

B.SC(Forestry)Honours

Class :- I Year Second Semester

Subject :- Plant Physiology

Course teacher :- Dr. Mahesh Shrivastava

Lecture Units :-(Topic Covered)

1:- Plant Water Relations

2:- Role Of Physiology in Forestry

3:-Growth Analysis

4:-Plant Growth Regulators

5:-Mineral Nutrition

6:-Stomatal Physiology

7:- Forest Canopy

8:- Tranpiration

9:-Water Stress

Regulation of stomatal conductance and transpiration

Processes regulating stomatal conductance, g_s , and transpiration, E , from forest canopies are reviewed. The first section deals with the response of g_s to environmental variables. Phenomenological models have been used to interpret field data and predict diurnal and seasonal variability in g_s , but models that couple stomatal conductance to photosynthesis at the leaf scale are now being used more widely. The vertical distribution of foliar nitrogen concentration is helpful for scaling these processes from leaves to canopies, and the analysis of data from many studies has led to the emergence of simplified, general relationships for estimating evaporation and carbon uptake by forests at stand and regional scales.

Evidence for the regulation of stomatal conductance by hydraulic and chemical signals is presented in the second section. Rapid and reversible changes in g_s following a perturbation to the water potential gradient in the flow pathway suggest that stomata respond directly to hydrostatic signals. Other evidence supports the contention that signals are transmitted by abscisic acid (ABA), possibly originating in the roots. For large woody plants, the short-term responses of stomata are probably brought about by hydraulic signals that affect g_s by triggering the release of ABA in the leaves. Tardieu and Davies (1993) developed an interactive model that incorporates hydraulic and chemical effects to describe the response of stomata to soil drying and evaporative demand.

In the third section, evidence is presented that short-term changes in g_s are linked closely to the hydraulic properties of the conducting system to minimize loss of hydraulic conductivity through xylem by cavitation. Examples of homeostatic mechanisms that operate to ensure the long-term balance between evaporative demand and the potential hydraulic conductivity of trees growing in different environments are described. Two hypotheses are examined: (1) height growth in trees is limited by the capacity of the conducting system; and (2) the decline in productivity with stand age is attributable to a decrease in conductivity.

Water moves from the soil through roots, xylem and leaves along a gradient of decreasing water potential that drives the flow. Ignoring capacitance effects, water flow along the branched pathway can be described by the sum of the partial flow rates. Accommodation can occur in response to short-term perturbations to the system over a time period of a few seconds, (e.g., when there is a sudden decrease in irradiance because of a transient cloud) or in response to long-term acclimation processes, (e.g., regrowth of a stand following thinning). Possible mechanisms for accommodating perturbations are identified explicitly in Equations 2--4 as: (i) canopy structural properties influencing g_a and the degree of coupling between the canopy and vegetation and the atmosphere; (ii) responses of stomata, and thus g_c , to environmental variables related principally to irradiance, D , and T ; (iii) properties of the conducting pathway from roots to leaves that modify the water potential gradient and G ; and (iv) direct response of stomata, and thus g_c , to changes in G . This review focuses on the last three topics because they are the most relevant to the regulation of stomatal and canopy conductance.

Stomatal response to environmental variables

Many field studies have shown that variability in diurnal and seasonal measurements of g_s can be accounted for by environmental variables (Jarvis 1980). Once stomata have opened in the morning as Q increases, there is often a close relationship between decreasing g_s and increasing D as the day progresses (Schulze and Hall 1982, Grantz 1990). However, the mechanism allowing stomata to sense changes in D has not been identified. For a range of C_3 and C_4 plants, Ball et al. (1987) found that the response of g_s to humidity with varying temperature and CO_2 concentration collapsed to a single relationship in an expression based on relative humidity at the leaf surface. Measurements of the response of g_s to changes in relative humidity and air saturation deficit at the leaf surface, D_s , to conclude that D_s was the appropriate variable for explaining changes in g_s . thickness of the boundary layer to vary the rate of transpiration from leaves. Leuning (1995) points out that relative humidity and D are equivalent when temperature is constant, but this is not usually the case for field measurements because diurnal variations in D are largely caused by changes in temperature, rather than absolute humidity. the response of stomata to humidity of several species in normal air and in a mixture of helium and oxygen (helox), which has a diffusion coefficient of more than twice the value for air, while maintaining temperature and CO_2 concentration at the leaf surface, c_s , constant. Thus, they were able to vary E and D_s independently. When air was replaced by helox, g_s increased but transpiration rate from the leaf remained the same, indicating that stomata were responding to water loss from the leaf, and not to D_s or the saturation deficit difference between the interior and the surface of the leaf.

that the majority of data showed a general linear dependence of g_s on transpiration rate, E (Regime A), supporting the conclusions). In some cases, E reaches a maximum beyond which any further increase in D_s results in decreases in g_s and E (Regime B). This could occur as a result of patchy closure of stomata in response to stress or decreased hydraulic conductivity. Monteith showed that a plot of $1/E$ against $1/D_s$ or a plot of g_s against E (if they are measured independently) allows estimation of g_m , a parameter that reaches an extrapolated maximum when E is zero (slope) and approaches zero when E reaches a maximum (intercept). Stomata are able to sense the water potential gradient between the guard cells and epidermal cells. This provides a mechanism that would result in a linear decrease in g_s with increasing E . However, the response of g_s to D while manipulating either leaf water potential or CO_2 concentration and concluded that stomata respond to the rate of peristomatal transpiration, rather than to E or leaf water potential. Because almost all of the evidence for this reasoning is derived from plants growing in controlled conditions, recently measured g_s (with a porometer) and E (sap flux measurements) independently in 15-m tall, field-grown *Populus* trees. Their results provide evidence to support the view that stomata respond to epidermal or cuticular transpiration rate, rather than to bulk leaf or stomatal transpiration rate, without the need to postulate patchy closure of stomata.

The phenomenological model described by Jarvis (1976) provides a practical approach for interpreting field measurements of stomatal conductance in relation to environmental variables. The response of g_s to each environmental variable is described by a nonlinear function and it is assumed that each variable acts independently, such that:

$$g_s = g_{smax} f_1(Q), f_2(T), f_3(D), f_4(\dots) \text{ etc.}, \quad (5)$$

where, for example, Q is irradiance, T is temperature, D is air saturation deficit, and \dots is the root zone water deficit. Maximum conductance, g_{smax} , is constrained by parameters in each

function, where the value of f_x varies between 0 and 1. This

$$g_s = g_{smax} \frac{aA}{1 + aA}, \quad (6)$$

creates a complex, multi-dimensional response surface. It is not possible to incorporate the mechanistic response to each

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1 D_s/D_{s0}

variable because these are not known, but the functions are chosen to represent observed responses based on measurements from controlled cuvette work or boundary line analysis of field data. Using this approach, much of the variability in g_s in forest canopies has been accounted for successfully with *Pseudotsuga menziesii* (Mirb.) Franco and *Picea sitchensis* (Bong.) Carr. with *Gmelina arborea* Roxb. and *Tectona grandis* L.f., with *Pseudotsuga menziesii*, *Tsuga heterophylla* (Raf.) Sarg. and *Abies amabilis* Dougl. with *Pinus radiata* D. derived surface conductances from Bowen ratio measurements above a *Pinus sylvestris* L. forest and used the approach in Equation 5 to explain the variability in surface conductance. The model was less successful for estimating g_c on an hourly basis than on a daily basis.

In a complex tropical forest canopy, a multi-layer approach incorporating decreasing Q through the canopy. Maximum g_s declined with depth in the canopy, but at high irradiance more leaves deeper in the canopy were able to reach light saturation for stomatal conductance. So, the relationship between g_s and g_c was dependent on irradiance. More complex radiation transfer models requiring considerable detail about canopy architecture have been used to estimate g_c for tree stands.

There has been some effort to identify the most appropriate form of the response of g_s to D and used a linear response of g_s to D , but this implies that g_s will become negative at some high value used a hyperbolic function, which is consistent

The close link between g_s and the rate of photosynthesis, A , for leaves (Schulze and Hall 1982) has led to the development of coupled models. A proposed mechanism for this link is that stomata respond to a chemical signal transported between the mesophyll and guard cells and that the rate of transport is dependent on A , regulated by proposed that stomata respond to environmental variables to optimize carbon uptake per unit rate of transpiration, and the inclusion of the term $g_s = f(D)$ by for *Macadamia integrifolia* Maiden & Betche is consistent with the

optimization hypothesis. However, evidence from laboratory and field measurements with *Pinus taeda* L., which shows that the sensitivity of E and A to changes in g_s is not constant, but changes throughout the day with increasing D_s . In a coupled model, an expression for stomatal conductance that is based on a modified version of the model. For well-watered conditions, stomatal conductance to CO_2 transfer, g_{sc} ($= g_s/1.6$ where 1.6 is the ratio of the diffusivities of water and CO_2 in air) is given by:

where g_{sc0} is the residual conductance at the light compensation point, Γ is the CO_2 compensation point, D_{s0} describes the sensitivity of g_{sc} to D_s , c_s is CO_2 concentration at the leaf surface and a is related to the intercellular CO_2 concentration, c_i , at saturating irradiance, where $1/a = (1 - c_i/c_s)$. The function, attributed to describe the response of g_{sc} to D_s is adopted and the term for Γ is included to ensure that g_{sc} does not decrease as A approaches zero when c_s is decreased. Combining this diffusion or supply function with the biochemical or demand function in the model of photosynthesis leads to the definition of a unique value for c_i where both functions describing limitation to photosynthesis are balanced.) the ratio of c_i/c_s varies with D_s such that c_i remains roughly constant with varying irradiance, which is consistent with field observations. Such coupled models are now used widely and have the advantage that the equations can be readily closed. However, coupled models cannot be fitted to all data sets, e.g., simulating stomatal conductance at low CO_2 concentrations

Responses of stomatal conductance to hydraulic and chemical signals

Substituting for E from Equation 4 in Equation 2 and expressing $(r - 1 - h_w g)$ as h/g where h is tree height, gives an expression that anticipates that g_s is sensitive to changes in G :

$$g_s = \frac{G}{hD_s L} \quad (7)$$

Hydraulic signals are transmitted rapidly, at the speed of sound in water, throughout the plant and a link between g_s and G may provide a mechanism to maintain leaf water potential within narrow limits and sustain water and carbon balances within the plant. However, although this implies that stomata are able to detect changes in G , the mechanism for such a response is unknown and Equation 7 should be reserved for descriptive purposes only.

There is inconclusive evidence for a link between g_s and G . the response of g_s in branches and small seedlings of *Abies amabilis*, when the stem was partially severed and when the roots were partially removed or cooled, was independent of evaporative demand and bulk leaf water potential. , stomatal and hydraulic conductances were coordinated in *Saccharum* spp. during development. there was a coordinated decrease in g_s and G with soil drying in *Macadamia integrifolia*. A similar relationship was evident for species growing in natural communities. For five woody hedgerow species with different responses of g_s to good seasonal relationship between stomatal conductance and hydraulic conductance. For two of the species, both g_s and G decreased during the summer, although this change was not attributable to decreasing soil water content. stomatal regulation of water loss

There is considerable evidence to suggest that stomatal conductance may be regulated by chemical signals that are independent of hydraulic signals. These chemical signals may be generated in roots and transmitted to leaves in the transpiration stream. Results from split root experiments with *Acer pseudoplatana* and *Zea mays* L. and observations showing that g_s in *Vigna unguiculata* (L.) Walp. is regulated by root water status and is independent of leaf water potential support the role of a chemical messenger acting between roots and leaves.

There is strong evidence for a relationship between stomatal conductance and ABA concentration in the xylem of woody plants, fourfold increase in xylem ABA concentration when well-watered seedlings of *Pseudotsuga menziesii* were subjected to drought. However, the flux of ABA into the leaves did not increase because g_s and E decreased.) showed that ABA concentration in the xylem sap accounted for differences in midsummer maximum values of g_s in *Vitis vinifera* L. growing in soil with contrasting water availabilities. However, diurnal changes in ABA concentration were not consistent with changes in g_s

A close relationship between g_s and ABA concentration does not provide conclusive evidence that stomata are regulated by the concentration of a chemical signal originating in the roots, because the same effect could be achieved

by dilution of the messenger with increasing g_s and E . Further, the rapidity of the response of g_s to a perturbation in the hydraulic pathway in large trees suggests that the mechanism is more complex than the generation of ABA in the roots and its transport in the xylem to the leaves induced reductions in conductance by making transverse cuts in the xylem of the stems of woody plants. The resulting decrease in g_s was not instantaneous, but occurred over a period of 20 min. The rooting environment was held constant during the experiments, suggesting that the response did not result from a chemical messenger from the roots.

The mechanism of the response of stomata to ABA is not known because a receptor for detecting changes in ABA concentration has not been identified

, the half-life of ABA in xylem sap is 36 min and concluded that turnover is sufficiently fast to allow regulation of its concentration in the leaf apoplast and guard cells without an apparent change in bulk leaf ABA concentration. The concentration of ABA in the guard cells is likely to result from both the rate of entry into the leaf and the concentration in the transpiration stream. This is consistent with a direct link between xylem sap ABA concentration and the concentration reaching the guard cells. Another explanation is that the source of ABA in the leaf and its release from the symplast of mesophyll cells results in a change in apoplastic ABA concentration. Perhaps hydraulic signals resulting from a change in either hydraulic conductance or water potential gradient trigger the release of ABA in the leaf. A decrease in epidermal water potential would sensitize stomata to ABA delivered to the apoplast in the transpiration stream.

The relative importance of the chemical and hydraulic signals is likely to depend on plant size and growing conditions. The short-term response of stomata to environmental variables could result from hydraulic signals related to evaporative demand and this could be moderated by changes in the sensitivity of g_s to ABA concentration in leaves throughout the day. The long-term response of decreasing g_s as the soil becomes progressively drier could be regulated by a gradual, more stable change in ABA concentration of the xylem sap. The concentration would remain reasonably constant and stomatal response to this messenger would depend on evaporative demand. In large trees, a rapid response of g_s to a change in the water potential gradient could be initiated by a hydraulic signal, but in small plants, stomata may be more sensitive to changes in ABA concentration, possibly originating in the roots.

To explain the response of stomata to changes in soil water status or air saturation deficit, and that incorporates both hydraulic and chemical signals. The model has three criteria: (i) if the production of the chemical messenger depends on root water status, it will also depend on the rate of transpiration and soil water potential; (ii) an increase in transpiration will dilute the concentration of the messenger in the xylem sap; and (iii) stomatal sensitivity to the messenger will be increased as leaf water potential falls. Transpiration from the plant is estimated from weather variables and aerodynamic properties by the Penman-Monteith equation and this determines the water potential gradients between the roots and leaves and between the soil and roots, for a given soil water status and set of hydraulic properties. The ABA concentration in the xylem sap is dependent on root water potential and transpiration rate. Stomatal conductance is simulated from a function combining the effects of xylem ABA concentration and leaf water potential.

Although the model employs only five equations with five unknowns (g_s , E , xylem ABA concentration, ψ_l and ψ_r), it is exceptionally good at reproducing the effects of two very different mechanisms that regulate stomatal response. For example, for *Zea mays* the model reproduces the reduction in g_s of drought-exposed plants in the afternoon, but not in the morning, with little apparent change in leaf ABA concentration.

The significance of hydraulic signals and the role of chemical signals in regulating stomatal conductance are still uncertain and the effects have not yet been incorporated into canopy scale models of transpiration. However, Tardieu's modeling approach provides a rigorous basis for developing concepts and elucidating mechanisms

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Water Stress in Plants: Effects and Responses

to abiotic stresses, for example, drought, salinity, extreme temperatures, chemical toxicity, oxidative stress, etc., cause imbalances in the natural status of the environment. Each year, stresses on arable plants in different parts of the world disrupt agriculture and food supply with the final consequence - famine. Factors controlling stress conditions alter the normal equilibrium, and lead to a series of morphological, physiological, biochemical and molecular changes in plants, which adversely affect their growth and productivity. The average yields from the major crop plants may reduce by more than 50% owing to stresses. However, plants also have developed innate adaptations to stress conditions with an array of biochemical and physiological interventions that involves the function of many stress-associated genes. In this chapter, we aim at the stresses related to water and the expression 'drought' which is derived from the agricultural context, is used as equal to water stress throughout the article.

Water, comprising 80-90% of the biomass of non-woody plants, is the central molecule in all physiological processes of plants by being the major medium for transporting metabolites and nutrients. Drought is a situation that lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological functions. However, a few groups of animals and a wide variety of plants are known for their tolerance to desiccation during the adult stages of their life cycle. Though our knowledge on plant's drought tolerance is ancient, the modern scientific study of drought tolerance started in 1702 with Anthony von Leeuwenhoek's discovery of the survival of rotifers without water for months.

2. Water stress – Why and how?

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Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense. Water stress is primarily caused by the water deficit, *i.e.* drought or high soil salinity. In case of high soil salinity and also in other conditions like flooding and low soil temperature, water exists in soil solution but plants cannot uptake it – a situation commonly known as ‘physiological drought’. Drought occurs in many parts of the world every year, frequently experienced in the field grown plants under arid and semi-arid climates. Regions with adequate but non-uniform precipitation also experience water limiting environments.

Since the dawn of agriculture, mild to severe drought has been one of the major production-limiting factors. Consequently, the ability of plants to withstand such stress is of immense economic importance. The general effects of drought on plant growth are fairly well known. However, the primary effect of water deficit at the biochemical and molecular levels are not considerably understood yet and such understanding is crucial. All plants have tolerance to water stress, but the extent varies from species to species. Knowledge of the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water limited conditions in higher plants.

3. Effects of water stress on plants

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Drought, as an abiotic stress, is multidimensional in nature, and it affects plants at various levels of their organization. In fact, under prolonged drought, many plants will dehydrate and die. Water stress in plants reduces the plant-cell’s water potential and turgor, which elevate the solutes’ concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure. This is followed by accumulation of abscisic acid (ABA) and compatible osmolytes like proline, which cause wilting. At this stage, overproduction of reactive oxygen species (ROS) and formation of radical scavenging compounds such as ascorbate and glutathione further aggravate the adverse influence. Drought not only affects plant water relations through the reduction of water content, turgor and total water, it also affects stomatal closure, limits gaseous exchange, reduces transpiration and arrests carbon assimilation (photosynthesis) rates. Negative effects on mineral nutrition (uptake and transport of nutrients) and metabolism leads to a decrease in the leaf area and alteration in assimilate partitioning among the organs. Alteration in plant cell wall elasticity and disruption of homeostasis and ion distribution in the cell has also been reported. Synthesis of new protein and mRNAs associated with the drought response is another outcome of water stress on plants. Under the water stress cell expansion slows down or ceases, and plant

growth is retarded. However, water stress influences cell enlargement more than cell division. Plant growth under drought is influenced by altered photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones.

3.1

Photosynthesis

Photosynthesis is particularly sensitive to the effects of water deficiency. Plants' resistance to water deficiency yields metabolic changes along with functional and structural rearrangements of photosynthesizing apparatus. Photosynthesis of higher plants decreases with the reduction in the relative water content (RWC) and leaf water potential. Lower

photosynthesis rate is a usual effect of water stress in plants and has been attributed primarily to stomatal limitation and secondarily to metabolic impairment. However, metabolic impairment is the more complex phenomenon than the stomatal limitation though the relative importance of stomatal or metabolic inhibitions is unclear. Some studies blamed stomatal closure for the inhibition of C_4 photosynthesis under water stress while others concluded that non-stomatal factors play the major role.

The photosynthesis rate of leaves in both C_3 and C_4 plants decrease under the drought conditions. Evidence indicates that C_4 photosynthesis is more sensitive to water stress and C_4 plants, such as corn (*Zea mays* L.) are more susceptible to water deficiency than C_3 plants, such as wheat. It explains the predominance of C_4 plants in hot, arid regions - areas prone to frequent drought. C_3 and C_4 plants are alike in the basic process of photosynthesis like Calvin cycle and electron transport chain components, yet significant differences exist between them, which make their responses to water stress differ at a number of levels.

There are some co-factors, which decrease plants' photosynthesis under water stress. Of them, qualitative and quantitative changes in the pool of photosynthesizing pigments, low CO_2 uptake due to stomatal closure and resistance, poor assimilation rates in photosynthetic leaves are prominent. Assimilation rates in photosynthetic leaves decreases due to reduced photosynthetic metabolites and enzymes activity, low carboxylation efficiency and inhibition of chloroplast activity at low water potential. Among other co-factors of water stress, the damage of the photosynthetic apparatus through the production of ROS such as superoxide and hydroxyl radicals, worth special mention.

Decrease in chlorophyll content of leaves under water stress is well known. Water stress inhibits chlorophyll synthesis at four consecutive stages: (I) the formation of 5-aminolevulinic acid (ALA); (II) ALA condensation into

porphobilinogen and primary tetrapyrrol, which is transformed into protochlorophyllide; (III) light-dependent conversion of protochlorophyllide into chlorophyllide; and (IV) synthesis of chlorophylls a and b along with their inclusion into developing pigment-protein complexes of the photosynthetic apparatus. In the majority of cases, carotenoids are less sensitive to water stress than chlorophyll, which has been demonstrated for several species of agricultural plants. However, unlike chlorophyll, increase in xanthophyll pigments such as zeaxanthin and antheraxanthin in plants under water stress have been reported. Xanthophyll pigments have a protective role on plants under stress, and some of these pigments are involved in the xanthophyll cycle which has inhibitory role on ROS production.

RuBisCO, the key enzyme for carbon metabolism in leaves, acts as a carboxylase in the Calvin cycle and as an oxygenase in the photorespiration which, however, frequently is viewed as an adverse process. RuBisCO is the most critical player influencing the physiology of plants under water-stressed conditions. Under the conditions of water stress, a rapid decrease in the amount of RuBisCO takes place in most plants which in turn leads to lower activity of the enzyme. This effect is evident in all plants studied though the extent is species-dependent. Water deficiency reduces the supply of carbon dioxide from the environment due to the closure of stomata. Consequently, photorespiration increases which ensure partial substrate replenishment and maintain the carboxylating function of RuBisCO. The end result is the utilization of excess reducing equivalents in chloroplast that causes a reduction in the oxygen-free radicals' production leading to the oxidative damage in chloroplasts. The reduction in chloroplast volume can also be linked to the desiccation within the chloroplast that leads to the conformational changes in RuBisCO. Moreover,

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drought stress conditions acidify the chloroplast stroma causing inhibition to the RuBisCO activity. In addition, decline in RuBisCO activity is also caused by the lack of the substrate for carboxylation, reduction in the amount and/or activity of the coupling factor - ATPase, loss of RBP recognition sites in RuBisCO, structural alterations of chloroplasts and RuBisCO, and release of RuBisCO from damaged plastids. In addition to RuBisCO, water stress can reduce activity of other photosynthetic enzymes to different extents such as NADP- dependent glyceraldehyde phosphate dehydrogenase, phosphoenolpyruvate carboxylase, NAD-dependent malate dehydrogenase, phosphoribulos kinase, fructose-1,6- bisphosphatase and sucrose phosphate synthase.

In addition to its negative effects on dark reactions of photosynthesis, water stress also disrupts the cyclic and non-cyclic types of electron transport during the light reaction of photosynthesis. The disruption is clearer in the oxygen-releasing complex and electron transfer from protochlorophyllide to P700. Lower electron transport rate negatively affects photophosphorylation process and decrease ATP synthesis as well as NADP⁺ reduction. ATPase inhibition under water deficiency is also responsible for the reduction in ATP levels in chloroplasts. All these factors cumulatively affect the intensity of photo-assimilation and the

stability of the photosynthetic apparatus under the conditions of water stress. Both of the PSs in chloroplasts are affected by water deficiency, however, PS I of some plants are more severely damaged compared to PS II, though there is an opposite conclusion as well.

3.2 Protein synthesis

Drought conditions bring about quantitative and qualitative changes in plant proteins. In general, proteins in the plant leave decrease during water deficiency due to the suppressed synthesis, more pronouncedly in C₃ than in C₄ plants. Water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs. The main proteins those synthesized in response to water stress are LEA, desiccation stress protein, proteins those respond to ABA, dehydrins, cold regulation proteins, proteases, enzymes required for the biosynthesis of various osmoprotectants,. In addition, protein factors involved in the regulation of signal transduction and gene expression, such as protein kinases and transcription factors are also synthesized. The majority of these stress response proteins are dehydrin-like proteins, which accumulate during seed production and embryo maturation of many higher plants as well as in water stressed seedlings. These proteins have highly conserved domain that linked to hydrophobic interactions needed for macromolecular stabilization.

Heat-shock proteins (Hsps) and late embryogenesis abundant (LEA)-type proteins are two major types of stress-induced proteins during different stresses including water stress. Protection of macromolecules such as enzymes, lipids and mRNAs from dehydration are well known functions of these proteins. LEA proteins accumulate mainly in the embryo. The exact functions and physiological roles of these proteins are unknown. Hsps act as molecular chaperones and are responsible for protein synthesis, targeting, maturation and degradation in many cellular processes. They also have important roles in stabilization of proteins and membranes and in assisting protein refolding under stress conditions. Expression of LEA-type genes under osmotic stress is regulated by both ABA-dependent and independent signaling pathways. Genes encoding LEA-type proteins are diverse - RD (responsive to dehydration), ERD (early response to dehydration), KIN (cold inducible), COR (cold regulated), and RAB (responsive to ABA) genes.

3.4 Morphological, anatomical and cytological changes

In the majority of the plant species, water stress is linked to changes in leaf anatomy and ultrastructure. Shrinkage in the size of leaves, decrease in

the number of stomata; thickening of leaf cell walls, cutinization of leaf surface, and underdevelopment of the conductive system - increase in the number of large vessels, submersion of stomata in succulent plants and in xerophytes, formation of tube leaves in cereals and induction of early senescence are the other reported morphological changes.

The root-to-shoot ratio increases under water-stress conditions to facilitate water absorption and to maintain osmotic pressure, although the root dry weight and length decrease as reported in some plants like sugar beet and *Populus*. Higher root-to-shoot ratio under the drought conditions has been linked to the ABA content of roots and shoots. Water stress is linked to decrease in stem length in plants such as *Albizzia*, *Erythrina*, *Eucalyptus* and *Populus* with up to 25% decrease in plant height in citrus seedling. Decreased leaf growth, total leaf area and leaf-area plasticity were observed under the drought conditions in many plant species, such as peanut and *Oryza sativa*. Although water saving is the important outcome of lower leaf area, it causes reduced crop yield through reduction in photosynthesis. Decrease in plant biomass consequences from the water deficit in crop plants, mainly due to low photosynthesis and plant growth and leaf senescence during the stress conditions. However, in some plants, higher yield was reported under-water deficit condition.

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ABA accumulatr

The plant hormone ABA accumulates under-water deficit conditions and plays a major role in response and tolerance to dehydration. Closure of stomata and induction of the expression of multiple genes involved in defense against the water deficit are known functions of ABA. The amount of ABAs in xylem saps increases substantially under reduced water availability in the soil, and this results in an increased ABA concentration in different compartments of the leaf. Another well-known effect of drought in plants is the decrease in PM-ATPase activity. Low PM-ATPase increases the cell wall pH and lead to the formation of ABA⁻ form of abscisic acid. ABA⁻ cannot penetrate the plasma membrane and translocate toward the guard cell by the water stream in the leaf apoplasm. High ABA concentration around guard cell results in stomata closure and help to conserve water.

3.6 Mineral nutrition

Water stress also affects plant mineral nutrition and disrupts ion homeostasis. Calcium plays an essential role in structural and functional integrity of plant membrane and other structures. Decrease in plant Ca²⁺ content was reported in many plants, for example,

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approximately 50% decrease in Ca^{2+} in drought stressed maize leaves, while in roots Ca^{2+} concentration was higher compared to control. Potassium is an important nutrient and plays an essential role in water relation, osmotic adjustment, stomatal movement and finally plant resistance to drought. Decrease in K^{+} concentration was reported in many plant species under water deficient condition, mainly due to membrane damage and disruption in ion homeostasis. K^{+} deficient plant has lower resistance to water stress. Nitrogen metabolism is the most important factor that influences plant growth and performance. Disruption in N- metabolism is a crucial in-plant injury under the water deficit conditions. Some studies showed the reduction of nitrate uptake and decrease in nitrate reductase activity under water stress.

4. Drought and oxidative stress in plants

Oxidative stress, which frequently accompanies many abiotic stresses like high temperature, salinity, or drought stress, causes a serious secondary effect on cells. Oxidative stress is accompanied by the formation of ROSs such as O_2^- , $^1\text{O}_2$, H_2O , and OH^- . ROSs damage membranes and macromolecules affect cellular metabolism and play a crucial role in causing cellular damage under drought stress.

