

THE UNIVERSITY OF CALGARY

Wind and plant growth. The influence of bending and reorientation of tissues on ethylene production, xylem flow and branch abscission.

by

Lukas de Wit

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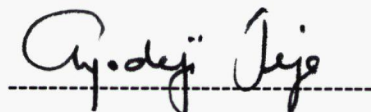
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ABSTRACT

The geometric forms developed by vascular plants are affected by the normal wind conditions at a habitat. Shorter and stouter plants are characteristically found at windy habitats. Winds may cause a plant to undergo temporary displacements such as bending and oscillations, or promote a net water loss from the leaves as in a drought. For some plants, ethylene is produced when they are subjected to such stresses. Ethylene may, in turn, induce growth responses in mature tissues. This study is directed to investigating the causal relationships. Gravistimulation and thigmostimulation are seen to result in increased ethylene production in stems of species that are self-supporting (notably sunflower and tomato). No increase in ethylene was observed in climbing plants (such as pea, bean, and cucumber) after similar treatments. Bending was not demonstrated to have any influence on the rate of ethylene production in any of the plants tested here, although there are certain technical difficulties in designing experiments that test for bending in the absence of gravistimulation. Drought stress caused consistent but moderate increases in ethylene levels; the increases became large in cases where drooping of the non-turgid stem induced gravistimulation. There was also a tendency for lower (presumably most expendable) leaves to show the highest increases in ethylene production when water stressed. Another series of experiments showed that bending of a plant stem results in a narrowing of the xylem vessels, and hence a decrease in flow. The possible reasons for the initial increase and subsequent decrease in the flow rate of excised stems were also

investigated. It appeared that the initial increase might be due to the replacement of air by water in some of the vessels. The decrease in flow rate was shown to have some relation to the formation of biofilm-forming bacteria on the inflow end of the stem section. The phenomenon of branch abscission in balsam poplar was also investigated. The pattern of branch fall was described for a three year period; other experiments suggested what factors may trigger the process. Comparison of daily maximum wind records with the phenology of branch fall suggested possible proximal and ultimate relations between branch abscission and wind.

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CHAPTER ONE - INTRODUCTION

Plants which grow in locations where they are exposed to strong winds characteristically develop stems and branches that are shorter and thicker than those of plants in sheltered areas. Stems or branches may also be permanently deformed, or they may be swept away from the prevailing wind direction. It is safe to say that these changes in form are not genetically determined, nor are they primarily the result of selection (survival) of certain individuals out of a population: they are the responses of individual plants to the climatic conditions that exist where the seeds germinated. The wind induces these characteristic shapes, but the mechanisms that are involved are not clear. The potential means by which wind affects plant growth might be placed into four categories: 1. alterations in the composition and temperature of the air surrounding the plant; 2. direct stimulation of epidermal tissues; 3. bending of leaves and stems; and 4. pruning of branches by breakage. The first is an indirect effect: moving air causes carbon dioxide to be supplied to the stomatal pore at a faster rate than it would be by simple diffusion. Likewise, water and oxygen molecules are swept away from the region surrounding the stomate, so that the water potential or vapor pressure gradients become steeper. These in turn affect the rates of transpiration and photosynthesis, and thus the ability of the plant to grow. Since the epidermis of most plants is constructed so as to prevent water and gas exchange except at the stomates, it is at these pores that the first kind of wind effect acts. The distribution of stomates in the plant is essentially that of the leaves, and to a lesser extent of the non-woody stems. This means that the result of windy conditions on growth of the plant

(considering only this first effect for the moment), is an increase or decrease in the overall growth of the plant, depending on the strength and duration of the moving air. The first category of wind-induced effects on plant form may alter the general level of activity in all the meristematic regions, but it will probably not affect the relative activities of the lateral versus the apical meristems: its effect will be to produce a plant that is larger or smaller than it otherwise would be, but whose shape is not greatly changed. For similar reasons, the second category of potential wind effects on plant growth, namely direct stimulation of epidermal tissues, is unlikely to cause differences in the form (as distinguished from the size) of plants grown in areas of varying wind. All regions of the epidermis (or of the periderm) are more or less equally massaged by the moving air, so that any effects on growth that might arise from this mechanism are likely to be distributed more or less uniformly over the stem and branches. It is to the last two categories above therefore that one's attention is directed in this search for the mechanism(s) of wind action on plant form. They are subtitled below as "Stem bending", and "Branch pruning".

1. Stem bending.

The need for some kind of mechanism to match up the architectural development of each portion of an organism with the usual forces that act on that point is not unique to plants, but it is one that is especially acute in larger land plants. It is perhaps no coincidence that this group is also the one which is most exposed and most vulnerable to the damaging effects of strong winds. By itself, the force of gravity demands that plants have a certain degree of structural strength, but it is when the static stresses caused

by gravity become dynamic stresses as a result of wind that severe demands are placed on the plant stem and branches. It might be hypothesized that the strains produced within each part of an individual plant are translated into sequences of physical and biochemical events whose result in each case is to strengthen those parts to an appropriate degree, especially during the period when they are in the growing stage. These strains are produced by the bending and twisting that result from the deflections of the branches, and by the oscillations that are set up throughout the tree by the natural frequencies of its parts. According to this idea, a plant adjusts its shape to the specific habitat in which it is growing, and to all those events (such as localized diseases, broken branches, changes in light and water availability) which alter the magnitude and direction of the forces acting on it, by the independent reaction of every lateral meristematic cell (and to a lesser extent non-meristematic cells as well) to these ever-changing conditions. (The apical meristems are discussed separately below.) But how can these forces be perceived by the growing cells? Three possible mechanisms suggest themselves: first, that the plastic, fragile cambial cells respond to the movements of the more rigid cells that surround them; second, that the strength-bearing cells cause the production of some messenger compound when their cell walls are stressed; and third, that bending and twisting of stems causes distortions of the tracheids and vessels, and thus restricts the water supply to distal tissues. Of these three possible mechanisms, the second and third are investigated in the experiments described here. The question being asked is: could these mechanisms cause localized changes in the growth processes so as to provide strength where it is needed? In the next few paragraphs, the three proposed mechanisms are introduced in

more detail. They will be identified as "Cambial stimulation", "Possible messengers produced by wounding of cell walls", and "Bending-induced restriction of xylem sap flow". Before continuing with the introduction of these two proposed mechanisms, however, let us return for a moment to the apical meristems.

The hypothesis advanced above (that plants produce reinforcing structures in proportion to the stresses imposed on their individual parts) has implications in the role of the apical meristems in determining plant form. By far the greatest stresses in a plant occur at the bases of stems and branches; conversely, the areas of least stress are the extremities, namely the apical meristems. Therefore, strong winds cannot regulate apical growth directly in the manner described above for cambial regions. There remain several other possible ways in which wind might have a more or less direct influence on axial growth. One is by the effect of air movement on the apex itself. This is unlikely to be important, since the apex is usually well protected by developing leaves. The other is by the action of wind in whipping about the portion of the stem just below the apex, namely the region of elongation. This possibility did not form part of the present investigation, but it can not be discounted. Besides these two relatively direct means, there are also some more indirect ways in which the growth of the apex may be influenced by the wind. They might be referred to as correlative controls of apical growth by proximal tissues. This is an unfamiliar way of envisaging physiological controls - usually the apices (both of the root and of the shoot) are thought of as primary sources of the hormonal regulators of growth, and therefore the areas that direct many of the activities of the regions in between. And yet in the present context, the idea of proximal (cambial) control has some appeal.

Certainly it is not difficult to imagine a possible mechanism for a degree of proximal control over the apex: if the lateral meristems are called upon to produce extra width and strength in keeping with the stresses placed on them, then the materials used for this will not be available for apical growth. This would seem to a natural kind of hierarchy for the prioritization of photosynthate utilization: that provision for adequate strength and stability should be ensured before the start of elongation growth. Again, this was not investigated in the present study, but the matter is raised in this introduction since plant form is the resultant of the relative growth rates of apical and lateral meristems, and because of the prominence of the apices in the literature of plant physiology.

Cambial stimulation

This area was not investigated in my work, but for completeness it too is mentioned briefly. A number of workers have suggested that pressure to a membrane may influence phospholipid/protein packing in the membrane, and hence physiological activity of the cell (Lintilhac, 1974; Halstead, 1984; Pickard, 1985; Boyd, 1985). Since the activity of the cambium determines the extent of lateral growth, it is entirely possible that deformations of the cambial cell membranes themselves regulate the relative growth of the lateral meristems throughout the plant.

Possible messengers produced by wounding of cell walls

It should perhaps be mentioned here that in this section as indeed throughout the rest of this thesis, both woody and herbaceous species are considered. Trees are often treated separately in physiological studies, but

they are included here with the other types of vascular plants, since the need for striking a balance between strength and height growth is common to higher plants in general. Busgen (1929) observed that plants have mutually opposing requirements for stiffness and strength on the one hand, and the expansion of the crown and roots, and the production of seed, on the other. Furthermore, since there are no clear demarcations between lignified and non-lignified taxa, it is doubtful whether the mechanisms for the perception of and responses to imposed dynamic stresses would be separate either. It was hypothesized above that one way in which localized stresses could be converted into signals that can be perceived by the cambium, is that strains or ruptures in the cell walls of load bearing cells might result in the production of a messenger substance. The mention of a wound hormone suggests the possible involvement of ethylene. Goeschl et al (1966) reported increased ethylene evolution when the growth of pea seedlings was obstructed by foam stoppers or by glass beads. Yu et al (1982) reported increased ethylene production in wheat seedlings when these were cut into segments. McAfee & Morgan (1971) found increased ethylene in cotton petioles and leaves after wounding. Elstner & Konze (1976) found that freezing of portions of sugar beet leaves caused ethylene production in the leaf area surrounding the frozen parts: thus it represented a wound response of physiologically perturbed cells, adjacent to the decompartmentalized (killed) cells. The effect of ethylene on growth is to reduce length and increase the diameter of the stem. Many workers, notably Jaffe (1976, 1980, 1985) have demonstrated that "mechanical perturbation" causes plants to become shorter and stouter. Usually this kind of experiment is done by rubbing the stems or leaves manually; sometimes

natural or machine-produced wind is used. The former type of experiment has usually been associated with increased levels of ethylene production. The latter type (where wind is the perturbing influence) in many cases described the effects on plant growth, but it seems that no one has reported the effect on ethylene production, although ethylene has often been suggested to be the mediator involved. It seems worthwhile therefore to measure the production of ethylene in plants exposed to wind.

There are other substances that might be considered as possible messengers for this proposed system. Kraus (1880, in Busgen, 1929) reported that mechanically caused shoot movements produced "an extraordinarily great influence" on the the composition of the sap in a very short time. When the shaking caused the stem to take a bow shape, the concentration of sugar on the concave side became immediately higher. Another possibility is water: distortion of the water-conducting cells of the xylem will place their contents under increased pressure. Since the xylem sap is in equilibrium with the porous matrix of the xylem walls and the apoplast this will result in a change in the water potential of the tissues adjacent to the vessels, including the cambium. Munch (in Busgen, 1929) found that a moderate bending of stems of various broad-leaved species resulted instantaneously in a great reduction of the turgor pressure of the sieve tubes, which lasted for about 15 minutes. At least some of the other plant hormones could be involved as well: Reches et al (1974) found gradients of endogenous gibberellins and of abscisic acid in twigs of weeping mulberry; Casperson (1968) found that cambial activity of horsechestnut epicotyls was stimulated by exogenous auxin or exogenous kinetin. Finally, electric fields in plants were investigated by Lund (1947) as

possible integrating mechanisms by which the cells of a system might operate collectively to a certain degree.

