA 80, 2591-2593.
5. Kuhn, D. N., Chappell, J., Boudet, A. & Hahlbrock, K. (1984) Proc. Natl. Acad. Sci. USA 81, in press.

1426 INDUCTION OF HYDROLASES AS A DEFENSE REACTION AGAINST PATHOGENS. Thomas Boller, Botanisches Institut der Universität Basel, Switzerland

Among the recognized defense weapons produced by plants against pathogens are hydrolases which can attack and lyse the cell walls of the invaders, like β-1,3-glucanase and chitinase (1). These two enzymes have initially been found to be induced by ethylene (2), a fact that may indicate a role for stress ethylene in defense reactions. We choose chitinase as a model to study the induction of such hydrolases further. We characterized and purified a highly active endochitinase in bean leaves (3). This enzyme was induced 30fold within 24 hours of an ethylene treatment, and it amounted to more than 1 % of the total protein after induction. We found no substrate for chitinase in the plant itself; however, it readily attacked isolated cell walls of *Fusarium solani*, a potentially pathogenic fungus, and it acted as a lysozyme when incubated with *Micrococcus lysodeikticus*. This supports the notion that chitinase functions as a defense enzyme against fungal and bacterial invaders.

Using immature pea pods, we investigated whether stress ethylene plays a role in the induction of chitinase and glucanase upon fungal infection (F. Mauch, L.A. Hadwiger and T. Boller, in preparation). Fungal infections or treatments with elicitors provoked an enhanced production of stress ethylene and strongly induced chitinase and β-1,3-glucanase. Incubation with exogenous ethylene induced the two enzymes also, albeit to a lesser degree than infection. However, when stress ethylene production was suppressed by specific inhibitors, the two enzymes were still fully induced after an elicitor treatment or infection. This indicates that elicitors and ethylene are independent stimuli for the induction of chitinase and β-1,3-glucanase, and that stress ethylene has no obligate role in this response to infection.

It is unknown, at present, to what extent chitinase and β-1,3-glucanase contribute to the actual defense potential of a plant against pathogens. However, their coordinate induction by elicitors and pathogens is reminiscent of the induction of the enzymes of phytoalexin production, and may serve as another interesting model to study the responses of plants to pathogen stress.

(1) Bell, A.A. (1981) Ann. Rev. Plant Physiol. 32, 21 - 81
(2) Abeles, F.B., Bosshart, R.P., Forrence, L.E., Habig, W.H. (1970) Plant Physiol. 47, 129 - 134
(3) Boller, T., Gehri, A., Mauch, F., Vögeli, U. (1983) Planta 157, 22 - 31

1427 INDUCTION OF GLYCEOLLIN BIOSYNTHESIS IN SOYBEAN; Hans Grisebach, Michael G. Hahn, Joachim Leube, Peter Moesta, Institut für Biologie II der Universität, D 7800 Freiburg, FRG

An important response of plants to infection by microorganisms is the accumulation of antibiotics called phytoalexins. Accumulation of the phytoalexin glyceollin in soybean infected by Phytophthora megasperma f.sp. glycinea or treated with a glucan elicitor isolated from this fungus is preceded by increases in the activity of a number of enzymes including those involved in the biosynthesis of glyceollin [1]. Infection with the incompatible race 1 of P. megasperma leads to a higher activity of phenylalanine ammonia-lyase [1] and dimethylallylpyrophosphate : 3,6a,9-trihydroxypterocarpan dimethylallyltransferase [2] than infection with the compatible race 3. Not all enzymes in the biosynthetic pathway increase after challenge. The activity of 3-hydroxyl-3-methylglutaryl CoA reductase decreased after elicitor treatment of cotyledons or hypocotyls. Infection leads to de novo enzyme synthesis [1]. We have investigated whether cAMP could be involved as a second messenger in the plant's response to infection. While cAMP could be detected in soybean hypocotyls using a radioimmunoassay, no correlation was observed between infection and cAMPlevels [3]. Furthermore, no cAMP could be detected in soybean cell suspension cultures. A specific radioimmunoassay for glyceollin I was developed which is linear in the range of 1 to 100 picomoles [4]. This radioimmunoassay permits quantitative determination of glyceollin I in 15 µm microtome sections of soybean tissue. The presence or absence of fungal hyphae in these sections was determined using an indirect immunofluorescent stain [5]. Correlations between glyceollin accumulation and extent of penetration of fungal hyphae in compatible and incompatible interactions will be discussed.

[1] Börner, H. & Grisebach, H. (1982) Arch.Biochem.Biophys. 217, 65-71.
[2] Leube, J. & Grisebach, H. (1983) Z. Naturforsch. 38c, in press.
[3] Hahn, M.G. & Grisebach, H. (1983) Z. Naturforsch. 38c, 578-582.
[4] Moesta, P., Hahn, M.G. & Grisebach, H. (1983) Plant Physiol. 73, in press.
[5] Moesta, P. & Grisebach, H. (1983) Eur.J.Cell Biol. 31, 167-169.

1428 BIOCHEMISTRY OF BARRIER LAYERS ERECTED IN RESPONSE TO STRESS, P. E. Kolattukudy and C. L. Soliday, Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340.

Cutin and suberin are structural components of barriers erected by plants to control diffusion of molecules and to protect plants against potential adverse effects of environmental factors. The cuticular polymer, cutin, is composed of hydroxy and hydroxy epoxy fatty acids derived from the common cellular fatty acids, palmitic acid, oleic acid and linoleic acid (1). Fungal pathogens break the cutin barrier with extracellular cutinase which has been purified and characterized (2). This enzyme uses a catalytic triad involving serine, histidine and a carboxyl group. It contains a single disulphide bridge which is essential for catalysis. Glucose-grown Fusarium solani pisi can be induced to produce cutinase as the major extracellular protein by the addition of submilligram quantities of cutin hydrolysate into the medium upon depletion of glucose. Poly(A)$^+$ mRNA from the induced cultures can be translated and the translation product of cutinase-specific mRNA can be isolated using antibodies prepared against cutinase. When cDNA prepared for mRNA from both induced and uninduced cultures were used to transform E. coli, 74 clones were found to be unique to the induced cultures. From them 15 were found to contain inserts coding for cutinase. A near full-length cDNA insert containing 950 bp was used for nucleotide sequencing. The complete amino acid sequence of cutinase was deduced from the nucleotide sequence. About 40% of the primary structure was confirmed by amino acid sequencing of tryptic peptides. The serine and histidine involved in catalysis are 68 amino acid residues apart and are probably held in close proximity by the two cys residues involved in the disulphide bridge. The degree of virulence on intact plants can be determined by the level of cutinase production. The molecular biology of regulation of expression of cutinase gene will be discussed. The noncuticular barrier erected in response to changing environmental conditions, physiological status and biological stress appears to be suberin and associated waxes. The suberin polymer, probably composed of aromatic and aliphatic components, is attached to cell walls. Suberization is induced by abscisic acid in potato tissue slices and tissue culture. This hormone induces the synthesis of a suberization-specific isoperoxidase which has been purified. The regulation of suberization will be discussed.

REFERENCES:
1. Kolattukudy, P.E., Science 208, 990 (1980). 2. Kolattukudy, P. E., Cutinases from fungi and pollen, in Lipases (B. Börgstrom and H. Brockman, eds.), Elsevier/North Holland, in press.

Plant Responses To Biological Stimuli V

1429 COMPLEX CARBOHYDRATES REGULATE STRESS PHYSIOLOGY AS WELL AS GROWTH AND DEVELOPMENT, P. Albersheim, A.G. Darvill, K.R. Davis, S.H. Doares, D.J. Gollin, M. McNeil, J.K. Sharp, B. Valent and W.S. York, Chem. Dept., Campus Box 215, Un. Colorado, Boulder CO 80309

The ability of complex carbohydrates to act as regulatory molecules is best exemplified by the hepta-β-D-glucopyranoside-alditol elicitor of phytoalexins isolated from the mycelial walls of a fungal pathogen of plants. Comparison of the structure of the elicitor-active hepta-β-glucoside alditol with the structure of seven similar elicitor-inactive hepta-β-glucoside alditols defined features required for activity. Such evidence established that the elicitor-activity depends on a highly defined structure. The elicitor-active hepta-β-D-glucoside has been chemically synthesized by a group at the University of Stockholm. We have obtained conclusive proof that the chemically-synthesized and the mycelial-wall-derived hepta-β-D-glucopyranoside alditols have identical structures and biological activity. The availability of milligram quantities of pure synthetic hepta-β-D-glucopyranoside elicitor will allow the mechanism of action of the elicitor to be investigated in greater detail. Toward this end, the pure elicitor has the advantage that, unlike crude β-glucan extracts, the pure elicitor does not appear to lead to cell death. The endogenous elicitors of phytoalexin accumulation, which are released from cell walls of plants by polygalacturonic acid-degrading enzymes, have been shown to be α-4-linked-oligogalacturonides. The oligogalacturonide elicitors stimulate the synthesis of the same phytoalexins as the hepta-β-glucoside alditol elicitor. In fact, the oligogalacturonide and hepta-β-glucoside alditol act synergistically, requiring less of each to stimulate phytoalexin accumulation when applied together. It will be possible, with the two pure elicitors, to determine whether genes encoding the same or different isozymes are activated. Another fragment of plant cell walls which is able to stimulate the death of plant cells may be involved in activation of the hypersensitive resistance response of plants, the most widely observed mechanism of defense against microbial and viral pathogens. Enzymes secreted by the rice blast pathogen, Pyricularia oryzae, can stimulate the release of the cell death-inducing fragment. Another example of a plant cell wall fragment capable of regulating a physiological process is a specific nonasaccharide fragment of xyloglucan, a hemicellulose of monocots, dicots, and gymnosperms. A concentration of 10^{-8} M or less of this fragment inhibits auxin-stimulated cell elongation in pea stem segments. Complex carbohydrates are also active regulatory molecules in animal systems, although evidence has not yet been obtained that their regulatory properties are mediated by regulation of gene expression. Nevertheless, the ability of complex carbohydrates to control physiological processes in plant and animal systems has established that complex carbohydrates are an important class of regulatory molecules. Supported by DOE Contract No. DE-AC02-76-ER01426.

1430 INDUCTION BY ELICITORS AND ETHYLENE OF PROTEINS ASSOCIATED TO THE DEFENSE OF PLANTS, Marie-Thérèse Esquerré-Tugayé, D. Mazau, B. Pelissier, D. Roby, D. Rumeau, and A. Toppan, Centre de Physiologie Végétale-LA 241 CNRS; Université Paul Sabatier, Toulouse 31062, France.

Plants respond to environmental stress by producing signals which, in turn, regulate the genome activity. In plant-microorganism interactions, the increased synthesis of ethylene and of a number of proteins are among the responses reported in the literature. Some of these proteins are associated to the defence of plants and can be also induced by treatment with ethylene (1). This has led to the hypothesis that ethylene might be one of the signals involved in their induction in infected plants.

In this lecture, we will report on the induction of three proteins whose levels are increased upon infection : cell wall hydroxyproline-rich glycoprotein (HRGP), chitinase and proteolytic inhibitors. It will be demonstrated that elicitors, either of fungal or of plant origin, induce both the synthesis of ethylene and of the three proteins. The inhibition of ethylene in infected plants (2) as well as in elicitor-treated plants, correlates with a decreased induction of these proteins. The role of ethylene as a possible signal will be discussed. This work provides a further model for studying at the molecular level, the regulation of proteins associated to the plant defense.

(1) M.T. Esquerré-Tugayé, C. Lafitte, D. Mazau, A. Toppan and A. Touzé 1979 Cell surfaces in plant-microorganism interactions. II. Evidence for the accumulation of hydroxyproline-rich glycoproteins in the cell wall of diseased plants as a defense mechanism. Plant Physiol. 70 : 320-326.

(2) A. Toppan, D. Roby, M.T. Esquerré-Tugayé 1982 Cell surfaces in plant-microorganism interactions. III. In vivo effect of ethylene on hydroxyproline-rich glycoprotein accumulation in the cell wall diseased plants. Plant Physiol. 70 : 82-86.

1431 PECTIC FRAGMENTS REGULATE THE EXPRESSION OF PROTEINASE INHIBITOR GENES IN TOMATO PLANTS. Clarence A. Ryan, Institute of Biological Chemistry and Biochemistry/ Biophysics Program, Washington State University, Pullman, Washington 99164

Wounding of leaves of tomato, potato and alfalfa plants by chewing insects or by other mechanical injury releases a signal that is systemically transported throughout the plants, but mainly acropetally, where it initiates the expression of proteinase inhibitor genes in the unwounded distal leaves (1). The signal, called the Proteinase Inhibitor Inducing Factor, PIIF, has been associated with pectic fragments that are released from wounded tissues, apparently by the enzymic fragmentation of the plant's cell walls by endogenous polygalacturonase activity (2,3). Pectic fragments (PIIF) have also been found to elicit localized synthesis of antifungal phytoalexins in castor bean (4), soybean (5) and pea tissues (6), indicating that plant cell wall fragments may be general signals to activate genes involved in plant protection. cDNAs prepared from mRNAs of two wound-induced proteinase inhibitors from tomato leaves have been cloned and characterized (7). These cDNAs have been used as probes to isolate genomic clones from potato and tomato gene libraries and to study specific inhibitor mRNA levels in leaves of wounded tomato plants. The techniques, strategies and results of these experiments will presented and discussed.

REFERENCES

1. Ryan, C.A. in Variable Plants and Herbivores in Natural and Managed Systems. (B. Denno and M. McClure) Academic Press pp. 43-60 (1983).
2. Ryan, C.A., Bishop, P., Pearce, G., Darvill, A., McNeil, M. and Albersheim, P. Plant Physiol. 686, 16 (1981).
3. Bishop, P., Makus, D.J., Pearce, G. and Ryan, C.A., Proc. Natl. Acad. Sci. (USA), 78, 3536 (1981).
4. Bruce, R.J. and West, C. Plant Physiol 69, 1181 (1982).
5. Hahn, M., Darvill, A. and Albersheim, P., Plant Physiol. 68, 1161 (1981).
6. Walker-Simmons, M., Hadwiger, L. and Ryan, C.A., B.B.R.C. 110 194 (1983).
7. Graham, J., Okita,T., Pearce, G., Marryweather, J., Titani, K., Walsh, K. and Ryan C.A., Fed. Proc. 42 1953 (1983).

1432 THE ROLE OF PECTIC FRAGMENTS OF THE PLANT CELL WALL IN THE RESPONSE TO BIOLOGICAL STRESSES, Charles A. West, Robert J. Bruce, Donald F. Jin, Augusto F. Lois, and Karen A. Wickham, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

Three lines of investigation have implicated pectic fragments derived from the plant cell wall as mediators during the elicitation of stress metabolites in higher plants by biological agents. (i) Fragments released from pectic materials of the cell wall by the action of a fungal endopolygalacturonase serve as heat stable elicitors of casbene synthetase activity in castor bean seedlings(1). (ii) Partial acid hydrolysis of cell wall preparations releases endogenous elicitors of glyceollin production in soybean tissues(2). The most active component with endogenous elicitor activity released from citrus pectin was identified as dodeca-α-1,4-D-galacturonide(3). (iii) The activity of proteinase inhibitor inducing factor (PIIF) released by wounding of tomato leaves has been shown to reside in pectic substances of the cell wall(4,5). Unpublished results from our laboratory indicate that either Rhizpous stolonifer endopolygalacturonase or pectic fragments can also elicit phytoalexin production in other plants. These results suggest that pectic fragments released from the endogenous cell wall of a plant may participate generally as initiators of metabolic responses to stresses that result in degradation of the cell wall.

Partial digestion of polygalacturonic acid with R. stolonifer endopolygalacturonase produces a mixture of α-1,4-D-galacturonide oligomers that are separable into size classes by DEAE Sephadex A-25 anion exchange chromatography. The nonamer is the smallest unit with casbene synthetase elicitor activity in the castor bean seedling bioassay; trideca-α-1,4-D-galacturonide was the most active of the oligomers tested. Methyl-esterification of the oligomers greatly diminishes their elicitor activity.

Mutants of Asgergillus niger lacking detectable extracellular endopolygalacturonase have been obtained by UV irradiation. The importance of this extra-cellular enzyme for elicitation of casbene synthetase activity in castor bean seedlings is being investigated with these mutants in comparison with wild type strains that produce the enzyme.

(1) R.J.Bruce and C.A.West. Plant Physiol.69:1181-88 (1982).
(2) M.F.Hahn, A.G.Darvill and P.Albersheim. Plant Physiol.68:1161-69 (1981).
(3) E.A.Nothnagel, M.McNeil, P.Albersheim and A.Dell. Plant Physiol.71:916-26 (1983).
(4) C.A.Ryan, P.Bishop, G.Pearce, A.G.Darvill, M.McNeil and P.Albersheim. Plant Physiol.68: 616-18 (1981).
(5) P.D.Bishop, D.J.Makus, G.Pearce and C.A.Ryan. Proc.Natl.Acad.Sci.USA 78:3536-40 (1981).

Environmental Stresses I

1433 LOSS IN CHLOROPLAST ACTIVITY AT LOW LEAF WATER POTENTIALS IN SUNFLOWER: THE SIGNIFICANCE OF PHOTOINHIBITION, Robert E. Sharp and John S. Boyer, University of Illinois and USDA/ARS, Urbana, IL 61801

It has been proposed that loss in chloroplast activity at low leaf water potentials (Ψ_w) may result from photoinhibition caused at least in part by low intercellular CO_2 concentrations (Ci) accompanying stomatal closure. We investigated this possibility in soil-grown sunflower using gas exchange techniques. After withholding water, two light treatments were imposed. Leaves received either exposures to a low photon flux density (40 μmol photons$\cdot m^{-2}\cdot s^{-1}$) or potentially photoinhibitory exposures to a high flux density (6 h at 2000 μmol photons$\cdot m^{-2}\cdot s^{-1}$). The rate of development of low Ψ_w was similar during both treatments. Determinations were made of the quantum yield of photosynthesis and the light- and CO_2-saturated rate of photosynthesis following these daily light treatments. Under high light, Ci at high Ψ_w was 200 ppm. As Ψ_w decreased, Ci fell to a minimum of 100 ppm before increasing to more than 200 ppm at a Ψ_w of approximately -20 bars. Despite the fluctuations in Ci, the measurements of quantum yield and of light- and CO_2-saturated photosynthesis indicated that the response of chloroplast activity to low Ψ_w was similar in the high and low light treatments. The sensitivity of the chloroplast response was also similar when the high light treatment was imposed while the leaf was maintained at the CO_2 compensation point. This study thus shows that, in sunflower, photoinhibition is of little consequence to the loss in chloroplast activity at low leaf water potentials.

1434 MECHANISMS OF ACCLIMATION OF PHOTOSYNTHESIS TO LOW LEAF WATER POTENTIALS, M.A. Matthews and John S. Boyer, University of Illinois, Urbana, IL 61801

Photosynthesis is less sensitive to low leaf water potential (ψ) in plants which have received water deficit pretreatments. The mechanism of this acclimation was investigated in sunflower (Helianthus annuus). The response of photosynthesis to decreasing ψ was shifted similarly to more negative ψ whether the pretreatment was a continual, mild soil water deficit or a series of more severe drying cycles. The acclimation was evident whether photosynthesis was expressed on a dry weight, chlorophyll, or leaf area basis. Analysis of the CO_2-response of photosynthesis at low ψ showed that the acclimaton was due more to the maintenance of chloroplast activity that to altered diffusive resistance at low ψ. The rate of photosystem II electron transport was greater in chloroplasts isolated from acclimated leaves than from controls at similar low ψ. The maintenance of chloroplast activity at low ψ by acclimated plants was associated with an increase in total solute content of leaves (osmotic adjustment) and a decrease in Mg^{++} content which occurred during the pretreatment. Photosynthesis was less sensitive to low leaf solute potential in acclimated plants than in controls. When osmotic adjustment was eliminated by shading, photosynthesis responded to low leaf solute potential similarly in acclimated and control plants. The osmotic adjustment resulted in acclimated leaves having a higher relative water content than controls at similar low ψ.