Drought creates an imbalance between light capture and its utilization, which inhibits the photosynthesis in leaves. In this process imbalance between the generation and utilization of electrons is created. Dissipation of excess light energy in photosynthetic apparatus results in generation of reactive oxygen species (ROS). Denaturation of functional and structural macromolecules is the well-known results of ROS production in cells. DNA nicking, amino acids, protein and photosynthetic pigments oxidation, and lipid peroxidation are the reported effects of ROS. As a consequence, cells activate some responses such as an increase in the expression of genes for antioxidant functions and production of stress proteins, up-regulation of anti-oxidants systems, including anti-oxidant enzymes and accumulation of compatible solutes. All these responses increase scavenging capacity against ROSs.

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5. Plant responses to water stress

Plants adapt themselves to drought conditions by various physiological, biochemical, anatomical, and morphological changes, including transitions in gene expression. The physiology of plants' response to drought at the whole plant level is highly complex and involves deleterious and/or adaptive changes. This complexity is due to some factors such as plant species and variety, the dynamics, duration and intensity of soil water depletion, changes in water demand from the atmosphere, environmental conditions, as well as plant growth and the phenological state in which water deficit is developed.

Plants' strategies to cope with drought normally involve a mixture of stress avoidance and tolerance strategies. Early responses of plants to drought stress usually help the plant to survive for some time. The acclimation of the plant to drought is indicated by the accumulation of certain new metabolites associated with the structural capabilities to improve plant functioning under drought stress. The main aspects of plant responses to water involve the maintenance of homeostasis (ionic balance and osmotic adjustment),

counter action to resulted damages and their quick repair such as scavenging of ROS and decrease oxidative stress and the regulation and recovery of growth.

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The complex plant response to water stress, alike other abiotic stresses, involves many genes and biochemical and molecular mechanisms. Sequentially, they are: signal sensing, perception and transduction by osmosensors like AtHK1, kinases and phospholipases as well as secondary messengers; transcriptional control by transcription factors such as DREB (dehydration-responsive transcription factors); and activation of stress responsive mechanisms such as detoxification of ROS by enzymes such as SOD and CAT; osmoprotection by compatible solutes and free radicals scavengers such as glutathione and proline; and water and ion homeostasis by aquaporins and ion transporters. The results of these responsive pathways are the re-establishment of cellular homeostasis and functional and structural protection and finally stress resistance or tolerance.

Stomata closure is the well-known first responsive event of plants to water deficiency. Stomatal closures are more closely related to soil moisture content than leaf water status, and it is mainly controlled by chemical signals such as ABA produced in dehydrating roots. A direct correlation between the xylem ABA content and stomatal conductance has been demonstrated. Changes in plant hydraulic conductance, plant nutritional status, xylem sap pH, farnesyl transferase activity, leaf-to-air vapor pressure deficit and decrease in relative water content are other factors working in stomatal regulation plants. Although CO₂ assimilation and net photosynthesis decreases due to stomatal closure but attainment of low transpiration rate and prevention of water losses from leaves is a good tradeoff for survival in exchange of growth. Stomata can completely close in mild to severe stress depending on plant species, and tolerant species control stomata opening to allow some carbon fixation and improving water-use efficiency. The increased stomatal resistance under stress levels indicates the efficiency of a species to conserve water.

6. Plants resistance to water stress

Plants optimize the morphology, physiology and metabolism of their organs and cells in order to maximize productivity under the drought conditions. The reactions of the plants to water stress differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development. Stress resistance in plant is divided into two categories, including stress tolerance and stress avoidance. Drought avoidance is the ability of plant to maintain high tissue water potential under drought conditions, while drought tolerance is a plant's stability to maintain its normal functions even at low tissue water potentials. Drought avoidance is usually achieved through morphological changes in the plant, such as reduced stomatal conductance, decreased leaf area, development of extensive root systems and increased root/shoot ratios. On the other hand, drought tolerance is achieved by cell and tissue specific physiological, biochemical, and molecular mechanisms, which include specific gene expression and accumulation of specific proteins. The dehydration process of drought-tolerant plants is characterized by fundamental changes in water relation, biochemical and physiological process, membrane structure, and ultrastructure of sub cellular organelles. Some plants are able to cope with arid environments by mechanisms that mitigate drought stress, such as stomatal closure, partial senescence of tissues, reduction of leaf growth, development of

Plant Water Relations: Absorption, Transport and Control

1. Introduction

Although water is abundant on Earth - covering 71% of the total surface - its distribution is not uniform and can easily cause restrictions in availability to vegetal production. At global scale, these restrictions are easily observed in dry climates and can appear in other regions which do not currently experience drought, as provided by the future backdrop of climate change .

The influences of water restriction on losses in the production and distribution of vegetation on the terrestrial surface are significantly larger than all other losses combined which are caused by biotic and abiotic factors. This striking effect of water on plants emerges from its physiological importance, being an essential factor for successful plant growth, involving photosynthesis and several other biochemical processes such as the synthesis of energetic composites and new tissue. Therefore, in order to characterise the growth and productive behaviour of plant species it is essential to have an understanding of plant water relations, as well as the consequences of an inadequate water supply. Broadly, the water state of a plant is controlled by relative rates of loss and absorption, moreover it depends on the ability to adjust and keep an adequate water status. This will be considered throughout this chapter.

2. Absorption and water flow through plants

Independent of the species, plants require from the soil a water volume that overcomes its metabolic necessities. Through the transpiration process plants transmit to the atmosphere the majority of the water absorbed from soil (generally around 90%). From this perspective, it is noted that the plant water requirements are defined primarily by the atmosphere evapotranspirative demand, which is a predominately passive process. Figuratively, and with some caveats, we can compare a plant water flow with the principles of oil flow in the wick of an old fashion lampion (Fig. 1).

When it is fired, the oil that is burned on the upper extremity of the wick is quickly replaced by new one that is situated just below, and so on - following the physical forces of interaction between liquid and tissue - until reaching the level of the fuel reservoir, in the basal extremity of the wick. Applying this example to the plant, the burning of oil can be analogous to the

process of the loss of water vapour through their leaves, i.e. the *transpiration*, which is caused by the pressure gradient of vapour between tissue saturated with water from the leaves and air, the "dry" atmosphere. The variations in this pressure gradient of the vapour will define the *evaporative demand* of the environment where the plant is. In the other extreme - where it represents the liquid reservoir of the lampion - we have water content present in the soil. In this scene, it is noted that the water flow through the plant is dependent on the energy formed by the gradient of the water content that is established between the soil and the atmosphere. However, we will see throughout this chapter that plants, unlike our lampion, can and must modulate this gradient in order to survive the wide variations of water availability between types of soils, weather and seasons.



Fig. 1. Schematic representation of water flow through the plant (arrows), by analogy with the oil flow through the wick of an old fashion lampion.

2.1 Water potential

The water content in the soil, plants and atmosphere is usually described as *water potential* (Ψ_w). This is based on the relation between the water content in the part of a system and pure water at the same temperature and atmospheric pressure, measured in pressure units (megapascal-MPa or bars-Bar). By definition, the potential of free pure water at atmospheric pressure and at a temperature of 25°C corresponds to 0 (zero) MPa. The contrast in the water potential between two points invariably determines the direction of water transport in a system. More precisely, the water potential represents all the water pressure in a given system and it is the sum of osmotic potential (Ψ_s), matrix potential (Ψ_m), hydrostatic pressure or the turgor potential (Ψ_p) and the gravitational potential (Ψ_g).

The osmotic potential (Ψ_s) is the chemical potential of water in a solution due to the presence of dissolved substances (solutes). This is always negative because the water moves

from one point with a lower concentration of solutes (for example, pure water) to a point with a higher concentration. So, the higher concentration of the solutes at a point which makes the system more negative will be the osmotic potential in this place. The water potential can also be influenced by a charged surface - mainly by soil components and cell walls - which compose the influence of the matrix potential (Ψ_m). In the soil, this influence of the matrix is so great that water potential is assumed negligible and therefore equivalent to the matrix potential. Concerning the potential of hydrostatic pressure (Ψ_p), it is noted that this component of the water potential can be positive or negative and it refers to the physical pressure that water exerts on a given system. For example, if we observe a turgid cell of a root cortex or a leaf mesophyll, the hydrostatic pressure is positive. However, in a xylem vessel subjected to a stressful condition - in a transpiring plant - this component of hydrostatic pressure is negative. Finally, we should emphasise that the gravitational potential (Ψ_g) - ignored in most cases - is very important in studies of the water potential of tree species, where plant height exerts a great influence on water flow. Considering that this gravitational component fluctuates at a rate of 0.1 MPa for every 10 meters of vertical displacement, it is suggested to consider if when plant height is 10 m or more.

2.2 Water dynamics in soil-plant-atmosphere system

From these components of water potential we return to our lampion scheme (Fig. 1) and show how the potential can vary over the continuum soil-plant-atmosphere, exposing the control points of each step of water flow from the soil to the atmosphere.

2.2.1 Soil water

The water potential in soil affects water reservoir and its availability for plants, hence it has a large impact on plant growth and production. Furthermore, the soil water content exerts a great influence on some physical and chemical properties of soil, such as the oxygen content, which interferes with root breathing, microbial activity and soil chemical status. Water potential is directly dependent on soil physical characteristics, and varies with time and space, depending on soil water balance. That balance is determined by input (rain, irrigation) and output of the soil (drainage, evaporation and root absorption). It is noteworthy that the amount of rain affecting soil water reservoir is only the *effective precipitation*. This is the amount of precipitation that is actually added and stored in the soil. For example, during drier periods less than 5 mm of daily rainfall would not be considered effective, as this amount of precipitation would likely evaporate from the surface before soaking into the ground.

It is important to emphasise that behaviour of water into soil differs from that in a pot, like the oil in the lampion reservoir (Fig. 1). That is, soil water interacts with the matrix and solutes, and it is under pressure or tension, resulting in various energy states, relative to free water (Kirkham, 2005). With regard to the physiological aspect, it is important to point out that the water content in soil is associated with three terms: *field capacity*, the *permanent wilting point* and the *available water content*.

The term “field capacity” corresponds to the maximum water content that a given soil can retain by capillarity, after saturation and gravity drainage, and it is conventionally estimated as the water content when the matrix potential is -0.03 MPa (-0.3 Bar). In spite of the great applicability of this term to irrigation management, field capacity has been recognized as an

imprecise term due to theoretical advances and precise irrigation techniques. It is because the capillary soil water constantly (even slowly) decreases (due to evaporation from soil surface or drainage losses) and never stabilises (Fig. 2), it turns the soil water potential decreases while the matrix potential increases. This is most evident with medium and fine texture soils (for example, those rich in clay and organic matter), which maintain a significant drainage rate over a long time. Therefore, there is no real and unique value for accurately characterising the field capacity of a given soil. Furthermore, the continuous drainage can induce an overestimation of the water consumption of the plant. Despite these uncertainties, the term *field capacity* is still useful for a qualitative understanding - rather than a quantitative understanding - of the water behaviour of a particular soil, providing an estimate of the maximum limit of water accumulation. It is noteworthy that the inaccuracy of the field capacity determination occurs mainly when analysis takes place on samples in the laboratory, which can be countered with evaluations directly in the soil, with specific sensors and considering together all characteristics of each site. In general, clay soils or those with higher content of organic matter (up to 5% of organic matter) present a higher soil water holding capacity (average field capacity ranging from 35 to 40% vol). In contrast, sandy soils have a lower water holding capacity and field capacity typically ranges from 10-15% vol. It is important to observe that *field capacity* cannot be regarded as a maximum limit of the water available to plants, due to the fact that plants also use free water that is in contact with the roots at the moment of soil drainage.

The *wilting point* (WP) is another important parameter in soil water dynamics as it dramatically affects plant physiology. This term is also known as the *permanent wilting point*, and can be defined as the amount of water per unit weight (or volume) of soil that is so tightly retained by the soil matrix that roots are unable to absorb causing the wilting of plant. In other words, it corresponds to the water potential of soil under which plants cannot maintain turgor pressure, even if a series of defence mechanisms have been triggered (e.g. increased ABA synthesis, stomatal closure, osmotic adjustment, leaf fall) (For more details see the Chapter by Mastrangelo et al.).

Similarly with FC, the value of water content in a soil at WP is not a unique and precise value despite it is conventionally measured at -1.5 MPa (-15 Bar) (Fig. 2). The WP is influenced by the physical and chemical characteristics of soil, but also by the plant species considered. This is because various plant species differ in their ability to deal with low soil water content due to differences in roots anatomy and depth, osmotic adjustment capacity and other defence drought mechanisms.

Conventionally, the *wilting point* is estimated as the water content when the matrix potential of the soil is -1.5 MPa (-15 bar). Nevertheless, some species of plants can absorb water from soil at a potential much smaller than this limit. For example olive

trees can set a water potential gradient between dry soil (-3 MPa) and leaf (-7 MPa) (Dichio et al., 2006). Similarly, *Larrea divaricata* may absorb water at -6.0 MPa soil water potential (Kirkham, 2005). Another species of the same genus of desert plant (*Larrea tridentata*) can survive with soil water potentials up to -11.5 MPa, maintaining the photosynthetic activity of leaves within the range between -5 and -8 MPa (Fitter & Hay, 2002). These examples serve to explain that the permanent wilting point does not exclusively depend on the soil but also on the plant species. At the *permanent wilting point*, the water potential of soil tends to be less than or equal to the osmotic potential of the plant, which is extremely low in plants adapted to dry environments.

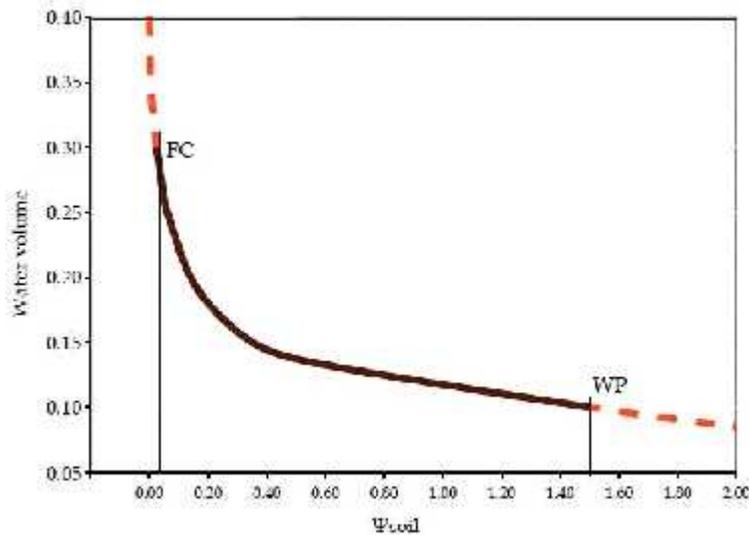


Fig. 2. Variation of the matrix of the water potential of soil (Ψ_{soil} , negative values) in relation to water volume ($\text{cm}^3 \cdot \text{cm}^{-3}$ of soil), characterising the limits of the field capacity (FC, -0.03 MPa) and wilting point (WP, -1.5 MPa) of a given soil. The curve was generated from results obtained by Santos, H.P., in Bento Gonçalves-RS, Brazil. 2010.

The indiscriminate use of a fixed value to estimate *field capacity* (FC) and the *permanent wilting point* (WP) can generate false interpretations. However, this reference to the water content in the soil is essential for calculating the *available water content* (AWC) for the plants. The AWC is calculated considering the soil volume explored by roots and the % of water content determined as the difference between FC and WP. Due to this interval of water availability, one may assume that water could be absorbed by the roots with the same facility in the range between FC and WP. For some plants this may be true, given that the energy to extract water from the soil is small, compared to the energy needed to transport the water from the root system to the atmosphere. However, with the reduction of soil water potential, there is also a reduction in its hydraulic conductivity (i.e. water moves slowly in the soil), limiting the water absorption capacity of the roots. In this scene - and for a majority of crops - the yields are reduced if the water content in the soil approaches the *wilting point*. Thus, the available water content should be considered as a relative value and, for the same soil water potential, it may have different proportions of accessibility, depending on the ability of each species to exploit or capture available water.

2.2.2 Water absorption by the roots

As was pointed out in Figure 1, the water flow of a plant is primarily controlled by the transpiration rate. In this flow system it is essential indeed that there are no limitations on water absorption by the root system. As the roots absorb water, there is a reduction in the water potential in the soil that is in contact with the roots (rhizosphere). This process establishes a water potential gradient between the rhizosphere and a neighbouring region of the soil which presents a higher water potential and which coordinates the water movement

towards the roots of a transpiring plant (Fig. 3). This water movement in the soil occurs mainly through mass flow due to the fact that the water filled micropores of the soil are interconnected. Therefore, water flows from soil to

root at a rate depending on the water potential gradient between soil and plant which is affected by plant water need, hydraulic conductivity of the soil, soil type and soil water content. Sandy soils have higher conductivity due to greater porosity, but they also retain less water in relation to clay soils or soils rich in organic matter.

At *field capacity*, water is initially removed from the centre of the largest pores (spaces ≥ 50 nm, that are too large to have any significant capillary force) between the soil particles, maintaining the water next to the particles due to adhesive forces. The reduction in water content causes a drastic decrease in soil hydraulic conductivity, because the water is replaced by air in the spaces between the soil particles (Fig. 3). Thus, the water movement in the soil is limited to the periphery of soil pores, which can promote restrictions in the hydraulic conductivity to the root surface and reach the *permanent wilting point* (discussed previously).

The water absorption by the roots is related to its surface directly in contact with soil. Thus, longer and younger (less suberised) roots with more root hairs are essential for increasing the contact surface and improve the water absorption capacity of the soil (Fig. 3). Moreover,

the distribution and proportion of the roots is very important for meeting the water demand of a plant. In humid regions, as tropical rain forest, plants usually do not require very extensive root systems (i.e. root:shoot ratio < 0.15 , Abdala et al., 1998), because a small volume of soil can meet the demands of transpiration. In addition, the water absorbed from that small soil volume is frequently (and easily) replenished by rainfall. This condition in turn induces a reduction of the root:shoot ratio. On the other hand, in dry regions, the plants invest more in their roots, increasing the root:shoot ratio such that the roots can represent up to 90% of a plant biomass in some species of a desert climate, such as observed in some species from open areas of the Bana woodland in southern Venezuela (i.e. root:shoot > 5 , (Ab. It is important to note that the use of this root:shoot relation in the classification of plants with respect to their *habitat* must be made with caution. In many species, a higher investment in roots is more related to the accumulation of reserves and not specifically to an increased root surface for water absorption (e.g. *Manihot spp.*). A higher investment in roots can also support a process called *hydraulic lift*, when the roots translocate the water from the soil positions with a greater water potential (for example, deeper) to soil positions with a less negative water potential. This process promotes a *hydraulic redistribution* (Burgess & Bleby, 2006) in the soil independently from plant transpiration, because it occurs when the stomata are closed (e.g. at night in C_3 and C_4 plants, and during the day in CAM - Crassulacean Acid Metabolism - plants).

With the water reaching the roots, the absorption process is directly dependent on the water potential gradient between the rhizosphere and the root xylem. There are two ways to establish this gradient, characterised by two absorption processes: 1) *osmotically driven absorption*, common in plants with low transpiration activity; and 2) *passive absorption*, which dominates in plants with high transpiration activity. The osmotically driven absorption occurs in plants under conditions of heat and non-limiting water availability in the soil, but with a restricted capacity of transpiration (for example, without leaves or with a limited vapour pressure deficit). In these cases, there is an accumulation of solutes in xylem vessels (for example, sucrose by degradation of starch reserves in the roots), reducing the xylem water potential in relation to the soil water potential. This condition results in water absorption and an increase in root pressure, which is itself responsible for the guttation that means the leaf water output through the hydathodes (pores located at the margins of the leaves) (Fig. 4). Moreover, increase in root pressure also promotes the water exudation in lesions of branches, easily observed in some species (e.g. exudation on branches of grape and kiwifruit plants after pruning in early spring).

In passive absorption and with an increasing rate of transpiration, the tension in the xylem vessels increases, indicating a predominance of the pressure potential influence upon the osmotic potential through the establishment of the water potential gradient between the root xylem and the rhizosphere. Under these conditions, the roots become a passive absorption organ, where the water is sucked into a mass flow promoted by the transpiration activity of aerial parts of the plant. A grapevine, for example, which during its annual growth and production cycle transpires between 650 to 900 mm of water, in accordance to environmental conditions where it is growing, and this volume corresponds to about 85% of all its absorbed water (Mullins et al., 1992).



Fig. 4. Detail of a leaf of a wheat plant (*Triticum aestivum* L.) presenting guttation in the morning. Photo: Ana Cláudia Pedersen.

The water intake in the roots can follow three ways into the root tissue in relation to the route of the epidermis to the endoderm of the root, called *radial water transport* (Fig. 3): 1) *apoplastic*, where the water moves through the intercellular spaces and does not pass through any membranes, exclusively occupying the continuous network of the cell walls; 2) *symplastic*, where the water moves exclusively from one cell to another through plasmodesmata connections; and 3) *transmembrane*, which corresponds to a mixed path between the first two, where the water goes in one direction through the root tissue, entering (symplastic) and exiting (apoplastic) cells. The relative importance of these pathways is still a cause of much discussion, but there is some evidence for the suggestion that plants displaying low transpiration activity predominantly witness symplastic transport, while those displaying high transpiration activity witness a greater proportion of apoplastic transport. Another important detail in relation to these different pathways is relevant only in the outer layers of the root tissue, because in the endoderm the water apoplastic flow is limited due to the Casparian strip (Fig. 3). In this hydrophobic barrier, the radial and transverse endodermal cell walls are impregnated with lignin, suberin, structural wall proteins and wax. Note that in many plants this barrier also occurs in the epidermal cells, forming a double layered hydrophobic barrier in the roots (Enstone et al., 2003). It is important to note that the Casparian strip does not always establish a barrier that is totally impermeable to water and solutes coming from the soil. This can be observed in - for example - the development of young roots where pericycle growth can break parts of the endoderm and allow free access to water until the reconstitution of the tissue.

With regard to water absorption control in the roots, plants also present a family of membrane water transporter proteins (water-channel proteins), called *aquaporins*. These proteins have a critical role in water absorption, reducing the resistance to the water flow along the transcellular path. The number of these proteins available for the root surface is variable throughout the day, being higher during the photoperiod due to the higher demands of photo-transpiration. The *aquaporins* are controlled by many **endogenous and exogenous** factors of the roots, such as pathogens, phosphorylation, pH, solute gradient, temperature and all environment factors that interfere in hydraulic conductance along the water flow by the plant.

2.2.3 Ascension of water through the plant: Vascular system

The presence of plants outside the water environment - among other factors - has been related to the evolution of the vascular system, which allows for the speedy upward movement of water to meet the demand of transpiration from the leaves. Water supply through cells by diffusion (difference in chemical gradient) alone is not able to maintain the hydration of a perspiring canopy plant. The need for a vascular system is more evident when we observe the hydraulic dynamic of a tree during a hot day, which demands a large flow of water (for example, 200 to 400 liters day⁻¹) to fit a transpiring surface that is situated along elevated positions, and in some species is higher than 100 meters (e.g. *Sequoiadendron gigantea*).

The water flows from the roots to the shoot of the plant through the xylem. The general mechanism to explain this upward movement of water is the *cohesion-tension theory*, which was proposed in the late 19th century. Basically, this

theory holds that the water evaporated in leaves establishes a tensile strength in the xylem, where the hydrogen bonds provide a continuous intermolecular attraction (cohesion) between the water molecules from the leaf to the root (Fig. 1). Thus, the water column in the xylem lumen is driven out of a region with a higher water potential, i.e. from the root and the stem, to a region with a lower water potential, as the leaves, and finally toward to the air that can reach very low water potential (e.g. -100 MPa, at 50% of air relative humidity).

Recently, the cohesion theory has been questioned as a result of assessments of tension in xylem vessels, which do not present a direct relation with tension values measured on leaves through pressure chambers. Furthermore, it is assumed that the hydrophobic interaction between the internal walls of the xylem and the sap composition (lipids, proteins, polysaccharides etc.) prevents the development of a tensile strength larger than 1 MPa, which is smaller than the estimated tension of rising water in a 30 m high tree (3 MPa). However, despite these questions, many studies argue that the fundamentals of the *cohesion-tension theory* are still valid for explaining the water flow in the continuous soil-plant-atmosphere. These elements support the idea that the water column of leaves to the roots provides an auto-regulation mechanism between the process of loss and absorption of water by the plants. Therefore, although the importance of the *cohesion-tension theory* has been neglected by some critics, this mechanism is considered to be essential for the survival of plants during the transpiration process, i.e. the loss of water.

With rising water in the trunk, in addition to pressure force, there is also capillarity strength in the vessels. In a perspiring plant, the water moves continuously from the xylem bundles to the intercellular spaces in the leaves, where the water potential is lower. Due to capillarity strength, water which evaporates through leaf stomata is replaced by the water contained in the lumen of the vascular bundles. In physiological temperatures (25°C), the cohesive forces between the water molecules are sufficient to prevent the disruption of the water column. This tension and the capillarity forces present in the vascular bundles also present resistance to the water flow along the plant by two major ways: 1) the inherent properties of the xylem flow and 2) the geometric aspects of the xylem conduits (*vessel elements* and *tracheids*). In this respect, it is notable that plants with the *vessel elements* of xylem can present a significantly lower hydraulic resistance than plants with *tracheids* (Tyree & Zimmermann, 2002). As such, the xylem diameter has a great influence on the hydraulic conductivity or water flow (J_v , mm s⁻¹), according to the Hagen-Poiseuille equation which describes the transport of fluids in ideal capillaries:

$$J_v = (R^4 \Delta \Psi) / 8 L \quad (1) \text{ In this equation, } \Delta \Psi$$

is the difference in the water potential (MPa) between two points of

observation throughout the capillary, R is the radius of the capillary (mm) with a determined length L (mm), through which occurs a flow with a constant viscosity (1,002 x 10⁻³ Pa second⁻¹, at 20°C). Accordingly, this equation shows that J_v is proportional to the fourth power of the vessel conductor diameter (the xylem). According to this logic, a trunk that presents few xylem vessels with large diameter has a greater J_v than a trunk with the same xylem area distributed in a larger number of vessels of smaller diameter. In general, xylem vessels with larger diameter are also longer. Xylem vessel diameter is also variable throughout the growth season, being larger in those vessels formed early in the growing cycle. This makes growth rings visible in transversal cuts of tree species. The vessels diameter is an important factor in preventing cavitation or embolism (formation of air bubbles by breaking the water column under high tensile strength, such as values close to -30 MPa), with thinner vessels being less susceptible to such a water column breakage. Generally, the cross-sectional area of vascular bundles is proportional to the transpiring leaf surface. This may be observed in plants adapted to arid environments, which have thinner vessels and a small transpiring surface, as reduced root:shoot ratio.

2.2.4 Leaf water and transpiration

Returning to our lampion scheme (Fig. 1), we emphasise that the leaves are the final frontier of the water flow in the continuous soil-plant-atmosphere system. In leaf mesophyll there is an extensive system of intercellular spaces - present in cell walls - which correspond to the internal surface of water contact with the air. By this interface between the cell walls and the intercellular spaces is established a water potential gradient, mobilising the water by the cell walls from the final extremities of the xylem bundles. This water flow by the cell walls occurs in an analogous way to the principles of water movement through the soil matrix, and the water interacts with cellulose microfibrils and other hydrophilic components of the cell wall (Fig. 5). Due to the high surface tension and as a result of water evaporation in the surface of the cell walls which are in contact with the air in the intercellular spaces, it is established that the tensile strength is transmitted to the xylem. Therefore, it is the tensile strength that drives the upward flow of the water column from the root and is produced in the internal evaporation process in the leaves. The maintenance of this process - as a result - depends on the output (in the atmosphere) of water vapour present in the intercellular spaces. As the leaf cuticle represents a

barrier to water outlet - allowing on average only 5% of water permeability - the water vapour moves from leaf intercellular spaces to the atmosphere predominantly through stomatal diffusion. This process of the loss of water

vapour by the leaves is called *transpiration* and corresponds to the majority (90%) of the volume of water absorbed by plants.

Transpiration has a number of positive effects (e.g. helps with mineral transport and leaf cooling) however it also may contribute to induce water stress when soil dry.