Bending-induced restriction of xylem sap flow

Jaccard (1919) based his research into tree form on the idea that the flow of water determines the extent of lateral growth. He did not include in his model any role for wind or bending, however. The idea that bending might cause a decrease in the rate of xylem flow is one that was proposed by Banks (1973), who suggested that when a stem bends, the tissue that has the greatest capacity to deform is the xylem, because the contents of most of its cells (the xylem sap) can be redistributed. Banks, an engineering student, did not have the opportunity to test his proposition. McIntyre (1987) has been arguing for some years in favor of water as an important regulator of plant development, based on the hypothesis that water is the factor that normally limits the rate of growth and metabolic activity in intact plants.

2. Branch pruning.

The precise prediction of any climatological parameter is apparently impossible for human beings, and it would be surprising to find an aspect of the weather that can be anticipated completely by other organisms. Plants, like meteorologists, play a game of probability: they develop into shapes and sizes that are sufficiently stable to resist the usual winds that occur in each locality. When an unusually strong wind comes along, the plant's shape is adjusted by breakage of the weaker branches. This results in a

loss of leaf area. The loss can be regarded as representing a certain amount of wasted effort, or it can be seen as the cost of gambling on the amount of strength put into a branch or trunk. One might imagine two kinds of strategies in plants with respect to how they adjust their load bearing members to their individual circumstances. One is to build in a high safety factor, so that losses from breakage are few. This strategy involves the allocation of resources to load bearing members, potentially to the detriment of reproductive activity, and potentially involving a loss of competitiveness where other species share the habitat. The other is to have low margins of safety, and to endure the consequent pruning of branches that will occur by breakage. Associated with this second strategy will be the cost of increased branch breakage, and the cost of an increased requirement for defense against invasions by pathogenic organisms. The likelihood that a branch will break during a heavy wind depends on the sail area of the branch, on the natural frequency of oscillation of the branch (as well as those of branches distal and proximal to it), and on the damping effect of the air in resisting oscillations. In fast-growing trees, it would be an advantage for an individual to be able to reduce its sail area, and in fact some species do this by abscising their non-productive branches. Members of the genus Populus are among the few trees native to Alberta that do this. As part of the present study, this phenomenon was studied to get some idea of the pattern of branch loss, and of the amount of sail area that is lost by the shedding of branches. Also, the extent of non-abscising branch breakage (wind pruning) was followed over a period of time.

CHAPTER TWO

Ethylene as a possible messenger in regulating cambial growth.

Introduction

When a plant sways under the influence of the wind, several things happen. Bending stresses are produced in certain of the tissues, and the orientation of branches and stems changes with respect to gravity: they are gravistimulated. Each of these can be considered separately to see their effect on ethylene production, and that is what the present study set out to do. In practice, the separation of the two is not always easy, as noted in the discussion below. Gravistimulation is easy to arrange in an experiment, but to design an experiment that tests bending in the absence of gravistimulation could be done ideally only in conditions of zero gravity. As a result, the effects of gravistimulation on ethylene production tended to be investigated much more thoroughly than those of bending in the experiments described below. In addition, certain of the experiments gave results that led to other experiments into the distribution of ethylene production in plants. For example, while testing for ethylene production in sunflower plants, it happened that one day several plants became droughted, and that their stems drooped over. Would the drooping portions of the stems produce increased ethylene levels, as did other non-horizontal stems in this species? The answer was yes, and on examining the literature on ethylene and drought, it appeared that the notion of gravistimulation (drooping) might help to resolve some of the contradictory reports on this

subject that had appeared in the past. Likewise, after a certain number of species had been tested for ethylene production, certain patterns began to emerge: some plants produced ethylene in response to a variety of stimuli, while others seemed to produce only a certain more or less constant level of ethylene. The responsive plants were basically free-standing species like sunflower and tomato, while the less responsive species were climbers like pea, bean, and cucumber. This seemed to tie in nicely with ideas about the need in some plants to have a mechanism of response to wind, and the absence of this need in others. In the course of this introduction, some of the experimental results have been anticipated somewhat so as to explain the way in which the experiments developed.

Materials and methods

Seeds were planted in 12 cm pots using a Terragreen medium. They were kept in growth chambers under a 16 hour photoperiod, and watered automatically for 2 minutes every hour with half-strength Hoaglands solution. Plants used in experiments were selected for uniformity of size and vigor. Throughout the text, English common names are used for the following species: Helianthus annuus L. var. D131, Pisum sativum var. Alaska, Phaseolus vulgaris L. var. Tender Green, Vicia faba L. var. Exhibition Long Pod, Cucumis sativus L. var. Straight Eight, Lycopersicon esculentum L. var. Vetomold #121, Populus balsamifera, and Picea glauca (Moench)

Voss. The common names used are sunflower, pea, bean, broadbean, cucumber, tomato, balsam poplar, and white spruce.

Gas samples were obtained from plants by the following the technique. Using a sharp razor blade, a portion of the plant was excised. The tissue sample was placed in a 10 ml plastic syringe, to which a 3-way stopcock was attached. The syringe plunger was inserted such that there was 2 ml of headspace above the sample. After exactly 20 minutes, the 2 ml of gas in the headspace was transferred to a second syringe, and analyzed for ethylene by gas chromatography. The machine used was a Varian 2000 with flame ionization detector. Temperature settings were 120°C at the detector, 40°C at the column, and 30°C at the injector. The samples were weighed afterwards, and the ethylene concentrations calculated from this. In all figures values of ethylene are given as nanoliters of ethylene per liter of air in 1 gram fresh weight of tissue.

For analysis of ethylene levels in poplar trees a hole was drilled into the wood of a tree to a depth of 5 cm using a 4 mm increment borer. The hole was then plugged with a cork, creating an air cavity continuous with the intercellular air space of the xylem. This cavity was monitored for ethylene levels by inserting the needle of a 5 ml syringe through the cork, and withdrawing 2 ml of air. This was then taken to the lab and analyzed by gas chromatography in the usual way.

Results and Discussion

This portion of the results is grouped into five sections, under the following headings:

- a. Distribution of ethylene in the plant
- b. Gravistimulation and ethylene
- c. Bending and ethylene
- d. Drought, gravistimulation and ethylene
- e. Thigmostimulation and ethylene

a. Distribution of ethylene in the plant.

The ease with which ethylene can be measured has resulted in the appearance of a large number of papers relating to this plant hormone. It is surprising, therefore, that there are very few among them that give any idea of the amounts of ethylene that are normally produced in various locations, and in the different organs throughout an individual plant. This is especially so for mature plants. Burg & Burg (1968) measured ethylene production along an etiolated pea stem: they found that the nodal areas produced greater amounts of ethylene than the internodes, and further that there was a gradient along the stem in both types of tissue, with the highest levels being toward the apex, and an especially high level in the hook region. In the great majority of the other cases, samples are either taken from one specific part of the plant (hypocotyl, first mature leaf, etc) or else the exact tissues

used are not specified (e.g. El Beltagy and Hall, 1974). These kinds of approach have both advantages and disadvantages. An advantage is that such things as time course studies, and multiple treatment regimens can be done without becoming too unwieldy. Another is that replicate treatments and statistical analysis are usually possible. A disadvantage is that in simplifying the situation down to a single response (perhaps assumed to be representative for the whole plant), the potential exists that an inaccurate picture of the events occurring throughout the plant can be developed. The different parts of a plant may respond to a treatment by producing different levels of ethylene. Furthermore, if gradients are found to exist within the plant, then this means that the rate of ethylene transport, or that of its precursor ACC (1-amino-cyclopropane-1-carboxylic acid), is not sufficiently great to equalize the observed production of ethylene in various locations, and that gradients in physiological response may also exist within the plant. This section seeks to put the rest of the experimental work of this thesis into perspective by showing the overall pattern of ethylene production in a normally growing, healthy plant.

Figures 1,2 and 3 give the rates of ethylene production in sunflower, pea, tomato, and cucumber. These results are from single plants: the production of ethylene by mature plants is of such variability that this method was chosen for presentation of the data. Such analyses were done for many individual plants of each species, and the results given here are typical. Although the absolute amount of ethylene might vary from individual to individual the relative amounts of ethylene in young leaves compared to old, and of leaves to stems followed the same patterns. These indicate the kind

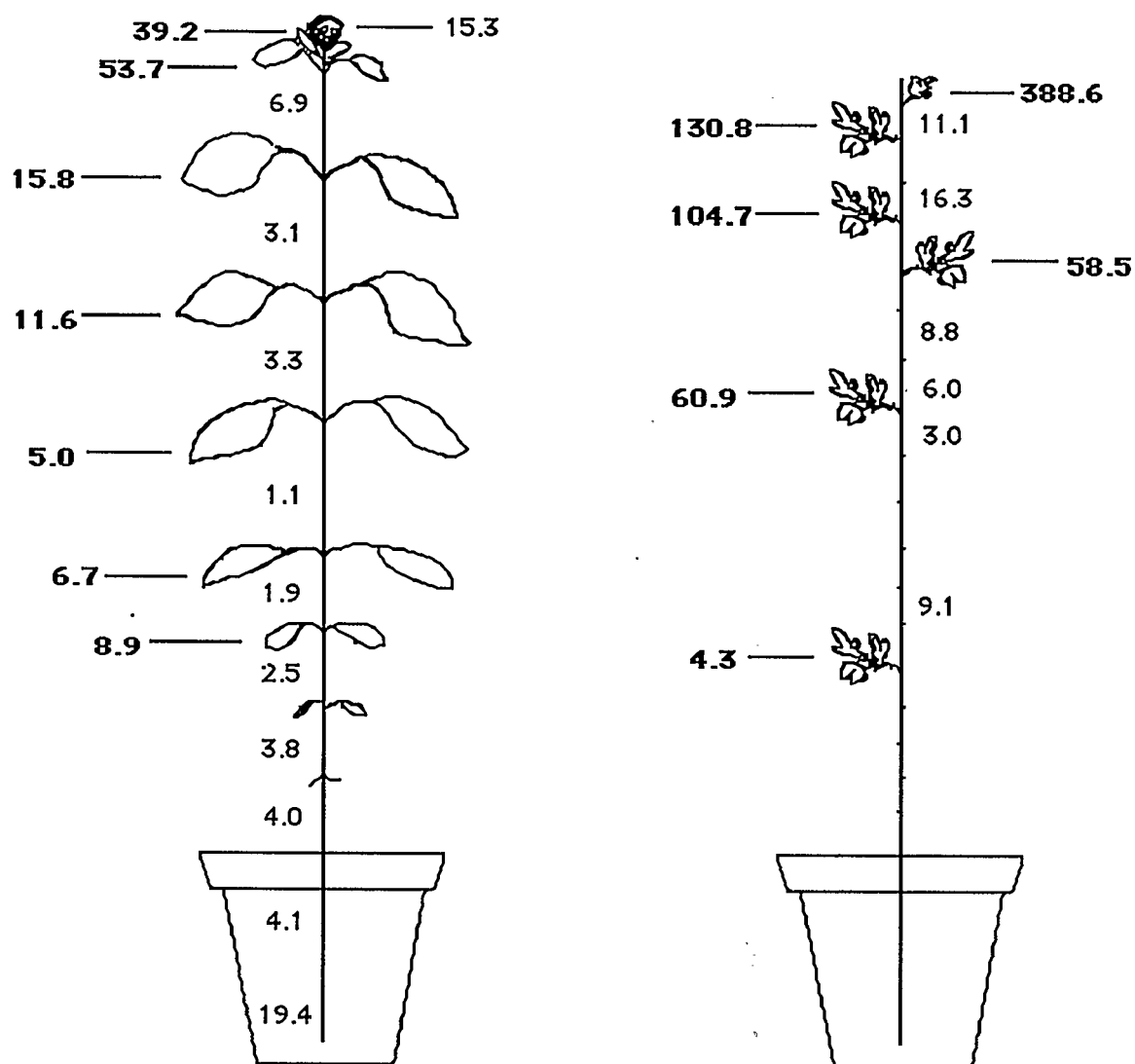


Figure 1. Ethylene production in the leaves, stem, and roots of (A) 56-day-old sunflower, and (B) 87-day-old tomato. All numbers represent ethylene production in nanoliters per gram fresh weight (FW) during a 20 minute period. The entire root mass of the sunflower plant was divided into two halves, a bottom half and a top half. Bold face numbers are for ethylene levels in leaves.

