

## Stress

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For better or worse, stress is an every day fact of life, not only for people, but also for plants. Stress, which is commonly denned as the wear and tear of life, occurs in varying degrees and in different forms that have impact, sometimes good, sometimes harmful, upon the health of people and plants. Stress can maximize productivity or it can have damaging side effects which may lead to disease, premature aging, or to shortened life expectancy. Therefore, it is essential that we understand the physiological changes caused by stress, not only in people, but also in plants, in order to improve our own health and longevity, and those of our crop plants.

*The wear and tear of life.* Avocado trees are subjected to varying degrees and different forms of stress. Stress can be caused by biotic factors, such as rodent and insect pests or bacterial and fungal pathogens. Stress can be caused by abiotic factors in the tree's environment: salinity, mineral nutrient deficiencies and toxicities, high or low temperatures, and water-deficit. Like humans, the avocado tree has a number of "built-in" mechanisms for coping with stress. These mechanisms are elicited in response to stress to compensate for the physiological changes it caused and to maintain the normal biochemical balance of the tree. These homeostatic mechanisms preserve tree health. The results of our current research suggest that we have identified a key homeostatic mechanism that compensates for a physiological change in plant tissues caused by a number of different abiotic stresses. We have been investigating the hypothesis that any stress that suppresses the growth of a plant or causes carbohydrate depletion will result in the accumulation of ammonia (measured as the combined pool of  $\text{NH}_3\text{-NH}_4$ ) in young and old leaves as an early response of the plant to stress. The reduction in growth can be as dramatic as inhibition of shoot growth or as subtle as failure of individual leaves to fully expand. As part of our hypothesis, we are testing the corollary that stress-tolerant plants maintain homeostasis by detoxifying their cells of ammonia through increased biosynthesis of arginine. When this homeostatic mechanism fails, ammonia accumulates to toxic levels and results in characteristic leaf symptoms; e.g., tip burn, margin necrosis, and increased leaf abscission (Rabe and Lovatt, 1986; Nevin and Lovatt, 1987).

*Why does ammonia accumulate? Why does arginine biosynthesis increase?* Avocado trees are 90% water, 9% carbohydrate (in plants the major carbohydrate is cellulose), 0.9% protein, and 0.1% fat, nucleic acid, and other compounds. All living organisms are comprised of these basic components. In plants, photosynthesis provides the reduced carbon and energy for the synthesis of (1) sugars which are linked to form

carbohydrates, (2) fatty acids used to make complex fats and lipids, and (3) the 21 amino acids which are combined to form proteins. The synthesis of amino acids, and hence proteins, also requires nitrogen. The nitrogen must be in a reduced form ( $\text{NH}_2$ ,  $\text{NH}_3$ , or  $\text{NH}_4$ ) to be metabolically useful to a living cell. Nitrogen is supplied to the avocado tree as ammoniacal fertilizer ( $\text{NH}_4$ ) or as nitrate ( $\text{NO}_3$ ) which must be reduced by the plant to  $\text{NH}_2$ ,  $\text{NH}_3$ , or  $\text{NH}_4$ . In a healthy tree, the synthesis and utilization of these basic compounds meets the needs of the tree for normal growth, maintenance, repair, and reproduction (flower and fruit production). Abiotic environmental stresses change this. Each stress prevents the plant or tree from achieving its full growth potential. (1) Water-deficit stress causes stomates to close thereby preventing photosynthesis from occurring. In addition, water entering individual cells exerts pressure and serves as the driving force for cell expansion and thus shoot elongation and leaf expansion. When water is limiting, growth ceases. (2) Salinity can reduce water pressure within plant cells by drawing water out of the cells and can thus mimic the effects of water-deficit stress. Specific ions present in a saline environment may be toxic to the plant, poisoning one or more metabolic pathways essential to growth. (3) Physiological processes have temperature optimums. Temperatures that are too high or too low cause metabolic processes and growth to stop. (4) Plants require 16 mineral elements for growth (Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorus, Potassium, Calcium, Magnesium, Manganese, Iron, Sulfur, Boron, Chlorine, Copper, Molybdenum, and Zinc). If any one of these essential nutrients is available at a less than adequate amount, growth is restricted or completely inhibited. When shoot production and growth is diminished or leaf expansion is less than normal due to any one of the above reasons, protein synthesis is reduced and the unused amino acids are catabolized. This releases  $\text{NH}_3$ - $\text{NH}_4$ . In addition, during several stresses  $\text{NO}_3$  uptake and reduction to  $\text{NH}_2$ ,  $\text{NH}_3$ ,  $\text{NH}_4$  continues, further increasing the ammonia content of the avocado tree. A plant that is able to cope with stress compensates for this effect of stress and maintains homeostasis by detoxifying its cells of the accumulating ammonia through increased biosynthesis of arginine. Removal of ammonia through increased biosynthesis of arginine is common to many different organisms. It is by this same metabolic pathway that humans rid their bodies of toxic ammonia. When this homeostatic mechanism fails, ammonia will accumulate to toxic levels causing leaf tip burn, leaf margin necrosis, leaf abscission, and shoot tip dieback. An abnormally high level of ammonia ( $\text{NH}_3$ - $\text{NH}_4$ ) in the leaves is a good indicator that the avocado tree is not coping with the prevailing stress.