In the *continuum* soil-plant-atmosphere of water flow, there are two major factors determining the water potential of a plant: 1) the water potential of the soil, which characterises the water supply; and 2) transpiration, which defines the loss of water. The plant, which is an intermediate in this process, may regulate the water potential gradient between the soil and the atmosphere primarily through the regulation of stomatal conductance. The stomata have a quick and fine control of the water relations of a plant, coordinating the control of the water potential gradient between the leaf and the air. In this interface of the leaf with the environment, it is important to note that small changes in the relative humidity of the air are reflected in major changes in the water potential gradient, which requires a stomatal control so as to maintain the water stability of the plant. A simple variation from

100% to 99% of the relative humidity already corresponds to a decrease of -1.36 MPa in the water potential of the air. This decrease becomes more evident in the water potential of the air in average (80%) and in extreme (50%) conditions of relative humidity, which respectively provide values of -14 and -93.6 MPa at 20°C (Nobel, 2009). If we compare the water potentials of the air with the average water potential of a mesophyte plant (-0.5 MPa), the high gradient always determines that the water is diffused from the leaves to the air (Fig. 4). Throughout this water route between the leaf and the air there are two components that can exert resistance to the diffusion process: 1) *stomatal resistance*, which is coordinated by the stomatal opening; and 2) *resistance of the air boundary layer*, which is located closest to the leaf surface (Fig. 5) and it is directly influenced by wind speed. The higher the speed of the wind, the greater is the frequency of air renewal in this layer surrounding the leaf,

restricting diffusion resistance for the maintenance of a major gradient of the water potential. Morphological and anatomical variations among leaves can interfere with the speed of displacement of this thin layer of air, restricting the rate of transpiration in dry environments. Among these modifications, we highlight the presence of hair, the stomata located at the lower surface of the leaf, and the shape and size of the leaves. Although these changes interfere directly with the transpiration rate, they do not exercise a variable and instantaneous control in relation to the ambient conditions, as is done by the stomata.

During the day, great changes occur in the water potential along the soil-plant-atmosphere system. Initially, let us consider a mesophyte plant in a constant atmospheric condition of

75% relative humidity at 20°C (-39 MPa of air water potential) and soil without water restriction (at *field capacity*). During the nocturnal period, the transpiration is virtually nil, by stomatal closure, promoting an equilibrium between soil, root and leaf water potentials leading the potential gradient to be near zero (Fig. 6). With the first rays of the sun, at dawn, the stomata opens, allowing the water diffusion of the leaf (transpiration) and, as a consequence, reducing the leaf water potential.

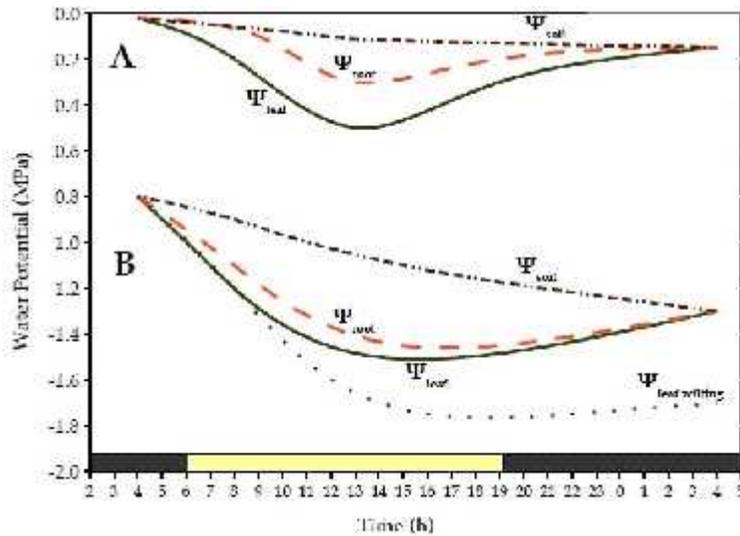


Fig. 6. Schematic daily variation of soil (Ψ_{soil}), root (Ψ_{root}) and leaf (Ψ_{leaf}) water potential of a plant well hydrated (A) and under water restriction (B).

When leaf water potential does not equilibrate with Ψ_{soil} at the end of day (case B), the permanent wilting point ($\Psi_{\text{leaf wilting}}$) is reached.

As a result - and with a delay that depends on the cohesion-tension forces of water and the water column size between the leaf and root - it begins the reduction of the root water potential. This reduction is slight (on average -0.3 MPa), due to high water availability of the soil. The reduction of the water potential in the plant reaches the minimum limits during the hottest times of the day, forcing the stomata to close for small intervals for extreme cases of transpiration demand (Fig. 6A). At dusk, these variations of the water potential in the leaf and the root are reversed.

In considering a plant under the same atmospheric conditions, but with severe water restriction (a soil water potential near to the permanent wilting point), the water potentials of the leaf and root necessarily reach values that are more negative for the sustainability of the water flow by the plant. At high limits of negative potential, the differences among the water potential of the leaf and root are smaller, but with great difference in relation to the soil water potential (Fig. 6B). If this condition persists, there will be a decrease in the turgor pressure of the leaves, causing temporary leaf wilting, which is recomposed during the nocturnal period. Mesophytic plants can tolerate this reduction in soil moisture up to the limit of -1.5 MPa, while some xerophyte plants can reach limits of -5.5 MPa .

3. Physiological and biochemical aspects of water in plants

In a plant cell, the water is predominantly located in the vacuole and represents the majority of the mass of growing tissue (on average 90%). This predominance is due to the importance that this universal solvent represents in the physiological and biochemical processes of a plant cell. At the cellular level - due to its polar structure - the water acts on the dissolution and mobilisation of ions and organic metabolites, such as amino acids, proteins, carbohydrates and hormones. These water properties are also crucial for the flow between parts of the plant, acting directly in the transportation of nutrients, carbohydrates and hormones. The water acts in membrane integrity and the support of herbaceous plants through the cells turgor pressure, which varies between 1 and 5 MPa. This turgor pressure is also essential in the expansion process of cell walls and in growing tissues. This subsection will expose some physiological and biochemical aspects of the water relations in plants.

3.1 Water deficit and its effects on plant growth

When a plant is under water deficit, responses at physiological, biochemical and molecular scale are triggered . Physiological responses are linked to a condition of recognition of stress by the root system, turgor changes and water potential and, consequently stomatal conductance, internal CO_2 concentration and photosynthetic activity decrease. In

biochemical terms, there will be a decrease in the photochemical activity of photosynthesis, rubisco enzyme activity and the accumulation of secondary metabolites linked to stress (such as glutathione and polyamines). From a molecular perspective, several genes expressed under stress conditions are activated, such as genes linked to the biosynthesis of abscisic acid and the synthesis of specific proteins.

As the cells hydration is reduced and the plant goes into a condition of water deficit, abscisic acid and solutes increase in the plant, especially in the root system (the increase in solutes occurs in a relative manner, due to water reduction). These factors will reduce stomatal conductance and, consequently, photosynthetic activity which ultimately will result in a reduction in the synthesis of proteins and cell walls, as well as a decrease in the rate of cell expansion. The sum of these responses to water deficit contribute to explain the reduction of plant growth.

3.2 Elements that define water demand

Basically, according the cohesion-tension theory already described, the water demand of plants is generated by a gradient at the top of the plant which generates negative hydrostatic

pressure that “suck” soil water through the plant into the atmosphere. Two main environmental factors will determine this *evaporative demand*: wind speed and solar radiation. Thus, those plants that exist in environments with high winds (e.g.

20 km h⁻¹ or more) or intense solar radiation (e.g. 2500 μE m⁻² s⁻¹ of photosynthetically active radiation) will suffer a greater water loss to the atmosphere. These plants need to make use of water control mechanisms in order to tolerate these environmental conditions. Plants can suffer morphological and anatomical alterations and osmotic regulation to improve stomatal resistance and increase water absorption through the root system.

3.3 Mechanisms of water status regulation

3.3.1 Morphological and anatomical characteristics associated with water control

Plants live with a constant dilemma, namely to undergo photosynthesis while preventing water loss. Every time plants open their stomata to allow the influx of carbon dioxide, they lose water by diffusion.

When plants achieved their “*terrestrial status*” and left behind the conditions of *algae*, they required several changes to adapt to this new environment. They acquired a root system for physical support and absorption of water and nutrients, vascularity for the movement of water and photoassimilates, and a stomatal complex and a wax layer for the regulation of water loss to the atmosphere.

It is known that intense solar radiation is accompanied by elevated temperatures, which can cause severe physical damage to leaves and lead to senescence and leaf abscission or, on small scale, reduce carbon assimilation. This situation forces plants to adopt strategies to minimise such negative effects.

In order to adjust the balance between water availability and atmospheric demand, plants can reduce the size and number of leaves. The organisation of the mesophyll and leaf dimensions is modified by water restrictions, and can provide a strategy for plants to affect the stomatal conductance and CO₂ diffusion (Evans et al., 2009). Plants which need greater efficiency in water use (μmol CO₂ fixed / H₂O transpired) have a strategic demand to retain higher CO₂ concentrations inside. Characteristics of leaf anatomy related to water deficit are reduction of thickness, higher cell density and smaller intercellular spaces, all of which try to mitigate the problems of excessive water loss. The reduction in cell size and, consequently, the reduction of tissues are associated with the turgor decrease of cells. However, smaller cells may stay more *turgid* when compared to larger cells, having better capacity to tolerate conditions of water restriction (Burghardt et al., 2008). These changes in cell size result in an increased internal surface to of CO₂ exchange per leaf area, seeking to maintain the photosynthetic rate with reduced stomatal conductance.

The relation of the root with shoots is also strongly modified due to conditions of water unavailability. One way that plants use to keep their water status stable is by reducing the growth of the shoot, in order to reduce leaf area and water loss to the atmosphere. At the same time, plants can invest in the growth of the root system, in order to increase the soil volume explored and in turn water absorption. All these mechanisms occur through hormonal changes as initiated by abscisic acid signals. Nevertheless, later on it will be the causal effects of interactions between auxins, cytokines and gibberellins which define the relation between root and shoot.

Anatomically, the change in vascular diameter may be a response to water deficit conditions and tends to decrease under water deficit. By reducing the radius of vessels, xylem conductivity is reduced (increased resistance) according to

equation 1. There are some situations of high *evaporative demand* which increase the tension in the xylem vessels, causing a disruption of the water column and the formation of air bubbles by embolism. The disruption of the water column can also occur under conditions where the water freezes inside the plant.

Another mechanism which protects the leaf from attack by insects and helps the plant to avoid water loss is the presence of leaf trichomes. Trichomes reduce water loss by: 1) reducing the arrival of solar radiation on the leaf surface; and 2) reducing the interference of wind on the boundary layer, which reduces the differences in water potential between the leaf and the atmosphere.

Leaf cuticle presents variations in anatomical position and chemical composition. Chemically, these cuticles are characterised by two specific groups of lipids: 1) cutin, which forms the support structure of the membrane; and 2) waxes deposited on the external surface - called *epicuticular waxes* - and also strongly dispersed within the matrix of the cutin (below the surface) called *cuticular waxes*. The most important function of epicuticular waxes is to avoid leaf water loss to the environment. In addition, they reduce leaf nutrient loss, prevent excessive solar radiation, pathogenic microorganisms, cooling, wind damage and physical abrasion; . Epicuticular waxes may be amorphous, have a flat format covering the entire surface area of the leaf, or have the shape of a crystal or blade. Involved in the formation of these waxes are alkanes, esters, ketones and alcohols.

An important aspect of plant morphology is the density of leaf veins. Angiosperms average 8 mm of vein per mm² of leaf area, while non-angiosperms have consistently averaged close to 2 mm mm⁻² throughout 380 million years of evolution. This was an important ecological strategy for the increment of photosynthesis, especially under conditions of higher temperatures and transpiration rates.

The most important anatomical tools to optimise the plant water use is the stomatal complex. Variations in opening, size and position of the stomata in the leaf help plants grow under conditions of water deficit.

3.3.2 Stomata metabolism

Stomatal complexes are present in green stems, flowers and fruits, averaging 30 to 400 per square millimetre. They are formed by an opening, called a *pore*; two *guard cells* that are responsible for opening and closing the pore; and in some situations neighbouring cells called *subsidiary cells*, whose function is to support the guard cells. The stomata liberate into the atmosphere around 95% of the water that exits from the leaves and causes 90% of gas exchange (in this case, also involving carbon dioxide), while only 5% of the water output is

through the cuticle. There are two types of stomata morphology: kidney-shaped and grass-like, sized between 5 and 15 µm wide and 20 µm long. The stomata works as a hydraulic valve regulated by water. When turgid, the stomatal pore opens, and when flaccid due to water loss it closes (Fig. 7). In the early morning, light in the blue band (440-490nm) indicates the arrival of solar radiation and possibility of photosynthetic activity. Thus, the plant opens the stomatal pores to allow influx of carbon dioxide. The first theory about stomatal opening control was called the "starch-sugar hypothesis," and it was widely accepted during the early 1940s. This hypothesis suggested that the hydrolysis of starch in soluble sugars decreased the osmotic potential of cell, promoting water absorption and stomatal opening. However, that theory lost strength as advances were made by studies of potassium movement in the guard cells. Currently, three modes covering the osmoregulation processes of guard cells are accepted: 1) the influx of potassium and chloride through proton pump activation and the synthesis of malate from starch breakdown; 2) sucrose synthesis by starch hydrolysis; and 3) sucrose synthesis by carbon dioxide fixation due to photosynthetic activity.

The blue light signalling process occurs through receptor pigments (phototropins). Thereafter, the opening process occurs by the reduction in the osmotic potential of guard cells by potassium input, consuming ATP by ATPase located in the plasmatic membrane. This ATPase release protons inside the guard cells, causing a variation in pH around 0.5 to

1, which enables membrane hyperpolarisation and, consequently, the opening of channels favours potassium absorption. The resulting osmotic gradient causes water movement towards these cells. Therefore, the guard cells become turgid and the stomatal pore opens, due to the action of cellulose microfibrils. However, it is important to emphasise that chloride and malate also contribute to the establishment of the osmotic gradient in guard cells exposed to blue light.

When solar radiation begins to decrease this affects the photochemical phase reducing levels of ATP and NADPH⁺ resulting in losses for the biochemical phase. Internally, the carbon dioxide content begins to rise as they are not being

used in the carboxylation process in the Calvin-Benson cycle (C_3). At the same time, calcium ions play an important role when they enter the guard cells, causing solute output and decreasing the osmotic potential of these cells, making the stomata close.

An atmosphere enriched with carbon dioxide can favour the photosynthetic activity of plants with the C_3 mechanism. However, excessive carbon availability will result in stomatal closure in some species, even with a C_3 mechanism. An example of natural CO_2 enriched conditions, where the plants can be submitted to excessive concentrations of carbon dioxide, occurs near volcanic activity.

3.3 Hormonal and molecular responses in different water conditions

The plants that display a higher production capacity, due to the morphological, physiological and metabolic changes of their organs and cells, tend to present higher demands on available resources and, consequently, possess greater vulnerability to conditions of water restriction. However, there are several strategies for the adaptation to dry environments that can be considered as a tool for progress in overcoming limiting conditions on growth and production. Generally, some plants can accumulate water to delay or escape such stress conditions, while others can deal with the stress through decreased metabolic activity (Bartels, 2005). The effect of water restriction depends on the degree and duration of the stress, the stage of plant development, the genotypic ability of the species and environmental interactions. In recent years, and mainly due to climate changes, several studies have looked to understand the biochemical and molecular basis of water stress. Water stress can influence a plant on several levels, with cell expansion and growth being the first processes to respond to water limitation. With the gradual increase of stress, other processes are also affected, such as photosynthesis and allocation of assimilates. At the cellular level, membranes and proteins can be damaged with the increment of reactive oxygen species or peroxidation. These responses are common with other abiotic stresses, such as the effects of salinity and low temperatures, resulting in the synthesis of a similar group of proteins.

Tolerance to water deficit can be manifested in four ways: 1) the seasonal adjustment of growth to avoid stressful conditions; 2) morphological adaptations, such as an increase in the root:shoot ratio, a reduction in the leaf area and wax accumulation on the leaf surface; 3) physiological adaptations, such as stomatal responses and leaf abscission; and 4) metabolic changes. Among the metabolic changes of adaptation, osmotic adjustment represents the most common change and results from the accumulation of certain metabolites.

The root has been generally accepted as the organ which acts on the perception of water stress, although so far there is no knowledge about how the cells in roots perceive the soil moisture content. However, when the plant water potential is influenced by the water restriction of soil or saline stress, the stomata must respond quickly to avoid water deficit. Signalling between the root and stomata is carried out by abscisic acid (ABA), which has been considered to be a “*plant hormone of stress*” due to its participation in the signalling

networks of other factors of stress. ABA is synthesized from carotenoid by the synthesising enzyme of ABA (zeaxanthin epoxidase, 9-cis-epoxycarotenoid dioxygenase and aldehyde oxidase) which is induced in the root apex or in the parenchyma cells of vascular bundles by water or saline stress. After the synthesis of ABA in the roots, it is transported through the xylem to the leaves alone or conjugated with glucose (the latter being more appropriate for transport over long distances). The proportion of each form of transport (alone or conjugated) is variable between species. Once in the leaf, conjugated ABA is hydrolysed into its free form by the apoplastic enzyme β -D-glucosidase, inducing stomatal closure through a signalling system in the guard cells of chloroplasts.

In addition to this long distance signalling between the roots and the stomata, recent studies also point out that the leaves must act as sensors of relative humidity in order to avoid desiccation. This is clear from observations of the extremely quick closure of stomata with increments in the vapour pressure gradient between the leaf and the air, even when there is adequate water availability in the soil. Recent works expose the possibility that leaf sensors of relative humidity are located in or near their own stomata guard cells. In addition, there is genetic evidence that leaf sensitivity to relative humidity (RH) is related to ABA metabolism, exerting a hormonal effect over a short distance. A recent study on genetic selection, based on infrared thermal imaging, identified two genes (*OST1* and *ABA2*) that are directly involved in the signalling route of RH sensing in guard cells. *OST1* codes a protein kinase that is involved with stomata closure, while *ABA2* codes an enzyme involved in ABA biosynthesis. This reinforces the involvement of ABA as a mediator in a signalling network of guard cells, which can be shared between different stimuli to control stomata closure.

ABA has been related to quantitative and qualitative variations in the gene expression and protein synthesis stimulated by water stress. Meanwhile, it is notable that some of the proteins that are *de novo* synthesized do not appear in the responses to the application of abscisic acid (ABA) and these signals/response routes to the water stress have ABA-dependent and ABA-independent routes. In ABA-independent routes, the signal molecule provided by the roots is still unknown. Bioinformatics analysis has promoted advances in the identification of several factors of transcription that are induced by water deficit, classified in six major groups: AP2/ERF (APETALA2/ethylene-response factor); bZIP (Basic leucine-zipper protein); MYB/MYC Zinc-finger protein; CDT-1; NAC and Dreb. In ABA-dependent routes, the promoters of genes containing a *cis*-sequence of six nucleotides are known as the *ABA response element* (ABRE). The genes' expression of ABA-dependence is activated by the AREB/ABF link - a transcription factor of the type bZIP - on the ABRE sequence. Further, in a gene ABA-dependent RD22 expression there is involvement with the transcription factors MYB and MYC, and they are related to the final stages of responses to drought that are ABA dependent. The promoters of genes related with responses to drought also have an alternative regulatory sequence of nine nucleotides called a *dehydration response element* (DRE). The DREs are involved in the ABA-independent expressions of genes that are induced under drought conditions. The *trans*-factors to the *cis*-elements are CBF/DREB1 and DREB2, which are expressed transiently after the detection of

Genes that are stimulated by drought can be categorised into two groups: 1) coding genes of those proteins responsible for protecting cells and organs against stress; and 2) coding genes of those proteins necessary for signals' translation and regulation of gene expression. The proteins of the first group act directly on membrane functions, the maintenance of water potential, proteins' protection and oxidative stress control. Stands out in this group the family of the *Embryogenesis Abundant protein* (LEA), which is formed by five types of proteins based on the structural domain and which are suspected of acting to protect the cell membrane. Beyond that, and by their hydrophilic properties, they act in water retention and prevent the crystallisation of other proteins and molecules during drying. Within the LEA family itself, the D-

11/RAB/Dehydrins group stands out, whose function has been related with the stabilisation of proteins and membranes. This group presents a wide distribution between plant species, and can be considered to be an alternative to the constitutive defence against rapid changes in the *water status* of tissue. The *aquaporins* family represents another important protein in cell protection against water stress, facilitating water absorption by the plasmatic membrane. Its importance in water relations was recently evidenced by the differential accumulation of aquaporins in relation to the degree of drought tolerance in varieties of beans. With regard to membrane protection and the water status of the plant we may also highlight lipid transfer proteins (LTPs) that catalyse the transfer of several classes of phospholipid and glycoproteins for deposits in cell walls or between membrane vesicles. The results with LTPs show that these proteins are induced during different conditions of stress and that they can act to increase membrane fluidity, decreasing water loss by increasing tissue impermeability, and as a physical barrier to biotic.

Another protein family has been associated with responses to drought, namely Heat Shock Proteins (HSPs), which are widely distributed in nature. These proteins are known as molecular chaperones, acting in the folding and assembly of functional proteins and in the removal of non-functional proteins, facilitating the recovery of cellular functions after stress. Several HSPs - classified according to their molecular weight - are induced in conditions of water and saline stress, such as HSP70 (the DnaK family), the chaperones GroEL and HSP60, HSP90 and HSP100 and the small HSP (sHSP). Within these proteins, there is the cyclophilin, which is a chaperone protein with systemic properties and which is highly induced during water stress, conferring multiple tolerances to abiotic stress. During conditions of stress, the recycling of macromolecules which lose their function to maintain cellular homeostasis is essential. In this process, and under conditions of water stress, an increase of protease activity has been observed, which is important in the destruction of denatured proteins and in the recycling of amino acids necessary to synthesize proteins for water deficit responses. In addition, the ubiquitin and polyubiquitin proteins are also induced with water restriction, both of which act marking proteins for proteolytic degradation.

3.4 Osmotic regulation

As discussed above, the osmotic regulation process of stomatal opening occurs by the movement of solutes - called *osmolytes* - thereby influencing water movement between the cells. This water movement has several purposes in relation to cell hydration inside plants, such as the stomatal opening and higher water absorption via root system. Several compounds that work in the osmotic regulation of plants are known: carbohydrates (sucrose, sorbitol, mannitol, glycerol, arabinitol, pinitol), nitrogen compounds (proteins, betaine, glutamate, aspartate, glycine, choline, putrescine, 4-gamma aminobutyric acid and organic acids (malate and oxalate) and so on.

From the standpoint of thermodynamics, there are concepts relating to water potential that are elementary in the explication of water movement. In turgid cells the water potential (Ψ_w) is composed by pressure (Ψ_p) and osmotic (Ψ_s) potentials.

The greatest possibility of interference in the Ψ_w of plant cells is by varying the amount of internal solutes. Thus, osmotic regulation is a process in which variation of the amount of solutes - and consequently in osmotic potential - interferes in water movement.

The higher the concentration of solutes in a solution, the greater will be the disorder of the system (entropy). Furthermore, this condition leads to more negative osmotic potential and, consequently, to a more negative water potential. The water inside the plant flows from the sites with higher water potentials to those with lower ones. Thus, an increase in solute concentration in the cell favours the entry of water.

The solutes that plants use to decrease the water potential and control the water flow must have a low molecular weight. Among these solutes, the best-studied is potassium (K^+), the lack of which is related to tissue dehydration. However, most references point out that the main role of potassium within the osmoregulation process is the regulation of stomata opening. It also acts in water absorption by the roots, in transpiratory control and in the cells' ability to resist low temperatures. In particular, under conditions of potassium deficit, sugars from starch hydrolysis become more relevant in osmotic regulation than in the process of stomatal opening.

The proline amino acid also stands out as an osmotic regulator, and is linked to stress both by water deficit and salinity. Nevertheless, proline does not only exert the function of osmoregulation in plant cells during periods of water deficit, but it can also protect against the activity of free radicals, regulate the pH in the cytoplasm, protect against the denaturation of macromolecules and also act as a source of carbon and nitrogen under conditions of stress. Another compound with importance in osmotic regulation is glycinebetaine, which, like proline, does not just exert or exercise its function in osmotic potential. These compounds also act in the reduction of reactive oxygen species (ROS) produced under conditions of stress in plants. With the process of tissue dehydration, there is production of ROS, such as the singlet oxygen (1O_2), the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). Reactive oxygen species (ROSs) are produced from oxygen metabolism and play an important role as indicators in the stress process of water deficit. Among these are oxygen ions, peroxides and free radicals, and these compounds will cause oxidative stress in cells and prejudice their operation.

The role of physiology in forestry

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Summary

Although a great deal of information concerning the physiology of trees has been accumulated, physiology has made a smaller contribution to forestry than it should. This is partly because of a lack of communication between field and laboratory workers and partly because of a lack of a general understanding of the role of physiology in forestry. The physiological processes of trees are the machinery through which the genetic potential and the environment operate to determine the quantity and quality of growth. Actual wood production usually is far below the genetic and physiological potential because important physiological processes are often inhibited by environmental stresses such as drought, mineral deficiencies, unfavorable temperatures, and air pollution.

The most useful contribution that physiologists can make to forestry is to determine which physiological processes are inhibited by particular stresses and suggest to tree breeders what characteristics will minimize the inhibitory effects of these stresses. Cooperative screening studies with geneticists to identify differences among families and provenances that result in differences in stress tolerance should be very productive. Perhaps as the cost of establishing forest stands increases, more attention will be paid to the selection of genotypes with physiological characteristics best suited to local environments.

Because of the complex interactions among stresses there is need for long-term interdisciplinary research programs in which soil scientists, meteorologists, and physiologists cooperate with foresters and forest geneticists in identifying stresses and their inhibitory effects on forest productivity. Because of the increasing specialization in science there also is great need for broadly trained generalists who can understand research in several fields, identify important contributions, and show their significance to investigators in other fields.

Introduction and historical review

The general theme of this paper is the role of plant physiology in forestry. However, I propose first to review briefly the history of forest botany and physiology because some knowledge of the past aids in understanding the present and predicting the future. The systematic study of plants is often said to have begun with the Greek philosopher-scientist, Theophrastus, a student of Aristotle and Plato, who lived about 300 BC. He classified plants as trees, shrubs and herbs, and noted the importance of weather in relation to plant growth and crop yield. Other Greeks and Romans wrote about plants, but forest botany received little attention for 2000 years. For many centuries after Greek and Roman days the recorded work on plants dealt chiefly with their real and imaginary medicinal properties, resulting in the 15th century herbals that appeared after the invention of printing. The influx of strange plants from the New World stimulated the study of taxonomy that culminated in the classification system of Linnaeus, published in 1753. During this long period the slow development of plant physiology is indicated by the fact that Aristotle's idea that plants absorbed their food from the soil ready for use persisted for 2000 years and was not seriously questioned until Liebig's work about 1840.

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Development of the microscope in the 17th century made possible the anatomical work of Grew published in 1671 and 1682 and that of Malpighi in 1675 on trees and other plants. Discovery of the circulation of blood in animals by Harvey in 1628 stimulated speculation concerning the possible circulation of sap in trees and several papers were published on this topic in the Proceedings of the Royal Society of London (1668–1671), but no conclusions were reached.

The first important quantitative work on the physiology of trees was done by Stephen Hales and published in his *Vegetable Staticks* in 1727. He measured root and stem pressures and transpiration, showed that water moves upward in the wood rather than in the bark, and decided that there is no circulation of sap in trees comparable to the circulation of blood in animals. Hales' work was largely neglected and nothing comparable on tree physiology appeared until more than a century later.

Two interesting but nonphysiological books on trees appeared during this period. Evelyn's "Sylva" published in 1670 was an attempt to interest English landowners in planting more trees and it mentioned sap flow of birch and the site requirements of several species. "La Physique des Arbres," published by Duhamel Du Monceau in 1758, attempted to put forestry on a more scientific basis and Enderlin in 1767 discussed the growth of trees and the characteristics of forest soils. G. Heyer in 1852 discussed the importance of light and shade in the growth of trees, a topic that became very important in the 20th century.

Modern plant physiology

Modern plant physiology, like silviculture, is often said to have begun in Germany about the middle of the 19th century with the work of Sachs and his successors such as Strasburger, Jost, Renner, and others. Investigations by the Hartigs, Büsgen, Münch, and others in the 19th and early 20th century resulted in important contributions to tree physiology. Out of this work came Th. Hartig's, "Anatomie und Physiologie der Holzpflanzen" (1878) and the classic "Bau und Leben unserer Waldbäume" published by Büsgen in 1897, revised by Münch in 1927, and better known to most of us in its English translation of 1931. Huber made important contributions in the 20th century, as did other investigators in Europe, but lack of space prevents discussion of their contributions.