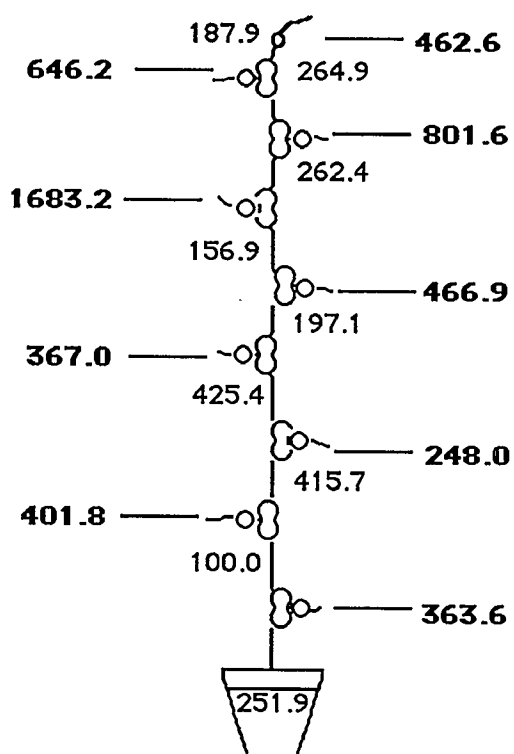


Figure 2. Rate of ethylene production in the leaves and stems of 28-day-old pea. (Ethylene in nl/gFW during 20 min.)

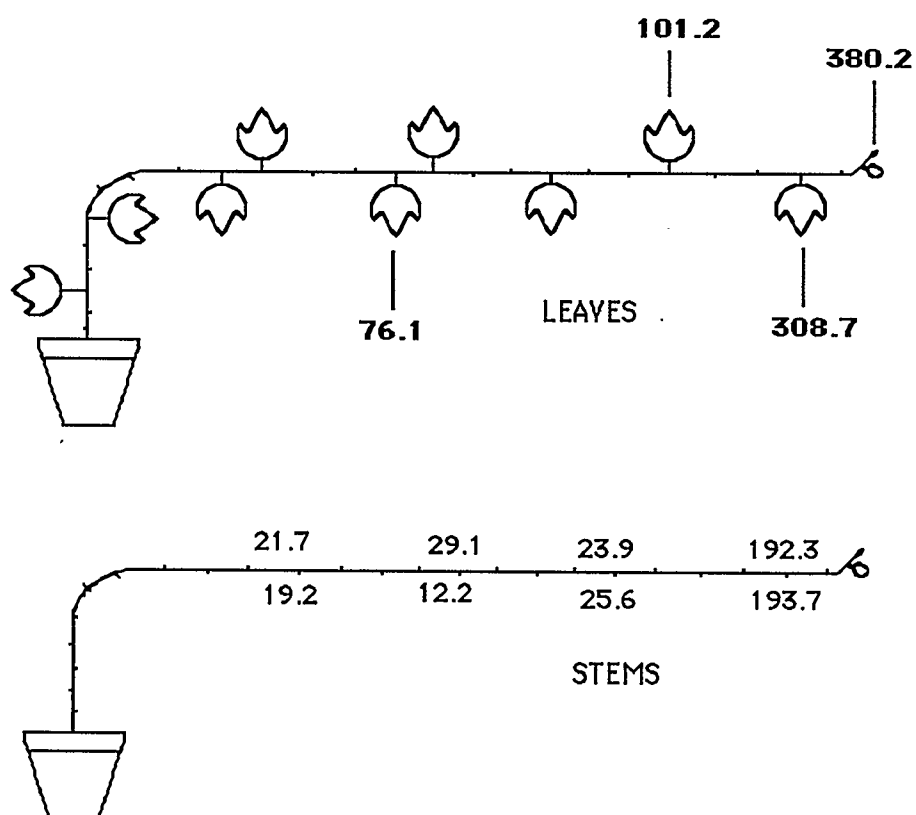


Figure 3. Rate of ethylene production in 45-day-old cucumber. (Ethylene in nl/l/gFW during 20 minutes).

of ethylene levels in normal, unstressed plants, and can be compared with similar diagrams that are presented later in the other kinds of experiments.

Fujino et al (1988) measured ethylene production in 90-day old tomato plants, and found that the apex produced the highest amounts of ethylene, with progressively lower amounts produced by each lower internode. They used the nodal area of the stem together with its attached leaf for their determination of ethylene production at each level, thus they did not distinguish between stem and leaf ethylene.

In all four of the herbaceous species shown in Figures 1-3, ethylene production was in general higher in the leaves than in the stem. The levels of ethylene produced by leaves were highest in the upper leaves. In cucumber, tomato, and sunflower, it was the youngest leaves that produced most ethylene, while in pea, highest ethylene was found in the third youngest leaf. There was an apparent gradient in the stem ethylene for the first three mentioned species, where upper parts of the stem had the highest levels. Pea was atypical in this respect as well - here the gradient if anything was opposite to that of the other species.

Figure 4 suggests that the areas of the lateral branches that produce highest levels of ethylene are somewhere near the middle of the branch, with lower levels near the trunk and towards the branch ends. A lateral branch is, in engineering terms, a cantilever (a beam which has one of its ends built-in to a wall or other supporting structure). The bending stress depends linearly on weight, length, and angle, and therefore the greatest

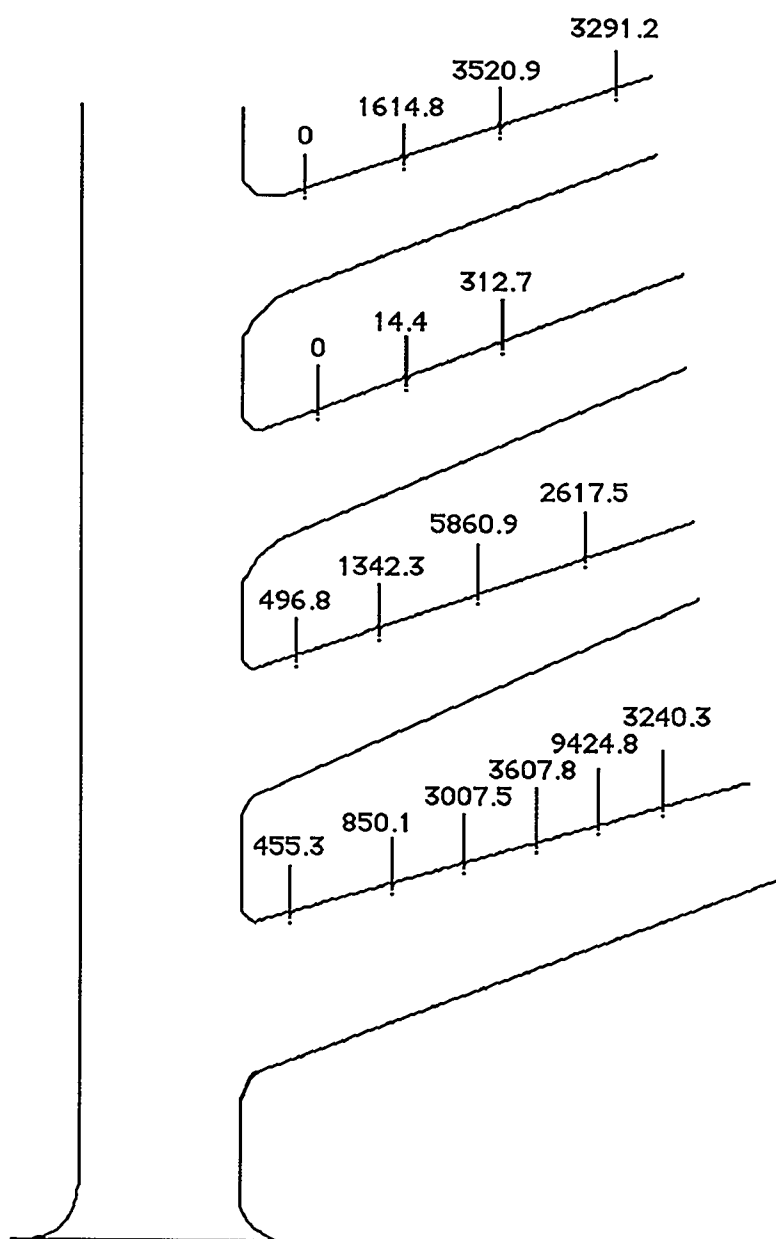


Figure 4. Ethylene production in poplar (nl/l of air sampled): distribution along the upper sides of four branches of one tree. Tree is drawn approximately to scale (1:20), but the branches are drawn to one side for clarity. (May 5, 1986).

stress is at the base of the branch. The ethylene levels seen here certainly are not greatest at the branch bases, and this was interpreted as evidence against the hypothesis that ethylene is a messenger by which cambial activity can be matched to the degree of stress experienced by a region. The possibility remains that at this time and in this season, the branch base is already sufficiently strong, and that the weak link is indeed the middle of the branches. However, a second piece of evidence that argues against this idea came from a comparison of ethylene levels and wind speeds - these results are shown in the section on Bending. No discernible gradient was found in the vertical distribution of ethylene production (Figure 5).

b. Gravistimulation and ethylene

The literature linking ethylene and gravistimulation is extensive, and dates back many years. Exactly what the role of ethylene in gravitropism might be is still not clear (eg. Clifford et al, 1983; Harrison & Pickard, 1986), but increases in the production of this hormone are consistently seen in many species subsequent to a change in orientation. That gravistimulation can produce ethylene, and that it induces compensatory growth in plants, independently of any flexion of plant parts, seem probable in the light of experiments with potted plants (eg. Wheeler & Salisbury, 1980), in which the orientation of the whole plant is altered, as well as experiments with explants (eg. Phillips, 1972; Hart & Macdonald, 1984). The effect is not merely a transitory one: it is seen in the horizontal branches of apple trees, as