1435 WATER STRESS EFFECTS ON VARIOUS COMPONENTS OF PHOTOSYNTHETIC CAPACITY OF INTACT LEAVES, Thomas D. Sharkey, C. Barry Osmond, Biological Sciences Center, Desert Research Institute, P.O. Box 60220, Reno, Nevada 89506

Whereas photosynthesis of cells and chloroplasts only responds to severe osmotic stress (below -15 bar) leaf photosynthesis is inhibited when leaf water potential is reduced by mild atmospheric or soil water stress (-10 bar). Analysis of intact leaf photosynthesis as a function of intercellular CO_2 pressure and light allow identification of the contributing biochemical responses. In mesophytic C_3 crop plants and weeds mild water stress initially reduces the light and CO_2 saturated rate of leaf photosynthesis. When stress occurs in full sunlight, quantum yield is also reduced. Subsequent prolonged or more severe stress reduces the CO_2 limited rate of leaf photosynthesis. In molecular terms, these changes can be interpreted as follows:

(1) water stress initially reduces the capacity for RuBP regeneration, especially during stress in full sunlight when quantum yield is reduced and/or
(2) water stress reduces the utilization of triose phosphates, so that chloroplast metabolism becomes phosphate limited;
(3) longer term responses of leaf photosynthesis to water stress presumably involve reduction in many components of the photosynthetic apparatus, including reduction in RuBP carboxylase and electron transport activities through reduced synthesis or accelerated degredation.

Although responses may differ between species and with the conditions under which water stress is experienced, progress in the interpretation of the potential rate, and the stability, of intact leaf photosynthesis in molecular terms is essential.

1436 THE MOLECULAR BASIS OF LIGHT DAMAGE TO CHLOROPLAST MEMBRANES. David J. Kyle, Itzhak Ohad and Charles J. Arntzen. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing MI 48824, and Department of Biological Chemistry, Hebrew University, Jerusalem, Israel.

High light stress results in a loss of photosynthetic capacity in higher plants and algae known as photoinhibition. Indeed, moderate light intensities may enhance damage potentiated by other environmental stresses such as chilling, overheating and drought. A first strategy to overcome overexcitation of the photosynthetic membrane is a biochemical modification of the reaction center allowing a non-damaging deexcitation to occur. Protein phosphorylation results in both an increase in excitation energy transfer from PS II to PS I as well as an increased back reaction cycling around PS II. If this modification is unable to handle the excess light load, damage is imminent. The primary process of light-induced damage involves the destruction of the 32 kilodalton apoprotein of the secondary acceptor of photosystem II (PS II). The protein damage may be a consequence of the generation of an oxygen radical by the semiquinone anion radical which is formed as an intermediate step in electron transport through the secondary acceptor complex. Repair of the photoinhibition damage requires chloroplast directed protein synthesis. A loss of the overall plant photosynthetic capacity will occur when the rate of damage to this protein exceeds its rate of repair. This balance can be affected by any environmental influence on the rate of protein synthesis (i.e. temperature) or the rate of protein damage (i.e. light intensity, or availability of the terminal acceptor of electron transport).

1437 INACTIVATION BY HIGH LIGHT OF PHOTOSYSTEM II REACTION CENTRE FUNCTION, Robyn E. Cleland and Christa Critchley, Botany Department, Australian National University, Canberra A.C.T. 2601, Australia.

The effect of high light intensity on isolated spinach thylakoids and O_2 evolving particle (OEP) preparations was examined. Inactivation of PSII electron transport in thylakoids or OEP exposed to excessive light was time-dependent and proceeded exponentially when FeCN and DMQ were used to assay activity after treatment. The ability of thylakoids to reduce silicomolybdate, however, decreased linearly with time of high light treatment. SDS-polyacrylamide gels revealed the loss of 2 minor (63 and 47 kd) and the reduction of 1 major (55kd) polypeptide from the OEP after photoinhibitory treatment. The variable component of room temperature chlorophyll *a* fluorescence, measured at 690nm, decayed in parallel with O_2 evolution in both thylakoids and the OEP exposed to high light stress. Fluorescence yields remained low in photoinhibited samples even after additions of DCMU or dithionite or when measured at 77°K. This suggests that a very efficient fluorescence quencher is generated by photoinhibition. We propose that this quencher is a modified form of Q_A, the first stable electron acceptor of PSII, the modification resulting from either attack by a hydroxyl radical with subsequent hydroxylation or a reaction between Q_A and a thiol group on the polypeptide to which it is bound. In either case the redox potential of the quinone would be altered preventing electron transport, its capacity to quench fluorescence would be increased and associated polypeptides affected.

1438 USE OF *LEMNA GIBBA* TO STUDY LIGHT STRESS EFFECTS ON HIGHER PLANT PHOTOSYNTHETIC APPARATUS Gary Peter and J. Philip Thornber Dept. of Biology and Molecular Biology Institute, UCLA, L.A., Cal. 90024

Lemna species are excellent organisms with which to study the direct effects of light, temperature and other stresses on higher plants. The organism's short generation time on defined media, its ability to grow in the dark on sucrose supplemented media, and its ease of fractionation make it a particularly useful higher plant to study the effects with biochemical and molecular biological techniques. We are growing *Lemna Gibba* under various light intensities and qualities. We are examining the plant's response with respect to a) the pigment-protein compositions of the photosynthetic apperatus and b) the relative proportion of each polypeptide in the chloroplast's stroma and thylakoid compartments. In particular we are interested in the effects of stess on the chlorophyll *a* and *b* containing protein complexes of photosystem I and photosystem II. These have been resolved by an electrophoretic procedure which retains essentially all the chlorophyll with the protein it is associated with in the intact membrane. The relative proportions of the four apoproteins of the LCHII chlorophyll *a*/*b* protein seem to respond to changes in white light intensity. In addition, high light intensities are somewhat inhibitory to the synthesis (or stability) of PSI and PSII core chlorophyll *a* - protein (i.e. CPI, CPIII, and CPIV).

1439 PHOTOINHIBITION OF PHOTOSYNTHESIS INDUCED BY ENVIRONMENTAL STRESS, Stephen Powles, Waite Agricultural Research Institute, The University of Adelaide, South Australia.

Photoinhibition of photosynthesis can occur as one response to the imposition of water, low temperature, high temperature or nutrient stress. Photoinhibition is the result of an interaction between incident sunlight and a stress factor to damage photosynthetic reactions which does not occur as a response to either sunlight or the stress factor alone. The damage in photoinhibition appears centered within, or very close to, the photosynthetic reaction centers with widely varying studies identifying a similar lesion site.

Specific examples of the occurrence of photoinhibition induced by temperature stress (low or high temperature), water stress, carbon stress and nutrient stress will be presented. Consideration will be made of mechanisms to avoid photoinhibition damage.

1440 THE EFFECT OF LIGHT ON THE EXPRESSION OF THE FLAVONOID-SPECIFIC CHALCONE SYNTHASE GENE IN FLOWERS OF PETUNIA, J.N.M. Mol*, A.G.M. Gerats, H.J. Reif**, F. Kreuzaler*** and T. Veltkamp*

*Dept. of Genetics, Free University, Amsterdam, The Netherlands. ** Bayer AG, Leverkussen, FRG. *** Max-Planck-Institut für Züchtungsforschung, Cologne, FRG.

Flowers of the Petunia mutant Red Star are uniformally coloured in dimmed light but develop alternating coloured and white sectors upon illumination. From enzyme measurements and Western blotting experiments, using specific antibodies, it is concluded that chalcone synthase (CHS), the key enzyme of flavonoid biosynthesis is absent in white segments of Red Star flowers. Northern blotting analysis using CHS cDNA reveals the presence of CHS mRNA in coloured sectors but not in white sectors. This indicates that transcription of the CHS locus is suppressed. Analysis of the CHS promotor region in the different tissues and gene transfer studies using chimeric genes (CHS promotor + selectable marker) may elucidate the active components of the CHS promoter (methylation, rearrangements etc.?). Such an analysis is in progress.

1441 A PHYSIOLOGICAL BASIS FOR PHOTOINHIBITION AT CHILLING TEMPERATURES IN RICE, Benjamin A. Moll and Katherine E. Steinback, Advanced Genetic Sciences, Inc., Oakland, CA 94608

A characteristic feature of photoinhibition at chilling temperatures is damage to Photosystem II (PS II). Analysis of room temperature and low temperature fluorescence in whole leaves or isolated chloroplasts of sensitive plants subjected to chilling temperatures has led to a general characterization of the site of damage at or very near the reaction center itself. We have investigated the physiological basis of this chilling damage in rice. Analysis of oxygen evolution in rice and barley, a chilling tolerant plant, indicated that rate limitations caused by low temperature were comparable in both plants and thus not the basis for the differential photoinhibitory damage in rice. Analysis of State change characteristics due to protein phosphorylation in rice and barley indicated that chilling temperatures did not interfere with modulation of energy distribution by this mechanism in either plant. However, in rice, an enhanced State change response at low temperature was observed, suggesting increased excitation energy arriving at PS II. Both *in vivo* and *in vitro* studies of fluorescence characteristics show changes with chilling independent of State changes brought about by protein phosphorylation. These studies suggest that the cause of chilling sensitivity in rice is an increased energy transfer to PS II at chilling temperatures.

1442 COLLIGATIVE AND NON-COLLIGATIVE FREEZING DAMAGE TO THYLAKOID MEMBRANES Jürgen M. Schmitt, Jutta E. Schmidt, Ulrich Heber and Dirk K. Hincha, Botanisches Institut der Universität, D-87 Würzburg, FRG.

When spinach thylakoid membranes were frozen in vitro in solutions containing constant molar ratios of cryotoxic to cryoprotective solute, maintenance of functional integrity strongly depended on initial osmolarities. Optimum cryopreservation of photophosphorylation was observed if the membranes were suspended in solutions of intermediate osmolarities (approximately 100 mM NaCl, 150 mM sucrose). Both higher and lower initial osmolarities were found to result in decreased cryopreservation. During freezing, proteins dissociated from the membranes, and the amount of the released proteins was correlated linearly with inactivation of photophosphorylation. The electrophoretic pattern of proteins released at low initial osmolarities differed from that released at high initial osmolarities. Cryopreservation was also found to depend on membrane concentration. Concentrated membrane suspensions suffered less inactivation than dilute suspensions. When thylakoids were frozen in solutions containing low concentrations of NaCl (2 mM), the ratio of sucrose to salt necessary to give full protection was high (up to 50). When the salt concentration was high, much lower ratios were sufficient for maintaining membrane integrity. In the absence of salt, more than 100 mM sucrose were needed for full cryopreservation of the membranes. It is proposed that damage at low initial osmolarities is caused predominantly by mechanical stress, probably by osmotic contraction/expansion. Damage at high initial osmolarities is thought to be basically colligative but in addition is influenced by the final volume of the unfrozen solution in coexistence with ice.

1443 THE BASIS OF THE CHLOROPLAST LOCALIZED INHIBITION OF PHOTOSYNTHESIS DUE TO CHILLING TEMPERATURES, Donald R. Ort, Bjorn Martin and Chuan Kee, Department of Plant Biology, USDA/ARS, University of Illinois, Urbana, IL 61801

Exposure of tomato plants in the dark to cool temperatures (1°C for 16 h) resulted in a large inhibition in the rate of light- and CO_2-saturated photosynthesis. However, at low light intensity, the inhibition was no longer evident and the absolute quantum yield of CO_2 reduction and of photosystem II electron flow was barely affected. These and other data demonstrate that inhibition of photosynthesis brought on by exposure to low temperature in the dark is 1) largely attributable to chill-induced malfunction of biochemical processes in the chloroplast but 2) is not accounted for by impaired PSII capacity. On the other hand, the simultaneous exposure of tomato plants to low temperature and bright light does result in significant inhibition of photosystem II activity. The recovery of photosynthesis subsequent to low temperature exposure in the dark is largely completed within 24 hrs after return to room temperature but is prevented by exposure to high intensity illumination.

1444 ANALYSIS OF COLD ACCLIMATION AND FREEZE INJURY IN SPINACH THYLAKOIDS, K. Niemi and J. Carter, Univ. of Minn, St. Paul, MN 55108

Thermal analysis by Differential Scanning Calorimetry of thylakoids from nonhardened and cold-hardened spinach reveals major differences, due to cold acclimation, in the number of endotherms found in the thylakoid membrane and the temperature at which they occur (Plant Physiol. 72:S96, 1983). Irreversibility of all endotherms implicates protein involvement. PAGE analysis is currently underway to determine whether changes in thermal properties of the thylakoid membrane caused by cold acclimation are due to synthesis of different proteins or to post-translational modification of existing proteins. PAGE analysis is also being done to identify which polypeptides are denaturing in the various endotherms.

Varying responses of thylakoids to in vivo and in vitro freezing were also found. Cold-hardened thylakoids are much less affected by freezing. Individual endotherms display different responses to the freezing regime. Some endotherms are absent from the thermograms following the freeze indicating denaturation of all proteins involved in that endotherm. Other endotherms are not markedly changed by freezing. Again, electrophoresis is being conducted to identify the proteins involved in the endotherms displaying the differential response to the freezing stress.

1445 SEARCH FOR AN ENDOTHERM IN CHLOROPLAST LAMELLAR MEMBRANES ASSOCIATED WITH CHILLING-INHIBITION OF PHOTOSYNTHESIS, P. S. Low, D. R. Ort, W. A. Cramer, J. Whitmarsh and B. Martin, Depts. of Chemistry and Biological Sciences, Purdue Univ., W. Lafayette, IN 47907 and Dept. of Plant Biology and USDA/ARS, Univ. of Illinois, Urbana, IL 61801

The phase transition of chloroplast lamellar membrane lipids has been proposed to be the underlying cause of chilling-induced inhibition of photosynthesis in sensitive plants. We have used differential scanning calorimetry to search for any endotherms arising from lipid state changes in chloroplast lamellar membranes of the chilling-sensitive plants, cantaloupe, kidney bean, domestic tomato and soybean. For comparison, calorimetric scans of chloroplast lamellar membranes from the chilling-insensitive plants, spinach, pea, and wild tomato were made. A large reversible endotherm, extending from below 10° to nearly 40°C, was observed in chloroplast membranes from tomatoes of both chilling-sensitive (Lycopersicon esculentum Mill. cv. Floramerica) and chilling-insensitive (Lycopersicon hirsutum LA 1361) species. A much smaller endotherm, approximately 5 to 10% of the area of that seen in the two tomato species, and extending over a similar temperature range, was detected in chloroplasts from chilling-insensitive spinach and peas, and also was generally observed in chloroplasts from chilling-sensitive cantaloupe, kidney bean and soybean. The enthalpy of these smaller endotherms indicate that, if the endotherm arose entirely from a lipid transition then they corresponded to the melting of less than 8% of the total membrane polar lipid. On the basis of these data we conclude that there is no correlation between chilling-sensitivity of photosynthesis and the presence or absence of a phase transition of bulk membrane lipids of the chloroplast lamellar membrane at temperatures above 5°C.

1446 CHLOROPLAST BIOGENESIS AT COLD-HARDENING TEMPERATURES AFFECTS THE DEVELOPMENT OF PHOTOCHEMICAL ACTIVITY AND THYLAKOID MEMBRANE ASSEMBLY, M. Krol and N. Huner, Dept. of Plant Sciences, Univ. of Western Ontario, London, Canada N6A 5B7.

An investigation of the effects of cold-hardening temperatures on chloroplast biogenesis was initiated. Puma rye seeds were germinated in vermiculite and kept in the dark for 5 days at 20°C or 21 days at 5°C after which the etiolated seedlings (ES) were transferred to continuous light at either 5° or 20°C. During illumination at 20°C, the 20° ES exhibited PSI activity after 1 hr which increased 10-fold after 2-3 hr. During this time, PSII activity was undetectable and chlorophyll accumulation was in its lag phase. After 3 hr in the light, the rate of chlorophyll accumulation increased and PSII activity increased to a maximum steady-state level level after 6 hr. However, PSI activity decreased during this time period to a steady-state after 30 hr illumination. The 5° ES exhibited similar kinetics for the development of PSI and PSII activities at 20°C. Thus, the development of PSI activity did not parallel chlorophyll accumulation during illumination at 20°C. In contrast to greening at 20°C, the development of PSI activity at 5°C ES parelled chlorophyll accumulation with maximum activity occurring after 80 hr. PSII activity was detectable only after 24 hr and increased to a maximum after 40 hr in the light. The 20° ES exhibited similar kinetics for PSI and PSII development at 5°C except that PSI and PSII activity was significantly lower. We conclude that thylakoid membrane assembly is significantly altered during growth and development at low temperature and that greening of 20° ES at 5°C may be more stressful than greening of 5° ES at 20°C.

1447 THERMAL ACCLIMATION OF PHOTOSYNTHESIS IN ARCTIC PLANTS MONITORED BY GAS ANALYSES, LIPID ANALYSES, WHOLE LEAF FLUORESCENCE AND EPI-FLUORESCENCE FROM SINGLE CELLS. W. Raymond Cummins, Bruce T. Mawson, and Stephen E. Jones, Erindale College, University of Toronto, Mississauga, Ontario, Canada L5L 1C6.

The arctic plant, Saxifraga cernua, shows thermal acclimation of its photosynthesis following increases or decreases of its growth temperature. By infra-red gas analysis, shifts in the rates of photosynthesis, respiration and photorespiration are shown to occur during acclimation. Changes in the unsaturation index of total saponifiable fatty acids also follow shifts in growth temperature but occur at rates shown to be slower than the acclimation of photosynthetic rates. Examination of whole leaf fluorescence kinetics shows that a feature coincident with photosynthetic thermal acclimation is the change in the rates of chlorophyll fluorescence induction and quenching. This rapid, non-destructive technique is shown to be an efficient and reliable method to follow the course of stress acclimation. The fluorescence technique has been extended for use in microscopy and low (77°K) temperature spectroscopy (Mawson, Franklin, Filion & Cummins, 1983, Comparative studies of fluorescence from mesophyll and guard cell chloroplasts in Saxifraga cernua: analysis of fluorescence kinetics as a function of excitation intensity. Plant Physiol. In press) to compare responses of individual guard cells and mesophyll cells and thereby compare stress responses of stomata with other photosynthetic cells in the same plant.

1448 MAGNESIUM CHELATASE AND THE REGULATION OF CHLOROPHYLL BIOSYNTHESIS BY ENVIRONMENTAL FACTORS, Thomas P. Fuesler and Paul A. Castelfranco, University of California, Davis, CA 95616

The chelation of magnesium into protoporphyrin IX constitutes the first committed step in chlorophyll biosynthesis. It stands at the branch point in metalloporphyrin synthesis, controlling the amount of protoporphyrin IX apportioned between the chlorophyll and the cytochrome pathways. As such, magnesium chelatase is of interest from the standpoint of enzyme regulation. Magnesium chelatase was studied in intact, developing chloroplasts isolated from cucumber cotyledons. The enzyme has an absolute requirement for ATP and is inhibited by AMP. Subplastidic localization studies using penetrant and non-penetrant sulfhydryl-alkylating agents have shown that the chelatase is located on the plastid surface accessible to the medium.