In the United States, among the early investigators were Samuel Williams (1794) who estimated the water loss from a maple forest, W. S. Clark who published on sap flow in trees in 1874, and Jones, Edson, and Morse who published on maple sap flow in 1903. No doubt, many other interesting observations are hidden in old books and journals. In the 20th century ecologists and silviculturists such as Bates, Burns, Pearson, Toumey, and Korstian began to study the effects of water, light, and temperature stress on competition and natural distribution of trees. Horticulturists such as Auchter, Chandler, Murneek, and Heinicke, working on fruit trees, also began to make important contributions to tree physiology, although foresters seem to have neglected their contributions. Heinicke's

measurements of gas exchange of apple trees are still noteworthy (Heinicke and Childers 1937).

Clarence Korstian, first Dean of the Duke University School of Forestry, directly and indirectly had considerable effect on the development of forest tree physiology. He not only pointed out various interesting problems that needed investigation, but also funded research on some of them. As a result of Korstian's efforts many students became aware of important problems in the field of tree physiology and they and their students are contributing to that field today.

Lessons from the past

In making this brief review of the literature of forest botany and tree physiology several general ideas developed. The most important are (1) the slow but accelerating rate of progress, (2) the failure to make use of available information, and (3) the numerous factors that contribute to progress.

Slow but accelerating progress

For many centuries progress was very slow. In fact it was nearly 2000 years from the speculations of Aristotle and Theophrastus to the anatomical observations of Grew and Malpighi and a century and a half from the work of Hales (1727) to that of Th. Hartig (1878). However, since World War II progress in understanding the complexities of physiological processes has proceeded more rapidly on a broad front, chiefly because of more workers, better instrumentation, and better communication among workers.

The slow adoption of new concepts

Slow progress resulted in part from the slow acceptance and limited use of new concepts. The cohesion theory of the ascent of sap in trees was proposed about 1895 and was supported by both experimental and theoretical evidence during the early part of the 20th century, yet it was being questioned as late as the middle of the 20th century. The concept of what is now termed water potential was proposed under the name of "Saugkraft" by Renner (1912) and others in the second decade of the 20th century, but the concept only came into general use under the term "diffusion pressure deficit" after the appearance of the Meyer and Anderson text in 1939. Use of the more appropriate "potential" terminology was discussed by Tang and Wang in an overlooked paper published in 1941, but it only became widespread after Slatyer and Taylor published their paper in 1960. Pressure flow porometers were described by Darwin and Pertz in 1911 and modifications were used by Gregory and Pearse, Wilson, and Alvim (see Kramer 1983, pp 328-330), but relatively little use was made of them until 60 years later when Fiscus (1984) combined them with computerized electronic equipment to monitor the water status of corn for the purpose of controlling irrigation. These examples lead the writer to conclude that progress in science is hindered as much by failure to use existing information as by lack of information.

Factors controlling progress in science

It is obvious that many factors control the rate of progress in science although historians and philosophers of science differ concerning their relative importance. I will suggest curiosity, social pressure, economic pressures, national prestige, and improvements in instrumentation as important factors. Natural human curiosity probably was the first pressure that led to the collection and naming of plants. This doubtless was strengthened by social pressures resulting from the use of plants for food and medicinal purposes, and economic pressures resulting from commerce in useful plants. Economic pressures are increasingly powerful in agriculture and forestry and we hope that in the future they will bring about the financing of much additional physiological research. In recent decades although national prestige has been a strong factor in terms of defense, technology, and Nobel Prizes it has had little effect on research on plants.

The most easily evaluated factor affecting scientific progress is instrumentation. The dependence of an understanding of the process of photosynthesis on development of instrumentation is a good example. Although Priestley observed in 1771 that green plants change the composition of the air in containers enclosing them, it was several decades later that improvements in analytical methods permitted quantitative studies of gas exchange. It was another 60 years before Sachs established the role of chloroplasts in gas exchange and still another 75 years before the availability of radioactive carbon isotopes made study of the reductive carbon cycle possible. Few physiological-ecological studies were made of photosynthesis until after World War II because there was no convenient way to measure gas exchange in the field. During the 1950s infrared gas analyzers became available and their improvement has resulted in a flood of measurements of gas exchange under almost every possible condition. The introduction of porometers to monitor stomatal conductance, and psychrometers and pressure chambers that permit evaluation of plant water status, combined with the use of miniaturized infrared gas analyzers, is resulting in a great increase in research on the carbon and water relations of plants. Unfortunately, it has probably resulted in too much dependence on instantaneous measurements of photosynthesis. Other examples of useful instrumentation are gas chromatography, fluorescence measurements, oxygen and ion-specific electrodes, desktop computers, and nuclear magnetic resonance spectroscopy and imaging. In fact the pace of development of new instrumentation is so rapid that it is becoming impossible for individual scientists to keep informed about new techniques.

The situation today

Today much information about the physiology of trees and other plants exists, but it is too poorly organized to solve problems of plant production. One reason for this is that physiologists are largely ignorant of the problems in forestry and foresters do not know how physiology can help solve these problems. The first

need seems to be a general philosophy about the role of physiology in relation to the practice of forestry, and I shall try to develop this in the next section.

The role of physiology in increasing forest productivity

The general objective of forestry is to grow trees efficiently, but to do this foresters must understand how trees grow, and this requires some understanding of tree physiology. Thus physiology should occupy a central position in forestry because, as shown in Figure 1, the physiological processes are the machinery through which heredity and environment operate. The hereditary potential or genotype determines the nature and limitations of the physiological machinery, but its actual productivity is determined by the environment in which it operates.

The only way geneticists can increase growth is by providing genotypes with a more efficient combination of physiological processes for a particular environment, and the only way that silvicultural treatments such as thinning or fertilization can improve yield is by improving the efficiency of essential physiological processes.

It is said that well managed plantations of Douglas-fir and loblolly pine produce less than 50% of their theoretical potential yield (Farnum et al. 1983) and the yield of agronomic crop plants is also far below the record farm yields (Boyer 1982). As indicated in Figure 1, there are three areas in which limitations to forest productivity can occur, (1) the genetic potential, (2) the physiological processes of the trees, and (3) the environment in which they grow. We will discuss each of these

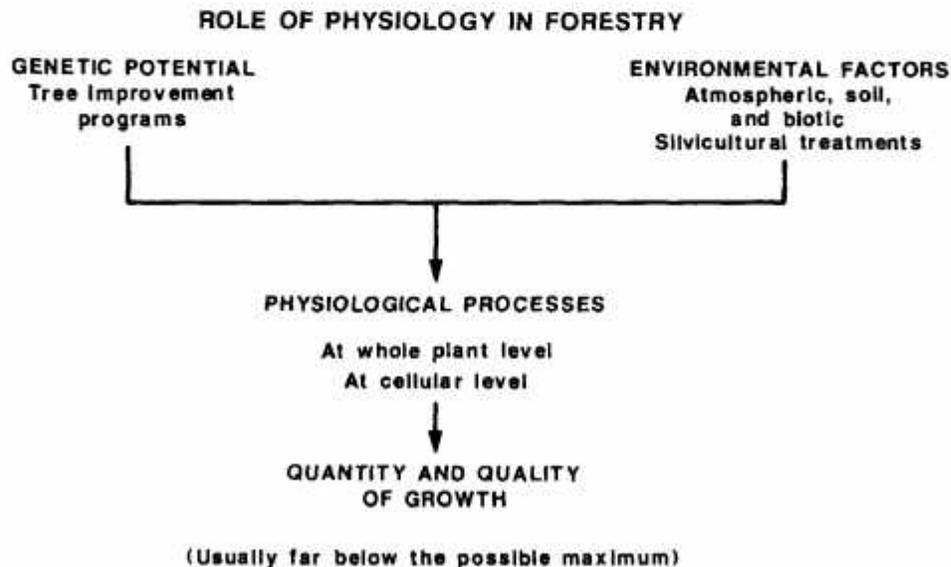


Figure 1. Diagram showing the role of physiology in forestry. This emphasizes that the physiological processes are the machinery through which the genetic potential and the environment operate to determine the quantity and quality of growth.

areas briefly and finally attempt to establish some research priorities.

Genetic limitations on yield

In nature, selection operates in terms of species survival rather than in terms of quantity and quality of wood, and a tree that is successful in nature is not necessarily what the forest industry desires. It may be crooked, divert too much dry matter into branches and roots, produce wood of low specific gravity, be slow growing, or suffer from other defects by commercial forestry standards, but if it reproduces it is successful in nature.

Fortunately, there is enough genetic variability among trees to provide many opportunities for improvement by phenotypic selection for characters such as rapid growth rate, wood quality, and resistance to certain diseases, and many opportunities for further improvement exist. For example, tolerance of cold soil is desirable for early planting of seedlings and Carlson (1986) observed wide differences among seedlings of various families of loblolly pine in ability of the roots to grow at low temperatures. A program is being started to screen loblolly pine for genotypes tolerant of ozone and other air pollutants and there are opportunities to find other desirable characteristics by screening programs.

In the future, production of plantlets by tissue culture and the screening of tissue cultures for cell lines tolerant of stresses such as dehydration, salinity, heavy metals, disease, and for the presence of viruses may become useful. However, it should be remembered that stress tolerance sometimes depends on whole plant characteristics that cannot be identified in cell cultures. Techniques used in genetic engineering such as recombinant DNA and protoplast fusion may provide combinations of desirable characteristics not now existing in nature, but many problems must be solved before these procedures can be used on a large scale. Some of the possibilities are discussed by Farnum et al. (1983) and Karnosky (1981), and in books by Bonga and Durzan (1982) and Dodds (1983).

Physiological limitation of yield

The interaction of physiological processes such as photosynthesis, respiration, translocation, water dynamics, and the metabolism of carbohydrate, fat, and nitrogen really determine the rate of growth. The processes that have received the most attention are photosynthesis, dark respiration, and the allocation of photosynthate to various processes and organs.

Photosynthesis

Because photosynthesis provides most of the material used in growth it is often assumed that an increase in the rate of photosynthesis should result in an increase in growth (Zelitch 1975). This has led to a demand for the development of plants with increased efficiency in photosynthesis, such as that resulting from the C₄ carbon pathway, and reduced photorespiration. However, attempts to correlate

growth and yield of trees (Ledig 1976; Helms 1976) and agronomic crop plants (Boyer 1982, Gifford and Evans 1981) with rate of photosynthesis have been disappointing. This is not surprising when it is realized that total dry matter production depends not only on the rate of photosynthesis per unit of leaf area but on total leaf area, leaf duration, and canopy exposure, and the amount of wood produced depends on how much photosynthate is used in respiration and how the remainder is partitioned among the various organs of the tree.

It was established 35 years ago that agronomic crop yields are seldom limited by lack of photosynthetic potential and that such cultural practices as fertilization and irrigation increase crop yield chiefly by increasing leaf area (Watson 1952). There is some debate about how fertilization affects the photosynthesis of a forest stand. Tamm (1977) stated that the principal effect of fertilization on Scandinavian boreal forests is through increase in leaf surface. Brix (1983) found that increase in amount of foliage accounted for over half the increase in stem wood of Douglas-fir fertilized when 27 years old, the remainder resulting from increase in rate of photosynthesis, and Miller and Miller (1976) found that the increase in leaf area was more important than the increase in rate of photosynthesis in Corsican pine. This problem is discussed in other papers in this volume.

In conclusion, it seems unlikely that important increases in the rate of photosynthesis will be achieved in the near future, and if they are achieved it is doubtful if they will significantly increase tree growth. It probably will be more productive to consider the benefits from increase in leaf area, at least before stand closure, a longer duration of photosynthesis in the autumn, and a photosynthetic machinery more tolerant of water and other stresses. Consideration also might be given to selection for the conversion of more photosynthate into cellulose and less into compounds requiring more energy, such as lipids, lignin, and phenolics (Chung and Barnes 1977). Perhaps trees grown for pulpwood where cellulose is the desired product should have a different biochemistry from that of trees grown for timber where strength is desired.

Dark respiration

In simple terms the carbohydrate produced in photosynthesis minus that used in respiration represents the amount available for use in growth. According to Möller et al. (1954) 40 to 50% of the product of photosynthesis in 10- to 85-year-old stands of beech is used in respiration. The rate of respiration approximately doubles with each increase in temperature of 10°C so warm nights decrease the amount of carbohydrate available for growth. The temperature dependence of net photosynthesis of various species is discussed at length by Larcher (1980, pp 111–117). Kramer (1957) found that decreasing the night temperature from 23 to 17°C resulted in a large increase in height growth of loblolly pine seedlings, and Decker (1944) found that, although the rate of dark respiration of loblolly and red pine seedlings increased rapidly in response to an increase in temperature from 20 to 35°C, the rate of photosynthesis decreased at higher temperatures, greatly reducing the net amount of photosynthate available for growth.

The lower shaded branches of trees in closed stands often have such a low rate of photosynthesis that they can be regarded as parasitic, and should be removed. Relevant to this suggestion is the observation of Labyak and Schumacher (1954) that a crown comprising the upper third of the tree height can support normal stem growth in loblolly pine plantations, a view supported by data of Young and Kramer (1952) and others. Perhaps we need trees with more rapid natural pruning of their lower branches as they become shaded. In conclusion, the benefits from reducing dark respiration may be more important than those obtained by attempting to increase photosynthesis.

Translocation and partitioning of photosynthate

In agriculture much of the increase in yield obtained in recent decades resulted from an increase in the proportion of total photosynthate allocated to the marketable product, such as fruits, seeds, tubers, or leaves, known as the "harvest index" (Gifford and Evans 1981, Gifford et al. 1984). For example, in wheat this proportion has risen to over 50%. Less attention has been given to the possibility of increasing the percentage of photosynthate incorporated into stem wood in trees. This may not be important if the entire tree is harvested for pulp, but it certainly is very important where saw timber is the chief marketable product. There is often considerable competition for carbohydrate among various organs and tissues which have different sink "strengths." Wood formation seems to be a relatively weak sink, whereas fruits and seeds are very strong sinks, and a heavy crop of flowers, fruits, and seeds often significantly reduces diameter and root growth, and leaf area of trees (Morris 1951, Nutman 1933). Over 50% of the total photosynthate goes into root production in some trees (Kramer 1983, pp 152-155) and Harris et al. (1977) reported that the dry weight of roots produced by southern pines and deciduous trees was much greater than the weight of aboveground wood produced. Caldwell (1976) discussed root extension and found it difficult to justify the energy cost of annual replacement of a large fraction of the fine root population of perennial plants. The rapid turnover of fine roots is particularly puzzling because significant amounts of water and minerals can be absorbed through suberized roots (Chung and Kramer 1975).

Thus it appears that an excessive amount of photosynthate is diverted into root and branch growth. Perhaps trees produce more roots than are necessary for good growth, except on dry sites deficient in minerals. They may also produce or retain more branches than necessary (Labyak and Schumacher 1954, Young and Kramer 1952). It seems probable that research on the control of partitioning of photosynthate and selection for allocation of a larger percentage into growth of the trunk might increase productivity. The problem of photosynthate allocation is discussed in other papers in this volume.

In summary, it appears likely that research dealing with allocation of photosynthate between respiration and new tissue, and among the various organs of trees will be useful. Investigation of differences in physiological processes among species and among families and provenances within a species may identify types

useful for planting in specific environments. Thus it seems possible that in the future there will be increasing use of strains with special physiological characteristics that adapt them to particular environments instead of simply planting a species over a large area without consideration of local environmental conditions. For example, selection might emphasize tolerance of poor aeration for planting on wet sites, tolerance of aluminum on acid soils, and root systems that grow well at low temperatures for areas where planting is done in the winter. Perhaps genotypes tolerant of air pollutants and more effective in using the increasing concentration of CO₂ will be found.

Lack of space prevents discussion of other important areas of tree physiology such as nitrogen metabolism, dormancy of seeds and trees, and growth regulators, for which readers are referred to Kramer and Kozlowski (1979). The physiological factors affecting the quality of pine seedlings were reviewed by Kramer and Rose (1986).

Environmental limitations on growth

The discussion of physiological limitations assumed that trees were growing in a favorable environment, but this is seldom true. In fact it seems safe to state that forest productivity is limited more often and more severely by environmentally-induced stresses than by deficiencies in the potential physiological processes of the trees themselves. Formerly the most common environmental stresses have been lack of water, minerals, and nitrogen, and temperature perturbations such as unseasonable frosts. To these must now be added the effects of the increasing concentration of CO₂ in the atmosphere and the presence of various atmospheric pollutants. These new stresses greatly complicate the problem of evaluating the relative importance of various environmental stresses on forest productivity.

Also, the losses in production caused by unfavorable environmental conditions become more serious as the genetic potential for growth improves. Thus more effort needs to be expended on identification of environmental constraints and of the physiological processes through which they operate to reduce growth.

Water stress

Physiological processes of forest trees are inhibited more often by water stress than by any other single factor. Growth is reduced directly by decreased cell enlargement and indirectly because of decreased leaf area, stomatal closure, and damage to the photosynthetic machinery. All of these effects reduce the photosynthetic production of the whole plant and decrease the amount of carbohydrate available for growth. Information is needed to elucidate how mild water stress affects enzyme-mediated processes, the reasons for differences in tolerance of protoplasmic dehydration, the importance of osmotic adjustment, and the advantages and disadvantages of prompt stomatal closure under mild water stress. The relationship between water stress and susceptibility to attacks by pathogens and insects also deserves investigation. Also, one may ask if irrigation will become

economically worthwhile, especially for plantations of trees with a high growth potential planted on fertile soil in areas subject to droughts.

Many aspects of the water relations of woody plants are discussed in Volumes II and VI of the series on Water Deficits and Plant Growth, edited by Kozlowski, and Tyree (1976) discussed some characteristics that might be important in screening for drought tolerance.

Although water deficits are the most common problem in the water economy of trees, flooded soil sometimes causes injury. An excess of water resulting in the possibility of injury to root systems from deficient aeration influences silvicultural practices and the choice of species for planting in the lowlands of the southern United States, where flooding is common. Excess soil water also often causes injury to ornamental trees and shrubs native to dry habitats, partly by increasing the incidence of injury to root systems by fungi when lawns are irrigated intensively. The physiological effects of flooding are discussed in a recent book edited by Kozlowski (1984) and in other papers in this volume.

Temperature

The injury caused by late spring and early autumn frosts is well known and largely unavoidable, except by choice of tolerant species in areas subject to freezing. However, the more subtle effects of a few degrees of difference in temperature caused by differences in altitude or latitude or by local microclimates are seldom considered. The writer found that cool nights increased the growth of loblolly pine seedlings in a phytotron (Kramer 1957) and it is well established that different amounts of chilling are necessary to break dormancy of various kinds of trees of the temperate zones. Perhaps it would be worthwhile to learn if there are differences in growth related to temperature among genotypes or families within a species of sufficient importance to select one family for a warm site and another for a cool site. It is already established that there are large differences in rate of root growth at low temperatures among families of loblolly pine (Carlson 1986). Considerable differences might also exist with respect to the effects of temperature on bud break and shoot growth (Carlson 1985). If so, families with different temperature requirements could be planted on the sites where temperatures are most favorable for them.

Soil conditions

Forest soils often are deficient in nitrogen and sometimes in phosphorus and other elements. However, forest fertilization is too large and complex a problem to be discussed here, except to point out that mineral deficiencies operate by inhibiting physiological processes. This may result in decreased leaf area, reduction in chlorophyll, interference with stomatal reactions, injury to membranes, and other metabolic disturbances. An excess of toxic elements such as aluminum, copper, and manganese can also reduce growth, and air pollution can cause indirect injury by acidification of soils low in buffering capacity.

Physical properties of the soil that affect water-holding capacity, aeration, and

depth of root penetration also are important. For example, Coile (1948) found that site quality for pine in the lower Piedmont of North Carolina was closely correlated with depth of the A horizon and the physical properties of the B horizon that affect root growth.

Carbon dioxide

There is much interest in the effects of the increasing concentration of atmospheric CO₂ on tree growth. Experiments in controlled environments indicate that it decreases stomatal conductance and transpiration, but increases photosynthesis, leaf area, and seedling growth (Sionit et al. 1985, Kramer and Sionit 1986). A high concentration of CO₂ will partly compensate for high temperature, water stress, and low irradiance, and increase water use efficiency (Lemon 1983, Tolley and Strain 1984). Thus there are important interactions between CO₂ concentration and other stresses that deserve intensive investigation, as shown in Figure 2. Predictions of increasing temperatures and decreasing rainfall in some parts of the United States suggest that there may be important indirect effects of increasing concentration of CO₂ on the range of some tree species.

Air pollution

The newest environmental stress, air pollution, is becoming widely publicized under the term "acid rain." Acidification may cause direct injury in lakes and soils low in buffering capacity and it may also result in indirect injury by causing increased concentration of toxic elements such as aluminum. In addition to the indirect effects of acidification on soil, the injury from ozone and oxides of nitrogen and sulfur may be increased by acidification. Thus the effects of air pollutants are very complex and require more investigation because they may soon become a major constraint on forest productivity. It seems possible that an interac-

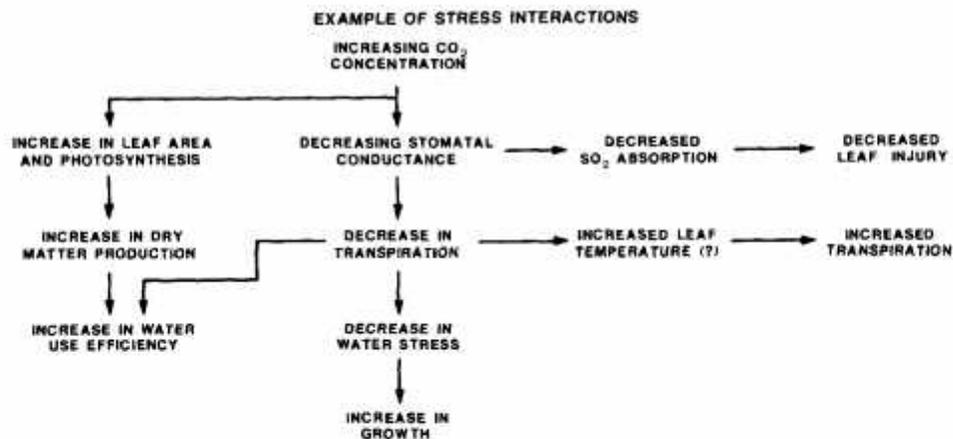


Figure 2. Diagram showing some interactions between increasing concentration of CO₂ in the atmosphere and photosynthesis, transpiration, and injury by air pollutants.

tion between atmospheric CO₂ and pollutants may occur because decreased stomatal conductance resulting from increasing CO₂ concentration (Figure 2) may decrease susceptibility to injury by air pollutants (Carlson and Bazzaz 1982, Shanklin and Kozlowski 1985).

Injury by pathogens and insects

Trees are always subject to attack by insects and pathogenic fungi. However, occasionally insect infestations such as the recent gypsy moth infestation in the Northeast or the current outbreak of the southern pine beetle in Texas and Louisiana create catastrophic conditions. A fungus disease wiped out the chestnut early in this century, Dutch elm disease eliminated elms in some areas and some readers can recall the little leaf disease of shortleaf pine a few decades ago. Fusiform rust is a continuing threat to pine seedlings. At least one paper on tree physiology in relation to factors affecting southern pine beetle attack appears in this volume (Lorio and Sommers 1986), and much more research needs to be done on the ecology and physiology of insect pests and diseases of trees. For example, the increasing concentration of atmospheric CO₂ may affect the feeding habits of leaf-eating insects (Lincoln et al. 1985).

Interaction of Stresses

The evaluation of the relative importance of various stresses is complicated by the frequent interaction among them. The conditions favorable for development of water stress often include abnormally high temperatures, and drying soil not only decreases water uptake, but also reduces mineral uptake. As mentioned earlier, increasing concentration of atmospheric carbon dioxide increases leaf area and photosynthesis, but decreases transpiration per unit leaf area and sometimes decreases injury from air pollution. Often it is desirable to conduct experiments in controlled environments where various factors can be controlled separately in order to evaluate their importance. An example of some interactions is shown in Figure 2.

Modeling

Sometimes modeling provides a helpful approach to complex problems such as the effects of stress. Ledig (1976) discussed the problems of modeling tree growth in some detail and considered some of the approaches, including the roles of tree canopies, respiration and the allocation of photosynthate. Several papers involving the use of models are found in this volume.

Future Developments

Limitations on the contributions from physiology

Although plant physiologists have accumulated much information concerning plant processes, mineral nutrition, water relations, growth regulators, and herbicides, plant physiology has contributed less to forestry than it should. One reason

is that physiologists have been more interested in exploring the mechanisms of physiological processes than in using their knowledge of physiology to assist in solving the problems of growing plants. To phrase it another way, physiology tends to be retrospective rather than predictive, but in forestry we need to predict the effects of various factors on growth.

Another difficulty is that much useful physiological information never reaches the potential users in the field. There are several reasons for this. One is administrative barriers that hinder communication among scientists working on different problems or even on the same problem, but in different administrative units. Another is the psychological barrier that sometimes develops between field and laboratory workers. We need more farsighted, scientifically trained administrators who can bridge these gaps. A third problem is that useful information is often lost in the flood of scientific literature. Even modern computerized systems for the retrieval of information cannot entirely solve this problem because important information is often buried in tables and discussions and its presence is not revealed by the title or even in the abstract.

An example of the problem of specialization is the current tendency to separate cell physiology from whole plant physiology. The important advances made in biology by research at the cellular and subcellular level have led some administrators and scientists to conclude that it is the only area deserving support. As a result of this overemphasis some young scientists are well trained in cell physiology but know little about whole plant physiology. This is unfortunate because the goal of research in plant science presumably is to provide a better overall understanding of plant growth, and cell and whole plant physiology should play complementary roles in this. Agricultural and forestry problems are recognized first in the field at the whole plant level, and it then becomes the task of physiologists to explain them in terms of disturbances of essential physiological processes. Although ultimate explanations of injury are often found at the cellular or subcellular level such as injury to membranes or disturbance of enzyme-mediated processes, the remedies are usually found at the whole plant level in terms of silvicultural treatments such as thinning, pruning, fertilization, or irrigation. Drought tolerance depends more often on postponement of dehydration by extensive root systems and good stomatal and cuticular control of transpiration than on protoplasmic tolerance of dehydration. Yield is limited more often by leaf area, leaf area duration, and the partitioning of photosynthate than by deficiencies in carboxylation and electron transport. Thus from the standpoint of agriculture and forestry cell physiology is valuable chiefly as a supplement to whole plant physiology.

Emphasis on the importance of whole plant physiology in forestry and agriculture in no way denies that ultimate explanations of processes generally are found at the cellular or subcellular level. An analogous situation exists in the physical sciences where quantum mechanics is necessary to explain the nature of matter but classical Newtonian physics explains such every day affairs as the operation of autos, airplanes, and refrigerators.

How to increase the usefulness of physiology

The most important step that can be taken toward increasing the usefulness of physiology in forestry is to develop a better understanding of its role. Nearly 40 years ago the writer (Kramer 1948) pointed out what is shown in Figure 1 of this paper, that the physiological processes of trees are the machinery through which genetic potential and environment operate to determine the quantity and quality of growth. Unfortunately, neither foresters nor physiologists have paid much attention to the concept. To reemphasize the importance of that concept, it is argued again that changes in genotype and in silvicultural practices are effective only to the extent that they increase the efficiency of the basic physiological processes. This indicates the need for better cooperation among forest geneticists, silviculturists, and physiologists in identifying the physiological limitations to growth and in finding remedies for them. Only when cooperation becomes routine will physiology attain its full usefulness in forestry.