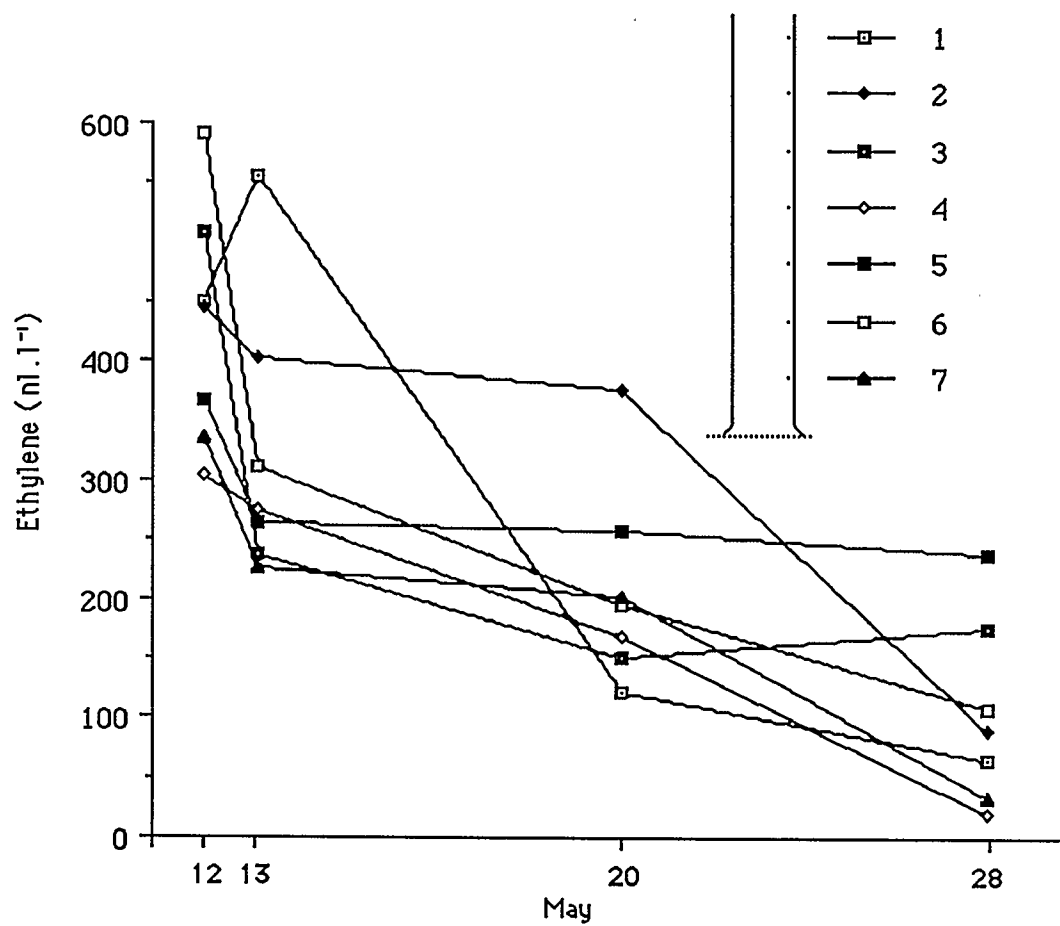


Figure 5. Ethylene production (nl/l of air sampled) in poplar: vertical distribution up one side of a main trunk (May 12-28, 1986).

reported by Robitaille (1975). Is the amount of gravistimulation that occurs when plant stems are bent by the wind sufficient to cause the plant to respond? Robards (1966) found that as little as 5 degrees of movement from the vertical position in S. fragilis was enough to produce an orientated eccentricity, and that each part of the cambium is influenced independently by the gravitational stimulus. Johnsson & Pickard (1979) found that the minimum presentation time for gravistimulation of coleoptiles, hypocotyls and roots is about 10-20 seconds. Van Haareveld (1907, in Larsen, 1962) found that when a clinostat axis made one revolution in 6 minutes, and was stopped in a certain position for one second during each revolution, a clear preference for downward curvature during the stop could be recorded after 16 hours rotation.

Included in this section are the results of an experiment that compared ethylene production in sunflower plants that were gravistimulated in conditions of light or dark. Abeles and Rubenstein (1964) found small differences in ethylene production in plants that were illuminated at different levels of light intensity. Since a gravistimulated stem receives more light than a vertical one (generally speaking), it is important to determine whether increases in ethylene are due to gravity, to light, or to both.

In this section as well are the results of another experiment that relates to responsiveness of stems of different growth habits. In this experiment, small plastic rings were placed next to the upper internodes of young plants of several species. The stems were bent so that a toothpick could be inserted between it and the plastic ring, the result being that a 90 degree bend was

created in the stems. The growing apices quickly turned up again of course, but as the plants matured, this slight deviation created by the ring and the toothpick remained. In several species, the toothpicks were eventually broken by the tendency of the stem to straighten itself; in the plants with climbing habits on the other hand, the toothpicks were seldom broken. As long as the stems of these latter plants were allowed to transport xylem and phloem without interruption, it seemed that they had no interest in any bends and detours the stems were subjected to. By using various kinds of toothpick-like objects, it was possible to measure roughly the forces generated by the various species as they attempted to straighten out.

Figure 6A shows that the ethylene production of a gravistimulated hypocotyl continues to remain elevated for many days after the initial gravistimulation. Figure 6B indicates that the region of greatest ethylene production is the outer portion of the lower side of gravistimulated stems. The tissue slices were obtained by using a device constructed for the purpose. It was made of plexiglas and allowed a double-edged razor blade to be clamped under compressive strain, such that the blade developed a bend in its mid portion. The blade could also be adjusted to produce slices of varying thickness. This allowed rates of ethylene production by outer and inner tissues to be compared. Iwami and Masuda (1976) found that gravistimulation of pea stems resulted in increased levels of IAA in the epidermis of the lower stem side. Wheeler et al (1986) reported that virtually all newly synthesized ethylene appeared in the bottom halves of horizontal tomato stems, where ethylene concentrations were up to 100 times those in

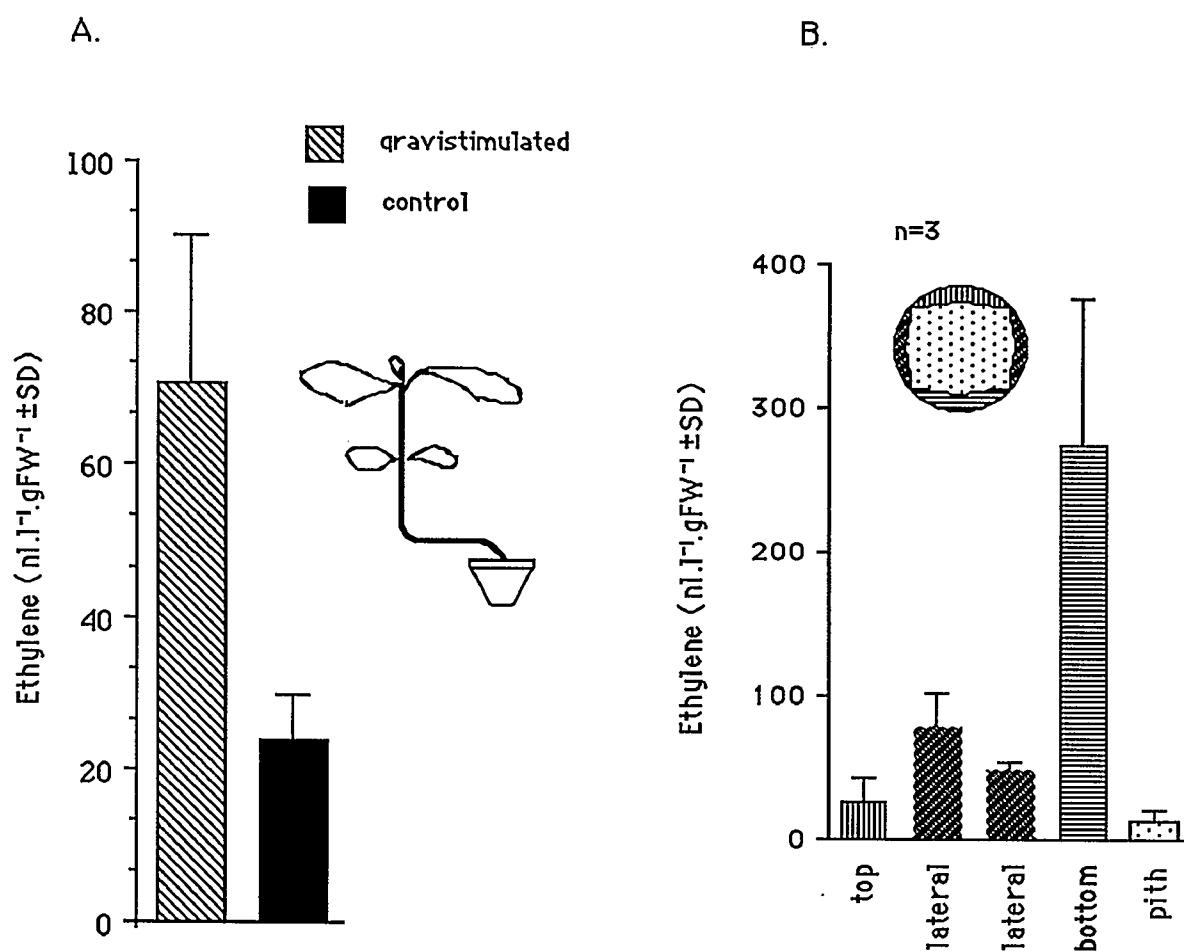


Figure 6. (A) Ethylene production in gravistimulated sunflower hypocotyls. The plants were bent over by means of plexiglas rods on day10, and harvested on day 18. (B) Distribution of ethylene-producing tissues in cross section of gravistimulated stems of sunflower. The circle indicates the tissues sampled. Plants were 29 days old, and gravistimulated for 3 hours by placing the pots at a 45° angle. Error bars represent standard deviation.

upright stems or in top halves of horizontal stems.

Figure 7 indicates that the rise in ethylene in a gravistimulated stem occurs after about 2 hours, that it can be clearly seen in non-elongating stem tissue, and that it is not transitory. Clifford et al (1983) using dandelion peduncles likewise reported that ethylene increases began about 2 hours after gravistimulation. They suggested that ethylene may control the later stages of gravibending and influence the straightening processs of autotropism. An additional possible role of ethylene in horizontal stems - may be to induce the lignification and increased radial growth necessary for support due to the disadvantageous orientation of the plant. The persistence of higher levels of ethylene far beyond the initial period of gravistimulation might thus be explained. Non-vertical stems are always more expensive than vertical ones, and there must be a system by which extra resources are marshalled and deployed in these organs.

Figure 8 suggests that ambient levels of ethylene have little influence on the rate of ethylene production in the vertical (control) plants, and a slight stimulatory effect in the case of the gravistimulated plants. Lyon (1970) reported that high ambient ethylene was associated with higher levels of IAA on the upper side of tomato and pepper hypocotyls. Palmer and Halsall (1969) found that gravistimulation inhibited polar transport of IAA in sunflower stems. The enhancement of the gravity-induced ethylene production in conditions of high ambient ethylene is reminiscent of the autocatalytic production of ethylene in fruit ripening (Burg & Burg, 1961).

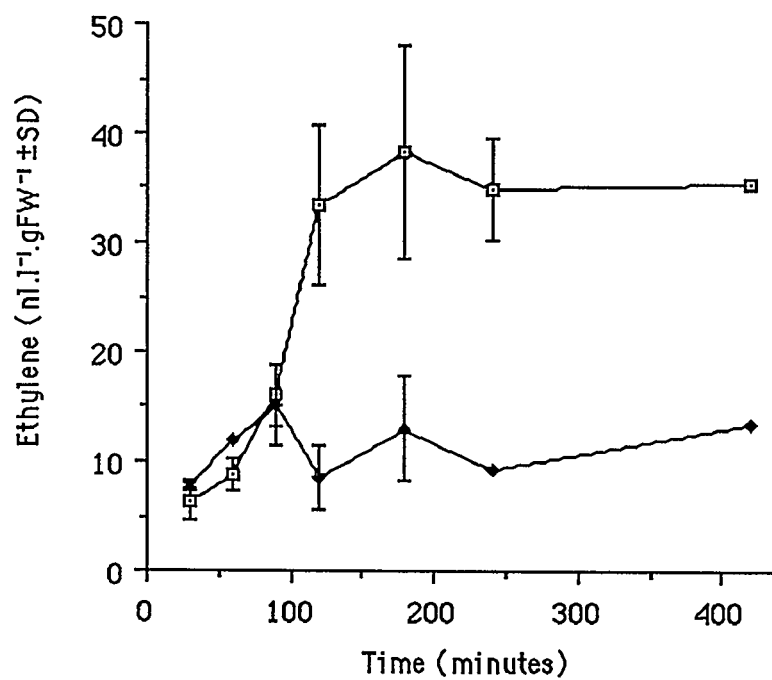


Figure 7. Time course of ethylene production in gravistimulated sunflower hypocotyls. Plants were 21 days old, and gravistimulation was by placing the pots at 45°. Upper line is gravistimulated stem; lower line is a control stem.