*Are there reasons of practical importance for studying the accumulation of ammonia during abiotic stress?* Water costs are now as high as 50 per cent of the gross revenue in some avocado-growing areas of California. To cut costs, growers have reduced the number of irrigations applied later in the season. This leads to the accumulation of levels of ammonia toxic to the leaves, resulting in leaf damage, reduced photosynthesis, and leaf abscission (Nevin and Lovatt, 1987). The concentration of ammonia in the youngest, fully expanded leaves of Hass avocado trees (*Persea americana* Mill.) on clonal Duke and rootstock increased during water-deficit stress: compare  $1508 \pm 102 \mu\text{g NH}_3\text{-NH}_4$  per g dry wt leaf tissue from well-watered control trees to  $2061 \pm 248 \mu\text{g}$  per g dry wt leaves from water-deficit stressed trees (Nevin and Lovatt, 1987). Secondly, the availability of irrigation water of good quality is becoming increasingly limited. High

boron and saline soils are becoming impediments to avocado production in California.

Salinity alters nitrogen metabolism and causes  $\text{NH}_3\text{-NH}_4$  to accumulate to toxic levels in leaves of herbaceous annuals (Lovatt, 1986). For example, transferring eight-day-old squash plants (*Cucurbita pepo* L. cv Early Prolific Straightneck) from aerated hydroponic culture in Shive's nutrient solution to aerated Shive's nutrient solution plus 30 mM or 60 mM NaCl-CaCl<sub>2</sub> (2:1 molar ratio: the salt is added at the rate of  $\frac{1}{3}$  the total amount every other day) resulted in a marked reduction in the growth of the plants. Despite the fact that the nitrate content of the young leaves (five-days-old) declined 50 per cent ( $P < 0.05$ ), there was a dramatic increase in the concentration of ammonia in the leaves of the stressed plants at the end of only 10 days of treatment ( $P < 0.05$ ). In addition, the amount of ammonia that accumulated increased in parallel with the increased amount of salt added. Mature leaves, which were exposed to the stress five days longer than young leaves, accumulated a greater net amount of ammonia. When compared to leaves from the healthy control plants, there was a net accumulation of 200 and 250  $\mu\text{g NH}_3\text{-NH}_4$  per g dry wt youngest, fully expanded leaves from the 30 and 60 mM treatments, respectively. A net increase of 250 and 350  $\mu\text{g NH}_3\text{-NH}_4$  occurred per g dry wt mature leaves for the two salt treatments, respectively. It was observed for squash that symptoms of ammonia toxicity appear when the concentration of  $\text{NH}_3\text{-NH}_4$  in the leaf exceeds the normal level for the tissue by more than 150  $\mu\text{g NH}_3\text{-NH}_4$  per g dry wt. Removal of ammonia through the synthesis of arginine decreased as the severity of the salt treatment increased. This work needs to be extended to woody perennials, especially to avocado, which is very sensitive to salinity. Supplying nitrogen for crop production by application of commercial nitrogenous fertilizers represents a significant expense to the grower. The accumulation of  $\text{NH}_3\text{-NH}_4$  in leaves of avocado trees grown under reduced irrigation or under saline conditions suggests that nitrogen fertilization probably needs to be managed differently if growers are going to reduce irrigation or if salinity problems exist in a grove. Certainly, it is essential to determine if the ammonia that accumulates during stress comes from the reduction of nitrate fertilizer to ammonia, from diminished protein synthesis and subsequent degradation of unused amino acids, or from some other source in order to develop the best cultural practice to prevent the accumulation of ammonia to a toxic level during stress.