We suggest that magnesium chelation occurs on the chloroplast envelope and that it responds to the energy charge of the cytosol. It has long been known that anaerobiosis, CN^-, N_3^- and uncouplers of mitochondrial ATP production inhibit the greening of etiolated plant tissues. It is probable that one reason for this inhibition has to do with the control of magnesium chelatase. Under conditions of low oxidative phosphorylation, the available protoporphyrin is switched from chlorophyll to heme production, thus favoring respiration at the expense of photosynthesis.

1449 WATER DEFICIT STRESS,ETHYLENE PRODUCTION AND ANTHOCYANIN SYNTHESIS IN SENESCENT LEAVES OF TERMINALIA CATAPPA,Rosario Fraino Pannier,Universidad Central de Venezuela.Facultad de Ciencias.Caracas.Venezuela.

Terminalia Catappa,a deciduous woody plant of tropical shorelines,shows a striking anthocyanin colouration of its leaves at the onset of senescence during the flowering period.Therefore,the cause of anthocyanin formation in dying leaves and its relationship with the senescence process was studied. During the vegetative growth stage,fully expanded leaves were excised at the base of the leaf blade with the petiole and placed,under controlled conditions in containers with the cut end of the petiole in solutions of Mannitol,NaCl and PEG,ranging 0 to -1.8 MPa in order to accelerate leaf senescence by water stress. Anthocyanin formed only in stressed leaves with its highest content in Mannitol and NaCl (-0.9 to-1.8 MPa). Ψ-leaf and phenylalanine ammonia-lyase (PAL)-activity was shown to be related.Time course of anthocyanin synthesis in Mannitol and NaCl (-1.2 MPa) showed a lag phase of 30 hrs.Afterwards,rapid formation continued until 96 hrs.Accumulation did not continue beyond when Ψ-leaf-values marked leaf dehidration.Application of (2-chloroethyl)phosphonic acid (Etephon) to non stressed leaves showed striking anthocyanin formation, as well as sigmoidal PAL-activity increase. Endogenous ethylene estimated by Gas-Liquid-Chromatography was greatest in stressed leaves.An increase in ethylene production during water stress in excised leaves could indicate that ethylene may be responsible for inducing anthocyanin synthesis in the plant.

1450 ETHYLENE BIOSYNTHESIS IN DROUGHT-STRESSED PLANTS, Neil E. Hoffman[1], Thomas A. McKeon[2], Yu Liu[3], and Shang Fa Yang[3], [1]MSU-DOE Plant Research Lab, E. Lansing, MI 48824, [2]USDA-WRRC, Albany, CA 94710, [3]Dept Veg Crops, Univ California, Davis, CA 95616

In 8 day old excised wheat leaves, water deficit stress resulted in a rapid increase followed by a decrease in ethylene production rates and in the levels of 1-aminocyclopropane-1-carboxylic acid (ACC). Since aminoethoxyvinylglycine (AVG), which blocks the conversion of S-adenosylmethionine (SAM) to ACC, inhibits stress induced ethylene and ACC application stimulates ethylene production in nonstressed tissues, the rate limiting reaction must be the conversion of SAM to ACC catalyzed by ACC synthase. Though the enzyme which converts ACC to ethylene is constitutive, wilting enhances while rehydration rapidly reduces this activity to the constitutive level. In response to water deficit, the level of 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC), the major metabolite of ACC, increased gradually then leveled off. Once formed, MACC levels did not decrease even after stressed tissues were rehydrated. Repeated wilting treatments following the first wilting and rehydration cycle resulted in no further increase in ethylene production and in the level of ACC and MACC. This data is consistent with the observations that the level of abscisic acid (ABA) also rises in wilted leaves and ABA pretreatment of drought stressed leaves reduces whereas BA stimulates both ACC and ethylene production. When benzyladenine (BA) was supplied during the preceding rehydration process, subsequent wilting treatment resulted in a rise in MACC level and a rise followed by a decline in ethylene production rates and in the level of ACC. Thus MACC levels are closely related to ACC synthesis which is in turn modulated by plant hormones.

1451 THE PATTERN OF LATEX FLOW FROM RUBBER TREE (HEVEA BRASILIENSIS) IN RELATION TO WATER STRESS, M.R. Sethuraj and A.S. Raghavendra, Rubber Research Institute of India, Kottayam 686 009, India.

The pattern of latex flow from rubber tree (Hevea brasiliensis) was examined, during depletion of soil moisture in drought tolerant (RRII 105, G1 1) and drought susceptible (Tjir 1) clones. Latex flow has two distinct phases, an initial expulsion of latex as a direct result of release in turgor pressure of latex cessels, followed by a slow capillary flow during which the latex cessels are plugged. During drought, the latter 'slow' phase was shortened and the point of deflection between the two phases was delayed. Plant water stress accelerated latex vessel plugging, effect being more in suspectible than in tolerant ones. Rubber particles and lutoids (vacuoles), sheathed by neutral and phospholipid layers respectively, are suspended in latex (cytoplasm of the vessels). Under water stress, the phospholipid and neutral lipid contents of latex decreased, particularly in drought sensitive clone Tjir 1, which resulted in instability of rubber particles and lutoids respectively. Such disruption in the membrane integrity of organelles appeared to be due to enhanced lipase and phospholipase activity during drought.

1452 DROUGHT STRESS EFFECTS ON APICES OF WINTER WHEAT EVALUATED BY 2-D GEL ELECTROPHORESIS WITH SILVER STAINING. B.D. Dunbar, D.C. Nielsen and B.S. Dunbar. USDA-ARS, Akron, Colorado, Baylor Med. College of Medicine, Houston, Texas.

It would be desirable to detect polypeptide shifts that occur in apices of wheat plants under various levels of stress. High resolution two dimensional gradient SDS polyacrylamide gel electrophoresis with a sensitive silver stain was used to separate the proteins found in shoot tips of growing winter wheat at various stages of development and water stress. Several hundred 'spots' were separated for each sample and each sample was replicated in the field and in the laboratory. No polypeptide change was detected between stressed and unstressed plants at any growth stage.

1453 WATER RELATIONS AND SOLUTE ACCUMULATION OF TWO WHEAT GENOTYPES WITH DIFFERENT DROUGHT RESISTANCE. Richard C. Johnson, Oklahoma State University, Stillwater, OK 74078.
Field observations have indicated that the wheat (Triticum aestivum L.) cultivar 'TAM-W101' is relatively drought resistant compared to the cultivar 'Sturdy'. A growth chamber experiment was conducted to determine if differences in leaf osmotic adjustment, water use efficiency (WUE), and tissue elasticity between TAM-W101 and Sturdy could be demonstrated. Pressure-volume curves showed prestressed leaves of both cultivars had significantly lower solute potential at full turgor (ψ_s^f) and zero turgor (ψ_s^o) than unstressed leaves, indicating osmotic adjustement. But prestressed TAM-W101 apparently adjusted more, with significantly lower values for ψ_s^f (-1.61 MPa) and ψ_s^o (-2.44 MPa) compared to prestressed Sturdy (-1.40 and -1.98 MPa, respectively). Lower leaf tissue elasticity was associated with significantly higher relative water content at zero turgor and lower WUE (mg total dry matter/g H_2O lost per pot) in TAM-W101 than in Sturdy, suggesting that TAM-W101 had greater water extracting capability than Sturdy. Nonreducing sugars were greater in prestressed (3.9%) than in unstressed (1.4%) leaves, but there was no evidence that a breakdown of nonstructural polysaccharides to free sugars contributed to osmotic adjustment because nonstructural polysaccharides did not differ between prestressed and unstressed treatments. Leaf K content was 0.8% higher in TAM-W101 than in Sturdy, but did not increase in prestressed leaves and thus did not contribute directly to osmotic adjustment. Free amino acids were greater in prestressed TAM-W101 than Sturdy leaves, but there did not appear to be sufficient difference in the amino acids to explain the difference in ψ_s^f between prestressed treatments. The data indicate that TAM-W101 may owe part of its drought resistance to both water extraction and osmotic adjustment capability.

1454 EFFECTS OF WATER STRESS ON STARCH METABOLIZING ENZYME ACTIVITIES AND STARCH CONTENT IN COTTON LEAVES. Chong W. Chang and John A. Wetmore. Western Cotton Research Lab, ARS, USDA 4135 E. Broadway Road, Phoenix, AZ 85040.
Cotton leaf starch was reported to accumulate during water stress (Ackerson and Hebert, 1981) but, no enzymic information was included. We investigated soluble, bound α-amylase, bound glucan synthetase and starch content in cotton plants during water stress. Four glandless cotton plants were grown in each pot for 32 days in a controlled growth chamber. At this time, one set of plants (CONTROL) was kept well watered by a daily supply of 250 ml H_2O. Another set (STRESS) was subjected to a gradual dehydration until leaf water potential reached -2.8 MPa at the end of 7 days. All daily leaf samplings for water potential and enzyme assays were made after 4-hr light exposure when maximum enzyme activities occurred. During the initial decrease in leaf water potential from -1.1 to -1.3 MPa, soluble α-amylase activity increased to approximately 250% of control, whereas both bound α-amylase and glucan synthetase activities decreased to approximately 50% of control. Thereafter the two bound enzymes increased gradually, with the further decrease in leaf water potential, to 450% (α-amylase) and 200% (glucan synthetase) of control at the end of this period of water stress (-1.8 MPa). Starch content of 32 day old leaves declined steadily to about 50% of control, probably because α-amylase activities (free and bound) greatly exceeded glucan synthetase activity. Starch content then increased sharply to 250% of control, presumably because of a corresponding increase in glucan synthetase activity to 200% of control. With a further decrease in leaf water potential to -2.8 MPa, however, both glucan synthetase activity and starch content decreased rapidly, while soluble and bound α-amylase activities continued to increase.

1455 MEMBRANE ORGANIZATION OF THE DESICCATION TOLERANT MOSS TORTULA RURALIS IN SEVERAL DEHYDRATED STATES, Jas Singh, Barbara A. Blackwell, Richard W. Miller and J. Derek Bewley, Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6 and Dept. of Biology, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

The moss Tortula ruralis is able to withstand repeated cycles of extreme desiccation, freezing to -197°C and plasmolysis. Since rehydration of the dried moss is complete within seconds, compartmentation of the cell has to be either maintained in the dry state or restored in a short period of time during cell expansion. Thus, an understanding of membrane organization in the desiccated moss cell is of importance to the study of dehydration tolerance. Membrane organization of the moss was studied in several dehydrated states (dry, 75% RH, frozen to -20°C and plasmolysed in 4M salt) by ^{31}P NMR and ultrastructural analyses. Both methods revealed that even at 75% RH (-400 bars), cellular membranes of the moss retained extended phospholipid bilayers. Fully hydrated moss showed an extensive proliferation of cytoplasmic vesicles. During dehydration, these vesicles form layers of membranes under the plasmalemma and even appear to fuse with the surface membrane. This suggests that these vesicles may serve as a reservoir of membranes to accomodate for membrane surface area changes during desiccation and subsequent rehydration.

1456 Cellular and Subcellular Structural Changes in Wheat Crop of Oklahoma Under Water Stress. Rajen S. Mehta, Agri Res. Center, Langston University, Langston, OK 73050

Statement of Research Interest

The Stress Physiology Study was undertaken in April '83 for a 5 year term under the guidelines of Cooperative State Research Services for the 1890 Land-grant Universities and Tuskegee Institute. The fund for the research is provided by the U.S. Department of Agriculture. For the Stress Physiology Study following areas will be studied: (1) Cellular and Subcellular Structural Changes in Wheat Triticum aestivum L, of different Geno-types vary in drought resistance, with the help of Electron Microscope (E.M.); (2) Cell-size variation among different Geno-types with the aid of E.M. Stereology; (3) Comparative study on stressed caused changes in relation to senescence; and (4) Evaluation of effects of environmental stimuli on stress, photosynthesis, and photosynthesis partitioning between total biological study (of plant) and yield of wheat crop.
Stereology is based on integral geometry and Geometric probability theory. Since the sections of plant tissues are randomly selected, every profile of the structure will be different, but the collection of all possible profiles will have the precisely known properties of the organelles being studied. Thus, the stereological study, may help to understand a contributing factor in cell size variation between Geno-types vary in drought resistance.
The author wishes to participate in Poster Session and would like to present data on Genotypic variation in growth and ultrastructural changes.

1457 DETERMINATION OF BETAINES BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY, Gene C. Jamieson, Ann C. Myers, William L. Fitch and David Rhodes, Zoecon Corporation, 975 California Avenue, Palo Alto, CA 94304, U.S.A.

A rapid, sensitive and selective method for the determination of glycine betaine (N,N,N-trimethylglycine) and related quaternary ammonium compounds is described. The method entails derivatization of glycine betaine to the n-propyl ester; this imparts a permanent positive charge to the molecule. Upon fast atom bombardment mass spectrometry (FABMS), intense signals from the glycine betaine propyl ester (m/z=160) are observed (limit of detection = 0.05 nmol/µl glycerol). Deuterium labeled N,N,N-tri(CD_3-methyl)glycine propyl ester is used as internal standard (m/z=169). We illustrate the application of this technique in the determination of glycine betaine levels in a range of halophytic plant species. In some tissues with abundant levels of glycine betaine (e.g. Cuscuta salina, parasitic on Salicornia depressa), glycine betaine propyl ester is the only detectable component in crude methanolic extracts subjected to esterification and FABMS. Further applications of this technique in heavy isotope tracer studies on the pathway of glycine betaine synthesis in spinach leaf discs will be reported. This approach may be useful in rapid screening for genotypic variability in rates of glycine betaine synthesis as a function of salinity or water stress. Proline betaine (stachydrine) is shown to be amenable to similar techniques.

1458 REGULATION OF ARGININE DECARBOXYLASE ACTIVITY IN STRESSED OAT LEAVES, Nevin D Young and Arthur W. Galston, Yale University, New Haven, CT 06511

Exposure of oat leaf segments to acid stress by incubation on a medium of pH 5.0 or less leads to a rapid, massive, and reversible increase in the titer of putrescine (1,4-diaminobutane; Put). A similar response occurs in leaf segments exposed to osmotic stress, e.g., 0.4 M sorbitol, or in plants on low-K nutrition. Put accumulation is accompanied by an increase in the activity of the Put biosynthetic enzyme, arginine decarboxylase (ADC), and is inhibited by the addition of cycloheximide. These results suggest that oat leaves respond to stress be synthesizing ADC de novo.

ADC was purified from 18 day old K-deficient oat leaves. In the oldest leaves of such plants, ADC activity was more than 30 times that in normal plants. Starting with this tissue, ADC was purified 670-fold, yielding an apparently homogeneous preparation which migrated as a single band on SDS-PAGE with M_r 39,000.

Leaf segments were then exposed to 0.4 M sorbitol, to pH 4.0, or to no external stress along with ^{35}S-methionine and labeled proteins were analyzed by SDS-PAGE and fluorography. Six bands in osmotic stressed and two bands in acid stressed leaves were enhanced. One of these bands, enhanced by both treatments, comigrated with purified ADC. The increase in labeling of this band due to osmotic stress was 28%.

1459 Mechanism of Salt Tolerance in the Halophyte Salicornia pacifica var. Utahensis Darrell Weber, Dept. of Botany, Brigham Young University, Provo, Utah 84602

Salicornia pacifica var. Utahensis is an inland halophyte that can grow in soils containing 12,000 ppm of NaCl. The halophytes in the genus, Salicornia are among the most salt-resistant higher plants. Osmotic measurements, wavelength dispersive x-ray microanalyses and energy dispersive x-ray microanalyses indicated that high concentrations of Na^+, K^+ and Cl^- were compartmentalized in the center cortex region of the stem. The outer region of the stem was much lower in Na^+, K^+ and Cl^- and photosynthesis readily occurred. The location of Cl^- was determined using an electron microscopy histochemical method. The Cl^- concentration was low in the chloroplasts. ATPase was isolated and found to be salt tolerant. Using electron microscopy histochemical methods it was observed that the ATPase was commonly located on the plasma membranes of the cells in the cortex region. Other enzymes such as RuBPCase were salt sensitive. Salicornia pacifica var. Utahensis can survive high salt concentrations by compartmentalization of the NaCl in the cortex region. Also the Na^+ concentration was kept low by the Na^+ pump associated with ATPase. During the growth season of Salicornia pacifica the NaCl concentration sometimes becomes too high and the lower nodes shrivel and die but the xylem system continues to conduct water to the green nodes above the shriveled node.

1460 SALINITY-SHOCK PROTEINS IN TOMATO ROOTS, Robert F. Sacher and Richard C. Staples, Boyce Thompson Institute, Cornell University, Ithaca, NY 14853

Studies have been made of proteins synthesized by tomato roots in response to salinity shock. For this purpose we have exposed 14-day-old tomato plants (Lycopersicon esculentum L. cv New Yorker) to 200 mM NaCl. At 6, 24, and 48 h, [^{3}H]leucine was added to the nutrient solution for one hour. Plants were then frozen and proteins extracted. Separation of the proteins by 2D-PAGE revealed that several new proteins were synthesized 6 h after exposure to salt, and a smaller number of additional new proteins appeared at 24 and 48 h after salt treatment. Some normally present proteins were absent after salt treatment. A salt tolerant tomato breeding line derived from L. esculentum x L. pennellii also exhibited salt induced proteins, but the pattern differed from that of similarly treated 'New Yorker' plants. Salt tolerance data on 18 accessions of L. pennellii show the potential of several as sources of salt tolerance germplasm.

1461 EFFECT OF SALINITY ON GROWTH AND MAINTENANCE COSTS OF PLANT CELLS, Suzan J. Stavarek and D. William Rains, Department of Agronomy and Range Science, University of California, Davis, CA 95616

Plants exposed to environmental stress show alterations in physiological processes. This involves diversion of metabolic energy to maintain cellular processes and provide tolerance to the stress. An understanding of the changes in energy costs associated with salinity can provide insight into the potential productivity of growing crops in a saline environment.

Alfalfa cells were selected which grow on normally lethal levels of NaCl. Studies between the unselected and NaCl selected cells under varying NaCl stress were used to determine differences in growth efficiency and maintenance cost of the cells.

Increase in biomass, glucose consumption, and changes in stored sugars and starch were measured. Growth yields and maintenance coefficients were calculated under several levels of NaCl stress. Oxygen consumption and CO_2 evolution were also determined to evaluate the respiratory efficiency of the alfalfa cells.

The nonselected cells were found to have decreases in growth, glucose uptake, stored carbohydrate utilization and oxygen consumption rates with increases in NaCl levels. The salt selected cells, on the other hand, showed little effect of NaCl on these processes except at very high levels of NaCl.

Growth yields and maintenance coefficient values will be discussed in relation to the efficiency of the two cell lines and their ability to grow under stress conditions.