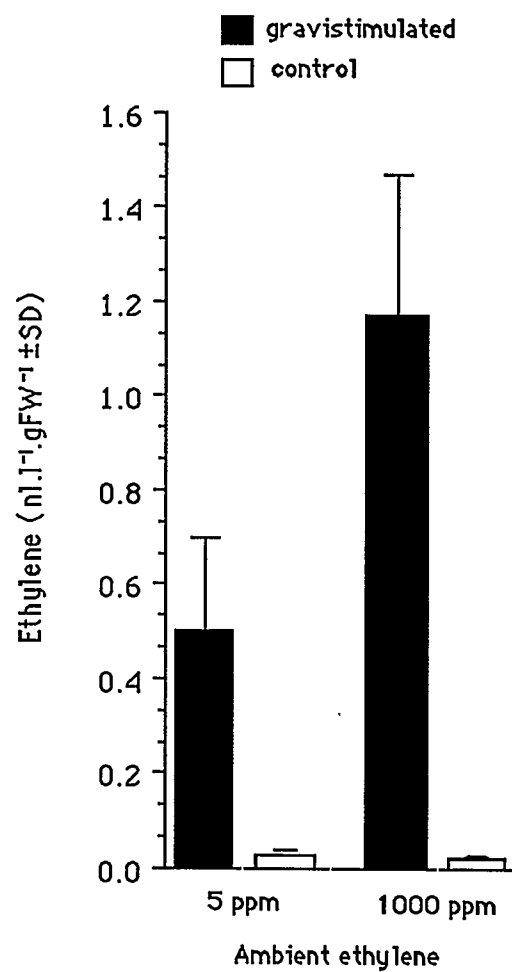


Figure 8. Effect of ambient ethylene levels on the rate of ethylene production by gravistimulated sunflower stems. Plants were 10 days old, gravistimulated for 3 hours.

Figure 9 shows that the effect on ethylene production in a gravistimulated sunflower stem is not a transitory one, nor is it restricted to the apical region. Seven days after the portion of the stem above the cotyledons had been bent over horizontally, the apical region had regained a vertical direction. Still, there was a substantial difference between the rates of ethylene production of the upper and lower stem halves, the difference being most pronounced in the youngest portion of the gravistimulated stem. There was no marked change in the ethylene production of the leaves. In this case, the increased ethylene in the stem is consistent with a hypothesis that a structure that has been placed under increased stress might produce ethylene as a signal to increase radial growth, or decrease axial growth, or increase lignification, or all of these.

These results seem to argue against the suggestion of Hart and Macdonald (1964) that the gravitropic response is restricted to the apex. Additional evidence for extra-apical responsiveness to gravity can be found in Britz and Galston (1982) who reported that removal of the apical bud of seedling peas strongly inhibited nutation, but did not affect gravitropism.

Figure 10 indicates the hypertrophy that results from prolonged gravistimulation of a sunflower stem. The seedlings were gravistimulated at 10 days by placing a plexiglas rod against the stems at the level of the cotyledons. The rod was left in place until day 45, when the plants were harvested. The gravistimulated hypocotyls were thicker than those of the control plants; the hypertrophy was most marked in the vertical plane, (where the increased size would be of most use mechanically).

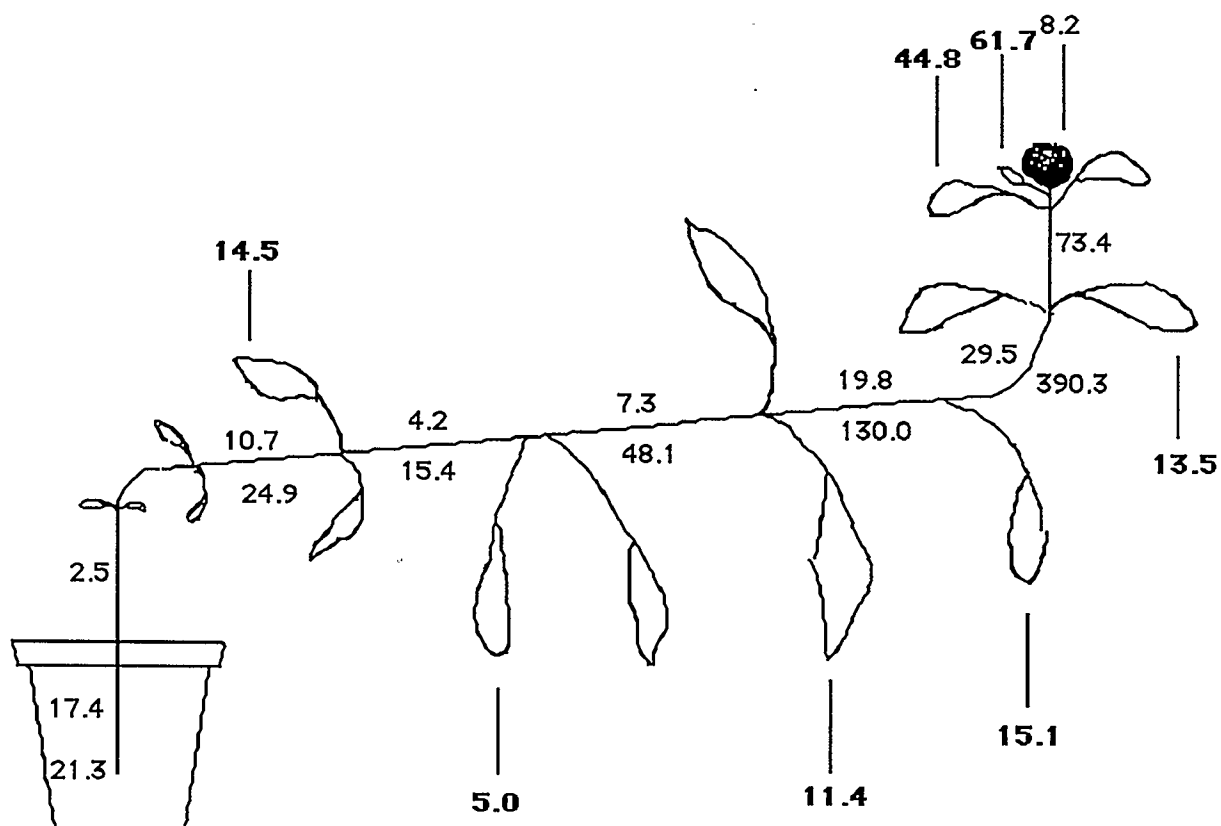


Figure 9. Effect of gravistimulation on production of ethylene in 58-day-old sunflower. Numbers represent rate of ethylene production in nl/l of air sampled over a 20 minute period. The plant grew upright for the first 48 days, then horizontally from day 48 to 58.

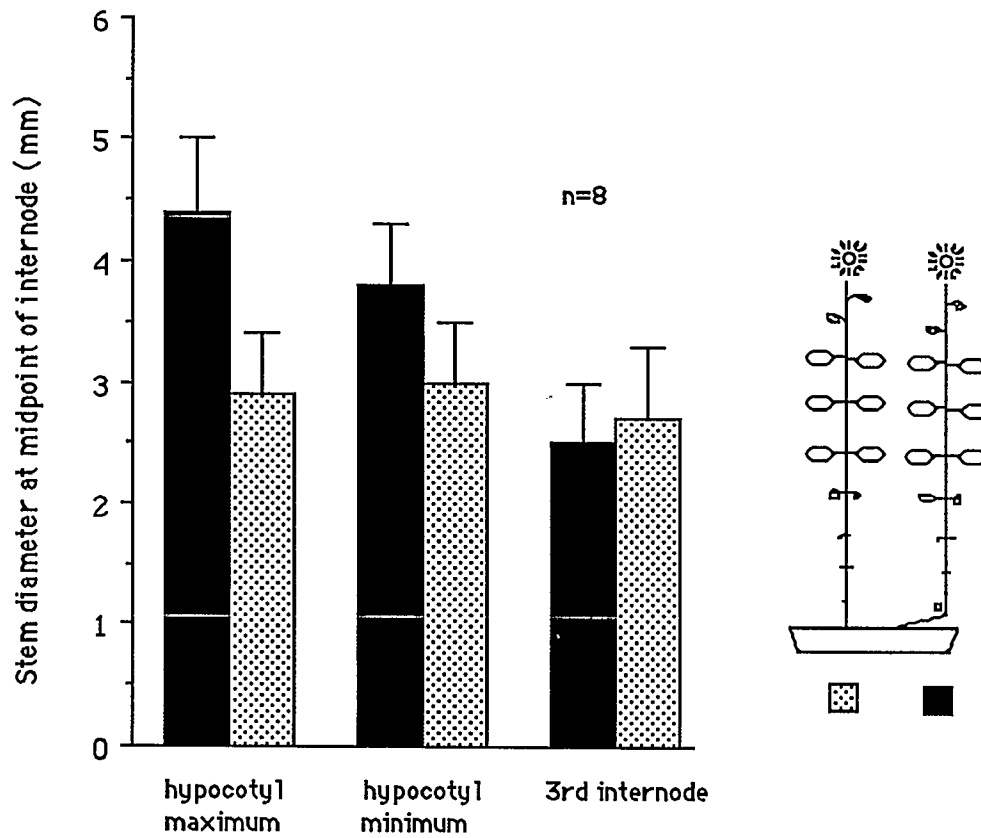


Figure 10. Effect of hypocotyl gravistimulation on stem diameter in sunflower. Error bars represent standard deviation.

Is the increase in ethylene production in a gravistimulated stem partly due to the greater amount of light now falling on the stem? Figure 11 suggests that it is not - rates of ethylene production were not different in light as compared to dark conditions. Abeles and Rubenstein (1964) reported decreased ethylene production in etiolated bean hypocotyls kept in the light for 5 hours as compared to dark treated plants. Kang and Burg (1969) likewise reported inhibition of ethylene production in pea stem sections after illumination with red light. They suggested that in some cases (hypocotyl hook opening, for example) the regulation of ethylene formation by light may be exerted by way of influences on tissue levels of phenolic inhibitors of ethylene biosynthesis. Vangronsveld et al (1988) also found that red light caused a decrease in ethylene production in 8-day-old etiolated bean seedlings, but that in plants younger than 6 days or older than 11 days, the response was absent. It may be therefore that the phytochrome-mediated effect on ethylene production is more important in etiolated plants, and in seedlings of a particular age, and that in older plants (such as those used in my experiments) light no longer plays a large role in regulating ethylene production.