References

- Bonga, J.M. and D.J. Durzan. 1982. Tissue culture in forestry. M. Nijhoff/W. Junk, The Hague.
- Boyer, J.S. 1982. Plant productivity and environment. *Science* 218:443-448.
- Brix, H. 1983. Effects of thinning and nitrogen fertilization on growth of Douglas fir: relative contribution of foliage quantity and efficiency. *Can. J. For. Res.* 13:167-175.
- Büsgen, M. 1897. *Bau und leben unserer Waldbäume*. G. Fischer, Jena.
- Büsgen, M. and E. Münch. 1931. *The Structure and Life of Forest Trees*. 3rd Ed. (Eng. transl. by T. Thomson). J. Wiley & Sons, New York.
- Caldwell, M. M. 1986. Root extension and water absorption. In *Water and Plant Life*. Eds. O.L. Lang, L. Kappen, and E.-D. Schulze. Springer-Verlag, Berlin. pp 63-85.
- Carlson, W.C. 1985. Effects of natural chilling and of cold storage on bud break and root growth potential of loblolly pine (*Pinus taeda* L.). *Can. J. For. Res.* 15:651-656.
- Carlson, W.C. 1986. Root system considerations in the quality of loblolly pine seedlings. *Sou. J. Appl. For.* 10: 87-92.
- Carlson, R.W. and F.A. Bazzaz. 1982. Photosynthetic and growth response to fumigation with SO₂ at elevated CO₂ for C₃ and C₄ plants. *Oecologia* 54: 50-54.
- Chung, H.H. and P.J. Kramer. 1975. Absorption of water and ³²P through suberized and unsuberized roots of loblolly pine. *Can. J. For. Res.* 5:229-235.
- Chung, H.H. and R.L. Barnes. 1977. Photosynthate allocation in *Pinus taeda*. I Substrate requirements for synthesis of shoot biomass. *Can. J. For. Res.* 7:106-111.
- Coile, T.C. 1948. Relation of soil characteristics to site index of loblolly and shortleaf pines in the lower Piedmont region of North Carolina. *Duke Univ. School of Forestry Bull.* 14.
- Decker, J.P. 1944. Effect of temperature on photosynthesis and respiration of red pine. *Plant Physiol.* 19: 679-688.
- Dodds, J.H. 1983. *Tissue culture of trees*. Cambridge University Press, U.K.
- Farnum, P., R. Timmis, and J.L. Kulp. 1983. Biotechnology of forest yield. *Science* 219:694-702.
- Fiscus, E.L. 1984. Integrated stomatal opening as an indicator of water stress in *Zea*. *Crop Sci.* 24:245-249.
- Gifford, R.M. and L.T. Evans. 1981. Photosynthesis, carbon partitioning and yield. *Ann. Rev. Plant Physiol.* 32:485-509.
- Gifford, R.M., J.H. Thorne, W.D. Hitz, and R.T. Giaquinta. 1984. Crop productivity and photoassimilate partitioning. *Science* 224:801-808.
- Hales, S. 1727. *Vegetable staticks*. W. and J. Innys and T. Woodward. London Scientific Book Guild, London. 1961.

- Harris, W.F., R.S. Dinerson, Jr., and N.T. Edwards. 1977. Comparison of belowground biomass of natural deciduous forests and loblolly pine plantations. *In* The Belowground Ecosystem. Ed. J.K. Marshall. Range Sci. Dept. Sci. Ser. No. 26, Colorado State University, Fort Collins, Colorado, pp 29–37.
- Hartig, T. 1878. *Anatomie und Physiologie der Holzpflanzen*. J. Springer, Berlin.
- Heinicke, A.J. and N.F. Childers. 1937. The daily rate of photosynthesis, during the growing season of 1935, of a young apple tree of bearing age. Mem. 201. Cornell Univ. Agr. Exp. Sta. Ithaca, New York.
- Helms, J.A. 1976. Factors influencing net photosynthesis in trees: an ecological viewpoint. *In* Tree Physiology and Yield Improvement. Eds. M.G.R. Cannell and F.T. Last. Academic Press, London, pp 55–78.
- Jones, C.G., A.W. Edson, and W.J. Morse. 1903. The maple sap flow. Bull. 103. Vermont Agr. Exp. Sta. Burlington, Vermont.
- Karnosky, D.F. 1981. Potential for forest tree improvement via tissue culture. *BioScience* 31: 114–120.
- Kozlowski, T.T. 1968–81. *Water deficits and plant growth*. Vols. I–VI. Academic Press, New York.
- Kozlowski, T.T. 1984. *Flooding and plant growth*. Academic Press, Orlando, Florida.
- Kramer, P.J. 1948. Plant physiology in forestry. *J. For.* 46:918–921.
- Kramer, P.J. 1957. Some effects of various combinations of day and night temperatures and photoperiod on the height growth of loblolly pine seedlings. *For. Sci.* 3:45–55.
- Kramer, P.J. 1983. *Water relations of plants*. Academic Press, New York.
- Kramer, P.J. and T.T. Kozlowski. 1979. *Physiology of woody plants*. Academic Press, New York.
- Kramer, P.J. and R.W. Rose, Jr. 1986. Physiological characteristics of loblolly pine seedlings in relation to field performances. *In* Proc. Int. Symp. on Nursery Management for Southern Pines. Ed. D.B. South. Alabama Agric. Expt. Station, Auburn, Alabama. pp 416–440.
- Kramer, P.J. and N. Sionit. 1986. Effects of increasing CO₂ concentration on the physiology and growth of forest trees. *In* The Greenhouse Effect, Climate Change, and Forest Management in the United States. Eds. W.E. Shands and John E. Hoffman. The Conservation Foundation, Washington, D.C. (in press).
- Labyak, L.F. and F.X. Schumacher. 1954. The contribution of its branches to the main stem growth of loblolly pine. *J. For.* 52:333–337.
- Larcher, W. 1980. *Physiological plant ecology*. 2nd Ed. Springer-Verlag, Berlin.
- Ledig, F.T. 1976. Physiological genetics, photosynthesis and growth models. *In* Tree Physiology and Yield Improvement. Eds. M.G.R. Cannell and F.T. Last. Academic Press, London. pp 21–54.
- Lemon, E.R. 1983. *CO₂ and plants*. Westview Press, Boulder, Colorado.
- Lincoln, D.E., N. Sionit, and B.R. Strain. 1985. Growth and feeding responses of *Pseudoplusia includens* (Lepidoptera, Noctuidae) to host plant growth in controlled carbon dioxide atmosphere. *J. Environ. Entom.* 13:1527–1530.
- Lorio, P.L. and R.A. Sommers. 1986. Evidence of competition between growth processes and oleoresin synthesis for available photosynthates in *Pinus taeda* L. *Tree Physiol.* 2:000.000
- Meyer, B.S. and D.B. Anderson. 1939. *Plant physiology*. D. Van Nostrand Co., New York.
- Miller, H.G. and J.D. Miller. 1976. Effect of nitrogen supply on net primary productivity in Corsican pine. *J. Appl. Ecol.* 13:249–256.
- Möller, Carl Mar, D. Müller, and Jörgen Nielsen. 1954. Ein Diagramm der Stoffproduktion im Buchenwald. (A diagram of the production of matter in beech forest.) *Ber. Schweiz. Bot. Ges.* 64:487–494.
- Morris, R.F. 1951. The effects of flowering on the foliage production and growth of balsam fir. *For. Chron.* 27:40–57.
- Nutman, F.J. 1933. The root system of *Coffea arabica*. Part II The effect of some soil conditions in modifying the "normal" root-system. *Empire J. Exp. Agr.* 1:285–296.
- Renner, O. 1912. Versuche zur Mechanik der Wasserversorgung. 2. Über wurzeltätigkeit. *Ber. deut. bot. Ges.* 30:642–648.
- Shanklin, J. and T.T. Kozlowski. 1985. Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO₂. *Environ. Pollut. (Ser. A)* 38:199–212.
- Sionit, N., B.R. Strain, G.H. Riechers, and C.h. Jaeger. 1985. Long-term atmospheric CO₂ enrichment affects the growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. for. Res.* 15:468–471.

- Slatyer, R.O., and S.A. Taylor. 1960. Terminology in plant-soil-water relations. *Nature* 187:922-924.
- Tamm, C.O. 1977. Factors limiting primary production in the boreal forest—long-term and short-term considerations. *In* Bicentenary Celebration of C.P. Thunberg's visit to Japan. Royal Swedish Embassy and Bot. Soc. of Japan, Tokyo. pp 52-59.
- Tang, P.S. and J.S. Wang. 1941. A thermodynamic formulation of the water relations in an isolated living cell. *J. Phys. Chem.* 45:443-453.
- Tolley, L.C. and B.R. Strain. 1984. Effects of CO₂ enrichment and water stress on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. Bot.* 62: 2135-2139.
- Tyree, M.T. 1976. Physical parameters of the soil-plant-atmosphere system: breeding for drought resistance characteristics that might improve wood yield. *In* Tree Physiology and Yield Improvement. Ed. M.G.R. Cannell and F.T. Last. Academic Press, New York. pp 329-348.
- Watson, J.D. 1952. The physiological basis of variation in yield. *Adv. Agron.* IV:101-145.
- Williams, S. 1794. The natural and civil history of Vermont. Walpole, Vermont, USA.
- Young, H.E. and P.J. Kramer. 1952. The effect of pruning on the height and diameter growth of loblolly pine. *J. For.* 50:474-479.
- Zelitch, I. 1975. Improving the efficiency of photosynthesis. *Science* 188:626-633.

CHAPTER 12

MINERAL NUTRITION

12.1 *Methods to Study the Mineral Requirements of Plants*

12.2 *Essential Mineral Elements*

12.3 *Mechanism of Absorption of Elements*

12.4 *Translocation of Solutes*

12.5 *Soil as Reservoir of Essential Elements*

12.6 *Metabolism of Nitrogen*

The basic needs of all living organisms are essentially the same. They require macromolecules, such as carbohydrates, proteins and fats, and water and minerals for their growth and development.

This chapter focusses mainly on inorganic plant nutrition, wherein you will study the methods to identify elements essential to growth and development of plants and the criteria for establishing the essentiality. You will also study the role of the essential elements, their major deficiency symptoms and the mechanism of absorption of these essential elements. The chapter also introduces you briefly to the significance and the mechanism of biological nitrogen fixation.

12.1 METHODS TO STUDY THE MINERAL REQUIREMENTS OF PLANTS

In 1860, Julius von Sachs, a prominent German botanist, demonstrated, for the first time, that plants could be grown to maturity in a defined nutrient solution in complete absence of soil. This technique of growing plants in a nutrient solution is known as **hydroponics**. Since then, a number of improvised methods have been employed to try and determine the mineral nutrients essential for plants. The essence of all these methods involves the culture of plants in a soil-free, defined mineral solution. These methods require purified water and mineral nutrient salts. *Can you explain why this is so essential?*

After a series of experiments in which the roots of the plants were immersed in nutrient solutions and wherein an element was added / removed or given in varied concentration, a mineral solution suitable for

the plant growth was obtained. By this method, essential elements were identified and their deficiency symptoms discovered. Hydroponics has been successfully employed as a technique for the commercial production of vegetables such as tomato, seedless cucumber and lettuce. It must be emphasised that the nutrient solutions must be adequately aerated to obtain the optimum growth. What would happen if solutions were poorly aerated? Diagrammatic views of the hydroponic technique is given in Figures 12.1 and 12.2.

12.2 ESSENTIAL MINERAL ELEMENTS

Most of the minerals present in soil can enter plants through roots. In fact, more than sixty elements of the 105 discovered so far are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium. There are techniques that are able to detect the minerals even at a very low concentration (10^{-8} g/mL). The question is, whether all the diverse mineral elements present in a plant, for example, gold and selenium as mentioned above, are really necessary for plants? How do we decide what is essential for plants and what is not?

12.2.1 Criteria for Essentiality

The criteria for essentiality of an element are given below:

- The element must be absolutely necessary for supporting normal growth and reproduction. In the absence of the element the plants do not complete their life cycle or set the seeds.
- The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element cannot be met by supplying some other element.
- The element must be directly involved in the metabolism of the plant.

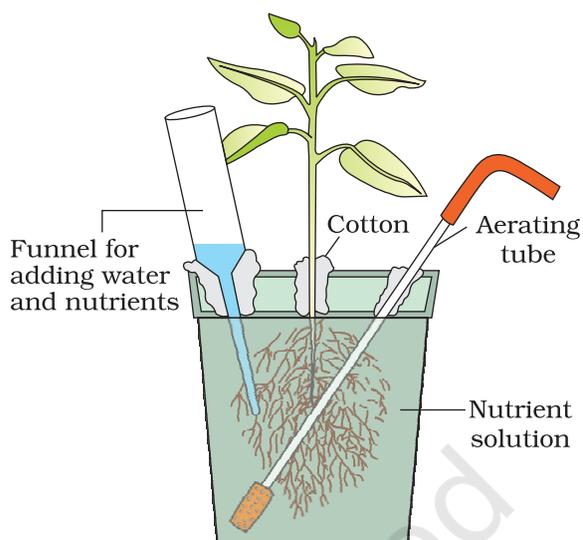


Figure 12.1 Diagram of a typical set-up for nutrient solution culture

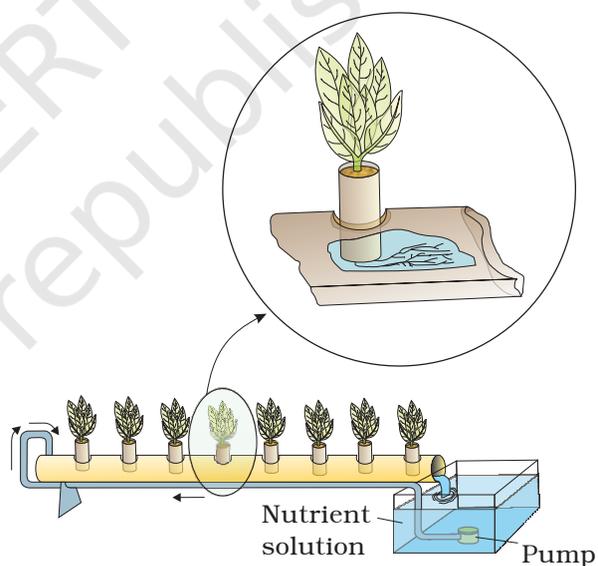


Figure 12.2 Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots are continuously bathed in aerated nutrient solution. The arrows indicate the direction of the flow.

Based upon the above criteria only a few elements have been found to be absolutely essential for plant growth and metabolism. These elements are further divided into two broad categories based on their quantitative requirements.

- (i) Macronutrients, and
- (ii) Micronutrients

Macronutrients are generally present in plant tissues in large amounts (in excess of 10 mmole Kg^{-1} of dry matter). The macronutrients include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium. Of these, carbon, hydrogen and oxygen are mainly obtained from CO_2 and H_2O , while the others are absorbed from the soil as mineral nutrition.

Micronutrients or trace elements, are needed in very small amounts (less than 10 mmole Kg^{-1} of dry matter). These include iron, manganese, copper, molybdenum, zinc, boron, chlorine and nickel.

In addition to the 17 essential elements named above, there are some beneficial elements such as sodium, silicon, cobalt and selenium. They are required by higher plants.

Essential elements can also be grouped into four broad categories on the basis of their diverse functions. These categories are:

- (i) Essential elements as components of biomolecules and hence structural elements of cells (e.g., carbon, hydrogen, oxygen and nitrogen).
- (ii) Essential elements that are components of energy-related chemical compounds in plants (e.g., magnesium in chlorophyll and phosphorous in ATP).
- (iii) Essential elements that activate or inhibit enzymes, for example Mg^{2+} is an activator for both ribulose biphosphate carboxylase-oxygenase and phosphoenol pyruvate carboxylase, both of which are critical enzymes in photosynthetic carbon fixation; Zn^{2+} is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism. *Can you name a few more elements that fall in this category?* For this, you will need to recollect some of the biochemical pathways you have studied earlier.
- (iv) Some essential elements can alter the osmotic potential of a cell. Potassium plays an important role in the opening and closing of stomata. You may recall the role of minerals as solutes in determining the water potential of a cell.

12.2.2 Role of Macro- and Micro-nutrients

Essential elements perform several functions. They participate in various metabolic processes in the plant cells such as permeability of cell

membrane, maintenance of osmotic concentration of cell sap, electron-transport systems, buffering action, enzymatic activity and act as major constituents of macromolecules and co-enzymes.

Various forms and functions of essential nutrient elements are given below.

Nitrogen : This is the essential nutrient element required by plants in the greatest amount. It is absorbed mainly as NO_3^- though some are also taken up as NO_2^- or NH_4^+ . Nitrogen is required by all parts of a plant, particularly the meristematic tissues and the metabolically active cells. Nitrogen is one of the major constituents of proteins, nucleic acids, vitamins and hormones.

Phosphorus: Phosphorus is absorbed by the plants from soil in the form of phosphate ions (either as H_2PO_4^- or HPO_4^{2-}). Phosphorus is a constituent of cell membranes, certain proteins, all nucleic acids and nucleotides, and is required for all phosphorylation reactions.

Potassium: It is absorbed as potassium ion (K^+). In plants, this is required in more abundant quantities in the meristematic tissues, buds, leaves and root tips. Potassium helps to maintain an anion-cation balance in cells and is involved in protein synthesis, opening and closing of stomata, activation of enzymes and in the maintenance of the turgidity of cells.

Calcium: Plant absorbs calcium from the soil in the form of calcium ions (Ca^{2+}). Calcium is required by meristematic and differentiating tissues. During cell division it is used in the synthesis of cell wall, particularly as calcium pectate in the middle lamella. It is also needed during the formation of mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes. It activates certain enzymes and plays an important role in regulating metabolic activities.

Magnesium: It is absorbed by plants in the form of divalent Mg^{2+} . It activates the enzymes of respiration, photosynthesis and are involved in the synthesis of DNA and RNA. Magnesium is a constituent of the ring structure of chlorophyll and helps to maintain the ribosome structure.

Sulphur: Plants obtain sulphur in the form of sulphate (SO_4^{2-}). Sulphur is present in two amino acids – cysteine and methionine and is the main constituent of several coenzymes, vitamins (thiamine, biotin, Coenzyme A) and ferredoxin.

Iron: Plants obtain iron in the form of ferric ions (Fe^{3+}). It is required in larger amounts in comparison to other micronutrients. It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochromes. It is reversibly oxidised from Fe^{2+} to Fe^{3+} during electron transfer. It activates catalase enzyme, and is essential for the formation of chlorophyll.

Manganese: It is absorbed in the form of manganous ions (Mn^{2+}). It activates many enzymes involved in photosynthesis, respiration and nitrogen metabolism. The best defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis.

Zinc: Plants obtain zinc as Zn^{2+} ions. It activates various enzymes, especially carboxylases. It is also needed in the synthesis of auxin.

Copper: It is absorbed as cupric ions (Cu^{2+}). It is essential for the overall metabolism in plants. Like iron, it is associated with certain enzymes involved in redox reactions and is reversibly oxidised from Cu^+ to Cu^{2+} .

Boron : It is absorbed as BO_3^{3-} or $\text{B}_4\text{O}_7^{2-}$. Boron is required for uptake and utilisation of Ca^{2+} , membrane functioning, pollen germination, cell elongation, cell differentiation and carbohydrate translocation.

Molybdenum : Plants obtain it in the form of molybdate ions (MoO_4^{2-}). It is a component of several enzymes, including nitrogenase and nitrate reductase both of which participate in nitrogen metabolism.

Chlorine : It is absorbed in the form of chloride anion (Cl^-). Along with Na^+ and K^+ , it helps in determining the solute concentration and the anion-cation balance in cells. It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.

12.2.3 Deficiency Symptoms of Essential Elements

Whenever the supply of an essential element becomes limited, plant growth is retarded. The concentration of the essential element below which plant growth is retarded is termed as **critical concentration**. The element is said to be deficient when present below the critical concentration.

Since each element has one or more specific structural or functional role in plants, in the absence of any particular element, plants show certain morphological changes. These morphological changes are indicative of certain element deficiencies and are called deficiency symptoms. The deficiency symptoms vary from element to element and they disappear when the deficient mineral nutrient is provided to the plant. However, if deprivation continues, it may eventually lead to the death of the plant. The parts of the plants that show the deficiency symptoms also depend on the mobility of the element in the plant. For elements that are actively mobilised within the plants and exported to young developing tissues, the deficiency symptoms tend to appear first in the older tissues. For example, the deficiency symptoms of nitrogen, potassium and magnesium are visible first in the senescent leaves. In the older leaves, biomolecules containing these elements are broken down, making these elements available for mobilising to younger leaves.

The deficiency symptoms tend to appear first in the young tissues whenever the elements are relatively immobile and are not transported out of the mature organs, for example, element like calcium is a part

of the structural component of the cell and hence is not easily released. This aspect of mineral nutrition of plants is of a great significance and importance to agriculture and horticulture.

The kind of deficiency symptoms shown in plants include chlorosis, necrosis, stunted plant growth, premature fall of leaves and buds, and inhibition of cell division. Chlorosis is the loss of chlorophyll leading to yellowing in leaves. This symptom is caused by the deficiency of elements N, K, Mg, S, Fe, Mn, Zn and Mo. Likewise, necrosis, or death of tissue, particularly leaf tissue, is due to the deficiency of Ca, Mg, Cu, K. Lack or low level of N, K, S, Mo causes an inhibition of cell division. Some elements like N, S, Mo delay flowering if their concentration in plants is low.

You can see from the above that the deficiency of any element can cause multiple symptoms and that the same symptoms may be caused by the deficiency of one of several different elements. Hence, to identify the deficient element, one has to study all the symptoms developed in all the various parts of the plant and compare them with the available standard tables. We must also be aware that different plants also respond differently to the deficiency of the same element.

12.2.4 Toxicity of Micronutrients

The requirement of micronutrients is always in low amounts while their moderate decrease causes the deficiency symptoms and a moderate increase causes toxicity. In other words, there is a narrow range of concentration at which the elements are optimum. Any mineral ion concentration in tissues that reduces the dry weight of tissues by about 10 per cent is considered toxic. Such critical concentrations vary widely among different micronutrients. The toxicity symptoms are difficult to identify. Toxicity levels for any element also vary for different plants. Many a times, excess of an element may inhibit the uptake of another element. For example, the prominent symptom of manganese toxicity is the appearance of brown spots surrounded by chlorotic veins. It is important to know that manganese competes with iron and magnesium for uptake and with magnesium for binding with enzymes. Manganese also inhibits calcium translocation in shoot apex. Therefore, excess of manganese may, in fact, induce deficiencies of iron, magnesium and calcium. Thus, what appears as symptoms of manganese toxicity may actually be the deficiency symptoms of iron, magnesium and calcium. Can this knowledge be of some importance to a farmer? a gardener? or even for you in your kitchen-garden?

12.3 MECHANISM OF ABSORPTION OF ELEMENTS

Much of the studies on mechanism of absorption of elements by plants has been carried out in isolated cells, tissues or organs. These studies

revealed that the process of absorption can be demarcated into two main phases. In the first phase, an initial rapid uptake of ions into the 'free space' or 'outer space' of cells – the apoplast, is passive. In the second phase of uptake, the ions are taken in slowly into the 'inner space' – the symplast of the cells. The passive movement of ions into the apoplast usually occurs through ion-channels, the trans-membrane proteins that function as selective pores. On the other hand, the entry or exit of ions to and from the symplast requires the expenditure of metabolic energy, which is an **active** process. The movement of ions is usually called **flux**; the inward movement into the cells is influx and the outward movement, efflux. You have read the aspects of mineral nutrient uptake and translocation in plants in Chapter 11.

12.4 TRANSLOCATION OF SOLUTES

Mineral salts are translocated through xylem along with the ascending stream of water, which is pulled up through the plant by transpirational pull. Analysis of xylem sap shows the presence of mineral salts in it. Use of radioisotopes of mineral elements also substantiate the view that they are transported through the xylem. You have already discussed the movement of water in xylem in Chapter 11.

12.5 SOIL AS RESERVOIR OF ESSENTIAL ELEMENTS

Majority of the nutrients that are essential for the growth and development of plants become available to the roots due to weathering and breakdown of rocks. These processes enrich the soil with dissolved ions and inorganic salts. Since they are derived from the rock minerals, their role in plant nutrition is referred to as mineral nutrition. Soil consists of a wide variety of substances. Soil not only supplies minerals but also harbours nitrogen-fixing bacteria, other microbes, holds water, supplies air to the roots and acts as a matrix that stabilises the plant. Since deficiency of essential minerals affect the crop-yield, there is often a need for supplying them through fertilisers. Both macro-nutrients (N, P, K, S, etc.) and micro-nutrients (Cu, Zn, Fe, Mn, etc.) form components of fertilisers and are applied as per need.

12.6 METABOLISM OF NITROGEN

12.6.1 Nitrogen Cycle

Apart from carbon, hydrogen and oxygen, nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls and many of the vitamins. Plants compete with microbes for the limited nitrogen that

is available in soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural eco-systems. Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond ($N \equiv N$). The process of conversion of nitrogen (N_2) to ammonia is termed as **nitrogen-fixation**. In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO , NO_2 , N_2O). Industrial combustions, forest fires, automobile exhausts and power-generating stations are also sources of atmospheric nitrogen oxides. Decomposition of organic nitrogen of dead plants and animals into ammonia is called ammonification. Some of this ammonia volatilises and re-enters the atmosphere but most of it is converted into nitrate by soil bacteria in the following steps:



Ammonia is first oxidised to nitrite by the bacteria *Nitrosomonas* and/or *Nitrococcus*. The nitrite is further oxidised to nitrate with the help of the bacterium *Nitrobacter*. These steps are called **nitrification** (Figure 12.3). These nitrifying bacteria are **chemoautotrophs**.

The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of denitrification. Denitrification is carried by bacteria *Pseudomonas* and *Thiobacillus*.

12.6.2 Biological Nitrogen Fixation

Very few living organisms can utilise the nitrogen in the form N_2 , available abundantly in the air. Only certain prokaryotic species are capable of fixing nitrogen. Reduction of nitrogen to ammonia by living organisms is

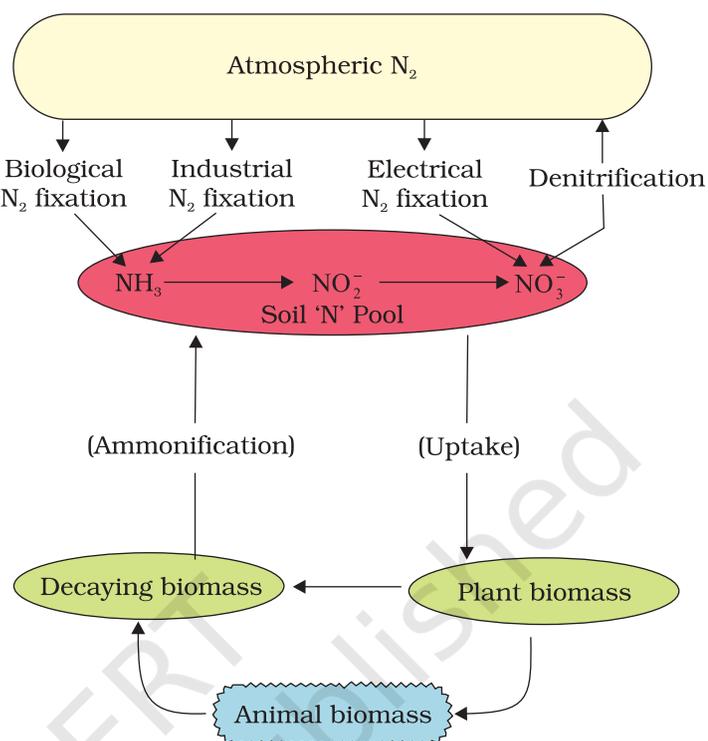
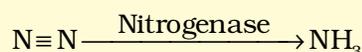


Figure 12.3 The nitrogen cycle showing relationship between the three main nitrogen pools – atmospheric soil, and biomass

called **biological nitrogen fixation**. The enzyme, nitrogenase which is capable of nitrogen reduction is present exclusively in prokaryotes. Such microbes are called N_2 -fixers.



The nitrogen-fixing microbes could be free-living or symbiotic. Examples of free-living nitrogen-fixing aerobic microbes are *Azotobacter* and *Beijernickia* while *Rhodospirillum* is anaerobic and *Bacillus* free-living. In addition, a number of cyanobacteria such as *Anabaena* and *Nostoc* are also free-living nitrogen-fixers.

Symbiotic biological nitrogen fixation

Several types of symbiotic biological nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* has such relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, *Frankia*, also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., *Alnus*). Both *Rhizobium* and *Frankia* are free-living in soil, but as symbionts, can fix atmospheric nitrogen.

Uproot any one plant of a common pulse, just before flowering. You will see near-spherical outgrowths on the roots. These are nodules. If you cut through them you will notice that the central portion is red or pink. What makes the nodules pink? This is due to the presence of leguminous haemoglobin or leg-haemoglobin.

Nodule Formation

Nodule formation involves a sequence of multiple interactions between *Rhizobium* and roots of the host plant. Principal stages in the nodule formation are summarised as follows:

Rhizobia multiply and colonise the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair. An infection thread is produced carrying the bacteria into the cortex of the root, where they initiate the nodule formation in the cortex of the root. Then the bacteria are released from the thread into the cells which leads to the differentiation of specialised nitrogen fixing cells. The nodule thus formed, establishes a direct vascular connection with the host for exchange of nutrients. These events are depicted in Figure 12.4.

The nodule contains all the necessary biochemical components, such as the enzyme nitrogenase and leghaemoglobin. The enzyme nitrogenase is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia, (Figure 12.5) the first stable product of nitrogen fixation.