1462 ALUMINUM TOXICITY AND THE SELECTION OF RESISTANT VARIANTS IN CELL CULTURES OF NICOTIANA PLUMBAGINIFOLIA, Carole P. Meredith and Anthony J. Conner, University of California, Davis, CA 95616

A culture system has been developed that permits the expression of aluminum toxicity in plant cell cultures and closely simulates the chemical environment found in aluminum-toxic soils. In order to keep the aluminum soluble in the medium, the phosphate concentration was lowered to 10 μM (from 1250 μM) and the pH lowered to 4.0. Two additional modifications to the medium were the use of unchelated iron and a reduction in the calcium concentration to 0.5 mM (from 3.0 mM). Since the gelling properties of agar are inhibited at pH 4.0, cells were cultured on filter paper supported by polyurethane foam saturated with liquid medium. The filter paper with adhering cells was transferred to fresh medium every 2 days to replenish the phosphate and maintain the medium pH at 4.0. Although cell growth of Nicotiana plumbaginifolia was reduced in the modified medium (due to phosphate deficiency), sufficient growth occurred to clearly demonstrate toxicity in the presence of aluminum. Experiments characterizing aluminum toxicity in cell cultures of N. plumbaginifolia will be described along with preliminary results on the selection of aluminum-resistant variants.

1463 SEEDLING GROWTH AND THE ONSET OF SALT ACCUMULATION IN HALOPHYTES, David K. Stumpf and James W. O'Leary, E.R.L., U of Az., Tucson, Az., 85706

Seed germination and seedling growth under saline conditions is a prerequisite for successful plant establishment. We have monitored dry weight accumulation, succulence, salt accumulation and levels of glycinebetaine (the primary compatible osmoticum in seedlings of the halophyte Salicornia europaea). Salt accumulation in S. europaea begins 12 days after germination and occurs in a relatively narrow time frame, the % ash of the tissue rising from 6% to 25% in 48 hours.

Accumulation of salt occurs in both the hypocotyl and cotyledon. At day 16 the accumulated salt is evenly distributed between the two tissues but by day 35, the cotyledon has accumulated 66% of the total salt in the seedling. Glycinebetaine (gb) profiles for the hypocotyl and the cotyledon are dramatically different with a loss of greater than 60% of the gb in the hypocotyl while there is a peak of gb content in the cotyledon followed by a decline and apparent transport of gb to the newly developing shoot tissue.

We will discuss this data further and also propose the concept of the "onset of halophytism" in relation to seedling growth in halophytic plants from different ecotypes.

1464 NaCl TOLERANCE IN ADAPTED TOBACCO SUSPENSION CELLS, Gretchen J. King, Victoria A. Turner, and Timothy G. Helentjaris. NPI, Salt Lake City, Utah 84108.

Nicotiana tabacum L. var Wisconsin 38 which had been adapted to 25 g/l of NaCl (received from P.M. Hasegawa and R. Bressan, Purdue University) were examined to determine what molecular events are triggering the adaptive cellular responses. Stress proteins are defined as those proteins identified by SDS - PAGE which are present in the adapted cells and absent in the unadapted cells. Antibodies were prepared to the major stress protein (26kd, approximately 10% of total cell protein) to determine any cross reactivity between it and other stress proteins as well as any normal proteins. Differential centrifugation was used to prepare three cell fractions: cell walls, membranes, and soluble enzymes. The major stress protein and two of the minor stress proteins have been localized to these three fractions. Differences in the mRNAs present in the unadapted cells and the adapted cells have been demonstrated by in vitro translation. These data in conjunction with physiological data should provide a better understanding of tolerance to water stress.

1465 PLANT RESPONSES TO OZONE STRESS--ANALYSIS OF ASCORBIC ACID IN O_3-SUSCEPTIBLE AND O_3-RESISTANT CULTIVARS BY HPLC, E.H. Lee & J. A. Jersey, USDA, BARC, Beltsville, MD

The mechanism of the processes that lead to plant injury from ozone (O_3) absorption is not well known. Ascorbic acid (AA) has been reported to be an effective compound in preventing or reversing the effects of O_3 damage to leaf tissues. However, very little research has been conducted towards establishing, with greater precision, levels of AA in O_3-susceptible (O_3-S) and O_3-resistant (O_3-R) tissues. The influence that AA may have on O_3 phytotoxicity remains unclear. As a consequence, the accumulation and degradation of AA in O_3-S and O_3-R cultivars of soybean (Glycine max L.) cv. 'Hark' (O_3-S) and 'Hood' (O_3-R), as well as snapbean (Phaseolus vulgaris L.) cv. 'BBL-290' (O_3-S) and 'Astro' (O_3-R) were compared and studied using high-performance liquid chromatography (HPLC). Isocratic separation of AA for O_3-stressed and non-stressed leaf tissues was accomplished in 5 min on a uBondapak C-8 reverse column using 2% NH_4PO_4 (pH 2.8) as the solvent. Tissues were extracted with 6% metaphosphoric acid containing 1 x 10^{-6}M EDTA. Our results show O_3 tolerant soybean and snapbean varieties to have higher concentrations of AA than do sensitive varieties. In response to O_3 stress (0.3 to 0.45 ul/l), an optimal concentration (1,000 ug/g fresh wt. AA) is required for protective function in leaf tissues. AA is a free radical scavenger and at physiological levels it reduces the extent of lipid peroxidation and leaf damage. O_3 stress has been shown to induce production and accumulation of AA. There are noticeable increases in AA levels after the beginning of the stress period, followed by rapid increases just prior to leaf lesion appearance. The concentration of AA in O_3-S leaf tissues increases to a lesser extent than O_3-R tissues.

Environmental Stresses II

1466 IDENTIFICATION OF MAJOR ANAEROBIC STRESS PROTEINS AS GLYCOLYTIC ENZYMES, Philip M. Kelley and Michael Freeling, University of California, Berkeley, California 94720

Anaerobic treatment of maize seedlings results in the selective expression of 10 major and 10 minor polypeptides designated as anaerobic polypeptides (ANPs) (Sachs et al.(1980) Cell 20: 761-768). We found an increase in specific activity of several glycolytic enzymes following anaerobiosis. We have identified two cytoplasmic enzymes corresponding to major anaerobic polypeptides: glucose phosphate isomerase I (ANP55) and fructose 1,6-diphosphate aldolase (ANP35.5, ANP33A).

1467 ALCOHOL DEHYDROGENASE ACTIVITY IS REGULATED BY OXYGEN TENSION IN CELL SUSPENSION CULTURES OF TOMATO, Joan M. Rejda, Bruce R. Thomas* and Bill G. Williams, ARCO Plant Cell Research Institute, Dublin, CA 94568 and *Calgene, Davis, CA 95616

Alcohol dehydrogenase (ADH) is one of the proteins induced by anaerobiosis in maize (Cell (1980) 20:761). Preliminary to a molecular biological test of the adaptive potential of ADH to anaerobic stress, we have analyzed ADH activity in tomato cell suspension cultures. ADH-2 is the inducible ADH activity in the tomato plant (Lycopersicon esculentum), and it is this isozyme which we find expressed in cell cultures; the levels of which vary with the phase of growth of the cultures, the surface to volume ratio of the cultures, and the oxygen partial pressure of the atmosphere above the culture.

The ADH activity was determined to be ADH-2 by activity staining of starch gels. The high levels of ADH in dense cell cultures facilitated the purification of the enzyme, which is a dimer of M_r 85,000 daltons and constitutes 5-7% of the total soluble protein.

Using minimal ADH activity as an empirical indicator of unstressed cells, we have found that cells growing in typical cell suspension culture set-ups require several cell doublings at low cell density to reach that minimum plateau. These "unstressed" cells have provided the baseline for lethal dose experiments designed to test whether cells induced by hypoxia are increasingly tolerant of a lethal challenge.

A wild tomato accession (L. cerasiforme) collected from a marshy environment produces an ADH protein which cross reacts with the polyclonal antibody to purified ADH-2 (from the cultivar VFNT Cherry), and comigrates on both native and SDS-PAGE gels. Comparative survival data for the L. cerasiforme cells will be presented.

1468 ACQUISITION OF THERMOTOLERANCE IN SOYBEAN SEEDLINGS: SYNTHESIS AND ACCUMULATION OF HEAT SHOCK PROTEINS AND THEIR CELLULAR LOCALIZATION, Chu-Yung Lin, Department of Botany, National Taiwan University, Taipei, Taiwan, and J. K. Roberts & Joe L. Key, Botany Department, The University of Georgia, Athens, Georgia 30602

Heat shock (HS) at 40°C or a brief HS at 45°C followed by a 28°C incubation induces the synthesis of heat shock proteins (HSPs) in soybean seedlings and provides thermoprotection to a subsequent prolonged incubation (2 h) at 45°C. During HS some HSPs become localized and stably associated with purified organelle fractions (e.g. nuclei, mitochondria, and ribosomes) while others do not. The relative amount of the HSPs which relocalize during a second HS increases with higher temperatures from 40°C to 45°C and this process completes within 15 min. Arsenite treatment (50 μM for 3 h) also induces the synthesis of HSP-like proteins and provides thermoprotection to a 45°C HS. Proteins induced by arsenite treatment also become selectively localized with organelle fractions during a subsequent HS (40°C to 45°C). There is a strong positive corelation between the acquisition of thermotolerance and the accumulation of HSPs which become localized with organelle fractions only during HS.

1469 HEAT SHOCK PROTEINS IN MAIZE: THEIR INDUCTION AND POSSIBLE PHYSIOLOGICAL ROLE, P. Cooper, A. Abu-Tabikh, A. Wang, T.D. Ho, University of Illinois, Urbana IL 61801

The pattern of protein synthesis is rapidly and significantly altered in most maize tissues when the temperature is increased from 28 to 40 C. In roots, 40 C induces a set of heat shock proteins (hsp) within 20 min of the transition. The synthesis is transient, not continuing beyond 4 h of continuous 40 C treatment. The hsp are synthesized in addition to normal cellular proteins, whose production is unaffected at 40 C. Returning the roots to 28 C results in a decline of hsp synthesis. Leaves, coleoptiles, and scutella are similar to roots in their heat shock response. In suspension-cultured cells, hsp synthesis is not transient. Germinating pollen grains are the only tissue that does not show inducible hsp synthesis. Hsp can also be induced by gradual temperature increase, a condition which occurs in nature. In order to investigate the physiological role of hsp, we have studied the relationship between hsp synthesis and the plant's resistance to high temperature. Root elongation is severely inhibited in plants subjected to 48 C, but growth can be protected by pretreatments at lower temperatures. Maximal thermoprotection is obtained by a pretreatment at 40 C. This thermoprotection increases for up to 4 h of pretreatment at 40 C, then declines. These results correlate well with the temperature optimum and time course of hsp synthesis. At the cellular level, H^+ efflux/K^+ influx, a membrane-related process, is strongly perturbed by 40 C heat shock. However, the rate of normal ion movement recovers in 20 min even if 40 C treatment is prolonged. Because the 72 kD hsp localizes in a microsomal membrane fraction, we suggest this hsp may have a membrane-related, thermoprotective function.

1470 **Adaptation to Thermal Tolerance Correlates with the Induction of Heat Shock Proteins in Tomato Cell Culture.** Lisa Staraci, Danny Alexander, Bruce Thomas*, Connie Wainwright, and Bill Williams, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

Tomato cells in suspension culture adapt to increased thermal tolerance after 1 hour at 38°C, rendering the cells immune to a 2 hour challenge of 44°C - the lethal dose for naive cells. This adaptation is similar to that we observe for young tomato plants (45°C adaptive, 55°C lethal) and to adaptations reported across the biological spectrum - from E. coli (Biocem. Biophys. Res Commun. 100: 894-900, 1981, and PNAS 79: 860-864, 1982) through mammalian cells (Cancer Res. 42: 2457-2461, 1982).

A comparison of the in vivo protein labelling patterns of cells and seedlings over the temperature range from the adaptive to the lethal treatments revealed that the proteins induced in both systems were indistinguishable by one dimensional SDS gels, and were invariant up to the lethal dose, where labelling became ineffective. A series of pulse-chase in vivo protein labellings done over the time the adaptation was seen to decay identified a sub-set of proteins that either diminish or disappear when thermal protection is lost.

A comparison was made between cell cultures at different phases of growth (measured by settled cell to media ratio, SCM) to their minimum lethal dose, which was invariant over the range of early log (SCM = 0.1) to stationary (SCM = 1.0)

Wild species accessions collected from stressful habitats have been put into cell suspension culture and compared for both their levels of naive minimum lethal dose and their levels of adapted tolerance to thermal challenge.

1471 REGULATION OF PROTEIN SYNTHESIS IN HEAT-STRESSED PLANT TISSUES, T.H.D. Ho, F. Belanger, D. Feickert and P. Cooper, University of Illinois, Urbana, IL 61801

In both corn roots and barley aleurone layers the profile of newly synthesized proteins is drastically altered when the tissues are subjected to high temperature stress at 40 C. While heat shock proteins (hsp) are rapidly induced in corn at 40 C, the synthesis of most of the other proteins continues. To investigate the regulation of hsp synthesis we have used the rabbit reticulocyte cell-free translation system to measure the level of mRNA coding for hsp. All the ten major hsp were synthesized in vitro when the reticulocyte lysate was primed with RNA from 40 C treated corn roots, but not with RNA from 28 C tissue. Since all the in vitro synthesized hsp are identical to the in vivo proteins in terms of MW and pI, there are no apparent post-translational modifications of these proteins. A time course study shows that the heat shock response at the mRNA level is also transient, i.e., the hsp mRNAs are abundant at 2-4 h, but totally absent by 10 h. When the tissue recovers from heat shock upon returning to 28 C, the level of hsp mRNA is also gradually reduced. In barley aleurone layers the 40 C treatment not only induces the hsp but also suppresses the synthesis of GA-induced α-amylase. However, the synthesis of α-amylase is resumed when hsp synthesis is reduced upon prolonged treatment at 40 C. This apparent competition between the synthesis of hsp and α-amylase is only partially regulated at the translational level. The stability of α-amylase mRNA is probably reduced at 40 C. The resumption of α-amylase synthesis is dependent on new transcription of α-amylase genes. The molecular mechanism underlying the regulation of protein synthesis in heat-stressed plant cells will be discussed.

1472 TRANSCRIPTS OF CHLOROPLAST DNA IN THE C_4 PLANT PEARL MILLET DURING HEAT SHOCK. Bernard Rutti and James R. Y. Rawson, Botany Department, University of Georgia, Athens, GA 30602 USA.

The effect of heat on transcripts in the chloroplast of the C_4 plant pearl millet (Pennisetum americanum) has been investigated. RNA was isolated from leaves of plants that were grown and subjected to heat in two different ways. One group of plants was germinated, grown in the dark for 4 days at 25°C and then illuminated for 10 hr at 30, 40 or 50°C. A second group of plants were grown for 4 weeks in a natural day-night cycle at 25°C and then placed in the light for 10 hr at 30, 40 or 50°C. Individual plasmids containing specific chloroplast DNA fragments from pearl millet were used as hybridization probes to Northern blots of RNAs from the different plants. The stable RNAs in the chloroplast after different heat treatments were identified on the basis of their size and their origin on the chloroplast genome. Transcripts in the chloroplast seem to be affected in at least three ways when pearl millet is placed at high temperatures. One, the majority of the RNAs are not greatly affected. Two, a few RNAs decrease substantially in concentration. Three, one RNA actually increase in concentration. Particularly interesting is the fact that the larger of the two transcripts originating from the gene for large subunit of ribulose 1,5-bisphosphate preferentially decreases in concentration when the plants are subjected to heat. Supported in part by grants from the USDA Competitive Grants Program (80-CRCR-1-0489) and the NSF (PCM-8200949).

1473 RuBP CARBOXYLASE SYNTHESIS DURING HEAT SHOCK. Elizabeth Vierling and Joe L. Key, Department of Botany, University of Georgia, Athens, GA 30602.

It is well established that plants respond to rapid elevation in temperature (heat shock) by inducing a specific set of proteins, the "heat shock" proteins (hsp's). Work to date has focused on non-photosynthetic tissues and therefore has not considered the effect of heat shock on chloroplast transcription and translation. We have employed highly chlorophyllous, soybean cell suspension cultures growing under photomixotrophic conditions to assess how heat shock effects the synthesis of RuBP carboxylase large (LS) and small (SS) subunits. Synthesis of LS and SS were examined by in vivo pulse labelling (^{35}S-methionine) and corresponding mRNA levels were examined by Northern blotting analyses. Results demonstrate that LS and SS synthesis are unchanged up to 37°C although hsp's are strongly induced at this temperature. At 38°C LS synthesis is depressed approximately 50% and SS approximately 60-75%. When cells are returned to 28°C following 2 hr at 38°C, LS and SS synthesis recover to control levels after 6 hr at which time hsp synthesis has declined to < 5% maximum levels. Preliminary data suggest that regulation of LS and SS production is exerted translationally or post-translationally rather than by changes in mRNA levels.

1474 THE PRESENCE OF HEAT SHOCK mRNAs IN GREEN SOYBEAN PLANTS DURING HIGH TEMPERATURE STRESS. Janice A. Kimpel and Joe L. Key, Botany Department, University of Georgia, Athens, GA 30602 USA.

Our laboratory has extensively defined many parameters of the heat shock response in etiolated, detached soybean hypocotyls, including the identification of cDNA clones for mRNAs encoding several low molecular weight heat shock proteins. We have now investigated the response of green soybean plants to a heat shock (hs) in growth chamber experiments and to high temperature stress under field conditions. One month old soybean plants, grown in a controlled environment (26°/22° D/N, 14 hr photoperiod, well-watered) show induction of the hs mRNAs when the temperature of the chamber is rapidly shifted to 43-45°C. This temperature of induction is significantly higher than the temperature needed to induce these messages in etiolated hypocotyls (38-40°C). This may reflect the ability of these plants to lower leaf temperatures below the ambient air temperature through transpirational cooling. Taking advantage of particularly warm weather in Georgia in August, we sampled soybean leaves from an irrigated and a non-irrigated field during a 24 hour period when midday temperatures reached 40°C. Several mRNAs for the low molecular weight hs proteins were present in samples from both fields although the levels of these messages were much higher in the non-irrigated leaves. The presence of these hs mRNAs in field grown plants suggests that hs proteins are produced as part of the normal plant response to high temperatures.

1475 PURIFICATION OF HEAT SHOCK PROTEINS FROM SOYBEAN SEEDLINGS, M. A. Mansfield, J. A. Kimpel, C. Y. Lin, J. L. Key, Department of Botany, Univ, of Georgia, Athens GA 30606

In soybean seedlings, heat shock proteins (hsp) are reversibly associated with various organelles after 2 hours at 40°C. After three hours of recovery at 28°C and one hour at 45°C, the amount of hsp associated with ribosomes is apparently enhanced over treatment at 40°C alone. Utilizing this incubation protocol in conjunction with a ribosome purification procedure, we have isolated a protein fraction containing ribosomal proteins and hsp. The hsp have been separated from the ribosomal proteins using CM-Sephadex chromatography. Two-dimensional electrophoresis of the hsp fraction demonstrates enrichment for a group of high molecular weight hsp (68-70 kD), several hsp in the range of 25-27 kD, and the entire spectrum of hsp at 15-18 kD. This fraction is being used to generate monoclonal antibodies for hsp. These antibodies will be used to quantitatively analyze the heat shock response in soybeans.

1476 ANALYSIS OF HEAT SHOCK GENES FROM SOYBEAN. Ronald T. Nagao, Ewa Czarnecka, Fritz Schöffl**, William Gurley* and Joe L. Key. Botany Department, University of Georgia, Athens, GA 30602, *Microbiology and Cell Science Department, University of Florida, Gainesville, FL 32611 and **Fakultät für Genetik, Universität Bielefeld, Bielefeld, FRG.