While gravistimulation in sunflower clearly results in increased ethylene production by the lower half of the stem, this is not the case in all plants. If ethylene plays some role in the response of the plant to the change in orientation, then it might be anticipated that those plants which do not respond to gravistimulation by increases in radial growth will not show increases in ethylene when their orientations are altered. Indeed, this pattern was seen in a number of climbing species, as shown below for

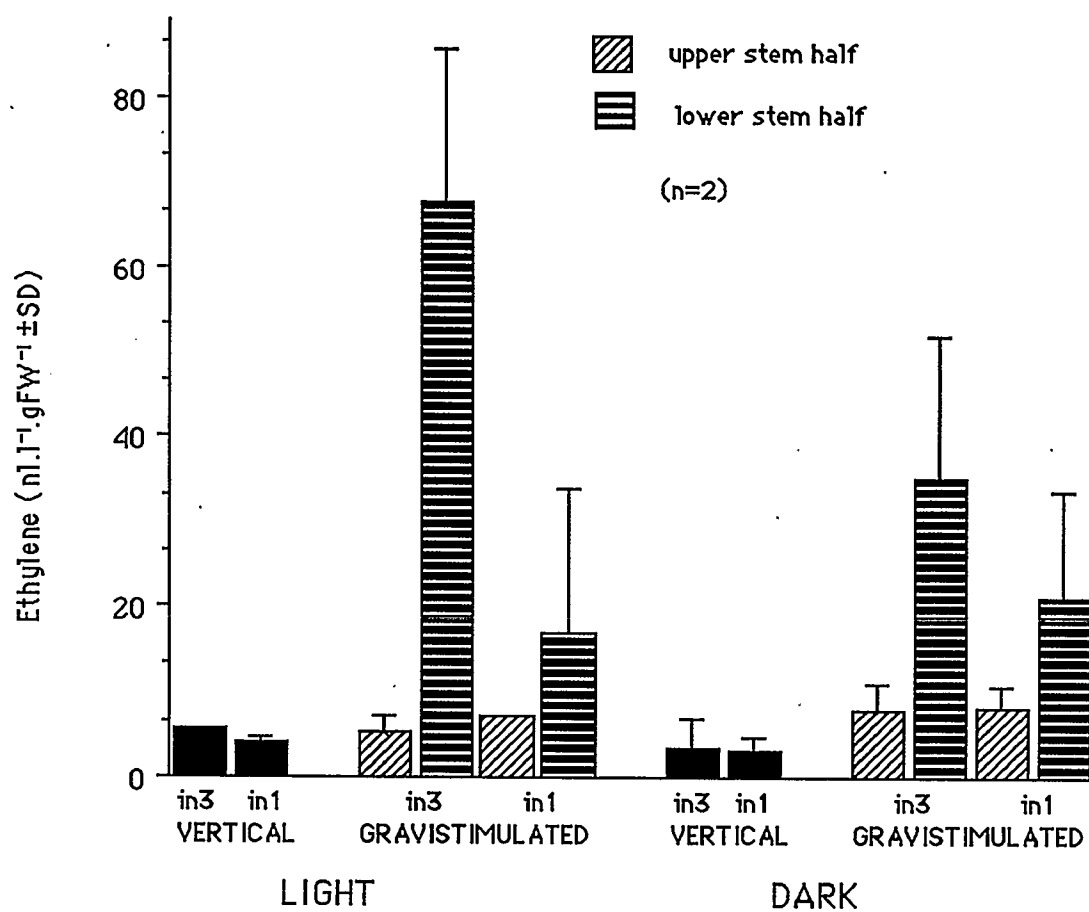


Figure 11. Effect of light on ethylene production in gravistimulated sunflower stems. The portion of the stem between the cotyledon and the first true leaf was named as the first internode (in1). Plants were 33 days old, and gravistimulated by placing the pots at a 45° angle for 2.5 hours.

cucumber and pea.

Figure 12 shows a clear increase in the ethylene produced by horizontal stems of tomato, the increase being entirely in the lower stem half. Harrison and Pickard (1986) on the other hand were unable to find any ethylene increase in gravistimulated tomato hypocotyls. Figure 13A indicates that gravistimulation does not //change the rate of ethylene production in mature cucumber stems.

In broadbean (Figures 13B and 14) there did appear to be an increase in ethylene production when plants were gravistimulated, but the increase was not particularly marked, nor was there a difference between upper and lower stem halves, as there was in sunflower (and in tomato). The overall ethylene increase in the gravistimulated broadbean stems was probably not related to wounding, since incubation time was 20 minutes; also, longitudinally split stems did not produce more ethylene than did intact stem sections.

Wheeler et al (1986) found that sunflower and tomato stems responded to unilateral application of ethephon by bending away from the side of application, but castor bean stems did not, which is consistent with the above argument concerning ethylene-responsive (free-standing) versus ethylene-nonresponsive (climbing) plants.

In poplar, the pattern of increased ethylene production in the lower half of a gravistimulated stem was reversed: ethylene was higher in the upper side of a large lateral branch, as shown in Figure 15.

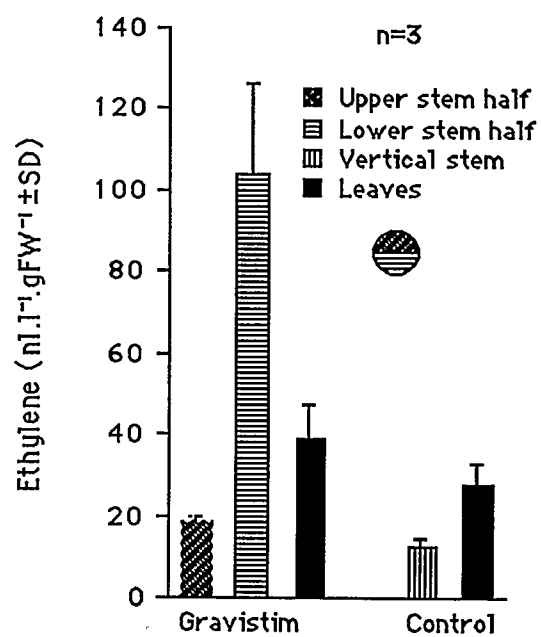


Figure 12. Effect of gravity on ethylene production in mature tomato plants. Plants were 41 days old, and gravistimulated for 4 hours by placing the pots at 45°.

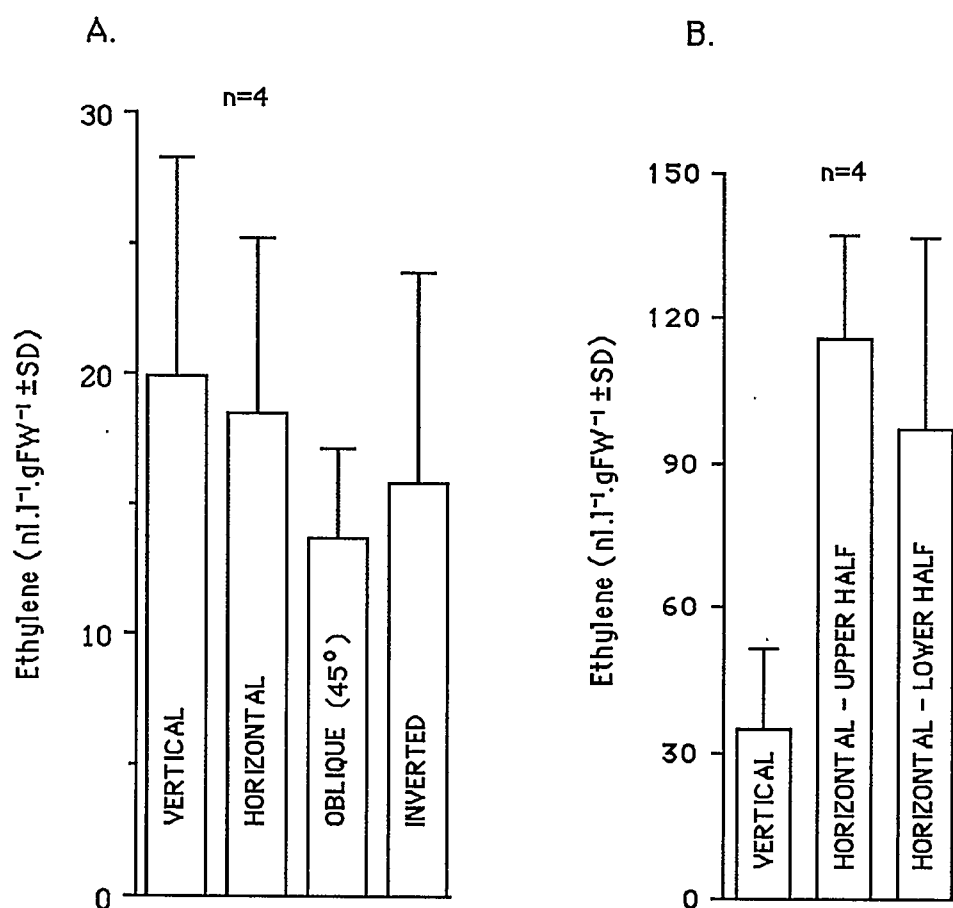


Figure 13. (A) Ethylene production in mature cucumber stems growing in various orientations. Plants were 41 days old. (B) Effect of gravistimulation on ethylene production in broadbean. Plants were 17 days old. They were gravistimulated for 5 hours by means of a plexiglas rod pushing laterally near the base of the stems.

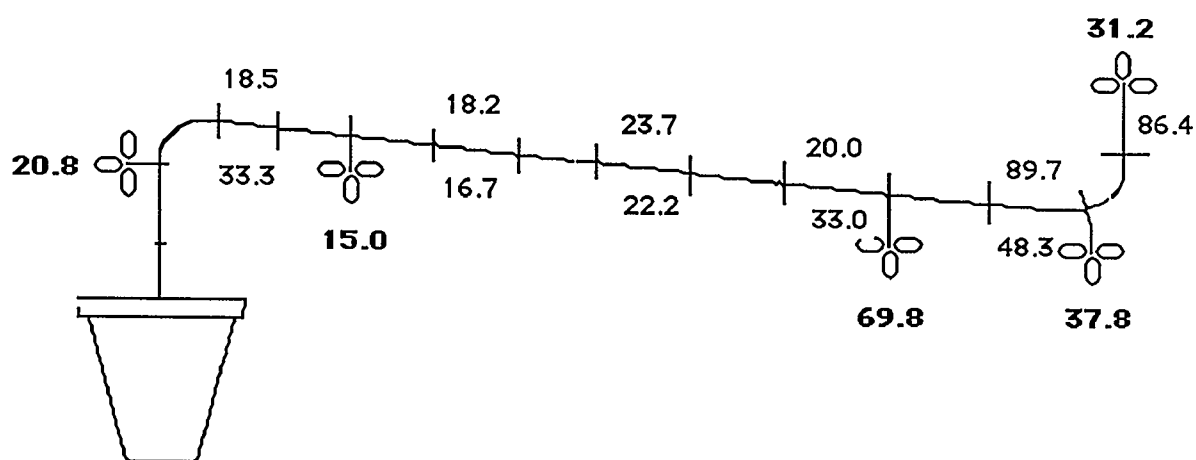


Figure 14. Rate of ethylene production in stems and leaves of broadbean. The plant was 60 days old. It fell over under its own weight at day 55: thus it was gravistimulated for 5 days.

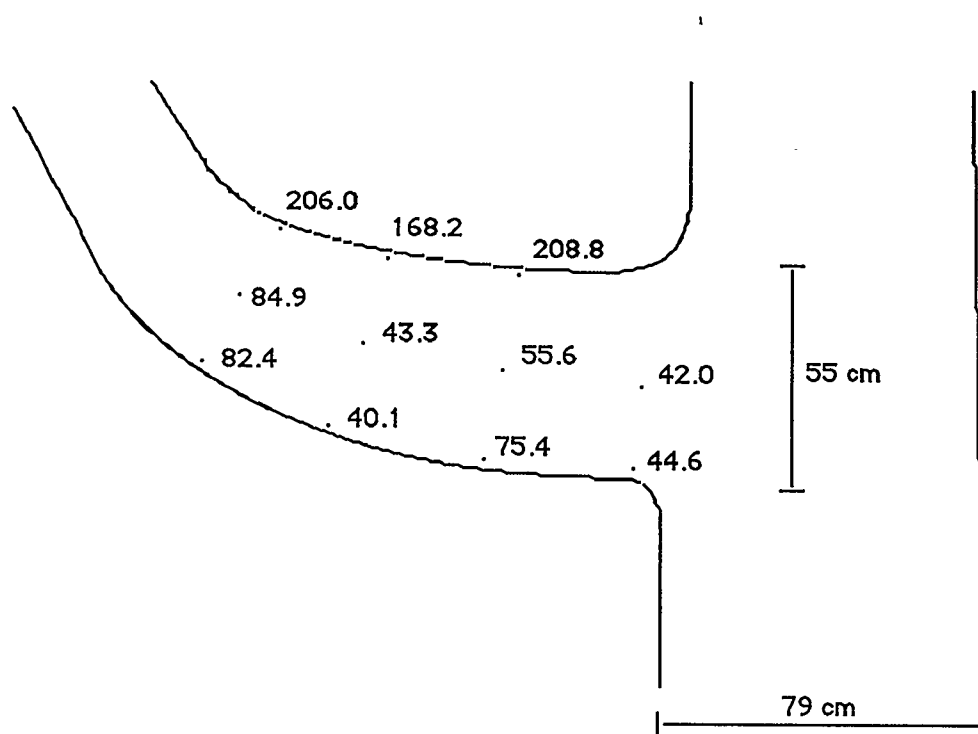


Figure 15. Pattern of ethylene production (nl/l of air sampled) at the basal portion of a large lateral branch of poplar (April 24, 1986).