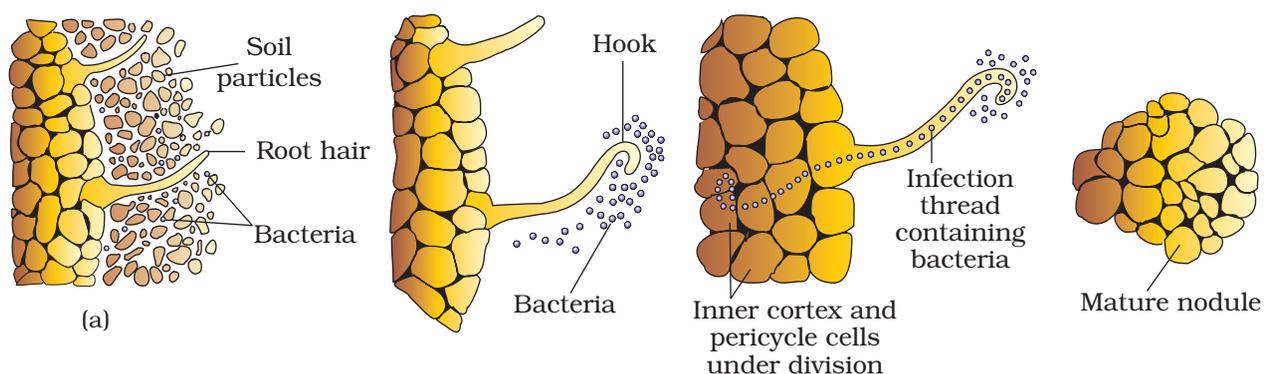
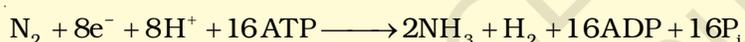


Figure 12.4 Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it, (b) Successful infection of the root hair causes it to curl, (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation, (d) A mature nodule is complete with vascular tissues continuous with those of the root

The reaction is as follows:



The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contains an oxygen scavenger called leg-haemoglobin. It is interesting to note that these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they become anaerobic (thus protecting the nitrogenase enzyme). You must have noticed in the above reaction that the ammonia synthesis by nitrogenase requires a

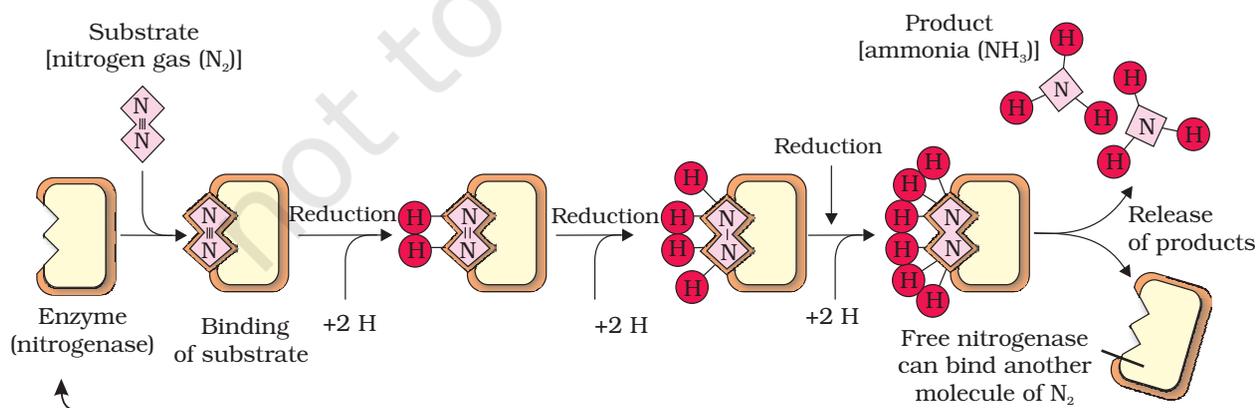
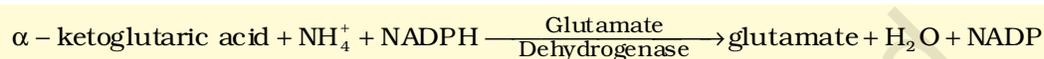


Figure 12.5 Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

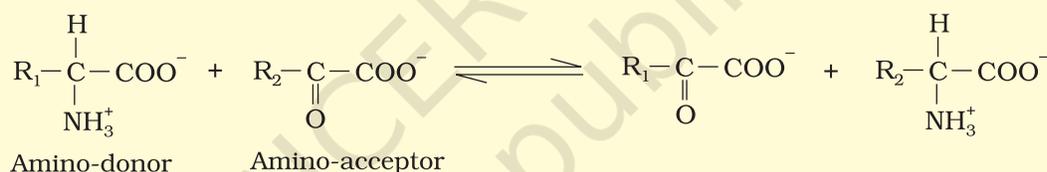
very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.

Fate of ammonia: At physiological pH, the ammonia is protonated to form NH_4^+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the latter is quite toxic to plants and hence cannot accumulate in them. Let us now see how the NH_4^+ is used to synthesise amino acids in plants. There are two main ways in which this can take place:

(i) Reductive amination : In these processes, ammonia reacts with α -ketoglutaric acid and forms glutamic acid as indicated in the equation given below :



(ii) Transamination : It involves the transfer of amino group from one amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH_2 , the amino group takes place and other amino acids are formed through transamination. The enzyme **transaminase** catalyses all such reactions. For example,



The two most important amides – asparagine and glutamine – found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid, respectively, by addition of another amino group to each. The hydroxyl part of the acid is replaced by another NH_2 radicle. Since amides contain more nitrogen than the amino acids, they are transported to other parts of the plant via xylem vessels. In addition, along with the transpiration stream the nodules of some plants (e.g., soyabean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

SUMMARY

Plants obtain their inorganic nutrients from air, water and soil. Plants absorb a wide variety of mineral elements. Not all the mineral elements that they absorb are required by plants. Out of the more than 105 elements discovered so far, less than 21 are essential and beneficial for normal plant growth and development. The elements required in large quantities are called macronutrients while those required in less quantities or in trace are termed as micronutrients. These elements are either essential constituents of proteins, carbohydrates, fats, nucleic acid etc.,

and/or take part in various metabolic processes. Deficiency of each of these essential elements may lead to symptoms called deficiency symptoms. Chlorosis, necrosis, stunted growth, impaired cell division, etc., are some prominent deficiency symptoms. Plants absorb minerals through roots by either passive or active processes. They are carried to all parts of the organism through xylem along with water transport.

Nitrogen is very essential for the sustenance of life. Plants cannot use atmospheric nitrogen directly. But some of the plants in association with N_2 -fixing bacteria, especially roots of legumes, can fix this atmospheric nitrogen into biologically usable forms. Nitrogen fixation requires a strong reducing agent and energy in the form of ATP. N_2 -fixation is accomplished with the help of nitrogen-fixing microbes, mainly *Rhizobium*. The enzyme nitrogenase which plays an important role in biological N_2 fixation is very sensitive to oxygen. Most of the processes take place in anaerobic environment. The energy, ATP, required is provided by the respiration of the host cells. Ammonia produced following N_2 fixation is incorporated into amino acids as the amino group.

EXERCISES

1. 'All elements that are present in a plant need not be essential to its survival'. Comment.
2. Why is purification of water and nutrient salts so important in studies involving mineral nutrition using hydroponics?
3. Explain with examples: macronutrients, micronutrients, beneficial nutrients, toxic elements and essential elements.
4. Name at least five different deficiency symptoms in plants. Describe them and correlate them with the concerned mineral deficiency.
5. If a plant shows a symptom which could develop due to deficiency of more than one nutrient, how would you find out experimentally, the real deficient mineral element?
6. Why is that in certain plants deficiency symptoms appear first in younger parts of the plant while in others they do so in mature organs?
7. How are the minerals absorbed by the plants?
8. What are the conditions necessary for fixation of atmospheric nitrogen by *Rhizobium*. What is their role in N_2 -fixation?
9. What are the steps involved in formation of a root nodule?
10. Which of the following statements are true? If false, correct them:
 - (a) Boron deficiency leads to stout axis.
 - (b) Every mineral element that is present in a cell is needed by the cell.
 - (c) Nitrogen as a nutrient element, is highly immobile in the plants.
 - (d) It is very easy to establish the essentiality of micronutrients because they are required only in trace quantities.

19. GROWTH ANALYSIS

Growth analysis can be used to account for growth in terms that have functional or structural significance. The type of growth analysis requires measurement of plant biomass and assimilatory area (leaf area) and methods of computing certain parameters that describe growth. The growth parameters that are commonly used in agricultural research and the name of the scientists who proposed the parameters are given below.

LAI	-	Williams (1946)
LAR	-	Radford (1967)
LAD	-	Power <i>et al.</i> (1967)
SLA	-	Kvet <i>et al.</i> (1971)
SLW	-	Pearce <i>et al.</i> (1968)
NAR	-	Williams (1946)
CGR	-	Watson (1956)
RGR	-	Williams (1946)
HI	-	Nichiporovich (1951)

i. Leaf Area

This is the area of photosynthetic surface produced by the individual plant over a period of interval of time and expressed in $\text{cm}^2 \text{ plant}^{-1}$.

ii. Leaf Area Index (LAI)

Williams (1946) proposed the term, Leaf Area Index (LAI). It is the ratio of the leaf of the crop to the ground area over a period of interval of time. The value of LAI should be optimum at the maximum ground cover area at which crop canopy receives maximum solar radiation and hence, the TDMA will be high.

$$\text{LAI} = \frac{\text{Total leaf area of a plant}}{\text{Ground area occupied by the plant}}$$

iii. Leaf Area Ratio (LAR)

The term, Leaf Area Ratio (LAR) was suggested by Radford (1967), expresses the ratio between the area of leaf lamina to the total plant biomass or the LAR reflects the

leafiness of a plant or amount of leaf area formed per unit of biomass and expressed in $\text{cm}^{-2} \text{g}^{-1}$ of plant dry weight.

$$\text{LAR} = \frac{\text{Leaf area per plant}}{\text{Plant dry weight}}$$

iv. Leaf Weight Ratio (LWR)

It was coined by (Kvet *et al.*, 1971) Leaf weight ratio is expressed as the dry weight of leaves to whole plant dry weight and is expressed in g g^{-1} .

$$\text{LWR} = \frac{\text{Leaf dry weight}}{\text{Plant dry weight}}$$

v. Leaf Area Duration (LAD)

To correlate dry matter yield with LAI, Power *et al.* (1967) integrated the LAI with time and called as Leaf Area Duration. LAD takes into account, both the duration and extent of photosynthetic tissue of the crop canopy. The LAD is expressed in days.

$$\text{LAD} = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

L_1 = LAI at the first stage

L_2 = LAI at the second stage, $(t_2 - t_1)$ = Time interval in days

vi. Specific Leaf Area (SLA)

Specific leaf area is a measure of the leaf area of the plant to leaf dry weight and expressed in $\text{cm}^2 \text{g}^{-1}$ as proposed by Kvet *et al.* (1971).

$$\text{SLA} = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

Hence, if the SLA is high, the photosynthesizing surface will be high. However no relationship with yield could be expected.

vii. Specific Leaf Weight (SLW)

It is a measure of leaf weight per unit leaf area. Hence, it is a ratio expressed as g cm⁻² and the term was suggested by Pearce *et al.* (1968). More SLW/unit leaf area indicates more biomass and a positive relationship with yield can be expected.

$$SLW = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

viii. Absolute Growth Rate (AGR)

AGR is the function of amount of growing material present and is influenced by the environment. It gives Absolute values of biomass between two intervals. It is mainly used for a single plant or single plant organ e.g. Leaf growth, plant weight etc.

$$AGR = \frac{h_2 - h_1}{t_2 - t_1} \text{ cm day}^{-1}$$

Where, h1 and h2 are the plant height at t1 and t2 times respectively.

ix. Net Assimilation Rate (NAR)

The term, NAR was used by Williams (1946). NAR is defined as dry matter increment per unit leaf area or per unit leaf dry weight per unit of time. The NAR is a measure of the average photosynthetic efficiency of leaves in a crop community.

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where, W1 and W2 is dry weight of whole plant at time t1 and t2 respectively

L1 and L2 are leaf weights or leaf area at t1 and t2 respectively

t1 - t2 are time interval in days

NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time ($\text{g g}^{-1}\text{day}^{-1}$)

x. Relative Growth Rate (RGR)

The term was coined by Williams (1946). Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval in relation to the initial weight or Dry matter increment per unit biomass per unit time or grams of dry weight increase per gram of dry weight and expressed as unit dry weight / unit dry weight / unit time ($\text{g g}^{-1}\text{day}^{-1}$)

$$\text{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W_1 and W_2 are whole plant dry weight at t_1 and t_2 respectively

t_1 and t_2 are time interval in days

xi. Crop Growth Rate (CGR)

The method was suggested by Watson (1956). The CGR explains the dry matter accumulated per unit land area per unit time ($\text{g m}^{-2}\text{day}^{-1}$)

$$\text{CGR} = \frac{(W_2 - W_1)}{\rho (t_2 - t_1)}$$

Where, W_1 and W_2 are whole plant dry weight at time $t_1 - t_2$ respectively

ρ is the ground area on which W_1 and W_2 are recorded.

CGR of a species are usually closely related to interception of solar radiation

xii. Total dry matter production (TDMP) and its distribution

The TDMP is the biomass accumulated by the whole plant over a period of interval of time and its distribution (allocation) to different parts of the plant such as roots, stems, leaves and the economic parts which controls the sink potential.

xiii. Translocation percentage (TP)

The term translocation percentage indicates the quantum of photosynthates translocated from source (straw) to the grain (panicle/grains) from flowering to harvest.

$$TP = \frac{\text{Straw weight at flowering} - \text{straw weight at harvest}}{\text{Panicle weight at flowering} - \text{panicle weight at harvest}}$$

xiv. Light extinction coefficient

It is the ratio of light intercepted by crop between the top and bottom of crop canopy to the LAI.

$$K = \frac{\log_e I / I_0}{LAI}$$

Where, I_0 and I are the light intensity at top and bottom of a population with LAI

xv. Light Transmission Ratio (LTR)

It is expressed as the ratio of quantum of light intercepted by crop canopy at top to the bottom. Light intensity is expressed in K lux or $W m^{-2}$

$$LTR = I / I_0$$

Where, I : light intercepted at the bottom of the crop canopy

I_0 : light intercepted at the top of the crop canopy

xvi. Dry Matter Efficiency (DME)

It is defined as the percent of dry matter accumulated in the grain from the total dry matter produced over the crop growth period.

$$DME = \frac{\text{Grain yield}}{\text{TDMP}} \times \frac{100}{\text{Duration of crop}}$$

xvii. Unit area efficiency (UAE)

It is expressed as the quantum of grain yield produced over a unit land area for a specified crop growth period.

$$\text{UAE} = \frac{\text{Grain yield}}{\text{Land area}} \times \frac{1}{\text{Duration of crop}}$$

xviii) Harvest Index

The harvest index is expressed as the percent ratio between the economic yield and total biological yield and was suggested by Nichiporovich (1951).

$$\text{HI} = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

20. PLANT GROWTH REGULATORS

Plant growth regulators or phytohormones are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. Thimmann (1948) proposed the term *Phytohormone* as these hormones are synthesized in plants. *Plant growth regulators* include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Auxins are the hormones first discovered in plants and later gibberellins and cytokinins were also discovered.

Hormone

An endogenous compound, which is synthesized at one site and transported to another site where it exerts a physiological effect in very low concentration. But ethylene (gaseous nature), exert a physiological effect only at a near a site where it is synthesized.

Classified definition of a hormone does not apply to ethylene.

Plant growth regulators

- Defined as organic compounds other than nutrients, that affects the physiological processes of growth and development in plants when applied in low concentrations.
- Defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting.

Plant Hormone

When correctly used, is restricted to naturally occurring plant substances, there fall into five classes. Auxin, Gibberellins, Cytokinin, ABA and ethylene. Plant growth regulator includes synthetic compounds as well as naturally occurring hormones.

Plant Growth Hormone

The primary site of action of plant growth hormones at the molecular level remains unresolved.

Reasons

- Each hormone produces a great variety of physiological responses.
- Several of these responses to different hormones frequently are similar.
- The response of a plant or a plant part to plant growth regulators may vary with the variety of the plant.
- Even a single variety may respond differently depending on its age, environmental conditions and physiological state of development (especially its natural hormone content) and state of nutrition. There are always exceptions for a general rule suggesting the action of a specific growth regulator on plants.
- There are several proposed modes of action in each class of plant hormone, with substantial arguments for and against each mode.

Hormone groups

- | | | |
|--------------|---|---|
| Auxin | - | Substances generally resembles IAA and has the ability to stimulate the elongation of coleoptiles. |
| Gibberellins | - | are diterpenoids, which have the ability to elongate the stem of green seedlings especially certain dwarf and rosette types. |
| Cytokinin | - | Usually substituted Adenines, which resembles zeatin (Naturally occurring cytokinin in <i>Zea mays</i>) and have the ability to stimulate cytokinensis in cultures of tobacco cells. |
| Ethylene | - | Gaseous regulator that stimulate is diametric growth in the apices of dicot seedlings. |
| Inhibitors | - | are regulators of growth, which originally depress the |

Auxins

Auxins are a group of phytohormones produced in the shoot and root apices and they migrate from the apex to the zone of elongation. Auxins promote the growth along the longitudinal axis of the plant and hence the name (auxein : to grow). The term, auxin was introduced by Kogl and Haagen- Smit (1931). Went (1928) isolated auxin from the *Avena* coleoptile tips by a method called *Avena coleoptile or curvature test* and concluded that no growth can occur without auxin. Auxins are widely distributed through out the plant however, abundant in the growing tips such as coleoptile tip, buds, root tips and leaves. Indole Acetic Acid (IAA) is the only naturally occurring auxin in plants. The synthetic auxins include,

Avena Curvature Test

IBA : Indole Butyric Acid

NAA : Naphthalene Acetic acid

MENA: Methyl ester of Naphthalene acetic acid

MCPA: 2 Methyl 4 chloro phenoxy acetic acid

TIBA : 2, 3, 5 Tri iodo benzoic acid

2, 4-D : 2, 4 dichloro phenoxy acetic acid

2, 4, 5-T: 2, 4, 5 – Trichloro phenoxy acetic acid

Natural auxins may occur in the form of either *free auxins*- which freely move or diffuse out of the plant tissues readily or *bound auxins*- which are released from plant tissues only after hydrolysis, autolysis or enzymolysis.

Physiological effects of auxin

1. Cell division and elongation

The primary physiological effects of auxin are cell division and cell elongation in the shoots. It is important in the secondary growth of stem and differentiation of xylem and phloem tissues.

2. Apical dominance

In many plants, if the terminal bud is intact and growing, the growth of lateral buds just below it remains suppressed. Removal of the apical bud results in the rapid growth of lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the lateral buds to grow is known as *apical dominance*.

Skoog and Thimmann (1948) pointed out that the apical dominance might be under the control of auxin produced at the terminal bud and which is transported downward through the stem to the lateral buds and hinders the growth. They removed the apical bud and replaced it with *agar* block. This resulted in rapid growth of lateral buds. But when they replaced the apical bud with agar block containing auxin, the lateral buds remained suppressed and did not grow.

3. Root initiation

In contrast to stem, the higher concentration of auxin inhibits the elongation of roots but the number of lateral roots is considerably increased i.e., higher concentration of auxin induces more lateral branch roots. Application of IAA in lanolin paste (lanolin is a soft fat prepared from wool and is good solvent for auxin) to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of abscission

Natural auxins prevent the formation of abscission layer which may otherwise result in the fall of leaves, flowers and fruits.

5. Parthenocarpy

Auxin can induce the formation of parthenocarpic fruits (fruit formation without pollination and fertilization). In parthenocarpic fruits, the concentration of auxin in the ovaries is higher than in the ovaries of plants which produce fruits only after fertilization. In the later cases, the concentration of the auxin in ovaries increases after pollination and fertilization.

6. Respiration

Auxin stimulates respiration and there is a correlation between auxin induced growth and respiration. Auxin may increase the rate of respiration indirectly through increased supply of ADP by rapidly utilizing ATP in the expanding cells.

7. Callus formation

Besides cell elongation, auxin may also be active in cell division. In many tissue cultures, where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Eradication of weeds

Some synthetic auxins especially 2, 4- D and 2, 4, 5-T are useful in eradication of weeds at higher concentrations.

9. Flowering and sex expression

Auxins generally inhibit flowering but in pine apple and lettuce it promotes uniform flowering.

Distribution of auxin in plants

In plants, auxin (IAA) is synthesized in growing tips or meristematic regions from where; it is transported to other plant parts. Hence, the highest concentration of IAA is found in growing shoot tips, young leaves and developing auxiliary shoots. In monocot seedling, the highest concentration of auxin is found in coleoptile tip which decreases progressively towards its base.

In dicot seedlings, the highest concentration is found in growing regions of shoot, young leaves and developing auxiliary shoots. Within the plants, auxin may present in two forms. i.e., *free auxins* and *bound auxins*. Free auxins are those which are easily extracted by various organic solvents such as diethyl ether. Bound auxins on the other hand, need more drastic methods such as hydrolysis, autolysis, enzymolysis etc. for extraction of auxin. Bound auxins occur in plants as complexes with carbohydrates such as glucose, arabionse or sugar alcohols or proteins or amino acids such as aspartate, glutamate or with inositol.

Biosynthesis of auxin (IAA) in plants

Thimann (1935) found that an amino acid, tryptophan is converted into Indole 3 acetic acid. Tryptophan is the primary precursor of IAA in plants. IAA can be formed from tryptophan by two different pathways.

1. By deamination of tryptophan to form indole-3-pyruvic acid followed by decarboxylation to form indole-3-acetaldehyde. The enzymes involved are tryptophan deamination and indole pyruvate decarboxylase respectively.
2. By decarboxylation of tryptophan to form tryptamine followed by deamination to form indole-3-acetaldehyde and the enzymes involved are tryptophan decarboxylase and tryptamine oxidase respectively. Indole 3-acetaldehyde can readily be oxidized to indole 3-acetic acid (IAA) in the presence of indole 3-acetaldehyde dehydrogenase.

Transport of auxin in plant

The transport of auxin is predominantly polar. In stems, polar transport of auxin is basipetal i.e., it takes place from apex towards base. Polar transport of auxin is inhibited by 2, 3, 5 Triiodobenzoic acid (TIBA) and Naphthyl thalamic acid (NPA). The substances are called as antiauxins.

Destruction / Inactivation of auxin in plants

Auxin is destroyed by the enzyme IAA oxidase in the presence of O₂ by oxidation.

IAA Oxidase



Rapid inactivation may also occur by irradiation with x-rays and gamma rays. UV light also reduces auxin levels in plants. Inactivation or decomposition of IAA by light has been called as photo oxidation.

Mechanism of Action

IAA increases the plasticity of cell walls so that the cells stretch easily in response to turgor pressure. It has been suggested that IAA acts upon DNA to influence the production of

mRNA. The mRNA codes for specific enzymes responsible for expansion of cell walls. Recent evidences indicate that IAA increases oxidative phosphorylation in respiration and enhanced oxygen uptake. The growth stimulation might be due to increased energy supply and it is also demonstrated that auxin induces production of ethylene in plants.

Gibberellins

Discovery

A Japanese scientist Kurosawa found that the rice seedlings infected by the fungus *Gibberella fujikuroi* grow taller and turned very thin and pale. An active substance was isolated from the infected seedlings and named as Gibberellin.

Biosynthesis of gibberellins in plants

The primary precursor for the formation of gibberellins is acetate.

Acetate + COA Acetyl COA Mevalonic acid MA pyrophosphate
Isopentanyl pyrophosphate Geranyl pyrophosphate GGPP Kaurene Gibberellins.

Physiological effects of gibberellins

1. Seed germination

Certain light sensitive seeds eg. Lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of buds

In temperate regions the buds formed in autumn remain dormant until next spring due to severe cold. This dormancy of buds can be broken by gibberellin treatments. In potato also, there is a dormant period after harvest, but the application of gibberellin sprouts the tuber vigorously.

3. Root growth

Gibberellins have little or no effect on root growth. At higher concentration, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of internodes

The most pronounced effect of gibberellins on the plant growth is the elongation of the internodes. Therefore in many plants such as dwarf pea, dwarf maize etc gibberellins overcome the genetic dwarfism.

5. Bolting and flowering

In many herbaceous plants, the early period of growth shows rosette habit with short stem and small leaves. Under short days, the rosette habit is retained while under long days bolting occurs i.e. the stem elongates rapidly and is converted into polar axis bearing flower primordia. This bolting can also be induced in such plants by the application of gibberellins even under non-inductive short days.

In *Hyoscyamus niger* (a long day plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in long day plants the gibberellin treatment usually results in early flowering. In short day plants, its effects are quite variable. It may either have no effect or inhibit or may activate flowering.

6. Parthenocarpy

Germination of the pollen grains is stimulated by gibberellins; likewise, the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases, eg. pome and stone fruits where auxins have failed to induce parthenocarpy, the gibberellins have proven to be successful. Seedless and fleshly tomatoes and large sized seedless grapes are produced by gibberellin treatments on commercial scale.

7. Synthesis of the enzyme - amylase

One important function of gibberellins is to cause the synthesis of the enzyme - amylase in the aleurone layer of the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Distribution of gibberellins in plant

Gibberellins are found in all parts of higher plants including shoots, roots, leaves, flower, petals, anthers and seeds. In general, reproductive parts contain much higher concentrations of gibberellins than the vegetative parts. Immature seeds are especially rich in gibberellins (10-100 mg per g fresh weight).

In plants, gibberellins occur in two forms free gibberellins and bound gibberellins. Bound gibberellins usually occur as gibberellin – glycosides.

CYTOKININS (Kinetin)

Kinetin was discovered by Skoog and Miller (1950) from the tobacco pith callus and the chemical substance was identified as 6-furfuryl aminopurine. Because of its specific effect on *cytokinesis* (cell division), it was called as cytokinins or kinetin. The term, cytokinin was proposed by Letham (1963). Fairley and Kingour (1966) used the term, *phytokinins* for cytokinins because of their plant origin. Chemically cytokinins are kinins and they are purine derivatives.

Cytokinins, besides their main effect on cell division, also regulate growth and hence they are considered as natural plant growth hormones. Some of the very important and commonly known naturally occurring cytokinins are Coconut milk factor and Zeatin. It was also identified that cytokinin as a constituent of t-RNA.

Naturally occurring cytokinins

Cytokinins can be extracted from coconut milk (liquid endosperm of coconut), tomato juice, flowers and fruits of *Pyrus malus*; fruits of *Pyrus communis* (Pear), *Prunus cerasiferae* (plum) and *Lycopersicum esculentum* (bhendi); Cambial tissues of *Pinus radiata*, *Eucalyptus regnans* and *Nicotiana tabacum*; immature fruits of *Zea mays*, *Juglans* sp. and *Musa* sp; female gametophytes of *Ginkgo biloba*; fruitlets, embryo and endosperms of *Prunus persica*; seedling of *Pisum sativum*; root exudates of *Helianthus annuus* and tumour tissues of tobacco. According to Skoog and Armstrong (1970), at least seven well established types of cytokinins have been reported from the plants.

Biosynthesis

It is assumed that cytokinins are synthesised as in the case of purines in plants (nucleic acid synthesis). Root tip is an important site of its synthesis. However, developing seeds and cambial tissues are also the site of cytokinin biosynthesis. Kende (1965) reported that cytokinins move upwards perhaps in the xylem stream. However, basipetal movement in petiole and isolated stems are also observed. Seth *et al* (1966) found that auxin enhances kinetin movement (translocation) in bean stems.

Physiological effects of cytokinins

1. Cell division

The most important biological effect of kinetin on plants is to induce cell division especially in tobacco pith callus, carrot root tissue, soybean cotyledon, pea callus etc.

2. Cell enlargement

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Significant cell enlargement has been observed in the leaves of *Phaseolus vulgaris*, pumpkin cotyledons, tobacco pith culture, cortical cells of tobacco roots etc.

3. Concentration of apical dominance

External application of cytokinin promotes the growth of lateral buds and hence counteracts the effect of apical dominance

4. Dormancy of seeds

Like gibberellins, the dormancy of certain light sensitive seeds such as lettuce and tobacco can also be broken by kinetin treatment.

5. Delay of senescence (Richmand - Lang effect)

The senescence of leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. Senescence can be postponed to several days by kinetin treatment by improving RNA synthesis followed by protein synthesis.

Richmand and Lang (1957) while working on detached leaves of *Xanthium* found that kinetin was able to postpone the senescence for a number of days.

6. Flower induction

Cytokinins can be employed successfully to induce flowering in short day plants.

7. Morphogenesis

It has been shown that high auxin and low kinetin produced only roots whereas high kinetin and low auxin could promote formation of shoot buds.

8. Accumulation and translocation of solutes

Plants accumulate solutes very actively with the help of Cytokinin and also help in solute translocation in phloem.