Earlier work from this laboratory identified five sets of cDNA clones which reflected members of "different" multigene families for heat shock (hs) proteins ranging from 15 to 27 kD. We have isolated genomic clones from a soybean λ_{1059} library (constructed by J. Slightom and Y. Ma, Agrigenetics Advanced Research, Madison, WI) for each of these families. Restriction digestion mapping and cross-hybridization experiments indicated extensive homology between several groups of genomic clones and the possibility of hs gene clustering. DNA sequencing was undertaken to clarify the organizational relationship of these genes. The cDNAs from the various hs families have been sequenced along with several genomic subclones from the 15-18 kD hs protein family. Sequence analysis has located the cDNA homology within each of the genomic subclone sequences. Analyses of the 5' regions indicate extensive sequence homology between the presumptive classical regulatory sequences.

This work is supported by Agrigenetics Research Corporation.

1477 ISOLATION AND CHARACTERIZATION OF A MAIZE GENE ENCODING MAJOR HEAT-SHOCK PROTEIN, Hsp70. Dilip M. Shah, Dean E. Rochester, Gwen G. Krivi, Cathy M. Hironaka, Thomas J Mozer, Robert T. Fraley and David C. Tiemeier. Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167

Four-day old maize coleoptiles rapidly synthesize a set of heat-shock proteins (hsp's) when the incubation temperature is raised from 30°C to 42.5°C. One of the major heat-shock proteins has a molecular weight of approximately 70,000 daltons (hsp70). By *in vitro* translation of poly A$^+$ RNA's isolated from tissue grown at 30°C and 42.5°C, we have shown that heat-shock elevates the levels of translatable hsp70 messenger RNA (mRNA)in this tissue. Using the cloned hsp70 gene from *Drosophila melanogaster* we have been able to hybrid-select translatable hsp70 mRNa from both normal (30°C) and heat-shocked (42.5°C) tissue.

Genomic blotting of maize DNA has indicated that hsp70 is encoded by a small multigene family. One member of this family has been cloned and partial nucleotide sequence determined. The deduced amino acid sequence is 80% homologous to the amino acid sequences of *Drosophila* and yeast hsp70 polypeptides. Interestingly, the maize gene contains an intervening sequence of approximately 700 bp in the codon specifying amino acid 71. Although the inducible hsp70 genes of *Drosophila* lack intervening sequences, a closely-related and constitutively expressed *Drosophila* heat-shock-cognate gene (hsc1) contains an intervening sequence in exactly the same position. However, unlike *Drosophila* cognate gene, transcription of our cloned maize gene does appear by Northern analysis to be enhanced by heat-shock.

1478 ZEA MAYS DNA SEQUENCE HOMOLOGY WITH DROSOPHILA 83 AND 70 KILODALTON HEAT SHOCK GENES. R.M. Sinibaldi and P.S. Dietrich, Department of Molecular Biology, Zoecon Corporation, Palo Alto, CA.

When the incubation temperature of 3 day old maize seedlings is raised from 27° to 41°C the synthesis of a set of specific peptides is induced. These proteins have approximate molecular weights of 108, 89, 85, 81, 78, 74, 72, 62, and 18 kilodaltons, as determined by one dimensional polyacrylamide SDS gel electrophoresis. When a comparable temperature shift is performed on Drosophila cells a similar set of proteins having molecular weights of 83, 70, 68, 28, 27, 23, 22 kilodaltons is induced. The 72 kd maize heat shock protein appears to be one of the most abundantly induced in both in vivo protein synthesis experiments and in in vitro translations of 41°C mRNA. This protein is assumed to be homologous to the abundant 70 kd heat shock protein of Drosophila. We and others have previously reported that the Drosophila 70 kd heat shock gene cross hybridizes to sequences contained within the maize genome. We have extended this observation to the Drosophila 83 kd heat shock gene. Both Drosophila probes detect a set of hybridizing bands in blotted restriction enzyme cut genomic corn DNA. The sequence homology is sufficient to allow selection of genomic clones from a λ library of maize DNA. We have constructed a DNA library of corn DNA with the λ phage L47.1 and have begun to screen it with both Drosophila probes. We are currently analyzing some positive plaques for the presence of corn heat shock genes. This report is part of a project to understand and characterize the heat stress response in maize.

1479 TEMPERATURE-DEPENDENT ALTERATIONS IN THE DYNAMICS OF BARLEY ROOT PLASMA MEMBRANE-BOUND PROTEINS, Charles R. Caldwell, Plant Stress Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705

Fluorescence quenching and measurement of protein sulfhydryl group reactivity were used to investigate temperature-induced changes in the protein dynamics of barley root plasma membrane-enriched microsomes. The intrinsic tryptophan fluorescence of the membrane-bound proteins was modified by changes in temperature, detergents, and cross-linking reagents in a manner which suggests considerable aggregation of the proteins in the native membrane. Temperature-dependent alterations in the quenching of membrane protein fluorescence by extrinsic agents and the reactivity of membrane protein sulfhydryl groups to a fluorescent maleimide probe indicate major changes in membrane protein dynamics as the temperature is raised from 25 to 35 °C. The enzyme-substrate affinity of the Mg^{2+}-dependent ATPase of barley root plasma membranes decreased 8 fold over the same temperature range, indicating altered enzyme conformation or polymeric structure. These results suggest cooperative changes in membrane protein conformation, rates of lateral diffusion, vertical displacement, and/or aggregation occur over this temperature range. Since the temperatures which induce the measured changes in protein dynamics are dependent upon the growth temperature of the barley seedlings, temperature-induced changes in membrane protein dynamics could provide a primary sensor for plant responses to temperature variations and might determine the upper limits for barley seedling growth.

1480 Damage to membrane transport during ice encasement injury of isolated winter wheat cells. C.J. Andrews and M.K. Pomeroy. Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, K1A 0C6

Uptake of ^{86}Rb by isolated cells of winter wheat is rapidly inhibited by the low temperature anaerobic stress of ice encasement, but efflux of ^{86}Rb and amino acids is increased at a much lesser rate by the stress. Calcium (up to 10 mM) has a promotive effect on survival of cells in ice. After ice stress, Ca^{++} has an initial inhibitory effect on the uptake of label, but after more than 1 hr exposure to the label, there is a promotive effect of Ca^{++} on uptake. A number of inhibitors of plasma membrane activity decrease ^{86}Rb uptake, which is partially reversed by Ca^{++}, but the inhibitors have less effect on ^{86}Rb efflux. Respiratory inhibitors decrease overall cell survival, and reduce both uptake and efflux levels. These results indicate that damage to plasma membrane transport enzymes has a central role in ice encasement injury to cells. As cells have similar response to ice encasement as intact leaves and plants, the information is relevant to ice injury in the field, which is a major factor in winterkilling of cereals in eastern Canada.

1481 **A NEW SIMPLE AND EFFICIENT cDNA CLONING SYSTEM**, Danny C. Alexander, Thomas D. McKnight, and Bill G. Williams, ARCO Plant Cell Research Institute, Dublin, CA 94568-2685

A highly efficient, versatile, and simple-to-use system for cDNA cloning of polyadenylated mRNAs is presented. The system is a modification of the method of Okayama and Berg (**Molecular and Cellular Biol**, 1982, 2:161). In our system the final cDNA inserts are bordered on both sides by polylinkers, permitting easy subcloning and determination of insert sizes. The preparation of the vector-primer is simplified by elimination of the gel electrophoresis purification step and one of the restriction enzyme digestions. The linker piece, used to complete the cyclization of the cDNA-vector pieces, is easily prepared at a yield of two moles per mole of plasmid and isolated by acrylamide gel electrophoresis. Using a variety of templates, including rabbit globin mRNA, tomato leaf mRNA, and watermelon embryo mRNA, we have obtained efficiencies of cloning into HB101 of approximately 10^4-10^5 cDNA inserts per microgram of starting mRNA. Preliminary analysis revealed that a high percentage of the globin clones are full length or nearly full length. We have isolated several cDNA clones for the small subunit of RuBP carboxylase from tomato leaf mRNA. These plasmids were identified by virtue of their hybridization to a soybean small subunit probe. Partial DNA sequence analysis confirmed their identity as small subunit cDNA clones. A cDNA library made with mRNA from leaves of heat-adapted tomato plants is presently being screened using a new low-background colony screening method (Taub et al, DNA, 1983, in press) that allows differential hybridization analysis on a single filter.

1482 THE FLAX GENOME - ITS PLACTICITY AND RESPONSE TO STRESS, C. A. Cullis, John Innes Institute, Colney Lane, Norwich NR4 7UH

A number of DNA sequences which vary between the environmentally induced genotrophs of the flax variety "Stormont Cirrus" have been isolated. These include the ribosomal DNA, 5S DNA, a part of a sequence which acts like a transposable element and a number of uncharacterised repetitive sequences. In addition to these, a number of cloned sequences which did not vary between genotrophs have also been isolated. Examples of highly repetitive, tandemly arranged sequences are included in both variable and constant classes. Even within a sequence set, namely the 5S DNA, particular recognisable subsets appear to be differentially affected.

The DNAs from normal seed derived plants, callus tissue and the progeny of regenerated plants have been compared using a number of probes characterised in the genotroph study. The same two classes appeared, that is, sequences which varied between genotrophs also varied between plant, callus and regenerants, while those which were constant in the genotrophs were also invariant between plant, callus and regenerants. The two classes are also apparent when a comparison was made between the DNAs from various flax and linseed varieties and the supposed progenitor of flax Linum bienne.

From these results it is proposed that the flax genome is compartmentalised into 2 parts, one of which is variable and the other which is constant. The effect of "stress" is to cause change in the variable component which is responsible for the phenotypic variation subsequently observed.

1483 cDNA Sequence Comparisons of Developmentally Regulated Cottonseed Storage Protein, Caryl A. Chlan, Jana B. Pyle, Glenn A. Galau, Leon S. Dure, University of Georgia, Athens, Georgia, 30602

Throughout embryogenesis and germination of cottonseed, seven developmentally regulated gene families can be identified. These families differ in their relative abundance of mRNA and protein populations in response to development.

One subset, the cottonseed storage proteins, has been well characterized as to their in vitro protein products (60, 69 kD), the mature protein products (the 52 kD glycosylated and the 48 kD protein species) and relationships between the two forms.

Based on hybridization studies of cDNA clones, three subgroups of storage proteins have been identified. The 69 kD in vitro translation product corresponds to a single group of cDNA clones and the 69 kD protein form is represented by two hybridization classes of cDNA clones. DNA sequence comparisons between cDNA clones representing the 69 kD and 60 kD precursors are presented.

1484 Levels of Expression of Different mRNA Families During Cottonseed Embryogenesis, Jean C. Baker, Glenn Galau and Leon S. Dure, University of Georgia, Athens, Georgia, 30602

Seven differently regulated mRNA families become abundant at periods during cottonseed embryogenesis. Each of these mRNA families has subfamily members whose regulation is basically that of the family but is not strictly coordinate during the time of expression.

We have analyzed several of these families by solution and filter hybridization. Cloned probes representing specific members of mRNA subfamilies were hybridized to mRNA extracted from cottonseed embryos at different developmental stages and the relative levels of these mRNA sequences deduced.

One of the families studied contains mRNA for the two major cotton storage proteins. This family is coordinately regulated but differs as to the amount of maximum expression. Another family contains mRNA sequences that become abundant late in embryogenesis. $C_0t_{\frac{1}{2}}$ analysis shows that this family may be divided into subfamilies based on differences in coordination of their expression.

1485 TRANSCRIPTION OF THE LEGUMIN GENES IN PEAS (PISUM SATIVUM L.) : EFFECTS OF SULFUR DEFICIENCY. Larry R. Beach, D. Spencer, P.J. Randall and T.J.V. Higgins, CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

One consequence of sulfur nutrient deficiency in developing pea seeds is a selective reduction in the proportion of legumin, one of the two major storage proteins. The legumin mRNA levels in developing S-deficient pea seeds are less than 10% of the levels in normal seeds. In addition, levels of both legumin and legumin mRNA rapidly return to normal when S-deficient plants are supplied with sulfate (recovered plants). The aim of this work was to determine if the decreased level of legumin mRNA in S-deficient peas is due to decreased transcription of the genes or decreased stability of the mRNA or a combination of these factors. The relative transcription rate of the legumin genes was determined using a nuclear transcription assay. Pea seed cotyledon nuclei were isolated from S-deficient and 24 hr recovered plants and incubated in the presence of [α^{32}-P]GTP. The nuclear RNA was then purified and hybridized to cloned legumin cDNA immobilized on nitrocellulose filter discs. Incorporation into legumin mRNA was two-fold higher in recovered nuclei than in nuclei from S-deficient plants. This two-fold increase in the relative transcription rate is insufficient to account for the ten-fold increase in legumin mRNA levels in cotyledons after 24 hrs recovery. We conclude that legumin mRNA transcripts in S-deficient cotyledons are much less stable than in cotyledons from plants with ample S. The effect of suboptimal S supply on one major seed storage protein, legumin, is a reduced level of transcription of the legumin genes and more importantly, reduced stability of the legumin mRNA after transcription.

1486 THE ROLE OF THE PLASMA MEMBRANE IN CELLULASE ACTIVATION IN HIGHER PLANTS, Lowell N. Lewis, University of California, Berkeley, CA 94720

During the process of abscission or fruit ripening, ethylene causes the rapid synthesis of the enzyme cellulase. The enzyme is transported across the plasma membrane and released into the cell wall area where it hydrolyzes the cell wall material to allow abscission or, in the case of fruit ripening, to soften the walls of the fruit. In the abscission system, the isoenzyme of cellulase associated with the abscission process is nonexistent prior to the ethylene stimulation. It has been shown by the use of antibodies to the abscission cellulase that a form of this enzyme is embedded in the plasma membrane. This form can be detected only after solubilization of the membrane with Tritons X-100. The membrane bound cellulase has a larger molecular weight than the abscission cellulase which may suggest that an insertion protein is functioning in the transport of this enzyme across the plasma membrane. We are presently making monoclonal antibodies to the abscission cellulase to assist in following its transport from the cytoplasm through the plasma membrane into the cell wall area, and to better understand the relationship between the membrane bound cellulase and the form ultimately found in the cell wall area. Although the differentiation process being studied in my laboratory is found only in the differentiation of plants, the mechanism by which a specific enzyme is transported across the plasma membrane is of interest to all cell biologists.

1487 DEVELOPMENT OF INTRANUCLEAR MICROINJECTION INTO PLANT PROTOPLASTS, Terry J. Reich, Larry A. Holbrook*, Brian L. A. Miki*, and V. N. Iyer. Carleton University and *Agriculture Canada, Ottawa, Ontario, Canada.

The technique of intranuclear microinjection of plant protoplasts for the purposes of genetic transformation is currently being developed. Protoplasts of Medicago sativa, are attached to grid patterns photoengraved on cover slips after nuclei have been stained with fluorescent dyes. A specifically-designed syringe system can start and stop the flow of DNA after micropipettes are inserted into the nucleus. The entry of DNA is visualized by the swell of the nucleus. As compared to controls, 90% of the injected protoplasts go into first cell division. Single cell analytical techniques for following the short-term fate of injected Ti-plasmid of A. tumefaciens are under development. An environmental chamber that controls humidity, temperature and sterility enclose the microinjection apparatus and ensures long-term survival of protoplasts after injection.

1488 DIPHENYLETHER-CHLOROPLAST INTERACTIONS, Ruth G. Alscher and Scott H. Wettlaufer, Boyce Thompson Institute, Tower Road, Ithaca, NY 14853

The herbicidal diphenylethers (DPES) have an absolute light requirement for activity Evidence accumulated to date suggests two sites of DPE action within the cell. One of these sites is located in the chloroplast. The results of both competition studies using a photoaffinity analog and tests of physiological functions point to a stromal or envelope binding site for the DPEs. Experiments are underway to identify the protein or proteins involved in DPE binding.

1489 MANIPULATING FATTY ACID COMPOSITION OF CULTURED SOYBEAN CELLS, William B. Terzaghi, Pete D. Gardner, and Karl G. Lark, University of Utah, Dept. of Biology, Salt Lake City, UT 84112

Adaptive changes in membrane lipids are a major response to plant stress. We are studying the influence of fatty acid composition on stress responses of cultured soybean cells. We have inhibited fatty acid biosynthesis with cerulenin and supplied fatty acids to the medium. Cerulenin kills, but treated cells grow if fatty acids are added. A mixture of saturated and unsaturated fatty acids must be supplied, but cells will grow in the presence of cerulenin and a variety of fatty acid combinations. Cells grown in cerulenin and heptadecanoic acid (and an unsaturated fatty acid) incorporate this acid as a major component of their lipids, whereas it is not detected in cells grown without cerulenin or in cerulenin and other fatty acids. These results show that fatty acid composition may be altered in a directed manner. Characteristics of cells with altered fatty acid compositions will be described. We are also using these characteristics to devise selections for fatty acid auxotrophs.

This work was supported by NSF Grant PCM80-07711; WT was supported by NSF Predoctoral Fellowship SPE835-0132.

1490 ETHYLENE STIMULATION OF ETHYLENE INDUCTION BY CELL WALL DIGESTING ENZYMES, James D. Anderson, Edo Chalutz and Autar K. Mattoo, Plant Hormone Laboratory, PPHI, BARC, Beltsville, MD 20705.

Ethylene synthesis is induced in tobacco leaf discs by a variety of cell wall digesting enzyme mixtures (e.g. Cellulysin) routinely used in protoplast production. Preincubation of leaves for 6h to 18h in 60 ppm ethylene increases their responsiveness to active cell wall digesting enzyme mixtures by increasing ethylene production up to 10 fold over non-pretreated leaves. This induction appears to be at the level of the rate limiting enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, which converts S-adenosylmethionine to ACC. The sensitization of the leaf tissue by ethylene is inhibited by cycloheximide. Pulse labeling of tissues with [^{35}S]L-methionine revealed that several new proteins are induced by ethylene; however it is not known whether these proteins have anything to do with the potentiation of ethylene biosynthesis induction by the active fraction. A highly purified active fraction from Cellulysin that induces ethylene production has been isolated by a combination of membrane ultra-filtration, Sephacryl gel filtration and preparative isoelectric focusing. The purified fraction is not inactivated by proteinase K, trypsin or chymotrypsin but is totally abolished by treatment with 3% SDS or by incubation at 70C and 50% at 60C.

Plant Responses To Biological Stimuli

1491 TOBACCO MOSAIC VIRUS COAT PROTEIN ENCASIDATES CHLOROPLAST DNA TRANSCRIPTS, Albert Siegel and D'Ann Rochon, Wayne State University, Detroit, MI 48202

An efficient process exists to ensure that viral RNA is incorporated into tobacco mosaic virus particles; assembly is initiated by reaction of a capsid protein oligomer with a specific viral RNA encapsidation initiation site. Nevertheless, preparations of tobacco mosaic virus are found to contain pseudovirions, particles resembling virions but containing host rather than viral RNA. Several virus strains were tested and all were found to contain pseudovirions with the U2 strain containing more than the others. The encapsidated host RNA is protected from degradation by pancreatic ribonuclease and is composed of discrete sized species which anneal to chloroplast DNA. Almost every component of a petunia chloroplast DNA clone bank hybridizes both to one or more pseudovirion RNA species and to the same sized species present in an uninfected leaf extract. All chlorplast DNA transcripts, except for ribosomal RNA, appear to be encapsidated. Thus, the indications are that chloroplast messenger transcripts have a site in common which resembles the virion RNA encapsidation initiation site. Pseudovirion formation may be one of the factors governing plant response to infection.