It is the upper side of the lateral branches of woody angiosperms that produce reaction wood; this is consistent with the idea of ethylene as a messenger. On the other hand, it is the lower side of a lateral branch of poplar that experiences hypertrophy; the pattern of hypertrophy is opposite that of ethylene production in this case.

Experiments by Ziv et al (1976) on runner-type peanut plants indicate that ethylene is necessary to induce a negative gravitropic response. Similarly, the work of Jackson (1979) with the diageotropic mutant of tomato suggests that in this variety there is a deficiency in the response mechanism to ethylene and to gravity.

Prasad and Cline (1985a) reported that shoot inversion in Pharbitis nil caused no changes in the production of ethylene by the stem adjacent to the highest lateral bud. They hypothesized that shoot inversion indirectly releases apical dominance by inhibiting terminal bud growth in Pharbitis. This agrees well with the findings of the present study; Pharbitis, a climbing plant, should produce ethylene only in the growing parts, namely the apical internodes.

Another group of experiments in this section involved the creation of small bends in the stems of 5 species, and determining the forces generated in individuals of each species tending to straighten out these bends. The procedure was described briefly above - it involved the use of one plastic ring and one toothpick for each stem. The four types of "toothpick" used for this purpose were: 1) flat wooden toothpick; 2) round wooden toothpick;

3) 1.1 mm glass micropipette; and 4) 1.6 mm glass micropipette. A series of 10 trials was conducted for each type of toothpick to determine its approximate breaking strength. This was done simply by laying a plastic ring on a weigh scale, and placing a toothpick on the ring. A gradually increasing force was then applied to the toothpick with the thumb until the toothpick broke. The reading of the weigh scale just at the moment of failure was then recorded. The results of these trials are given in Table 1.

Table 1. Average force required to break two kinds of wooden toothpick and two sizes of glass pipette.

Type of strut:	Round	1.6 mm	Flat	1.1mm
	Toothpick	Micropipette	Toothpick	Micropipette
Average breaking force (kg)	2.37	1.03	0.61	0.32
Standard deviation (n=10)	0.37	0.16	0.09	0.03

Table 2. Number of days taken to break the four types of strut in six herbaceous species.

	Round toothpick	1.6 mm micropipette	Flat toothpick	1.1 mm micropipette
Pea	-	-	-	-
Bean	-	-	-	-
Cucumber	-	-	-	-
Broadbean	-	-	-	2
Tomato	-	21	6	2
Sunflower	15	10	7	3

The struts were inserted between a plastic ring and the stem of tomato, bean, pea, broadbean, cucumber, and sunflower plants. As indicated in Table 2, no struts broke in the case of pea, bean and cucumber. In broadbean, only the 1.1 mm pipette broke. In tomato, all but the round wooden toothpick eventually broke, and in sunflower all four types of strut broke within 15 days. These results further demonstrate the degree of what I have termed "responsiveness" in species of different growth habit. This responsiveness in the stems of upright species can also be seen in the

shape of the stem subsequent to the breaking of the strut. The stems of the plants whose struts broke first straightened out almost completely. These experiments did not fit neatly into any of the sections of this chapter. They have been included in the section on gravistimulation, because gravity may be the stimulus that these plants respond to in their attempts to reestablish a straight stem, although this has not been demonstrated here. There is an indirect piece of evidence that gravistimulation may be driving the plant's responses here, and that is the formation of adventitious roots on the lower side of a gravistimulated portion of stem. Woodrow et al (1988) found that application of Ethephon to mature tomato plants resulted in the development of adventitious buds on the lower stem. Gravistimulation in tomato plants results in increased ethylene production by the stem, thus it may be that both the new root formation and the lateral growth tending to straighten the stem are caused by ethylene.

c. Bending and ethylene

Mergen & Winer (1952) described compression failures in living trees without complete breakage of the stem, in cases where the load imposed by snow or wind is great enough to cause the leeward side of the bole to fail in compression, but not sufficient to cause tension failure on the windward side. Wardrop (1956) found numerous slip planes and minute compression failures in tension wood. Lyon (1971) found that increases in respiration

occurred when plants were rotated on a clinostat, and suggested that a combination of vibrational & shearing stresses may be responsible.

Wainwright et al (1976) cite Mark's (1967) prediction that the greatest stresses in load-bearing woody tissues will be shear stresses in the S1, and that after this, the next point of fracture will be a separation of the S1 and S2 layers. These types have been found in anatomical studies, and are termed brash fracture (secondary bonds broken), and tough fracture (covalent bonds broken). Winandy & Rowell (1984) note that cyclic or repeated loadings often induce fatigue failure at stress levels far lower than those required to cause static failure in wood. They point out that hydrogen bonds within the cellulose allow the molecule to absorb shock by breaking and reforming. At the proportional level of elasticity, covalent C-C and C=O bonds begin to rupture. Macroscopically, minute cracks initiate, propagate, and terminate throughout the cellular system in all directions.

The foregoing descriptions of damage to cell walls by bending refer mainly to woody tissues, in which the load-bearing cells are largely the dead cells of the xylem. If this is the region from which originate the signals that result in increased cambial activity, then the notion that ethylene is the messenger substance, has points both for and against. Ethylene has in its favor the fact that, being a gas, it is readily diffusable through the intercellular spaces, and thus can easily be transported to the cambium. On the other hand a strong argument against ethylene as messenger here is that the synthesis of ethylene, like that of all the other plant hormones, must take place in living cells (Eltner & Konze, 1976). Clifford et al (1982) reported bending responses in dandelion peduncles after applying lateral stress to vertically held peduncles of dandelion, and suggested that stress itself might

act as a trigger for geobending. Ridge and Osborne (1971) found that application of ethylene to etiolated pea stems altered cell-wall synthesis and deposition.

Figure 16 shows the rates of ethylene production in sunflower stems that were bent and tilted in a number of ways to distinguish between the effects of gravistimulation and bending. Although the bent portions of the stem did show slight ethylene increases in two of the treatments, the increases were not seen in all bent stem segments. It may be that in some cases there was some gravity-induced ethylene included in the bent portions.

The only clear trend that emerges from Figure 16 is for increased ethylene production in non-vertical sections of stem. Although the bent sections show a small increase in ethylene production, it is very likely that this is due largely to the fact that these samples also contain about 50% gravistimulated stem material. There is certainly no great increase in ethylene for the bent tissues above what one might expect from this gravity-related component. What does emerge very clearly from this experiment is the observation, already made several times in the section on gravistimulation above, that any non-vertical stem of sunflower, regardless of the age or developmental stage, will respond by producing increased levels of ethylene. The experiment described in Figure 16A resembles one by Prasad and Cline (1985b), in which plants of Pharbitis nil were bent into various positions. In that species, ethylene production increased significantly only in those treatments in which the apex (the responsive region in a climbing species) was not vertical, in contrast to this experiment

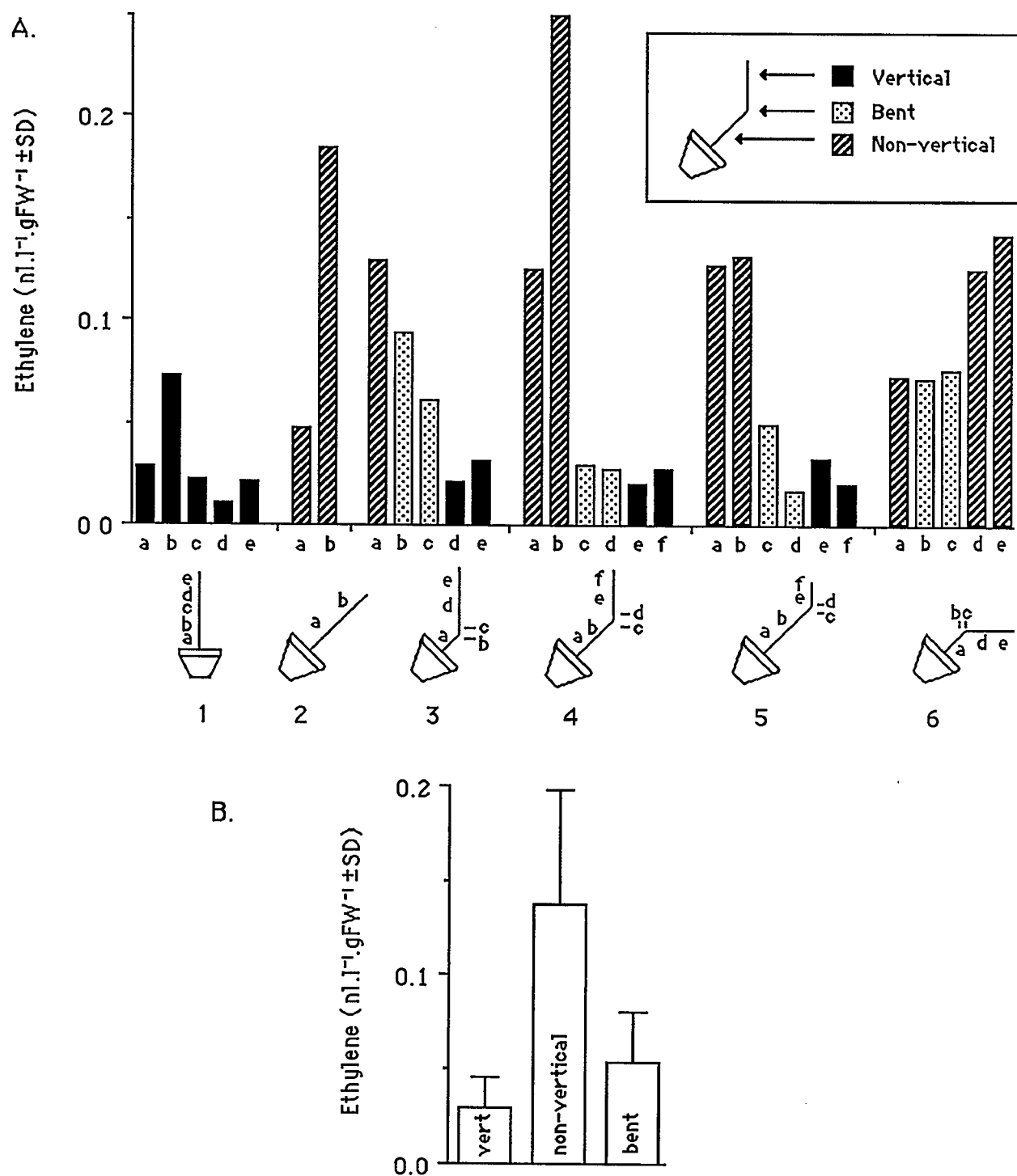


Figure 16. (A) Effect on ethylene production of gravistimulation and of bending in sunflower. (B) Summary of the results in (A).

with sunflower, where all non-vertical stems showed increased ethylene production. Prasad and Cline also reported higher ethylene levels than normal in the bent regions, and interpreted this as the result of bending stress. This I doubt, for two reasons. First, another paper by the same authors (1985a) found no increase in ethylene in this region of Pharbitis. The results presented in this chapter regarding the production of ethylene by mature regions of climbing plants also make it unlikely that bending causes ethylene production in mature Pharbitis stems (this being the second reason).