*Damaging side effects of stress.* The side effects of stress are damaging to the tree. Ammonia is toxic. At high concentrations it causes cell death in leaves and shoots. This results in premature leaf abscission and shoot dieback. The loss in tree canopy diminishes the photosynthetic capacity of the tree. The reduced photosynthetic area provides less carbohydrate and energy to the tree for growth, maintenance, repair, and reproduction. Knowledge of the homeostatic mechanisms operating during stress, especially an understanding of why specific coping mechanisms like arginine biosynthesis fail to remove ammonia during some stresses, but not others, or in some plants, but not others, may provide a key for improving avocado tree health and longevity.

*Stress can maximize productivity.* It is important to understand the physiological changes brought about by abiotic environmental stresses and the homeostatic mechanisms that are elicited by each stress because some stresses when not excessive in duration or severity do improve tree crop productivity. For tropical and

subtropical tree crops such as citrus, avocado, coffee, lychee, and mango, flowering is recurrent under tropical and subtropical conditions due to the tropical phylogenetic background of these species, unless synchronized into a well-defined period of concentrated bloom by external conditions. Flower formation in tropical and subtropical species is promoted by drought or low temperature followed by restoration of climatic conditions favorable for growth (Monselise & Halevy, 1964; Monselise & Goren, 1969; Monselise, 1978, 1985; Southwick & Davenport, 1986).

Results of research in my lab employing either low temperature or water-deficit stress to induce flowering in healthy trees of 'Washington' navel orange (*Citrus sinensis* L. Osbeck) and 'Frost Lisbon' lemon (*Citrus limon* L. Burm), respectively, demonstrated that floral intensity (flower number) increased with increased duration or severity of the stress ( $p < 0.01$ ). Of all the parameters measured, only the leaf concentration of  $\text{NH}_3\text{-NH}_4$  changed in a manner that paralleled both the degree of stress and flower number ( $p < 0.01$ ). Furthermore, under minimal, non-inductive stress conditions (either low-temperature or water-deficit), which result in a low flower number that is not significantly different from the unstressed control trees, floral intensity was increased 2.5-fold by artificially raising the leaf content of  $\text{NH}_3\text{-NH}_4$  through the foliar application of low biuret urea. The number of flowers produced by urea treated trees was significantly greater ( $p < 0.001$ ) than that of minimally stressed trees not treated with urea and equal to the number of flowers produced by trees receiving maximum stress. While leaf carbohydrate content did not change in a manner that was significantly correlated with flower number, results of experiments employing carbohydrate depleted trees provided evidence suggesting that there was a threshold level of starch that must be available for maximum flowering to occur.

Thus far, we have been able to induce flowering in avocado using low temperature but not water-deficit stress. The water-deficit stress treatment employed to induce flowering in lemons results in the accumulation of ammonia to a level toxic to Hass avocado leaves and shoots. Leaf tip burn, margin necrosis and leaf abscission, as well as shoot dieback, result when the Hass avocado is subjected to water-deficit stress. We suspect that the homeostatic mechanism of ammonia detoxification through increased arginine biosynthesis fails to take place during water-deficit stress of the Hass avocado. In addition, we think that this mechanism is elicited during low temperature stress and is essential to flower induction. It is important to determine if ammonia accumulation and the concomitant increased biosynthesis of arginine are essential components of flower induction in avocado. If this proves to be the case, we will be able to take the first steps in learning to manipulate the time and intensity of avocado bloom and the resulting harvest to bring varieties to market when the price is the highest.

*Knowledge provides a key.* With a greater understanding of the physiological changes caused by stress and the homeostatic mechanisms that maintain tree health during stress, we hope to be able to minimize the deleterious effects of abiotic environmental stresses on avocado fruit production and maybe, just maybe, make stress work for us in the market place.

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