1492 DISEASE INDUCING DETERMINANTS OF CAULIFLOWER MOSAIC VIRUS, Stephen D. Daubert, James Schoelz, and Robert J. Shepherd, Department of Plant Pathology, University of California, Davis, CA 95616

In an effort to determine if particular regions of the cauliflower mosaic virus (CaMV) genome could be associated with particular phenotypic characters, strains of CaMV differing in biological properties were recombined *in vitro* to produce hybrids. Segments of DNA from cloned infectious genomes were excised and recombined by ligation *in vitro*, or by simultaneous inoculation to turnip plants for ligation *in vivo*. Recombinants in infected plants were characterized by restriction mapping of their DNA to confirm the hybrid being sought. New hybrid strains that induced less severe disease, or more severe disease, than either parent could be produced. These experiments indicated that typical disease induction (leaf chlorosis and mottling) mapped to open reading frame VI (ORF VI). Insect transmissibility mapped to ORF II. The ability to infect solanceous plants, tested by recombination of an ordinary strain with one that infected *Datura stramonium* systemically, was controlled by ORF VI. In this case the systemic mobilization of virus, that otherwise replicated only locally, suggested ORF VI probably functions in cell-to-cell movement of the virus.

1493 THE T-REGION OF TI-PLASMIDS CODES FOR AN ENZYME OF AUXIN BIOSYNTHESIS. Joachim Schröder, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

In contrast to most normal cells, plant cells transformed with Ti-plasmids of Agrobacterium tumefaciens grow in tissue culture without addition of hormones like auxins and cytokinins. Genetic experiments show that auxin-independence is controlled by two different, cooperating genes. Our recent experiments show that these genes can be expressed into defined proteins in bacteria, e.g. E. coli and Agrobacteria. Since Agrobacteria as well as crown gall cells actively synthesize indole-3-acetic acid, we tested whether the genes code for enzymes of auxin biosynthesis.
E. coli and Agrobacteria expressing gene 2 from the T-region, an "auxin-gene" hydrolyze indole-3-acetamide into indole-3-acetic acid, and the plant hormone was unambiguously identified by mass spectrometry. Crown gall cells, but not normal cells, also possessed the enzyme activity, and the properties of the enzymes in bacteria and in plant cells are very similar. In both systems the K_m for indole-3-acetamide is about 1 µM.
The results suggest that at least one of the tumor genes of the T-DNA of Ti-plasmids codes for an amidohydrolase which is directly involved in auxin biosynthesis in transformed plant cells. We are currently testing whether other genes of the T-DNA also code for enzymes of hormone biosynthesis.

1494 REGULATION OF IAA PRODUCTION IN *PSEUDOMONAS SYRINGAE* PV *SAVASTANOI*, Steven W. Hutcheson and Tsune Kosuge, University of California, Davis, CA 95616

Pseudomonas syringae pv *savastanoi* is a phytopathogenic bacteria that causes hyperplasias (galls) at the infection site. The bacterial synthesis of indoleacetic acid (IAA) has been shown by genetic and molecular means to be a virulence factor in this host-pathogen interaction. In an effort to elucidate the factors controlling the synthesis of IAA, we have characterized the physical and regulatory properties of tryptophan 3-monooxygenase (TMO), an enzyme catalyzing the oxidative decarboxylation of trp to form 3-indoleacetamide (IAM) -- the initial step of IAA synthesis. TMO was purified from cell-free lysates of *E. coli* SK1592-pLUC2 (a pBR328-derived chimeric plasmid which contains a constitutively expressed TMO gene) by ammonium sulfate fractionation followed by sequential chromatography on DEAE-cellulose, hydroxyapatite, and phenyl-sepharose. The resulting preparation was homogeneous for a 62 kd polypeptide which contained a single catalytically-active FAD moiety. Immunological evidence indicates that the *E. coli* enzyme is similar to the *Ps.* enzyme. When tested for kinetic properties, TMO exhibited simple Michaelis-Menton kinetics with an apparent Km for trp of 50 uM. IAM was found to be a potent competitive inhibitor with an apparent Ki of 7 uM and an I_{50} at $[trp]_{km}$ of 230 uM. A proposed storage intermediate, IAA-lys, was ineffective as a regulatory metabolite. Other aromatic amino acids (tyr, phe) had no effect on the kinetic properties of TMO. As TMO is constitutively expressed in *Ps. savastanoi*, these results suggest that IAA synthesis is regulated by substrate availability and by IAM-IAA levels.
(Aided by grants from the USDA-CRGO and NSF)

1495 AN AMINO ACID CONJUGATE OF IAA AND VIRULENCE IN PSEUDOMONAS SYRINGAE PV. SAVASTANOI
N. Louise Glass and Tsune Kosuge, University of California, Davis, CA 95616

The phytopathogen, Pseudomonas syringae pv. savastanoi (Ps. savastanoi) incites the production of galls on olive and oleander plants. Gall formation is dependent upon the synthesis of the phytohormone, indoleacetic acid (IAA). IAA is further metabolized by Ps. savastanoi to an amino acid conjugate of IAA, 3-indole-acetyl-e-L-lysine (IAA-lysine).

The genetic determinants for IAA-lysine synthesis are localized on the pIAA plasmid, but are not part of the IAA operon based on analysis of insertion mutants in the first locus of the IAA operon. Evidence obtained utilizing deletion mutants in the pIAA plasmid suggest that the IAA-lysine conjugate synthetase determinants are located in close proximity to the IAA operon.

Ps. savastanoi mutants isolated following selection for resistance to aza-tryptophan accumulate as much as three times more IAA than the wild-types. Upon inoculation into oleander plants, these mutants produce consistently larger galls than the parental type. When analyzed for IAA-lysine production, these mutants either failed to produce any detectable IAA conjugate or produced it at reduced levels. These results suggest that conversion to the lysine conjugate helps to regulate free IAA pool sizes in the phytopathogen, and therefore, also helps to modulate virulence in Ps. savastanoi. (Aided by grants from the USDA-CRGO and NSF)

1496 REVERSION OF TUMORS INDUCED BY AGROBACTERIUM RHIZOGENES, Brian H. Taylor, Frank F. White, Richard M. Amasino and Milton P. Gordon, University of Washington, Seattle, WA 98195

Agrobacterium rhizogenes, the causative agent of hairy root disease, incites tumors on dicotyledonous plants by transferring bacterial DNA from its Ri (root-inducing) plasmid into the host plant genome. Unlike crown gall tumors induced by the closely related A. tumefaciens, hairy root tumors exhibit a high frequency of reversion in vitro, differentiating first into roots and then into intact plants. Revertants have been obtained in two species of Nicotiana: N. glauca and N. tabacum cv. Xanthi. A number of these revertants contain T-DNA and exhibit aberrant morphology. T-DNA inserts in these reverted lines were examined to determine if changes had occurred which might be responsible for the reverted phenotype. Transcription of the T-DNA in these lines was also examined.

1497 OPINE CATABOLISM AND PLANT-BACTERIAL INTERACTIONS, Stephen K. Farrand, Yves Dessaux and Jacques Tempe, Stritch School of Medicine and University of Paris-Sud.

Close interactions between a soil bacterium and the plant can prove beneficial for the growth and productivity of certain agriculturally important crops. It would be of value if such close interactions could be maximized. One strategy for insuring such a relationship involves the ability of the bacterial partner to specifically and efficiently utilize a nutritional source produced by the plant which is not available to competing soil flora. Agrobacterium tumefaciens exhibits such a selective advantage by specifically catabolizing novel carbon sources, called opines, produced by crown gall tumors. We are investigating the genetics of mannityl opine utilization by bacteria as a model system for the construction of selective plant-bacterial partnerships. The location of genes required for mannopinic acid catabolism was determined by cloning octopine-type Ti plasmid fragments in an Agrobacterium tumefaciens plasmid vector. Only KpnI fragment 1 conferred on strain NT1 the ability to utilize this opine as sole source of carbon. Cloning HindIII partials of this fragment into pKT231 yielded a broad host range plasmid encoding this catabolic trait. Neither clone allowed strain NT1 to utilize mannopine, agropinic acid, or agropine, the other opines of the mannityl opine family. However, when a recombinant plasmid harboring KpnI fragment 6 of the Ti plasmid was introduced into these strains, they acquired the ability to utilize mannopine and agropinic acid. The recombinant plasmids are being introduced into Pseudomonas and Rhizobium stains to determine if these organisms can be engineered to utilize the novel compounds.

1498 AGROBACTERIUM CHROMOSOMAL GENES INVOLVED IN ATTACHMENT TO PLANT CELLS, Carl J. Douglas, Walter Halperin and Eugene W. Nester, University of Washington, Seattle, WA 98195

An initial step in crown gall tumor formation by Agrobacterium tumefaciens is the attachment of Agrobacterium to plant cells. We have studied this process by measuring the attachment of Agrobacterium to suspensions of freshly isolated Zinnia leaf mesophyll cells. Analysis of avirulent mutants with Tn5 insertions in chromosomal DNA showed that nine such independently isolated mutants were defective in their ability to attach to plant cells. This phenotype was linked to the Tn5 insertions. Cosmid clones of Tn5 containing DNA from several mutants were constructed, and these clones were used to deduce a restriction map spanning 30 kb of the Agrobacterium (strain C58) chromosome. All nine independently isolated attachment defective mutants had Tn5 insertions clustered within an 11 kb portion of this region. Wild-type chromosomal sequences from this virulence region were obtained by screening a cosmid bank of total A. tumefaciens DNA, and are currently being subjected to transposon mutagenesis. Genetic analysis of this chromosomal region and biochemical analysis of attachment defective mutants should be usefull in establishing the molecular mechanism by which A. tumefaciens attaches to plant cell.

1499 TRANSFER OF A MUTANT proB GENE, THAT CONFERS PROLINE OVERPRODUCTION AND INCREASED OSMOTOLERANCE ON E. coli, INTO PLANT CELLS, Stanton B. Gelvin, Ray A. Bressan and Laszlo N. Csonka, Purdue University, West Lafayette, IN 47907

High intracellular levels of proline confer increased tolerance of osmotic stress on bacteria. Proline overproducing mutants of E. coli have been isolated which have, as a consequence of the mutation, acquired increased resistance to osmotic stress. The first enzyme of proline biosynthesis, γ-glutamyl kinase, which in E. coli is encoded by the $proB^+$ gene, is normally subject to feedback inhibition by proline. In the mutant strains, proline overproduction is the consequence of alterations in the proB gene rendering γ-glutamyl kinase less sensitive to feedback inhibition by proline. We have cloned, on a 1 kb DNA fragment, the wild-type E. coli $proB^+$ gene and also the corresponding gene from a proline overproducing osmotolerant mutant. We have introduced the wild-type and mutant proB genes into Agrobacterium tumefaciens. As in E. coli, the mutant gene confers resistance to 3,4-dehydroproline on A. tumefaciens, indicating that the E. coli gene is not only expressed but causes proline overproduction in the latter organism. The wild-type and mutant proB genes have been inserted into a plasmid which contains a cloned fragment from the T_R region of the octopine-type Ti-plasmid pTiA6 in such a way that they should be under direct T-DNA promoter control. After recombination into the entire Ti-plasmid in A. tumefaciens, tumors have been incited on tobacco and sunflower hypocotyls. We are currently determining whether or not the proB genes are expressed in the tumors, and if so, whether the tumors display increased levels of proline and are resistant to osmotic stress.

1500 TN5 MUTAGENESIS AND CLONING OF VIRULENCE FACTORS FROM PSEUDOMONAS SYRINGAE. Dallice Mills, Dirk Anderson and Frank Miepold, Oregon State University, Corvallis, OR 97331. Pseudomonas syringae pv. syringae and P. syringae pv. phaseolicola are the causal agents of bacterial brown spot and halo blight, respectively, in common bean, Phaseolus vulgaris. Transposition of Tn5 (kanamycin resistance, Km^r) from the suicide plasmid pSUP1011, into the genomes of these pathovars was achieved by conjugal transfer from Escherichia coli S10-1011. The frequency of mutation to auxotrophy was approximately 0.5 percent among nearly 4000 Km^r colonies analyzed. Reversion frequencies were determined to be low ($<10^{-8}$) in 16 of 20 mutants. Nine prototrophic Km^r colonies were shown to have mutations in virulence factors. They exhibited either attentuated or complete avirulence in bioassays on a susceptible cultivar of bean. Southern hybridization revealed that Tn5 inserted into a unique EcoRI fragment in each avirulent mutant. A 12 kb EcoRI fragment containing Tn5 was cloned from an avirulent mutant of P. syringae pv. syringae and used as a molecular probe to identify clones with the wild type gene(s) in a clone bank. A 20 kb insert in one cosmid clone restored the virulence phenotype by complementation when mobilized into the avirulent mutant, and the avirulent mutant phenotype reappeared when this cosmid was eliminated from the cells. Fine structure restriction maps have been developed of the fragment bearing Tn5. Further characterization of this virulence factor and others identified by Tn5 mutagenesis is expected to provide new information about the molecular mechanism of plant stress in this host-parasite interaction.

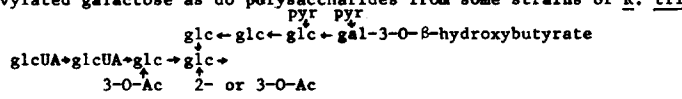
1501 β-GALACTOSIDASE, A SELECTABLE NON-ANTIBIOTIC MARKER FOR FLUORESCENT PSEUDOMONADS, Bruce C. Hemming and David J. Drahos, Monsanto Company, St. Louis, MO 63167

Species within the subgroup 1b pseudomonads (fluorescent species such as Pseudomonas aeruginosa, P. fluorescens, P. putida or P. syringae) are recognized as saprophytes and pathogens of plants. Examination of more than 300 rhizosphere fluorescent pseudomonad isolates has revealed the constant absence of the o-nitrophenylgalactoside hydrolysis positive phenotype in subgroup 1b pseudomonads. These isolates also have been found incapable of growth on lactose as a sole carbon source. Recombinant plasmids bearing E. coli LacZ and LacY genes have been constructed from derivatives of a broad host range vector plasmid, RSF1010. The vector and the construct plasmids are maintained in fluorescent pseudomonad strains. Plasmid vectors containing LacZ express the encoded β-galactosidase activity in fluorescent pseudomonad strains. In the presence of a chromogenic substrate, X-Gal (5-chloro-4-bromo-3-indolyl-β-D-galactopyranoside), colonies of these strains exhibit both their natural fluorescent pigment and the blue coloration of the hydrolyzed substrate, providing a unique, non-antibiotic marker for tracking the organisms or the plasmids themselves. The introduction of recombinant plasmids possessing these E. coli genes is shown to confer the metabolic capacity necessary for fluorescent pseudomonad growth on lactose minimal medium which permits the selection of such marked fluorescent pseudomonads.

1502 CAN EXTRACELLULAR POLYSACCHARIDES OF RHIZOBIA PLAY A ROLE IN HOST SPECIFICITY? Andrew J. Mort and Mao-Sung Kuo, Dept. of Biochemistry, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK 74078

There is evidence for and against the possibility that extracellular polysaccharides produced by rhizobia have some role in the specificity of the infection process of legumes by rhizobia. Until recently it was generally thought that the polysaccharides were species specific. However, it now has been reported that R. trifolii, R. leguminosarum, and at least one strain of R. phaseoli make seemingly identical polysaccharides (Dudman et al., Carbohydr. Res. 117, (1983) 169-183). Thus the potential specificity of the polysaccharides is in question. We now report that there are subtle differences between the polysaccharides of R. trifolii and R. leguminosarum.

By ^{1}H NMR and methylation analysis under neutral conditions of oligosaccharides generated from the polysaccharides by selective HF solvolysis, we have shown that there is an acetate group on the branched glucose of the polysaccharide only in the R. leguminosarum. There is an acetate group on O-3 of the nonbranched glucose in the polysaccharide backbone in both species. R. leguminosarum polysaccharide has a β-hydroxybutyrate esterified to O-3 of the terminal pyruvylated galactose as do polysaccharides from some strains of R. trifolii.



1503 RACE VARIATION IN PLANT PATHOGENS, Barbara Valent, Department of Chemistry, University of Colorado, Boulder, CO 80309

Plant breeders have incorporated genes conferring resistance to pathogens into agronomically useful cultivars. These genes are easy to incorporate because their pattern of inheritance is simple and they initially confer total resistance against the pathogen. However, genetic variability in agriculturally important fungal pathogens has prevented breeding enduring resistance to fungal disease in many crop plants. Physiologically distinct races of pathogens have been identified by their ability to overcome these genes. Variation in pathogens can be so great that races virulent on a new cultivar can appear immediately after introduction of the cultivar to the field. The genetic basis of resistance in cultivars of a host and of virulence in races of a pathogen was first determined by H.H. Flor, who studied flax rust. Flor's model suggested that resistance results from an interaction between specific gene products of both the pathogen and its host. The major limitation of the flax rust system, and other systems that have been studied genetically, has been the inability to grow the pathogen free of its host. This limitation has meant that the biochemical and molecular genetic mechanisms underlying the classical genetic model have not been elucidated. We are approaching the problem of race variation through studies of the rice blast fungus, Pyricularia oryzae, which grows on simple defined medium. Genetic analysis of virulence of this pathogen has been impeded by the low fertility of field isolates. We have increased the fertility of Pyricularia strains through a systematic program of inbreeding and selection. The strains we are developing will permit the study of a host-pathogen system using both biochemical and genetic techniques. Supported by DOE (DE-AC02-76ER0-1426), The Rockefeller Foundation (RF 81042), and Monsanto.

1504 A GENETIC SYSTEM FOR THE STUDY OF HOST SPECIFICITY IN A FUNGAL PATHOGEN, M.S. CRAWFORD, C.G. WEAVER, K.A. PARSONS, F.G. CHUMLEY, AND B. VALENT, Dept. of Chemistry, Campus Box 215, University of Colorado, Boulder, CO 80309

The Ascomycete Pyricularia is an important fungal pathogen of cultivated and wild grasses. Field isolates of Pyricularia infect one or a few different species. Strains of Pyricularia infecting rice exist as many races, defined by the cultivars of rice which the fungus can attack. The frequent occurrence of new races has hampered attempts to control the disease in rice through introduction of resistant cultivars. The biochemical and genetic basis for both race variability and host range are unknown. We have undertaken the genetic analysis of Pyricularia in order to answer these questions. We have observed that field isolates of Pyricularia vary in fertility according to host range. Isolates pathogenic to finger millet are moderately fertile while those pathogenic to rice have low fertility. We have increased the fertility of field isolates by a systematic program of inbreeding and selection. The 10% germination of ascospores from crosses involving field isolates has been increased to greater than 90%. It is now feasible to use tetrad analysis to study the genetics of pathogenicity. Crosses we have conducted between strains able and unable to infect rice indicate that there are several loci involved in pathogenicity towards rice. By constructing strains which lack a single "virulent allele" we will identify each of these loci. The long-term goal of this project is the isolation and characterization of the genes and gene products involved in pathogenicity. Supported by DOE (DE-AC02-76ER0-1426), The Rockefeller Foundation (RF 81042) and Monsanto.