9. Protein synthesis

Osborne (1962) demonstrated the increased rate of protein synthesis due to translocation by kinetin treatment.

10. Other effects

Cytokinins provide resistance to high temperature, cold and diseases in some plants. They also help in flowering by substituting the photoperiodic requirements. In some cases, they stimulate synthesis of several enzymes involved in photosynthesis.

11. Commercial applications

Cytokinins have been used for increasing shelf life of fruits, quickening of root induction and producing efficient root system, increasing yield and oil contents of oil seeds like ground nut.

Ethylene

Ethylene is the only natural plant growth hormone exists in gaseous form.

Important physiological effects

1. The main role of ethylene is it hastens the ripening of fleshy fruits eg. Banana, apples, pears, tomatoes, citrus etc.
2. It stimulates senescence and abscission of leaves
3. It is effective in inducing flowering in pine apple
4. It causes inhibition of root growth
5. It stimulates the formation of adventitious roots
6. It stimulates fading of flowers
7. It stimulates epinasty of leaves.

Abscisic acid

Addicott (1963) isolated a substance strongly antagonistic to growth from young cotton fruits and named Abscissin II. Later on this name was changed to Abscisic acid. This substance also induces dormancy of buds therefore it also named as Dormin.

Abscisic acid is a naturally occurring growth inhibitor.

Physiological effects

The two main physiological effects are

1. Geotropism in roots
2. Stomatal closing
3. Besides other effects

1. Geotropism in roots

Geotropic curvature of root is mainly due to translocation of ABA in basipetal direction towards the root tip.

2. Stomatal closing

ABA is synthesized and stored in mesophyll chloroplast. In response to water stress, the permeability of chloroplast membrane is lost which results in diffusion of ABA out of chloroplast into the cytoplasm of the mesophyll cells. From mesophyll cells it diffuses into guard cells where it causes closing of stomata.

3. Other effects

- i. Including bud dormancy and seed dormancy
- ii. Includes tuberisation
- iii. Induces senescence of leaves fruit ripening, abscission of leaves, flowers and fruits
- iv. Increasing the resistance of temperate zone plants to frost injury.

Growth retardants

There are synthesis compounds which prevent the gibberellins from exhibiting their usual responses in plants such as cell enlargement or stem elongation. So they are called as anti gibberellins or growth retardants. They are

1. Cycocel (2- chloroethyl trimethyl ammonium chloride (CCC))

2. Phosphon D – (2, 4 – dichlorobenzyl – tributyl phosphonium chloride)
3. AMO – 1618
4. Morphactins
5. Maleic hydrazide

STOMATAL PHYSIOLOGY1

It is now widely recognized that the major resistance to water movement in plants is located at the leaf surface where water moves in the vapor phase. It has been shown that the rate of water loss from a leaf is determined by the sum of a resistance proportional to the width of the stomatal aperture and an independent diffusion resistance due to the presence of a layer of still air at the surface of the leaf. Since at least 90 per cent of the water loss from a leaf occurs by diffusion through the stomata, fluctuations in stomatal aperture provide the mechanism for the regulation of transpiration. Stomata should be effective as regulators of transpiration at apertures of 50 per cent of maximal opening and smaller. Experimental evidence supports this notion

Stomata are not only the major pathway for transpiration, but they are also essential for gas exchange. Very little photosynthesis occurs when the stomata are closed. Rate of photosynthesis is controlled by stomatal aperture, particularly when CO₂ is limiting at relatively low values of the mesophyll diffusion resistance for CO₂. The function of stomata therefore is an important one and the mechanism and control of stomatal movement should be understood.

The literature dealing with stomatal movement has been reviewed a number of times in recent years between them, provide a complete bibliography. The purpose of the present review is not the presentation of a complete bibliography but rather an evaluation of the state of the art.

STRUCTURE AND FUNCTION

Information about the structure of the stomatal apparatus can be found in anatomy textbooks. The basis of movement is formed by the structural characteristics of the guard cell wall. The structure of the wall is such that changes in turgor lead to changes in shape of the guard cell. Not only is the gross structure important, but the fine structure of the walls is also involved. The cellulose micellae of the guard cell wall fan out from the ventral wall (next to the pore) towards the dorsal wall which is attached to the neighboring cell. The extensibility of a cell wall is greatest normal to the direction of

these micellae. The greatest extension, therefore, will take place parallel to the long axis of the guard cell. Due to the fan-like arrangement extensibility in a longitudinal direction will be very small at the ends of the guard cell where the micellae are oriented approximately parallel to the long axis of the wall. This fine structure may explain the movement of stomata, such as those of *Azolla*, which lack local secondary thickening of the guard cell wall. It has been shown that the greater extensibility normal to the direction of the micellae is a purely mechanical consequence of the construction of the wall and not the result of swelling mechanisms, etc. Therefore, micellar structure is involved in the turgor movement. The primary response due to micellar structure is modified by the presence of heavy secondary layers which are deposited in specific patterns along the wall. Such layers always occur along the ventral wall and prevent any appreciable extension of that wall. At least part of the dorsal wall is thin and elastic. An increase in turgor will cause extension of the dorsal wall. Since the ventral wall cannot extend appreciably, it must become concave. This explains why stomata open when the turgor increases. The picture is a simplified representation and may not apply in this exact form to all different types of stomata. Grasses, for example, have a different mechanism of opening.

Generally speaking, guard cells are surrounded by accessory cells which differ from regular epidermal cells in size, shape, and micellar structure of their walls. The accessory cells are involved in stomatal movement. They restrict the movement of the dorsal wall of the guard cells and exert pressure on the stomatal apparatus. When the accessory cells are punctured, the guard cells curve excessively and the aperture is excessively large. Complete closure is often impossible under these conditions.

CYTOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF THE GUARD CELLS

Guard cells differ from epidermal cells in many respects. One striking observation is the absence of plasmodesmata in the wall between guard and accessory cell. Plasmodesmata have been found in the outer walls of guard cells (19, 20), between accessory and epidermal cells (19), between epidermal cells (18, 19), and in the outer wall of the epidermis

Since the dorsal wall of the guard cell is very thin, it might be difficult to demonstrate plasmodesmata by the usual methods. However, the electron microscope does not reveal plasmodesmata in it (21). This absence of plas-

modestly has led to the belief that the guard cells are isolated from the rest of the leaf. This conclusion is premature. The dorsal wall is very thin or has thin areas. In grasses its thickness is only 100 to 150 m μ . Such a thin wall does not constitute a serious diffusion barrier unless it is cuticularized. Moreover, labeled sugars or p^{32} fed to leaves appear quickly in the guard cells. The chloroplasts in guard cells are much smaller than those in the mesophyll. They are often palincolor and not well developed although the chloroplasts of *Nelleborus helveticus* have well-developed lamellae. There is ample evidence that the chloroplasts of the guard cell contain chlorophyll. Freeland extracted and chromatographed the pigment and identified chlorophyll. Spectrophotometric methods have shown that both chlorophyll *a* and *b* are present

The ability of these chloroplasts to carry out photosynthesis has been a point of much contention. The plastids were earlier thought to function as amyloplasts or leucoplasts. It has definitely been shown, however, that guard cell chloroplasts fix a small but significant quantity of C^{14}O_2 in light. Earlier starch formation had been demonstrated in the guard cells of leaves made starch-free by starvation in darkness and then exposed to light. Starch formation occurred only in the presence of CO_2 , Scarth & Shaw covered the green areas of a starved variegated leaf with black paper. The covered green areas did not make starch in the light, but the guard cells in the white areas did. The guard cell chloroplasts also reduce AgNO_3 , as well as blue tetrazolium (30), in light. It is certain, therefore, that the chloroplasts in the guard cells are functional in photosynthesis.

Relative to starch formation in the guard cell, it is of interest that phosphorylase activity has been demonstrated in the guard cells. Guard cells of starch-free sections contain abundant starch after 4 to 5 hrs incubation with glucose-1-Phosphate. The activity is located in the chloroplasts. The discovery of phosphorylase activity in guard cells once gave new life to the idea that the starch-sugar conversion is involved in stomatal movement.

The increase in phosphorylase activity during differentiation of the stomatal apparatus is very striking. Many Liliaceae do not normally contain starch in the mesophyll or epidermis of adult leaves, with the exception of the guard cells. Very young epidermis and mesophyll cells have a slight phosphorylase activity, which disappears with increasing age. The activity increases rapidly in the developing guard cell. This observation suggests that the phosphorylase activity is essential for stomatal operation.

Guard cells differ from epidermal cells in many other ways. Stafelt has reviewed this aspect in detail and a short summary will be sufficient here. Guard cells are more resistant to toxic substances and to unfavorable environmental conditions than the rest of the leaf. They are, however, more sensitive to water stress. This has led to misinterpretation of experiments involving repeated stress and recovery periods. Guard cells may suffer irreversible injury when exposed to water stresses which have no lasting effect on the rest of the leaf. Protoplasmic properties, chemical composition, and cellular inclusions are different too. Such miscellaneous differences emphasize the unique character of the guard cell but the relation of these differences to the mechanism of movement is not at all clear.

THE DRIVING FORCE

Beginning with von Mohl it has been accepted that stomatal movement is a turgor phenomenon. Stomata can be isolated from the epidermis by cutting the surrounding cells or by micromanipulation. Such isolated stomata close in sugar solutions because the turgor of the guard cells decreases. In water the turgor increases and the stomata open. The structure of the guard cell wall causes the turgor changes to manifest themselves as changes in size and shape. Stomatal movement is thus a turgor movement, just as are, for example, the nyctinastic and seismonastic movements of leaves.

The problem then is to discover how such turgor changes come about.

Superficially, stomata appear to be excellent objects for the study of turgor movement because only two cells are involved and these cells are readily accessible. Unfortunately the methodology is complicated by the smallness of the object and its sensitivity to environmental factors. Moreover, the stomata are influenced by epidermis and mesophyll. This complicates the interpretation of experiments with stomata *in situ*.

The epidermis has a strong effect on the stomata. The immersion of leaves in water causes closure of stomata, in contrast to the behavior of isolated stomata which open in water. Von Mohl correctly concluded that the epidermal cells exert pressure on the guard cells. The order of magnitude of the epidermal effect depends on the nature of the stomatal apparatus. It varies from one species to another, but is always present. Stomatal movement, therefore, is the result

of an interaction between the guard cells and the surrounding tissue. The difference in turgor between the guard cells and the subsidiary cells determines the actual movement.

This is but one example of an interaction between guard cells and other parts of the leaf. Obviously such interactions are important for an understanding of stomatal movement and regulation in situ, but they confuse the issue in studies of the mechanism of turgor changes.'

Thus, the subject matter of stomatal physiology is really twofold: the regulation of stomatal aperture and (b) the cause of turgor changes in the guard cell. Both problems have been approached by studying the effect of environmental factors on stomatal movement. Further observations have been made on changes in the guard cell during opening or closing. Finally, the effect of environmental factors on the guard cell has been studied.

ENVIRONMENTAL FACTORS CONTROLLING STOMATAL MOVEMENT

The effects of environmental factors occupy a central position in stomatal physiology and a detailed discussion of these effects is in order. First, the role of environmental factors in stomatal regulation will be considered, in particular the effects of carbon dioxide concentration, light and

temperature. The water deficit of the leaf can also be considered as an environmental factor for the guard cells.

Carbon dioxide.-Linsbauer observed that CO₂-free air causes opening of the stomata both in light and in darkness. An increase in the external CO₂ concentration causes a decrease in aperture, even in light. Linsbauer's results were not generally accepted due to the crude techniques used, but later work has vindicated his conclusions. Renewed interest in CO₂ arose when it was discovered that the very wide opening of stomata under a porometer cup is caused by a low CO₂ concentration. Apparently the CO₂ in the small volume of air in the cup is rapidly consumed by photosynthesis. This phenomenon minimizes the value of the earlier work with porometers.

A study of the effect of different concentrations of CO₂ showed that the maximal opening effect is obtained with 0.01 per cent CO₂. A further reduction of the CO₂ concentration to zero does not cause further opening even though the stomata are able to open wider. The reason for this behavior is to be found in the existence of a CO₂ compensation point for photosynthesis. When the CO₂ concentration limits the rate of photosynthesis, the CO₂ concentration in the intercellular spaces is reduced to approximately 0.01 per cent, irrespective of the initial concentration (45, 46). Similarly, when CO₂-free air or 0.0024 per cent CO₂ is given to the plant, the final equilibrium concentration is 0.009 to 0.01 per cent. One cannot, therefore, expect the CO₂ concentration inside the leaf to drop much below 0.01 per cent unless CO₂-free air is passed through the leaf at a very high rate of flow. Such an air flow is physically impossible.

final CO₂ concentration for an experiment in which the substomatal cavities were washed out with CO₂-free air. He calculates that the mean CO₂ concentration of the air forced through the leaf should be about 0.0045 per cent. Unfortunately, no actual measurements are available. It is reasonable to assume that the CO₂ concentration inside a leaf in the light is 0.005 to 0.01 per cent over a range of external CO₂ concentrations. Apparently little photosynthesis takes place when the intercellular space CO₂ concentration is lower than 0.01 per cent. When the external CO₂ concentration is not limiting for photosynthesis, the concentration in the intercellular spaces can be expected to rise above the threshold value. Thus, the lack of a response of stomata to external CO₂ concentrations between zero and 0.01 per cent is the result of the physiology of the leaf. It is not necessarily characteristic for the turgor mechanism of the guard cell.

Recently, Meidner reported an exception to this pattern of CO₂ response. The stomata of *Zea mays* respond to external CO₂ concentrations between zero and 0.01 per cent. Reduction below 0.01 per cent causes further opening. At the same time, Meidner found that corn leaves are able to exhaust the atmospheric CO₂ content to zero. These results confirm the conclusion outlined in the previous paragraph.

It would be interesting to test the response of isolated stomata to different CO₂ concentrations. Isolated stomata of *Pelargonium* open in CO₂-free air in the light and isolated stomata of *Allium ursinum* open in CO₂-free air in both light and darkness. Concentrations of CO₂ between zero and 0.01 per cent have not been tried, however. Apparently, the CO₂ concentration inside the leaf, particularly in the substomatal cavity, determines the response, for CO₂-free air prevents closure when the light is switched off, but once the stomata are completely closed CO₂-free air cannot cause opening. This is true for *Pelargonium*. It is not known whether the same holds for other plant species.

CO₂ occupies a central position in the regulation of stomatal movement. The effects of light, temperature, and hydration are mediated, at least in part, through CO₂. The mechanism of action of CO₂ will be discussed later in this review.

Light.-Stomata are closed in darkness. When a turgid leaf which has been kept in darkness for a few hours is exposed to light, the stomata open after a short time lag. When the light is switched off, closing starts immediately. Closing is the more rapid process (55) and may be measurable 15 sec after the beginning of darkness. Recently, it has been reported for Pinto beans (*Phaseolus vulgaris*) that closing is much slower than opening. The stomata were not completely closed after 16 hr of darkness. This is an anomalous case which needs further confirmation.

The opening responds to light intensity. There is a minimal intensity for opening which varies with the species. Tomatoes require at least 275 ft-c, while 90 ft-c causes opening in *Pelargonium* (58). At each intensity the aperture reaches a steady-state value which is characteristic for that intensity provided the water deficit of the leaf is small. The higher the intensity is, the greater the final opening up to an intensity which causes maximal opening. The saturating intensity is around 105 erg cm⁻² sec⁻¹ for incandescent light sources. Sometimes very high intensities cause a decrease in opening. This is probably a secondary effect due to high leaf temperature or high rate of water loss.

The rate of opening is also influenced by light intensity. Virgin demonstrated this in an elegant series of experiments with wheat seedlings by measuring change in transpiration rate with time. The resulting curve is sigmoid. The precise shape of the curve and the duration of the initial lag depend on light intensity. The rate of opening increases with increasing light intensity, while the lag is shortened. Virgin's results are at the very early opening, including the lag period, is not affected by light intensity. This is true for apertures up to 2p. in *Helianthus*. The contradictory results may have been caused by differences in leaf turgidity or by the degree of closure at the beginning of the experiment. Differences in methods may also play a role, for measured stomatal conductance

directly when changes in transpiration rate. The relation between transpiration rate and stomatal opening is nonlinear during early opening, when transpiration increases very rapidly with small changes in aperture. Thus, estimates of stomatal aperture based on transpiration rates are not conclusive during early opening unless the relation between transpiration and aperture has been determined by simultaneous measurement of both in the same material.

Since, as will be seen later, the major function of light is the lowering of the CO₂ concentration in the leaf, the lag period probably represents in part the time required for photosynthesis to accomplish the lowering of the CO₂ concentration inside the leaf to the required level. This idea is supported by the fact that the duration of the lag depends on pretreatment: the longer the preceding dark period, the longer the lag. Synthesis of carbohydrates may also be involved. On the basis of these considerations one would expect the duration of the lag period to be dependent on light intensity.

Responses to light intensity may be related to the total amount of energy received or to the energy flow. If the stomatal response is determined by the amount of energy, the product law should hold and opening should be independent of intensity as long as the product of intensity and time of exposure is constant. The evidence is conflicting. A specific opening can be found for which the product rule holds. This aperture is (J. in *Vicia faba* and 4.75tJ. in *Helianthus*. In other words, the attainment of an aperture of 5tJ. is determined by the amount of energy received, independent of intensity. The product law does not hold for other apertures. The final steady-state aperture is determined by the energy flux. The final aperture is to some extent independent of intensity, however, for the same steady-state opening is reached in continuous light of 32,000 m-c and in intermittent illumination with 64,000 m-c (5 sec light, 5 sec darkness). One can hardly expect the product rule to hold ideally, for the epidermal resistance to movement is variable and water deficit may increase during opening, causing a closing movement.

The action spectrum of the light effect has generally been studied with rather broad spectral areas. The blue light (425 to 495 mJ.) has the highest efficiency, followed by red (631 and 690 mJ.). Far-red (733 mJ.) has no effect on stomata. Within the blue range the efficiency changes with wavelength and the highest efficiency in the blue was found with light which passed a filter with maximal transmission at 439 mJ. It is of interest that the maximal light absorption by leaves is also found at 439 mJ. (64). The most striking finding in Karve's experiments is the very high efficiency of blue light. Earlier workers who used quantitative methods in light experiments, also found blue light more effective than red, generally by 2 to 3 times. The only exception is

that red is twice as active as blue. The results obtained with light other than red or blue are generally confusing due to the relative impurity of the light used. Karve's experiments are not decisive either since he tested nothing between 495 and 568 mJ. Stomatal movement does not respond to infra-red radiation.

The action spectrum of stomatal opening resembles the action spectrum of photosynthesis of leaves (and the absorption spectra of leaves and chlorophyll. The efficiency peak at 680 mJ. strongly suggests that chlorophyll is involved in the light response of stomata. On the other hand, the high efficiency of blue radiation has been well established and the efficiency of blue is higher than that expected on the basis of absorption by chlorophyll. The high efficiency of blue light suggests that the carotenoids are involved in the stomatal light response. It is also possible to think in terms of light effects not mediated through photosynthesis. At present, however, the action spectrum data do not offer rigorous proof for the existence of light processes other than those involving photosynthesis. Furthermore, stomatal aperture is the same with different qualities of light if they are supplied at different intensities, such as to produce rates of photosynthesis which just balance respiration (This shows that the principal action is through photosynthesis. These experiments should be repeated and extended with better optical methods.

The question of whether chlorophyll is the light absorbing pigment for the stomatal response can be explored by investigating the light response of plants with little or no chlorophyll. The results obtained from such experiments are strongly suggestive but they do not constitute absolute proof. Albino mutants of barley contain only 1.2 [J.g chlorophyll per g fresh weight . The guard cells do not contain detectable amounts of chlorophyll and do not fix $C^{14}O_2$. The mesophyll fixes CO_2 at a very slow rate. Small quantities of carotenoids are present. The stomata of such plants do not open in light nor in CO_2 -free air. In fact, opening has never been observed in albino mutants of barley. The experiments suggest that chlorophyll is required absolutely for the light response of stomata. It is possible, however, that the stomata of these mutants are unable to open, possibly because the carbohydrate content of the guard cells is not sufficient to produce the required increase in turgor. Unless it can be shown that the stomata of albino barley can be made to open, the evidence regarding chlorophyll requirement is not fully convincing.

A vast number of experiments has been carried out with white leaves or white parts of leaves of variegated plants. Stomata located in the white areas of variegated leaves respond to light, but the movement is slower and more restricted than in the green parts of the same leaves. Unfortunately, the guard cells in the white areas contain appreciable quantities of chlorophyll and their chloroplasts are capable of photosynthesis.

the same number of chloroplasts in the guard cells of the white and green areas. Only two reports are available of guard cells without chlorophyll. Paetz could not detect chlorophyll in the stomata in the white parts of the leaves of a variegated *Caladium* species. He used fluorescence microscopy as a detection technique. The stomata in the white areas did not respond to light. This is an interesting case which needs confirmation and further work. Sawyer reported the absence of chloroplasts and starch for the guard cells of normal leaves of a number of *Vaccinium* species. This report also needs confirmation. Sawyer found that the stomata responded to light sluggishly or not at all. The value of experiments with variegated leaves has been further decreased by the finding that the opening stimulus of light can be translocated at a rapid rate.

the upper part was either illuminated or kept in darkness. When the light was switched on over the upper part of the branch, the stomata of the basal part, which is in darkness, open at the same time, although not as widely, as those of the illuminated leaves. Apparently the opening stimulus is transmitted rapidly over a considerable distance.

Transmission within a leaf also occurs. For example, the stomata in the white areas of a variegated leaf respond to light in a normal manner when they are located near a green area or when the white areas are small. The influence of a green area on stomata in a white area spreads with continuing exposure to light. The effect is probably transmitted by the lowering of the CO_2 concentration in the intercellular spaces.

Heath & Russell observed that a change in light intensity in one area of a leaf has an effect on the stomata in another area of the same leaf which has been kept under constant illumination. The light stimulus was translocated across an area of 1.7 cm which was kept in high intensity light, while the intercellular spaces were flushed with CO_2 -free air. This appears to exclude CO_2 as the transmitting agent. The mechanism of such transmission is not known.

The existence of various kinds of transmission of light effects complicates the interpretation of experiments with variegated leaves. The data strongly suggest that chlorophyll is essential for the stomatal light response, but they do not prove that there are light reactions which are independent of chlorophyll.

Finally, the problem has been approached by studying the light effect

in etiolated plants. no stomatal light response in etiolated plants until a certain amount of chlorophyll had been formed. There was a strong correlation between chlorophyll formation in the mesophyll and light response.

light has a strong opening effect on stomata. Chlorophyll is the light-absorbing pigment and there is little or no evidence for light reactions other than those involving chlorophyll.

The mechanism of light action. The important role of chlorophyll in the light response suggests that photosynthesis is involved. light induces opening through the photosynthetic removal of CO₂ from the intercellular spaces. This was supported by his observations on the opening effect of CO₂-free air. Linsbauer, and later Scarth thought that the role of photosynthesis in the guard cells was negligible. Therefore, in their opinion, the direct effect of light on the guard cell is very small. This is supported by the observation that CO₂-free air largely, but not entirely, overcomes the greater opening in green as compared with white areas of a variegated leaf (49). Moreover, the degree of opening in CO₂-free air and darkness is almost the same as that in normal air and high light. This again indicates that light has but little direct effect on the guard cells and that light induces opening through its photosynthetic activity.

The nature of the light-CO₂ interaction further supports this notion. Both light and low CO₂ concentration cause opening. Darkness and high CO₂ concentration cause closing. Light and CO₂ interact, for the higher the light intensity, the higher the external CO₂ concentration required for closing. The CO₂-operated mechanism is saturated at an external CO₂ concentration of 0.017 per cent in darkness and at 0.049 per cent in low intensity light (90 ft-c). The system is not completely saturated at 0.08 per cent external CO₂ when the light intensity is 800 ft-c. This is strong evidence that the photosynthetic reduction of CO₂ content of the leaf is the cause of opening in light.

The greater part of the CO₂ reduction takes place in the mesophyll. The sluggish and restricted opening of stomata in the white areas of variegated leaves is the consequence of the inability of the chlorophyll-less mesophyll cells to decrease the CO₂ concentration. This observation clearly demonstrates the importance of the mesophyll. Thus, the major part of the light effect is an indirect effect mediated through photosynthesis and lowered CO₂ concentration.

In addition to these indirect effects of light there is also a direct effect of light on the guard cells. It has already been stated that the guard cells carry out photosynthesis (page 251). This in itself is proof of a direct light effect. Moreover, the effect of CO₂ concentration in the intercellular spaces can be excluded by forcing air of known CO₂ concentration through the leaf. Even when CO₂-free air is passed through the leaf, a strong, intensity-dependent light effect is the most logical explanation of this phenomenon is that the intracellular consumption of part of the respiratory CO₂ results in a lower CO₂ concentration at the essential sites in the guard cell than would be possible by diffusion. As yet there is a

need to invoke a non-photosynthetic mechanism.

The available facts are in agreement with the hypothesis that the effect of light on stomatal movement can be explained quantitatively in terms of photosynthesis. Kuiper (47) offers additional arguments in favor of this hypothesis. In this connection it is interesting that hydroxylamine inhibits opening in light in normal air

Water content of the leaf.-Changes in water content or hydration of the leaf are expressed as changes in water deficit. Such changes will induce stomatal movement and the reaction is related to the magnitude of the deficit. Some of the movements are passive movements caused by turgor changes in the surrounding tissue. When the stomata are open the leaves lose water through transpiration and the deficit increases. The extent of the change in water deficit depends on the relative magnitudes of transpiration and water supply. When the water deficit reaches a critical value, characteristic for the species, the environmental conditions during development, and the age of the leaf, the stomatal aperture becomes smaller. The water deficit is a very powerful regulator. When the deficit rises above the critical value, it overrides all opening stimuli and causes closing even in high intensity light or in CO₂-free air. The water deficit is the only regulator of the aperture of the hydathodes in the wheat coleoptile

Little is known about the mechanism of action of hydration. The CO₂ concentration rises under conditions of moisture stress. However, the increase is too small to be responsible for the whole closing reaction unless water stress brings about an increased sensitivity to CO₂,

Efforts have been made to establish a correlation between photosynthesis and stomatal movement, with the idea that water stress might cause a decrease in the rate of photosynthesis. The resulting increase in intercellular or intracellular CO₂ concentration could then induce closing. In view of the results referred to in the previous paragraph this does not appear a likely explanation. However, photosynthesis is reduced under conditions of mois-

tur stress and the reduction can be independent of stomatal opening, decrease was not affected by removal of the epidermis. In this case the decrease in photosynthesis could not have been caused by the closing of the stomata. Parallelism between photosynthesis and stomatal opening during increasing water deficit. On this basis they concluded that the stomata are more sensitive to water stress than is photosynthesis and that, therefore, photosynthesis is not involved in the stomatal response to water stress, because the data show that the rate of photosynthesis in the opening phase is 2.4 to 3.5 times that in the closing phase for the same aperture. The relation between water deficit, stomatal movement and photosynthesis is complex and the

problem should be approached warily. An extensive discussion of the relation between stomatal resistance and photosynthesis and the conditions influencing -

Temperature.-Within a normal range of temperatures, for example, 10 to 25°C, temperature has little effect on steady-state stomatal aperture. Temperatures higher than 30 to 35°C have a closing effect which may cause the so-called midday closure. The level of CO₂ in the intercellular spaces rises with increasing temperature, probably due to increased respiration. In plant species which show midday closure the increase is very rapid when the leaf temperature is raised over 30°C. The CO₂ concentration in the air passages of coffee leaves, for example, rises from 0.012 per cent at 30°C to 0.025 per cent at 35°C. The same has been found for the leaves of onion and the palm *Phoenix reclinata*. The rise in CO₂ content is accompanied by closing of the stomata. The closing can be prevented by passing a stream of CO₂-free air through the cavity of an onion leaf while the temperature is raised. Apparently, high temperature causes closing of the stomata by increasing the CO₂ concentration in the intercellular spaces.

In the course of these investigations another temperature effect was found. When the CO₂ concentration in the cavity of the onion leaf is kept low by flushing with CO₂-free air, the rate of opening on transfer from darkness to 300 ft-c light and the final width are increased. The Q₁₀ for the increase in rate of opening is 2.2, indicating that some chemical reaction is involved in the opening process and that this chemical reaction is limiting, even at low light intensities.

Much of the work with temperature is difficult to evaluate because the temperature effects on the stomata cannot always be separated from the effects of temperature on the diffusion coefficient, on the water deficit of the leaf and on the relative humidity. This led to conflicting conclusions which for these reasons have not been discussed here.