1505 HETEROKARYOSIS, DIPLOID FORMATION, AND GENETIC ANALYSIS IN THE PYRICULARIA, F.G. CHUMLEY, M.S. CRAWFORD, C.G. WEAVER, K.A. PARSONS, AND B. VALENT, Department of Chemistry, University of Colorado, Boulder, CO 80309

The Pyricularia occur as fungal pathogens of a number of grasses. The fungus is a haploid Ascomycete with septate hyphae that normally contain a single nucleus per cell. We have isolated a variety of mutants with altered nutritional requirements, drug resistances, or pigmentation properties, and we are using them to study whether heterokaryons and true diploids can be formed. When small agar blocks containing mycelia of auxotrophic mutants with complementary growth requirements are placed in contact on minimal medium, vigorous prototrophic growth appears after 5-7 days' incubation. Heterokaryotic growth appears regardless of the mating type of the mutants. Heterokaryons can be subcultured by transferring mycelial plugs to minimal plates, but not by transferring hyphal tip segments. The heterokaryons give rise to sectors that include each of the parental auxotrophic types when cultured on complete medium. The conidia isolated from a heterokaryon are auxotrophs, including each of the parental types. Efforts are under way to isolate prototrophic conidia, which could possibly be true diploids. Results show that such diploid conidia are more rare than one in several thousand tested. Our objective in these studies is to perform genetic complementation and dominance tests of Pyricularia mutants, especially variants that differ in host specificity and pathogenicity. Information gained through these analyses will provide insight into the genetic basis of pathogenicity and will be essential to subsequent molecular cloning of genes that determine pathogenic characteristics. Supported by DOE (DE-AC02-76ER0-1426), The Rockefeller Foundation (RF 81042), and Monsanto.

1506 SOLUBILIZATION, RECONSTITUTION, AND SUBSTRATE SPECIFICITY OF PISATIN DEMETHYLASE FROM NECTRIA HAEMATOCOCCA, Anne E. Desjardins, David E. Matthews and Hans D. VanEtten, Cornell University, Ithaca, NY 14853-0331.

Some isolates of the fungus Nectria haematococca can demethylate pisatin, a pterocarpan phytoalexin from pea. Pisatin demethylation appears to be necessary for tolerance to pisatin and virulence on pea, and is catalyzed by a microsomal cytochrome P-450 monooxygenase system. We now report solubilization and further characterization of this enzyme. Pisatin demethylase activity was obtained in the high speed supernatant of detergent treated microsomes, if detergent was removed before assay. The CO-binding spectrum of the soluble enzyme preparation confirmed the presence of cytochrome P-450. Recovery of soluble activity was approximately 30% for pisatin demethylase and over 95% for NADPH-cytochrome c reductase, a normal component of cytochrome P-450 monooxygenases. Demethylase activity disappeared when reductase was removed by adsorption on 2',5'-ADP-agarose. The demethylase activity of reductase-free fractions could be restored by adding an approximately 100-fold purified reductase preparation. We conclude that pisatin demethylase requires NADPH-cytochrome c reductase for activity. The Km of the enzyme for pisatin was approximately 0.2 μM, indicating a relatively high substrate affinity. Other pterocarpans tested as competitive inhibitors showed lower affinity. The best competitor was (-)-pisatin, the enantiomer of the natural substrate.

1507 ROLE OF MONOOXYGENASES IN PISATIN BIOSYNTHESIS AND IN THE FUNGAL DEGRADATION OF MAACKIAIN, David E. Matthews, Eric J. Weiner, Patty S. Matthews and Hans D. VanEtten, Cornell University, Ithaca, NY 14853.

Some isolates of the plant pathogen Nectria haematococca detoxify the isoflavonoid phytoalexin (-)-maackiain by hydroxylation at carbon 6a (pterocarpan numbering). Precursor feeding studies strongly suggest that the penultimate step in (+)-pisatin biosynthesis by Pisum sativum is 6a-hydroxylation of (+)-maackiain. We have used ^{18}O labelling to test the involvement of monooxygenases in these two reactions. When fungal metabolism of maackiain was performed under $^{18}O_2$, more than 95% of the product was labelled; no label was incorporated by metabolism in $H_2{}^{18}O$. The increase in mass due to ^{18}O was observed for the molecular ion (M^+) but not for the abundant M^+-H_2O fragment, confirming that this characteristic fragment in the mass spectra of 6a-hydroxypterocarpans is produced by dehydration at carbon 6a. Pisatin synthesized by chitosan-treated pea pods in the presence of $^{18}O_2$ or $H_2{}^{18}O$ was a mixture of molecules containing up to 3 labelled oxygen atoms. To simplify the spectra, the samples were analyzed by tandem mass spectroscopy to visualize the fragmentation of the molecular ion in each isotopic class. The isotope distribution of the M^+-H_2O ion derived from each isotope-enriched molecular ion revealed the extent of labelling of the 6a oxygen atom. This analysis indicated that the 6a oxygen of pisatin was derived from H_2O and not from O_2. Labelling patterns for the other 5 oxygen atoms in smaller fragments were consistent with the proposed pathway for biosynthesis of pisatin and related isoflavonoids. We conclude that the fungal hydroxylation of maackiain is catalyzed by a monooxygenase, but the biosynthetic route to the 6a-hydroxyl of pisatin is unknown.

1508 THE ROLE OF NAPHTHAZARIN PRODUCTION BY NECTRIA HAEMATOCOCCA MP VI IN PATHOGENESIS ON PEA (PISUM SATIVUM). Kathryn Tegtmeier, Zoecon Corporation, Palo Alto, CA 94304

When grown in culture the plant pathogenic fungus Nectria haematococca MP VI (anamorph: Fusarium solani) synthesizes a family of compounds structurally characterized as naphthazarins. The phytotoxicity of several naphthazarins has led to the hypothesis that the virulence of the fungus is dependent on the production of these compounds. A screening of 43 field isolates collected from diverse habitats identified one isolate, isolate T217, that did not produce detectable levels of naphthazarins (nap^-). And indeed, T217 was weakly virulent on pea, a suscept of N. haematococca MP VI. To determine whether low virulence on pea was causally related to the nap^- phenotype, progeny were isolated from a series of crosses between isolate T217 or nap^- progeny derived from it and isolates that produce naphthazarins (nap^+). Four hundred thirty-five progeny from five crosses, segregating both for naphthazarin production and for virulence, were scored. Naphthazarin production segregated as a single pair of alleles in these crosses. The nap^- progeny had as broad a range of virulence as the nap^+ progeny. The recovery of highly virulent nap^- progeny shows conclusively that high virulence on pea is not related to the ability to produce naphthazarins in culture.

1509 MODE OF ACTION OF OPHIOBOLIN A, A FUNGAL PHYTOTOXIN, Carl L. Tipton, Pak C. Leung and Stephen G. Carter, Iowa State University, Ames, IA 50011.

Ophiobolin A is one of a series of sesterterpenes produced by phytopathogenic fungi of the genus Cochliobolus (Helminthosporium). It is toxic both to plants and to animals and we have shown recently that it is a potent inhibitor of calmodulin from brain, spinach and maize.

A series of structurally related compounds, including anhydrophiobolin A, 6-epi-ophiobolin A and 18-bromo-19-methoxyophiobolin A have been isolated or synthesized. Their effects on ion leakage from maize roots and on the activity of calmodulin have been measured and compared with the effects of ophiobolin A. 18-Bromo-19-methyoxyophiobolin A has effects identical to those of ophiobolin A both on calmodulin and ion leakage. Anhydro-ophiobolin A and 6-epi-ophiobolin A are less active than ophiobolin A in both assays. Additional derivatives of ophiobolin A are being synthesized and tested.

A (Ca^{2+}-Mg^{2+})-ATPase from maize coleoptiles, similar to an enzyme reported (Dieter, P., and Marmé, D., (1981) FEBS Lett. 125, 245-8) to be calmodulin-dependent, is inhibited by ophiobolin A at low concentrations.

These results support the hypothesis that the toxic action of ophiobolin A in vivo involves inhibition of calmodulin.

1510 HERBIVORY INDUCED STRESS AND THE PRODUCTION OF RUBBER AND RESISTANT FACTORS IN GUAYULE, Eloy Rodriguez, Phytochemical Laboratory, University of California, Irvine CA 92717

Cultivars and F_1 hybrids of the rubber plant guayule (Parthenium argentatum) were examined for the production of high molecular weight rubber and resisant factors. Hybrids of guayule with the tropical and temperate montane species resulted in hybrids producing potent insect repellents and good quality rubber. The repellents were identified as sesquiterpene lactones a class of compounds not present in guayule, but found in other species of Parthenium. The patterns of inheritance of rubber and resistant factors in guayule and hybrids will be presented and discussed. The effects of herbivory on terpenoid production in guayule and selected hybrids will be highlighted. (supported by NSF).

1511 INDUCTION OF A GLUCOSYLTRANSFERASE IN OATS IN RESPONSE TO THE ALLELOCHEMICAL SALICYLIC ACID, Nelson E. Balke, Carol C. Lee, and Michael P. Davis, Department of Agronomy, University of Wisconsin, Madison, WI 53706.

Salicylic acid, an allelopathic, phenolic acid, inhibits K^+ absorption in oat (Avena sativa) root tissue in a pH-, concentration-, and time-dependent manner. At pH 6.5 and 0.5 mM salicylic acid, the tissue is able to metabolize the allelochemical after a lag period of about 6 hr. We have studied this metabolism of salicylic acid. Protein extracts from root tissue incubated several hours in salicylic acid metabolized the chemical to the same polar compound as excised roots did. A rapid, sensitive, radioactive assay to measure in vitro metabolism of salicylic acid was developed. Uridine diphosphate glucose(UDPG) was required for this enzymatic reaction. The reaction product was the same whether ^{14}C-salicylic acid or ^{14}C-glucose in UDPG was included in the assay mixture. Greater amounts of this enzyme activity were extractable from tissues that had been incubated for longer periods of time in salicylic acid. Thus, this enzyme appears to be an inducible UDPG:salicylic acid glucosyltransferase (EC 2.4.1.35). Because such enzymes are important in the detoxification of both naturally-occurring and synthetic phytotoxic chemicals, we are currently developing procedures to purify this enzyme in anticipation of characterizing it. (Partially supported by USDA/CRGO Grant No. 5901-0410-8-0032-0.)

1512 WOUND SIGNALS IN PLANTS: A SYSTEMIC PLANT WOUND SIGNAL ALTERS PLASMA MEMBRANE INTEGRITY, Mary Walker-Simmons, Heike Hollander-Czytko, Julie K. Andersen and Clarence A. Ryan, Washington State University, Pullman WA 99164

Within 4 hr following wounding of the lower leaves of young potato and tomato plants, either mechanically with a hemostat or by chewing insects, a rapid and remarkable change was induced in the cells of upper undamaged leaves that resulted in extensive lysis of protoplasts during their isolation. When protoplasts were isolated four hours after wounding, recovery was decreased 25% below yields from leaves of unwounded plants. By 8 hr following wounding, protoplast yields were less than half of those from unwounded plants. Multiple woundings decreased yields even further as did chewing on the leaf by tobacco hornworms over a period of several minutes. Additionally, excision of young tomato plants with a razor blade caused, within 4 hr, a 90% decrease in protoplast yields with respect to yields from intact plants.

The major loss of protoplasts induced by wounding was primarily due to an increased cell lysis during protoplast isolation. Cell lysis was apparently due to a weakened cell membrane since newly recovered protoplasts, released from leaves of wounded plants, were extremely fragile and exhibited 70% lysis during low speed centrifugation, compared to 20% lysis of protoplasts recovered from control plants.

It is proposed that a signal is released by wounding that plays a role in inducing cellular changes in the plant cells as part of their responses to environmental stress such as pest attacks.

1513 ISOLATION AND CHARACTERIZATION OF cDNAs FOR WOUND-INDUCED TOMATO LEAF INHIBITORS I AND II AND IN VIVO STUDIES OF THE TRANSCRIPTIONAL REGULATION OF INHIBITOR SYNTHESIS. John Graham, Gregory Pearce, T. Okita, J. Merryweather, K. Titani, and C.A. Ryan. Institute of Biological Chemistry and Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164; Chiron Corporation, Emeryville, CA 94608, and Dept. of Biochemistry, University of Washington, Seattle, WA 98175.

Full length cDNAs for the wound-induced proteinase Inhibitors I and II from tomato leaves have been constructed and characterized. Complete amino acid sequences of the proteins, including their signal peptide regions, have been determined. The cDNAs have been employed to study the in vivo transcriptional regulation of each inhibitor mRNA synthesis in tomato leaf cells in response to wounding. Within four hr following a single wound mRNA levels for Inhibitors I and II rapidly increase and then slowly decrease over the next several hours. Repeated wounding, either initially or after several hours, causes a doubling of mRNA levels. The cDNAs have also been utilized as probes to identify genomic clones of both Inhibitors I and II from tomato and potato genomic libraries.

1514 INDUCTION OF ENZYMES OF PHYTOALEXIN SYNTHESIS IN CULTURED SOYBEAN CELLS BY FUNGAL ELICITOR, Jürgen Ebel, Universität Freiburg, 78 Freiburg, FRG

A glucan elicitor from the fungus P. megasperma f.sp. glycinea, a pathogen of soybean (Glycine max), induced rapid but transient increases in the activities of several enzymes associated with the synthesis of the isoflavonoid phytoalexin, glyceollin, in suspension cultured soybean cells. For two of the induced enzymes, phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), the changes in catalytic activity were shown to be preceded by large changes in their rates of synthesis as determined in vivo and in vitro. The earliest increases in the mRNA activities encoding these enzymes could be measured at about 1 h, with the highest activities occurring about 6 h after the onset of induction. Although diverse microbial compounds such as P. megasperma glucan elicitor, xanthan, an extracellular polysaccharide from X. campestris, and endopolygalacturonase, an extracellular enzyme from A. niger, induced PAL and CHS to a similar extent, only the glucan elicitor efficiently stimulated glyceollin accumulation in soybean cells. Little is known at present about the interactions at the molecular level between the glucan elicitor and soybean cells which will eventually lead to the induction of enzymes of phytoalexin biosynthesis. We observed recently that the presence of Ca^{2+} ions in the cell culture medium enhanced the elicitor-mediated induction of PAL and CHS which might suggest a role for Ca^{2+} in signal transmission.

1515 MOLECULAR BIOLOGY OF THE PHYTOALEXIN DEFENSE RESPONSE, Chris J. Lamb, John N. Bell, Thomas B. Ryder, Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138; Richard A. Dixon, University of London and John A. Bailey, University of Bristol.

Changes in the mRNA activity of chalcone synthase, the first enzyme of phenylpropanoid metabolism specific to flavonoid/isoflavonoid biosynthesis have been investigated in relation to expression of the phytoalexin defense response in race-cultivar specific interactions between hypocotyls of Phaseolus vulgaris and the partially biotrophic fungus Colletotrichum lindemuthianum, causal agent of anthracnose. In an incompatible interaction (host resistant) there is an early but localized increase in chalcone synthase mRNA activity prior to the onset of accumulation of the phenylpropanoid-derived phytoalexin phaseollin and expression of hypersensitive resistance. In contrast, in a compatible interaction (host susceptible) there is no induction of mRNA activity in the early stages of infection, but rather a delayed, widespread increase during attempted lesion limitation at the onset of symptom development. The data indicate that control of phytoalexin gene expression is likely to be a key early component in the defense responses of biologically-stressed cells during a race-cultivar specific host-pathogen interaction.

1516 cDNA CLONES OF PISUM SATIVUM AS PROBES FOR MONITORING GENE EXPRESSION IN SUSCEPTIBLE AND RESISTANT INTERACTIONS OF PEAS WITH FUSARIUM SOLANI, Robert C. Riggleman, Brian W. Fristensky, and Lee A. Hadwiger, Wash. State University, Pullman, WA 99164

Poly A-RNA from pea endocarp tissue extracted 8 h after inoculation with the non-compatible pathogen Fusarium solani f. sp. phaseoli was used to construct a cDNA library. Of 2400 recombinant clones screened by differential hybridization, 7 distinct homology classes were selected to assay mRNAs that increase in abundance following inoculation. Also, in vivo transcriptional activity of the corresponding plant genes was estimated by pulse-labeling endocarps with ^{3}H-uridine or $^{32}PO_4^{-}$ at 0, 2, 4, 6, 8, 10, 24 and 48 h after inoculation with H_2O, the non-compatible pathogen, or the compatible pathogen, F. solani f. sp. pisi. The in vivo labeled RNA was hybridized to filter-bound plasmid DNA from clones pI49, pI176, pI204, pI206, pI230 and pI259. In the incompatible interaction increases in labeling occurred from 4-48 h after inoculation which were 2X-7X those in water treated control tissue. Labeling increases of generally lower magnitude were observed in the compatible interaction at 4-8 h, further, there was a return to near-control levels 12-48 h after inoculation. Similar results were obtained using each of the clones as ^{32}P-labelled probes against Northern blots. Also similar correlations were observed for individual "resistance response proteins" labelled in in vitro translation system coded with accumulated mRNAs (Physiol. Plant Path. 23: 153, 1983). Thus there is an increased synthesis of specific pea mRNAs as the pea resists both F. solani f. sp. at 4-8 h and a subsequent decrease in labeling at 12-48 h as the pea tissue becomes susceptible to f. sp. pisi.

1517 DISEASE RESISTANCE INDUCED BY HEAT SHOCK: ASSOCIATION WITH ETHYLENE PRODUCTION AND CELL WALL HRGP ACCUMULATION, Bruce A. Stermer and Raymond Hammerschmidt, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

A brief heat shock of cucumber seedlings, normally susceptible to Cladosporium cucumerinum, induces resistance to subsequent challenge by the fungus. Immersion of seedlings in a 50 C waterbath for 40 seconds prior to inoculation results in a 60% or greater decrease in disease symptoms. A period of at least 12 hours between the heat treatment and the challenge inoculation is required for maximum resistance to be expressed. Heat shock also increases the peroxidase activity of the seedlings; there is a close correlation between the level of peroxidase activity and the degree of resistance induced in treated seedlings. An early event detected in heat shocked seedlings is an increase in ethylene production. By 6 hours after heat shock, seedlings produced twice as much ethylene as unshocked controls, and the heat shocked seedlings continued to produce ethylene at a higher rate for at least 24 hours. Increases in the amounts of 1-aminocyclopropane 1-carboxylic acid, an ethylene precursor, were similarly higher in heat shocked tissues, reflecting the higher rate of ethylene synthesis. Accumulation of hydroxyproline-rich glycoprotein (HRGP) in plant cell walls is implicated in the disease resistance of cucurbits. In cucumbers, the levels of HRGP were enhanced in seedlings after heat shock; the cell walls of shocked seeldings contained up to 100% more of the glycoprotein than did unshocked seedlings. Interestingly, HRGP may be crosslinked in the cell wall by peroxidase, an enzyme whose activity is closely related to induced resistance in cucumbers.

1518 ROLE OF EXTRACELLULAR POLYGALACTURONASE FROM ASPERGILLUS NIGER CULTURES IN THE ELICITATION OF CASBENE SYNTHETASE ACTIVITY IN CASTOR BEAN SEEDLINGS, Augusto Lois and Charles West, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

Aspergillus niger cultures have previously been shown to elicit the production of casbene synthetase activity in castor bean (Ricinus communis L.) seedlings (D. Sitton and C. A. West, Phytochemistry 14: 1921 (1975)). The factor responsible for elicitation in Rhizopus stolonifer culture filtrates has been shown to be an endopolygalacturonase (S. C. Lee and C. A. West, Plant Physiol. 67: 633 (1981).

A. Niger is known to secrete both an endopolygalacturonase and an exopolygalacturonase. In the present study we have irradiated A. niger with UV light and have selected mutants that appear to lack the ability to break down polygalacturonic acid when colonies are grown on indicator plates. An analysis of the viscosity-reducing activity of culture filtrates of two of these mutants in comparison with the culture filtrate of the wild-type indicated that the mutants lacked endopolygalacturonase activity but still produced exopolygalacturonase. Preliminary studies of these endopolygalacturonase$^-$ mutants show that they retain the capacity to elicit casbene synthetase activity, but at lower levels than the wild type. Further investigations of the mutants and enzyme preparations from their culture filtrates as elicitors are in progress.

1519 PURIFICATION OF CASBENE SYNTHETASE FROM INFECTED CASTOR BEAN SEEDLINGS, P. Moesta and C.A. West, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

Castor bean (Ricinus communis L.) seedlings respond to stress by producing the antifungal diterpene casbene. The enzyme catalyzing the production of casbene from geranylgeranylpyrophosphate, casbene synthetase, was purified approximately 5000-fold to a final specific activity of 250 nmoles casbene produced per min per mg protein. The purification procedure included ammonium sulfate fractionation, QAE-Sephadex ion exchange chromatography, dye-ligand chromatography using Matrex Gels Red A and Blue A, and preparative HPLC on a LKB Ultropac DEAE column. The purified enzyme yielded a single band of apparent molecular weight of 58000 by SDS polyacrylamide gel electrophoresis.

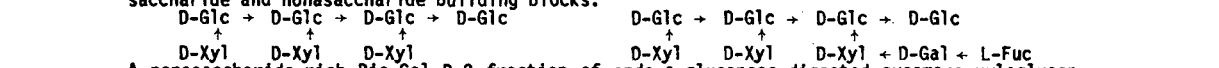
This is the first instance of availability of a highly purified enzyme that catalyzes the terminal step in the biosynthesis of a terpenoid stress metabolite. Antibodies produced against the purified enzyme will be used in further studies on the induction of casbene.

1520 TESTS OF RHIZOPUS STOLONIFER ENDOPOLYGALACTURONASE AND α-1, 4-D-GALACTURONIDE OLIGOMERS AS PHYTOALEXIN ELICITORS, Karen A. Wickham and Charles A. West, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

The pectinolytic enzymes that are commonly produced by phytopathogenic fungi and bacteris may play a general role in phytoalexin elicitation. In castor bean (Ricinus communis L.) seedlings, endopolygalacturonase (EPGase) isolated from Rhizopus stolonifer culture filtrates as well as a mixture of α-1,4-D-galacturonide oligomers produced by partial digestion of polygalacturonic acid with EPGase act to elicit casbene synthetase activity (R.J. Bruce and C.A. West, Plant Physiol. 69: 1181 (1982)).

Both R. stolonifer EPGase and the partial digest of polygalacturonic acid with this enzyme are being tested for elicitor activity in a number of other phytoalexin-producing plants to see how general this response may be. Both substances have been found to act as elicitors of phaseollin in bean (Phaseolus vulgaris) and of ipomeamarone in sweet potato (Ipomea batatas L.), but neither acts to elicit rishitin in potato (Solanum tuberosum). Further work is being done to quantify the results in these systems and to explore other systems.

1521 ENDOGENOUS PLANT ENZYMES MAY RELEASE BIOLOGICALLY ACTIVE XYLOGLUCAN OLIGOSACCHARIDES FROM PLANT CELL WALLS, Alan R. White, William S. York, Alan G. Darvill, and Peter Albersheim, University of Colorado, Campus Box 215, Boulder, CO 80309.
Xyloglucans are hemicelluloses in the primary cell walls of dicots, monocots, and gymnosperms. The structure of xyloglucan from the walls of suspension-cultured cells and from pea, bean, and other plants have been shown to be composed largely of the following heptasaccharide and nonasaccharide building blocks.



A nonasaccharide-rich Bio-Gel P-2 fraction of endo-β-glucanase-digested sycamore xyloglucan inhibits, at 10^{-8} M, 2,4-D-stimulated elongation of etiolated pea-stem segments. It has been hypothesized that the nonasaccharide plays a role in plant development by acting as a feedback inhibitor of auxin-stimulated growth or, after transport down the stem, by inhibiting the growth of axillary buds, thereby functioning as a regulator of apical dominance. Oligosaccharides acting as developmental regulators would presumably be released from their positions within the cell wall by specific endoglycanases. It has been shown that the activity of an endo-β-glucanase in etiolated pea epicotyls is stimulated 50-fold by auxin. This enzyme can convert pea xyloglucan to the hepta- and nonasaccharide fragments. We are examining this and other cell wall glycanases to determine whether plant cell walls possess enzymes capable of releasing specific biologically active oligosaccharide fragments from cell wall polysaccharides. This will help to determine whether these oligosaccharides act in vivo as regulators of developmental and stress physiology. Supported by NIH Postdoctoral Fellowship 5F32AM06982-02 and DOE Grant DE-AC02-76-ER01426.

1523 STRUCTURE AND ACTIVITY OF ELICITORS OF PHYTOALEXINS RELEASED FROM POLYGALACTURONIC ACID AND SOYBEAN CELL WALLS BY A BACTERIAL ENDOPOLYGALACTURONIC ACID LYASE, Keith R. Davis, Alan G. Darvill, and Peter Albersheim, Depts. of Molecular, Cellular, and Developmental Biology and of Chemistry, Campus Box 215, University of Colorado, Boulder, CO 80309
Plants often respond to microbial infection by producing a class of stress metabolites called phytoalexins. Other laboratories have shown that phytoalexin accumulation is due to the de novo synthesis of mRNAs encoding the enzymes responsible for phytoalexin synthesis. We have shown that a bacterial pectin-degrading enzyme, α-1,4-endopolygalacturonic acid lyase, is an elicitor of phytoalexin accumulation in soybean cotyledons. The lyase released elicitors of phytoalexin accumulation from soybean cell walls, from polygalacturonic acid, and from citrus pectin. Elicitor-active oligogalacturonides obtained by lyase treatment of polygalacturonic acid have been purified by anion exchange and gel filtration. At least 95% of the most elicitor-active fraction was found to be a decamer of α-1,4-linked galactosyluronic acid residues containing a 4,5-unsaturated galactosyluronic acid residue at the nonreducing terminus. The decamer exhibited half-maximal elicitor activity at 1 μg galactosyluronic acid equivalents/cotyledon (6 μM). The structures of elicitors released from soybean cell walls by the lyase is currently being investigated. Recent experiments indicated that the lyase and oligogalacturonide elicitors have a stimulatory effect on the elicitor activity of a pure hepta-β-glucoside-alditol elicitor derived from fungal cell walls. This synergism resulted in at least a 10-fold increase in phytoalexin accumulation above that expected by a simple additive response. [Supported by DOE (DE-AC02-76ER0-1426) and The Rockefeller Foundation (RF 79049)].

1522 PLANT CELL WALL FRAGMENTS ACT AS REGULATORY MOLECULES, David J. Gollin, Peter Albersheim, Alan G. Darvill, Steven H. Doares, William S. York. Dept. of Chemistry, Campus Box 215, University of Colorado, Boulder, CO 80309

Evidence has been obtained that plant cell wall fragments act as regulatory molecules in plants. Three examples of such activities are described below. First, acid-solubilized fragments of sycamore (Acer pseudoplatanus L.) cell walls, at 100 μg/ml, inhibited flowering (50-100%) and stimulated vegetative growth (50-100%) in the long day aquatic plant Lemna gibba G3 grown under long days. Second, cell wall fragments may be involved in eliciting the hypersensitive resistance response of plants to potential pathogens, a response that appears to result in the slowing down of the invading organism's growth, providing time for other defense reactions of the plant to stop an attempted infection. [^{14}C]Leucine incorporation into acid precipitable polymers of suspension-cultured sycamore cells is a convenient measure of the viability of the cells, and, therefore, perhaps of hypersensitive cell death. Acid-solubilized fragments of sycamore cell wall pectic polysaccharides inhibited the incorporation of [^{14}C]leucine into polymers. Invading microbes may elicit hypersensitive cell death by enzymically solubilizing these pectic fragments from plant cell walls. Third, a nonasaccharide-rich fraction of sycamore extracellular xyloglucan isolated from the filtrate of suspension-cultured sycamore cells inhibited 2,4-D-stimulated growth of etiolated pea epicotyls. The inhibitory activity of the nonasaccharide-rich fraction exhibited a sharp concentration optimum between 10^{-2} and 10^{-1} μg/ml (10^{-8} M). This suggested that the xyloglucan nonasaccharide fraction is involved in feedback inhibition of auxin-stimulated growth and/or of apical dominance in vivo. These results support the hypothesis that cell wall fragments have diverse regulatory functions in growth, development, and pathogenesis. Supported by DOE (DE-AC02-76ER0-1426).

1524 IMMUNOCHEMICAL IDENTIFICATION OF ANTIGENS INVOLVED IN PLANT/PATHOGEN INTERACTIONS, Arthur R. Ayers, Harvard University, Cambridge, MA 02138

Genetic studies of plants and their pathogens have provided many examples indicating that genes for disease resistance in the host are complemented by corresponding genes for avirulence in the pathogen. Both host and pathogen must have at least one of these pairs of corresponding genes in order for the pathogen to be detected. In the absence of detection, which normally leads to the induction of defensive responses, the pathogen can spread through host tissue and produce characteristic disease symptoms.

Polyspecific and monoclonal antibodies are now being used to identify the products of genes for resistance and avirulence. Antibodies specific for the glycomoities of extracellular glycoproteins secreted by a fungal pathogen are being used to examine the possibility that avirulence genes code for glycosyl transferases. A search for receptors for pathogen glycoproteins is in progress using antibodies specific for components of the plant plasma membrane.

Receptors for pathogen components are also being investigated using synthetic compounds that mimic the structure and activity of their natural counterparts. Elicitors, pathogen molecules that elicit the accumulation of toxic secondary metabolites in host tissues, are being examined by this approach.

1525 FLUORESCENCE-ACTIVATED CELL SORTING FOR LOCALIZATION OF PHYTOALEXINS, M.L. Pierce and M. Essenberg, Oklahoma State University, Stillwater, OK 74078; V. E. Scholes, Oral Roberts University, Tulsa, OK 74171

During the resistant response of cotton line OK 1.2 to Xanthomonas campestris pv. malvacearum, leaf cells next to a bacterial colony lose membrane semipermeability, collapse, become yellow-green fluorescent, and turn brown. The resistant leaf produces the two phytoalexins 2,7-dihydroxycadalene (DHC) and lacinilene C (LC) and their 7-methyl ethers. LC is yellow-green fluorescent. Are the phytoalexins predominantly located in the yellow-green fluorescent cells? If so, we calculate their concentrations to be high enough to account for the observed inhibition of bacterial growth. Using a fluorescence-activated cell sorter (FACS), we are sorting cells on the basis of yellow-green fluorescence for the purpose of analysis of phytoalexin content. Mesophyll cells are isolated from slices of infected leafy cotyledons by peeling away the abaxial epidermis, infiltrating and incubating the rest with a solution of Macerase, and gently brushing out the loosened cells with a toothbrush. Cells are separated from larger particles by filtration through mesh with 55 μm pores and from small debris by retention on mesh with 15-18 μm pores. Suspension of the cells in a near-isopycnic solution prevents clogging of the FACS nozzle. LC and DHC diffuse from peeled cotyledons into aqueous medium with half-times of 1.2 and 5.6 hr. Cells are isolated and sorted within 1 hr. Sorting deflects yellow-green fluorescent cells and non-fluorescent cells into different tubes. Microscopical analysis of cells we have sorted indicated that more than 90% of the visibly fluorescent cells were in the yellow-green fluorescent cell fraction.

1526 RESISTANCE-RELATED PROTEINS IN BARLEY-POWDERY MILDEW INTERACTIONS, John M. Manners, Andrew D. Davidson and Kenneth J. Scott, University of Queensland, Brisbane, 4067, Australia.

Patterns of protein synthesis associated with resistance to the obligate parasite, *Erysiphe graminis* f.sp. *hordei* in barley conditioned by single host genes will be described. Near-isogenic lines of barley differing for the Mla, Mlp and Mlk genes were pulse labelled with [^{35}S]-methionine at 24, 48 and 72h after inoculation. Two-dimensional fluorographic analysis of extracted proteins has demonstrated that resistance-specific polypeptides (RP's) are synthesised in barley carrying the Mla gene at 24h after inoculation whilst for the Mlp and Mlk genes synthesis of RP's commences at 48 hours. These different genes condition the synthesis of both common and unique RP's. Importantly, the net accumulation of at least one RP has been demonstrated by silver staining. Synthesis of RP's coincides with the cessation of fungal growth indicating an active role in resistance. Synthesis of RP's and inhibition of pathogen growth, are both delayed to 96h after inoculation in heat-shocked leaves of barley carrying the Mla gene. RP's are not heat shock proteins. Although RP's identified *in vivo* did not appear to be present amongst *in vitro* translation products of poly A$^+$ RNA some possible precursors to RP's associated with Mla and Mlp gene activity were identified. Experiments are underway to test whether the RP's of barley are coded for or regulated by these genes for resistance to the powdery mildew fungus.

1527 ON THE HYPERSENSITIVE RESPONSE AND PHYTOALEXIN PRODUCTION IN SOYBEAN CELL SUSPENSION CULTURES UNDER BIOTIC STRESS. R. M. Zacharius, Eastern Regional Research Center, ARS, USDA, NER, 600 E. Mermaid Lane, Philadelphia, PA 19118

Previously, we reported that soybean cell suspensions when exposed to crude fungal elicitor preparations produce the stress metabolite glyceollin accompanied by an atypical hypersensitive response (culture darkening but not death) and a sharp decline in cellular isoflavonoids, daidzein and genistein. Cell cultures, initially low in these isoflavonoids will neither darken nor produce glyceollin on biotic stress. Darkening and glyceollin production of the whole culture exposed to the elicitor was also inhibited with 2 x 10-3 M of the antiozonant N-/ 2-(2-OXO-1-imidazolidinyl)ethyl_/-N'-phenylurea.) Soy suspension cultures, were mechanically resolved into small and large cell aggregations and only the latter darkened on exposure to fungal elicitor. The small cell aggregates failed to darken but a reduction in the daidzein and genistein levels occurred concommitant with glyceollin production. The findings demonstrate that culture darkening with biotic stress is not a prerequisite to glyceollin production.

1528 COTTON PEROXIDASE PROFILES ELICITED BY FUNGAL-INDUCED STRESS, Jay E. Mellon, USDA,ARS,SRRC, P.O. Box 19687, New Orleans LA 70179

The role of peroxidase isozyme multiplicity in plant defense mechanisms of cotton was examined. Enzymes were extracted from carpel tissue and ovule cultures by homogenization in 0.05 M citrate buffer, pH 5.0, containing polyvinylpyrrolidone (insoluble). Crude peroxidase extracts were analyzed by isoelectric focusing gels with an activity stain. Cotton bolls, 30 days post-anthesis, were independently inoculated with the following fungi: Aspergillus flavus, Fusarium equiseti, F. moniliforme, F. semitectum, Rhizoctonia solani, and Verticillium dahliae. Six days after inoculation, test bolls were removed from plants for assay. Between 10 and 20 peroxidase isozymes were detected, depending upon the tissue being examined. Fungal-induced stress elicited an increase in total peroxidase activity of carpel extracts compared to controls. In addition, the levels of specific isozymes were significantly elevated in these extracts of fungal-stressed carpels. Examination of cotton ovule cultures demonstrated a secretion of peroxidase(s) into the growth medium. The peroxidase profiles of fungal-stressed tissue were compared with the culture-derived activity.

1529 INDUCTION OF RESISTANCE AGAINST BIOLOGICAL STRESS, Fritz Schönbeck and Heinz-W. Dehne, Institut für Pflanzenkrankheiten, University of Hannover, D-3ooo Hannover, West-Germany

Plants have - independent from the presence of specific genes for resistance - natural defence mechanisms against plant pathogens which can be activated. Nontoxic metabolic substances produced by some bacteria and fungi were able to induce a nonspecific resistance in plants against diseases caused by obligate biotrophic fungi. Without any direct, antibiotic activity these inducers altered the physiology of various host plants that rust, powdery and downy mildew developed not or only to a limited extent on their hosts. The biological stress due to the diseases could be reduced by the microbial metabolites even under field conditions, so that yield loss was prevented. Reduction of disease and damage could not be correlated to the formation of inhibitory compounds in treated tissues. As could be demonstrated by histological and biochemical investigations, disease development was decreased due to a reduced effectivity of the parasitic haustoria in the plant. On treated plants there was not only a reduction of disease incidence, but also a decreased development and reproduction of the pathogens.

1530 INFLUENCE OF VA MYCORRHIZAE ON ENVIRONMENTAL AND BIOLOGICAL STRESS, Heinz-W. Dehne and Fritz Schönbeck, Institut für Pflanzenkrankheiten, University of Hannover, D-3ooo Hannover, West-Germany

The symbiotic host-fungus-association, which is formed by endomycorrhizal fungi and a broad range of host plants, can influence the resistance of plants to environmental and biological stress. Mycorrhizal plants grow better in nutrient deficient soils and tolerate extreme temperatures and drought better than nonmycorrhizal plants. Under the influence of vesicular-arbuscular mycorrhizae plants compensate parasitic stress better than non-mycorrhizal hosts. In direct competition with pathogens in the root system there is a preference of host cells for mycorrhizal fungi. Due to an occupation of possible infection sites mycorrhizal roots become more resistant to plant pathogens and less sensitive to parasitic damage. Besides nutritional aspects morphological and physiological alterations in the entire host can be correlated to the increased stress tolerance of mycorrhizal plants. Respiration and dissimilatorial enzymes are stimulated in mycorrhizal root cells. Changes in the morphology of vascular tissue are accompanied by a stimulated assimilate transport into the mycorrhizal root system and changes in the osmotic pressure. Assimilation and photosynthetic capacity are stimulated by the formation of VA mycorrhizae in the root system.

1531 HOST-PATHOGEN INTERACTIONS IN PINUS SUSPENSION CULTURE, Joseph A. Laszlo and Peter F. Heinstein, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907

Adult pine trees produce copious amounts of resin, apparently as a phytoalexic response to physical damage and invasion by fungi. The resin is composed primarily of the monoterpenes, α- and β- pinene, and the dieterpenes, abietic acid and dehydroabietic acid. The fungus causing the resin induction in pine trees is believed to be Fusarium moniliforme var. subglutinans. We have established cell suspension cultures of P. elliottii (slash pine) and P. palustris (longleaf pine) in order to identify the resin-inducing elicitor component(s) from F. moniliforme var. subglutinans. The pine suspension cultures predominantly produce dehydroabietic acid, normally at 1.0 - 2.5 mg/l levels. The ability of heat-inactivated Fusarium, as well as various sub-cellular fungal fractions, to induce increased diterpene synthesis (up to 10 mg/l) in the cell suspension cultures and the molecular interaction of the pathogen and the host will be described.