STOMATAL REGULATION:

The information obtained about the effects of environmental factors on stomatal movement allows a fairly precise description of stomatal regulation. Light intensity and magnitude of the water deficit of the leaf have the greatest effect on movement. Light-induced movements photoactive, those caused by water deficit hydroactive. An increase in light intensity causes opening, an increase in the water deficit brings about closing. Generally, the balance between photoactive opening and hydroactive closing determines the aperture in light. The regulation, therefore, is achieved by the equilibrium between two antagonistic processes. The passive movements, caused by turgor changes in the epidermal cells when the leaf is close to water saturation, are accompanying phenomena which may at times upset the balance. The external CO₂ concentration does not play any large role under natural conditions, apart from reinforcing photoactive stage.

opening when the photosynthetic rate is high. High temperature has a regulatory function in those plants which show midday closing.

The water deficit is a very powerful regulator and an increase in the deficit will override all opening stimuli. This does not mean that each value of the water deficit causes a specific aperture when the light intensity is constant, for the rate of change of the deficit and the direction of the change are of great importance. Different values for stomatal resistance may be found for the same deficit in the same leaf under identical light conditions, depending on whether the deficit is increasing or decreasing. There is a distinct possibility, however, that such differences are due to injury to the guard cells caused by large water deficits. Also, stomata which are closing do not open to the same extent as before when they are induced to open again.

THE MECHANISM OF STOMATAL MOVEMENT

So far, little attention has been given to mechanisms at the level of the guard cells. What mechanism is responsible for the turgor changes required for movement? The classical approach is to consider opening the active phase. The problem then is to increase the water uptake of the guard cells so that the turgor will rise. This requires an increase in the suction force. The increase can be the result of an increase in osmotic value, a decrease in wall pressure, or an active process of water uptake. A wall mechanism is very unlikely in the case of stomatal movement (17) and the evidence for active, non-osmotic water absorption is equivocal (95). Moreover, substantial increases in osmotic value have been observed during opening. Attention will be focused, therefore, on possible mechanisms for an increase in osmotic value.

The characteristic presence of chlorophyll in the guard cells led to the formulation of the photosynthesis theory. According to this theory light causes opening by supporting the photosynthetic production of osmotically active materials. Thus osmotic value and suction force should be increased. Unfortunately the photosynthetic potential of the guard cells is not sufficiently large. By the most optimistic estimates photosynthesis can account for a rise in osmotic pressure of 0.6 to 0.7 atm/hr in the guard cells of *Tulipa gesneriana*. In the same plant the osmotic value may increase 2 to 10 atm/hr during rapid opening. Another objection against photosynthesis as the sole mechanism for opening is that it does not explain the opening in CO₂-free air in darkness. Since the light effect is completely (or nearly completely) mediated through the photosynthetic reduction of the CO₂ concentration, photosynthesis is involved in the opening process but hardly in the classical sense. Photosynthesis is probably required in order to provide an adequate pool of carbohydrates.

The guard cells of most plants contain starch. The starch content tends to be low when the stomata are open and high when they are closed. The enzyme theory proposes that the increase in osmotic value during opening is caused by the enzymatic conversion of starch into sugars. The reverse takes place during closing. In some cases, good correlation between starch content and stomatal movement; at other times, none. Midday closure in particular takes place without changes in starch content. Since most of the observations have been made under natural conditions, the diurnal variation in starch content is responsible for the majority of the positive correlations found. There are no obvious differences between plants with the stomata open in light and plants with stomata closed in darkness at the same time of the day. Moreover, the changes in starch content are not necessarily accompanied by reverse changes in the sugar concentration of the guard cells.

The presence of starch is not essential for the opening process. The guard cells of plants like *Allium cepa* do not normally contain starch, but the stomata are able to open and close nonetheless. Such plants contain large amounts of other polysaccharides, such as fructosans (1) which may be subject to conversions similar to the starch-sugar conversion. The water uptake may be independent of starch in other plants as well. Mouravieff reported that starch hydrolysis is strongly promoted by guard cells continues after all the starch has disappeared.

There is good evidence that starch has a function in stomatal movement and that the hydrolysis of starch may cause an increase in osmotic value (108). The idea is attractive because it allows osmotic changes without major loss or gain of substances. However, the starch-sugar conversion is not absolutely necessary for stomatal movement and there is little quantitative information on the matter. One can only come to the unsatisfactory conclusion that at present the relation between starch-sugar conversion and stomatal movement is not clear.

A number of hypotheses have been based on the changes in protoplasmic properties which occur during movement. The so-called permeability theory is an example of this approach. This hypothesis relates the turgor changes to changes in permeability. Such changes have been observed (110),

but the evidence is conflicting and inconclusive. Increased as well as decreased permeability has been reported during the same phase of movement. As has been pointed out (112), changes in permeability can only change rates, not the final equilibrium, unless the change is one from no permeability at all for a substance to a measurable permeability or the reverse. Increased permeability could facilitate the influx of osmotic material from other parts of the leaf into the guard cells. However, this type of approach always creates more problems than it solves.

The possibility of changes in permeability of intracellular membranes should not be excluded from these speculations. Such changes could allow new enzymatic reactions to proceed or increase the rate of existing conversions. This could be accomplished by increasing substrate availability or by admitting needed ions to active sites.

Apart from permeability, other protoplasmic properties may be changed as well. Stomatal movement is accompanied by pH changes in the guard cells. During photoactive opening the pH rises. The rise may be caused in part by a decrease in the CO₂ concentration. It has been suggested that such pH changes affect

imbibition or hydration of the protoplasmic colloids. This is the protoplasm theory. Alvim however, was unable to find a correlation between colloidal swelling and opening. Mouravieff, on the other hand concluded that the opening effect of pH 3.5 was due to increased imbibition. A pH of 3.5 is a rather drastic treatment. Moreover, the effect is dependent on the nature of the buffer used. It is well known that salts have complex effects on aperture. The interpretation of results obtained with varying pH is complicated by this fact. It is possible that many apparent pH-effects are really effects of the ions in the buffer.

In general, opening is obtained by alkaline external pH. For example, acetate buffers have a maximal opening effect when their pH is 6.9-7.3. Stomata also open very wide in ammonia vapor. It would appear that under normal conditions the effect of a change in the hydration of colloids is negligible in terms of the amount of water taken up or released. Enzymatic processes such as starch hydrolysis or rates of transport could still be affected.

So far, the discussion has dealt with attempts to explain the turgor movement in osmotic terms. It is obvious that these attempts have not been very successful. Much speculation is involved. There is evidence that the phenomena which form the basis for the various classical theories are real. However, their causal relationship to movement is not clear. Certainly none of the theories can explain the events. It is tempting to conclude that a combination of a few or all of the classical theories is necessary. The various processes probably proceed independently, although they may influence each other at times.

There is evidence for such interrelationships. For example, when stomata are exposed to CO₂-free air, the pH of the guard cell becomes more alkaline and the starch content decreases. These two effects may be related, for starch formation is affected by the pH of the external solution.

That starch formation occurs in guard cells immersed in acetate buffers with a pH between 4.1 and 7.3. The optimum is found between 5.0 and 6.0. Unfortunately this optimum corresponds to the pH found in opening guard cells. If phosphorylase is responsible for starch hydrolysis, pH should have a large effect: the ratio of inorganic phosphate to esterified phosphate drops from 10.8 at pH 5.0 to 3.1 at pH

There is little other evidence linking the various changes which occur during movement. In case the changing processes form part of a catenary system, their order is a matter of speculation. Commonly, the classical scheme as an explanation of stomatal movement: light photosynthesis reduction of CO₂ rise in pH starch-sugar conversion increase in osmotic value increase in turgor stomata open

In view of this disappointing state of affairs it is not surprising that attention has been turned towards energy-requiring, non-osmotic processes. Opening could be the result either of active absorption of water or of counter-gradient transport of osmotic material from the neighboring cells into the guard cells. During closing, loss of water or osmotic material must occur, perhaps as the consequence of a cessation of the active process. On the other hand, closing could be accomplished by either the active excretion of solute or the active pumping out of water (55). If active processes are the driving force, stomatal movement should be sensitive to temperature, oxygen tension and metabolic inhibitors. These factors will have complex effects. Some useful information concerning them has nonetheless been obtained.

The information about temperature effects does not allow a definite conclusion, but it is not at variance with the idea of active movement. Stomatal movement is inhibited below 10°C, but little effect of temperature is found between 10 and 25°C when the supply of water and CO₂ are adequate (page 260). When the CO₂ concentration is kept low the rate of opening of onion stomata increases with increasing temperature and the Q₁₀ is 2.2. This may suggest the existence of an active process, but more evidence is required.

The effect of oxygen tension has been studied but rarely. Opening of closed stomata in darkness in reduced partial pressures of oxygen has been reported suggesting that closing may require energy. It is well-established that the initial application of anaerobic conditions to open stomata causes closing. After prolonged anaerobiosis (2 to 4 hr) stomata may open again. Again, the evidence is not conclusive and more information is needed.

The effects of a number of metabolic inhibitors has been studied. Sodium azide inhibits or prevents opening and stomata which are open start closing. On the other hand, that azide also inhibits closing induced by darkness or CO₂, azide actually stimulated opening in light in the presence of 3 per cent CO₂. The simplest conclusion is that sodium azide inhibits stomatal movement in general, independent of direction. This is not at all unlikely, opening induced by light and closing caused by darkness and found that both were inhibited.

Stomatal opening is also inhibited by phenylurethane, potassium cyanide, sodium arsenite, dinitrophenol, and sodium fluoride. Apparently respiratory inhibitors, particularly those which are uncoupling agents, are effective inhibitors of stomatal opening. This suggests that oxidative phosphorylation is necessary for opening.

α -Hydroxy sulfonates also inhibit opening. Hydroxysulfonates are inhibitors of glycolic acid formation in leaf metabolism. They cause extensive changes in the products of photosynthesis. It is of interest that glycolic acid affects photophosphorylation. Perhaps photophosphorylation participates in the light-induced stomatal opening.

The evidence presented indicates that energy-requiring processes play a part in stomatal movement. The information is not sufficient to decide whether the energy is actually used for the non-osmotic transport of water or solutes or for some other purpose. Assuming that the energy is required to support active transport, it is not possible to implicate either water or solute movement.

The combination of the inadequacy of osmotic processes with the positive results obtained with inhibitors of energy-yielding reactions leads to the tentative hypothesis that some form of active transport is involved in the turgor movement of the guard cells. It is not possible to decide whether opening or closing is active, or whether perhaps both are. The evidence favors active opening, but this is to some extent due to the design of the experiments with metabolic inhibitors.

The major problem to be solved is the mechanism of action of CO_2 . Undoubtedly, the solution has to be found inside the guard cell, for guard cells isolated from the mesophyll or in contact with killed mesophyll are able to open. The major function of the mesophyll is to reduce the CO_2 concentration to a sufficiently low level. Once this has been done, the guard cells can take it from there. Such a function of the mesophyll is not surprising in view of the low photosynthetic capacity of the guard cells. The guard cells receive CO_2 from their own respiratory activity, from the surrounding epidermal cells, and from the atmosphere inside as well as outside the leaf. Photosynthesis in the guard cell will not be sufficient to cope with this steady influx. But once the external CO_2 concentration has been lowered sufficiently by photosynthesis in the mesophyll, the chloroplasts will be able to reduce the CO_2 concentration in the guard cell to the level required. One can only speculate on the effect of low CO_2 concentrations on the guard cell. Low CO_2 probably causes an increase in pH and hydrolysis of starch. However, the causal relation between these changes and CO_2 is not clear and their adequacy is questionable from a quantitative point of view.

When active processes are involved energy must be provided. Green

plants have two sources of energy: oxidative phosphorylation and photosynthetic phosphorylation.

Photophosphorylation, in contrast to oxidative phosphorylation, is an anaerobic process. It does not require either CO_2 or water. Actually there is a competitive relationship between photophosphorylation and CO_2 fixation. Therefore, at low partial pressures of CO_2 in the light more ATP will be available for energy requiring processes. This energy may be used for the active transport of water or solute during opening in the light. As yet there is no

direct evidence for the participation of photophosphorylation in photosynthetic active opening. Information might be obtained by studying the effect of inhibitors of photophosphorylation. It is quite clear that part of the light action is a direct effect on the guard cells, whether through photosynthesis or photophosphorylation. This is demonstrated by the reactivity of isolated stomata (51, 132). It is also a logical consequence of Liebig's finding that the opening of the stomata in the lower epidermis requires much higher light intensities when the upper epidermis is illuminated instead of the lower one.

Neither photosynthesis nor photophosphorylation can explain the opening caused by CO_2 -free air in darkness, or, alternatively, the closing caused by CO_2 in darkness. A study of this mechanism is highly desirable, the more

so because CO_2 occupies such a central position in the stomatal mechanism.

Very little is known about the effect of CO_2 -free air on the metabolism of the guard cells. There is an urgent need for an investigation of the effects of CO_2 on the metabolism of the guard cell and on the active transport of water or solutes, if the existence of such active transport mechanisms can be demonstrated.

The importance of forest canopy structure

If you are looking for a classic example of the circle of life, consider the forest canopy structure. A forest's canopy structure is influenced by environmental and soil factors, and it also influences these factors in return. It is an aspect of forest ecology that is receiving more attention in this age of climate change emergency. The canopy structure is often overlooked, with most of the focus being on biodiversity. One of the reasons could be that the forest canopy structure is a more complex concept, which cannot be easily captured in a number.

Understanding Forest Canopy Structure

A forest or any biome is made up of several trees that belong to many species. A tree has a stem and a crown of branches that bear leaves, flowers, and fruits. Though forest canopy structure can mean different things, it basically refers to the arrangement of tree crowns; it has two components: the vertical structure and horizontal structure.

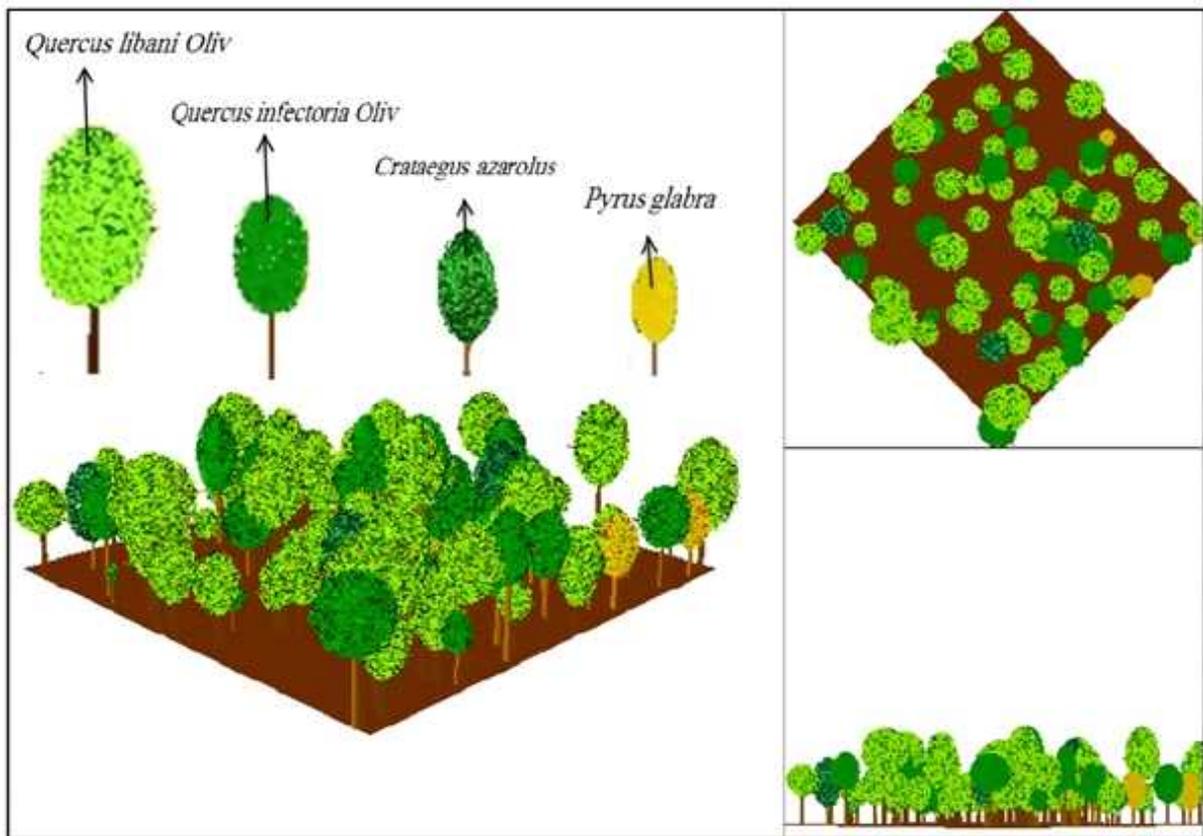


Figure 10. Stand visualization simulation of Blake forest (vertical and horizontal structure)

Figure 10 showed Blake forest have two story layers.

Figure 1: The figure at the top right shows the horizontal structure, while the bottom right shows the vertical structure. The figure on the left shows the different species making up the forest. (Image credits:<http://www.imedpub.com/articles/study-of-vertical-and-horizontal-forest-structure-in-northern-zagros-forest-case-study-west-of-iran-oak-forest.php?aid=11590>)

Horizontal structure is created by the placement of trees and how close their crowns are to each other. A collection of these crowns creates the canopy (See Figure 1).

Vertical structure is formed because trees and shrubs in a forest will have different heights. So, two or more layers of tree crowns can be found in forests. These layers are called forest storeys or strata. The top-most stratum is made of emergent trees; below them are the canopy trees, which are usually present as a continuous layer. The tree strata below them are called the sub-canopy and the understorey. Next, close to the ground could be shrubs, herbs, and grasses (See Figure 2).

Complicating this concept of forest structure is the issue of spatial scale, i.e., space or area that is considered for estimating the canopy structure.

Regional Scale

At a regional scale, the number of trees, shrubs, herbs, their species, and their arrangements give the forest canopy structure (See Figure 3). At this large scale, forest canopy structure defines the biome and its conditions, and it tells us whether the forest is

Continuous/patchy or pristine/disturbed.
Coniferous, scrub, savanna, grassland, broadleaved, etc.

Remote sensing is the preferred method of collecting data about forest canopy structure at large scales.

Local Scale

At the local scale, forest stand attributes, such as tree girth, the shape of a crown, height, and tree architecture, are the defining characteristics of forest canopy structure. Here, the forest canopy structure can also refer to the total quantity of leaves in a forest. Ground based methods are used to collect data on local forest canopy structure.

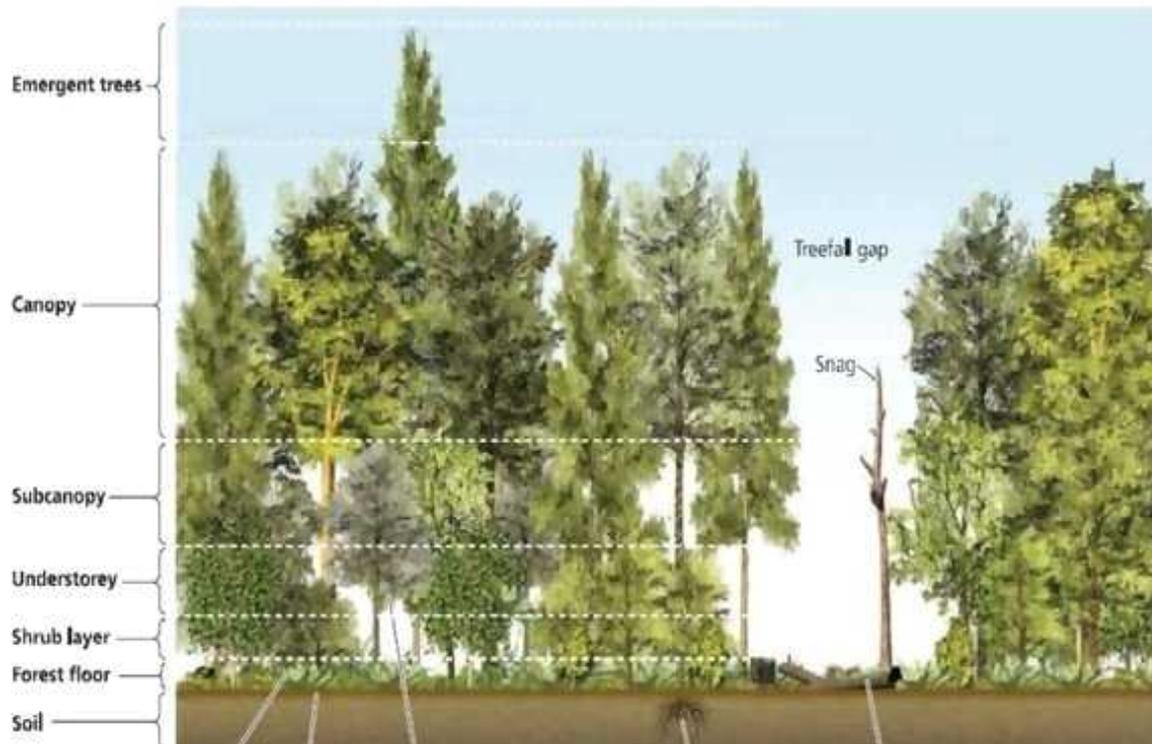


Figure 2: Vertical Forest Structure. (Image credits:<https://slideplayer.com/slide/13767425/>)

Role of Forest Canopy Structure

Forest canopy structure determines many of the environmental factors and functions of a forest and is, therefore, important for all the species living in a forest.

Light interception: Light distribution within the forest depends on the number of layers. The upper layers will get more light radiation, with little light coming through to the forest floor, if the canopy is dense. For this reason, there is little grass and herbs in well-developed and old forests. The amount of light reaching lower

parts of a tree is also important as photosynthesis depends on it. So, there is more photosynthesis in the upper layers of a forest canopy.

Moderate temperature: Temperatures within a forest vary with height. At the upper canopy layers, temperatures are extreme and fluctuate more, while in the forest interior, they are moderate and stable, making forest floors preferred homes of large animals like ungulates.

Wind and rain protection: Forest canopies act as a windbreak and intercept rainfall making the forest interior more hospitable.

Produces niches: The combination of environmental conditions at various heights creates a myriad of microclimates, which provide specialised niches for other plants, animals, birds, insects, etc. For example, epiphytes grow in branch nooks, and most insects live in the crowns of trees.

Structural diversity: Besides the microclimates, the forest canopy with its branches and arboreal connections creates structural diversity which is vital for the survival of many

animals. For example, many tree-dwellers escape ground predators by staying and moving in the canopy.

Biodiversity: As a result of its influences, the forest canopy structure is necessary to maintain the biodiversity of a forest. Differing forest canopy structures of biomes increase the overall biodiversity of the earth.

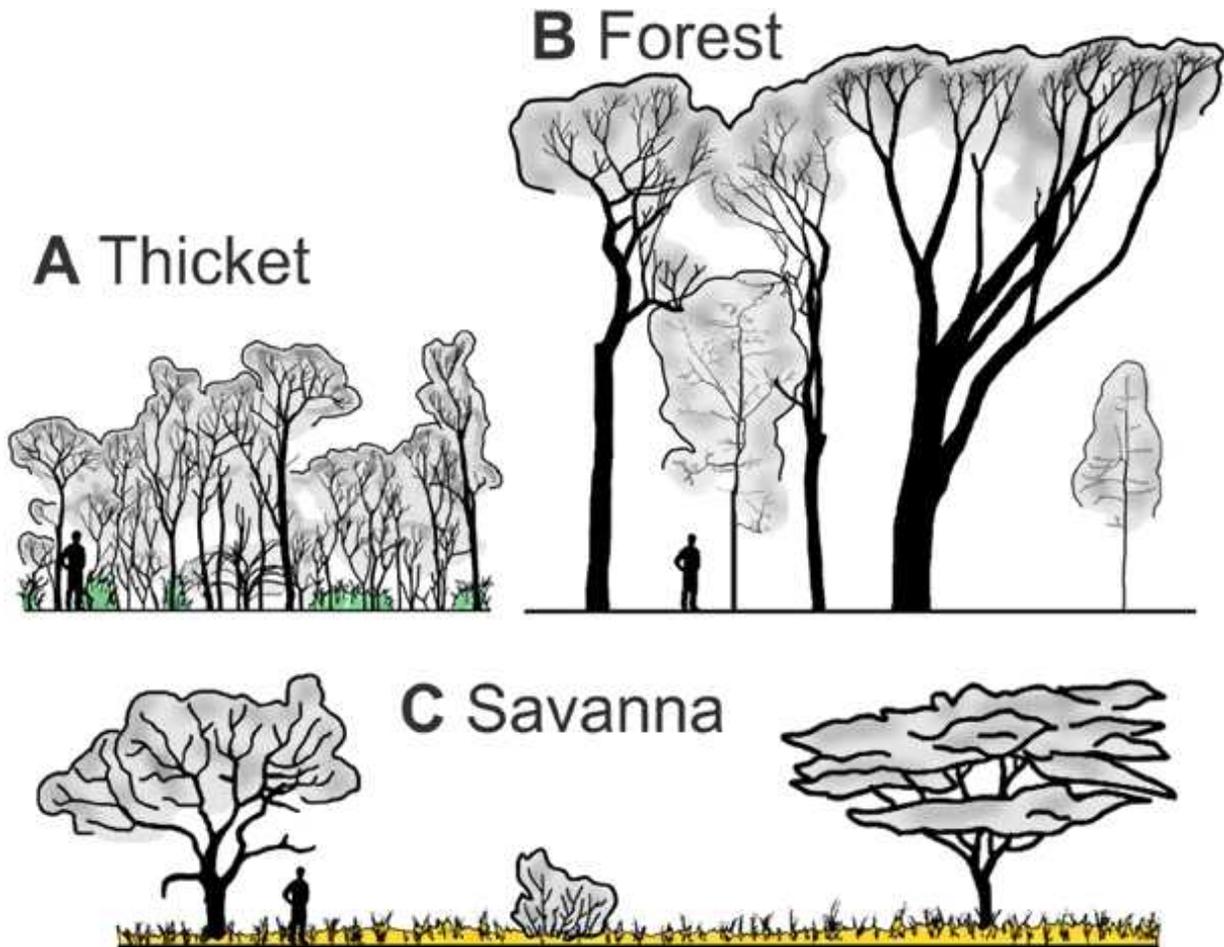


Figure 3: Biome vegetation structure. A Thicket with a low closed canopy and a discontinuous layer of shade tolerant grasses; B Forest with a tall closed canopy, no grass layer, and a mid-storey of shade-tolerant trees; C Savanna with a continuous C4 grasses layer and a discontinuous tree cover. From Charles-Dominique, et.al. (2015) (Image credit DOI: 10.1016/j.sajb.2015.05.005).

Uses of Canopy Structure

Besides the importance of forest canopy structure for the forest ecosystem, it is useful for people in many ways and is therefore monitored. Some of the most important uses are as follows:

Ecosystem Productivity: Forest canopy structure studies at the regional and local scales have often been used to determine carbon uptake, storage, and flux for carbon accounting and validation. Such productivity analysis is common in climate change mitigation studies.

Ecosystem Services: It is the canopy structure of the forest that determines many of the ecosystem services we associate with forests. These could be:

Intercepting and helping in percolation of 60-90% of rainwater/ snow gently into the soil and producing a watershed region important for the hydrology of a region.
Providing people and animals with clean drinking water.

Producing litter (leaves, fallen branches, etc.) that maintain soil fertility and soil carbon banks.

Providing a canopy or ground cover that protects soil from the force of rain and a mat of roots that hold soil to conserve it.

Forest canopies hold a lot of the water that trees absorb and recirculate it into the atmosphere through transpiration, providing input for rain, so the water cycle is maintained. For example, one-third of the rainfall in the Amazon is due to the forest canopy.

Agricultural productivity: Many cash crops have been traditionally cultivated as understorey crops and require a forest canopy to thrive. Examples are cardamom, coffee, or cacao.

Forestry: Forest canopy structure is also regulated because many commercial forests provide timber and wood.

Non-timber products: It is essential to maintain and monitor primary forests' canopy, as it provides non-timber products such as medicinal plants, nuts, fruits, resins, barks, etc. The tree crown provides most of these products.

Ecology studies: Forest canopies are a reflection of available moisture and soil types, and differences in canopy structure can establish gradients in site quality of forests.

Measuring Forest Canopy Structure

Forest canopy structure can be measured on the ground by quantifying the leaf area in the forest or through forest cover estimation. The Plant Canopy Imager CI-110, produced by CID Bio-Science captures wide-angle canopy images while estimating Leaf Area Index (LAI) and measuring Photosynthetically Active Radiation (PAR) levels. It has a GPS to provide the exact coordinates of a place, for repeat observations. The GPS makes it also possible to integrate ground observations with remote sensing data in Geographical Information System (GIS) to expand its efficiency.

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