Figure 17 shows the results of two of the experiments which were designed to test the effect on ethylene production of dynamic bending of plant stems. In Figure 17A, 21-day-old sunflower plants were placed at a 45° angle, so as to allow bending in the lateral plane (without additional gravistimulation). One half the plants were bent by grasping one cotyledon and the opposite first leaf, and pulling these in opposite directions 20 times. 30 minutes later the plants were bent again, this time using the other cotyledon and first leaf. The plants were harvested after 2.5 hours. At this time the apices were beginning to turn up again. Here again, no difference could be detected in ethylene production between bent and control plants. In Figure 17B, White Spruce branch tips were selected for uniformity of size and growth habit. The date was July 2. It was thought that perhaps by choosing a plant that naturally has horizontal branches, some of these could be bent in the horizontal plane, thus avoiding the complications of gravistimulation. In this case, the stems labelled "flexed" were manually flexed 20 times, and the ethylene production measured from excised stem

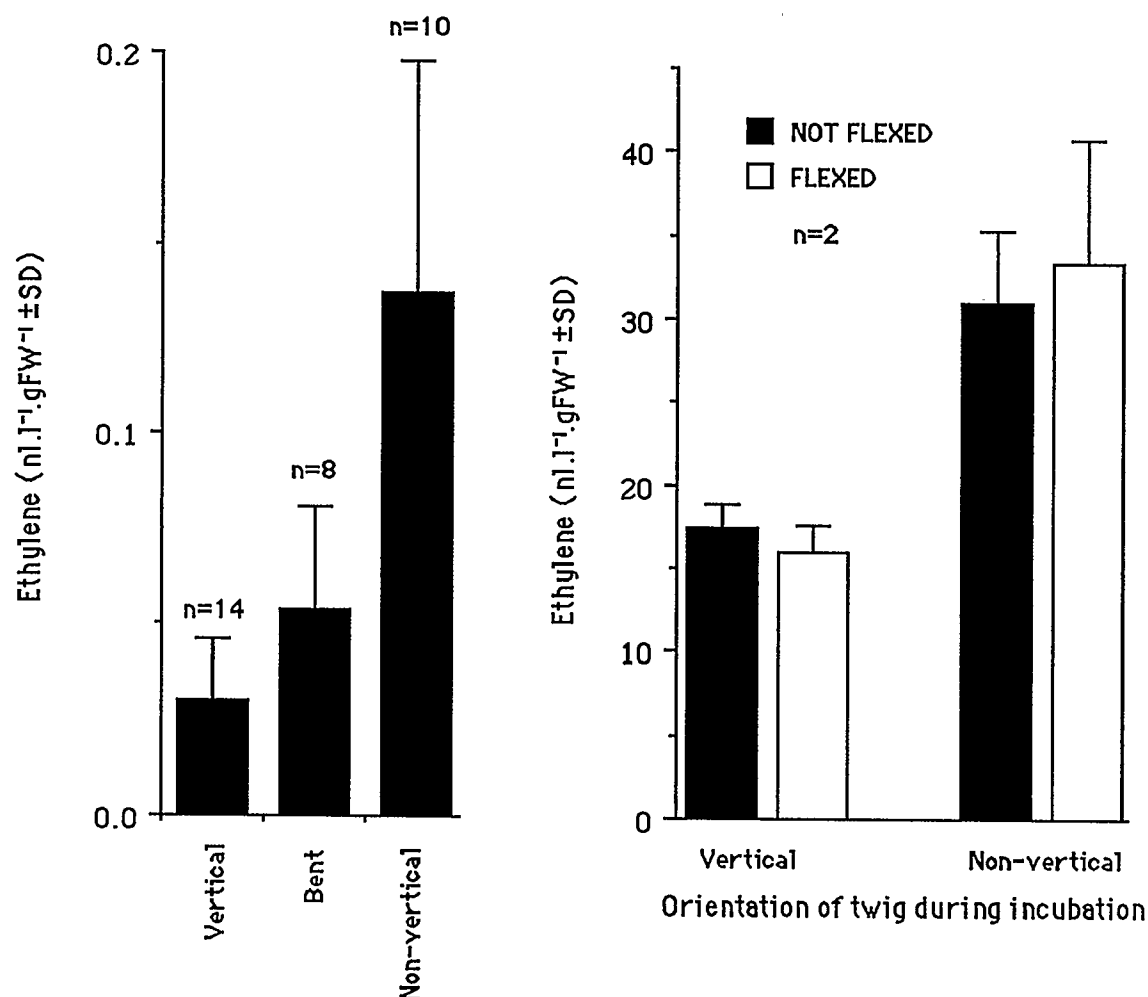


Figure 17. (A) Effect of bending on gravistimulated sunflower stems. (B) Effects of flexure and of gravistimulation on ethylene production in White Spruce. Horizontal branch tips 10 cm in length were excised and flexed manually 20 times in the horizontal plane, then enclosed in Plexiglas tubes (2 x 12 cm) and sealed with Teflon stoppers. Half the branch tips were incubated in a horizontal position, the other half in a vertical position. Incubation was for 4 hours.

segments one hour later. There was no detectable increase in the production of ethylene.

Figure 18 shows the effect on ethylene production of hanging a heavy weight (10 bottles of water with a total weight of about 200 kilograms) on a lateral branch of poplar. The experiment was done twice, and in each case there was no detectable effect on the production of ethylene, either at the time that the weights were hung, or when they were removed.

In the course of sampling the levels of ethylene in the outer xylem wood of a number of poplar trees on and near the university campus, it became apparent that on certain days the ethylene was especially high in all the trees sampled. Two examples are given in Figures 19 and 20.

These results suggested that perhaps the days in which ethylene levels were high (especially May 6 & 26) were days following heavy winds. This was not the case, however, as shown in Figure 21. Neither was the effect due to any recognizable pattern in the daily temperatures for that time period, as shown in Figure 22. Figures for various air pollution indices were obtained from Alberta Environment, Air Quality Division, to see if the high ethylene levels were due simply to a generally higher level of substances in the air. Here again, though, there was no correlation with the days of high ethylene production (data not shown).

The results reported here show no evidence for bending-induced increases in ethylene production. Nor has anyone else demonstrated that bending produces higher ethylene production. Robitaille (1975) measured

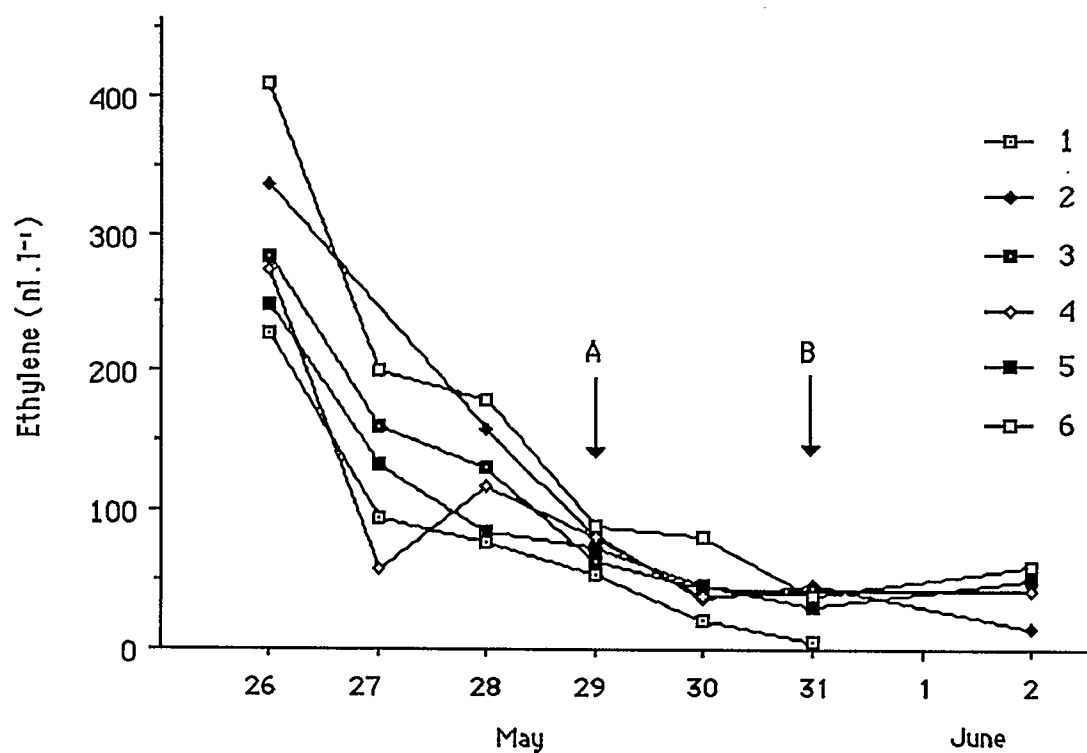


Figure 18. Effect on ethylene production (nl/l of air sampled) in outer xylem of poplar of hanging a heavy weight on a lateral branch. The weight was hung on May 29 (A), and removed on May 31 (B). The six numbers at right represent six locations along the upper side of the branch: 1 was closest to the trunk.

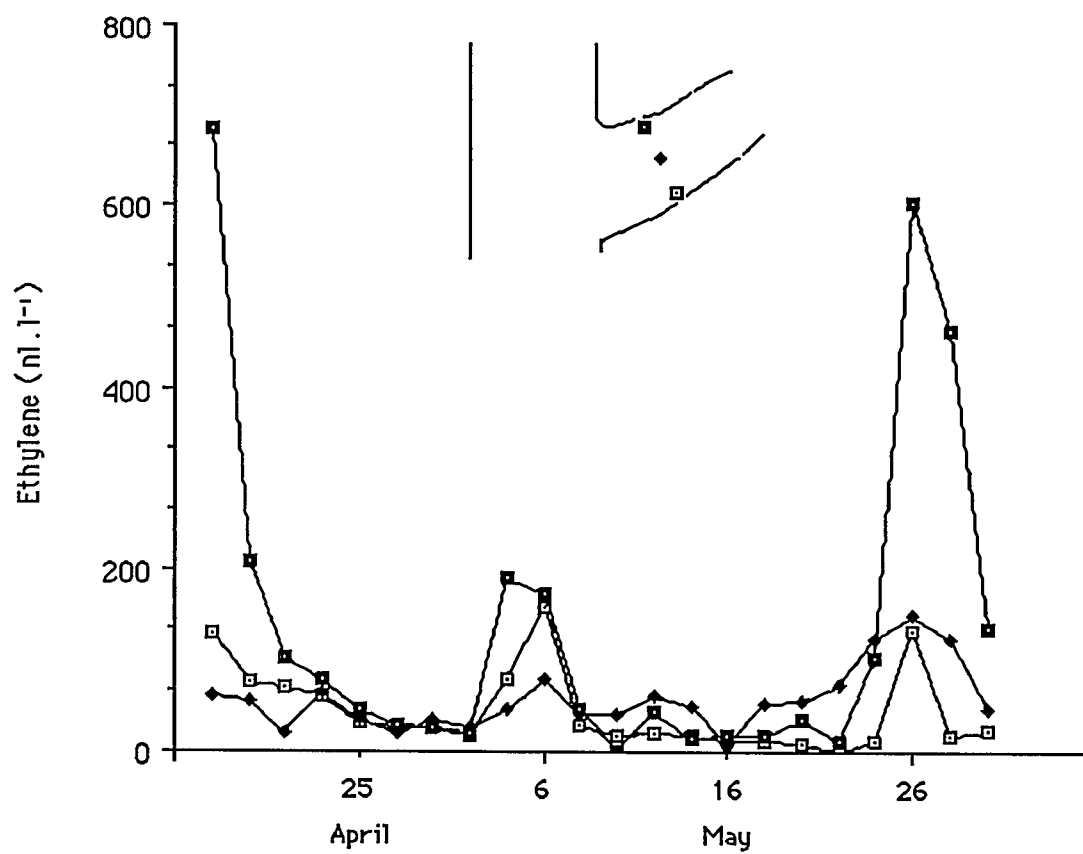


Figure 19. Ethylene production in outer xylem of lateral poplar branch.

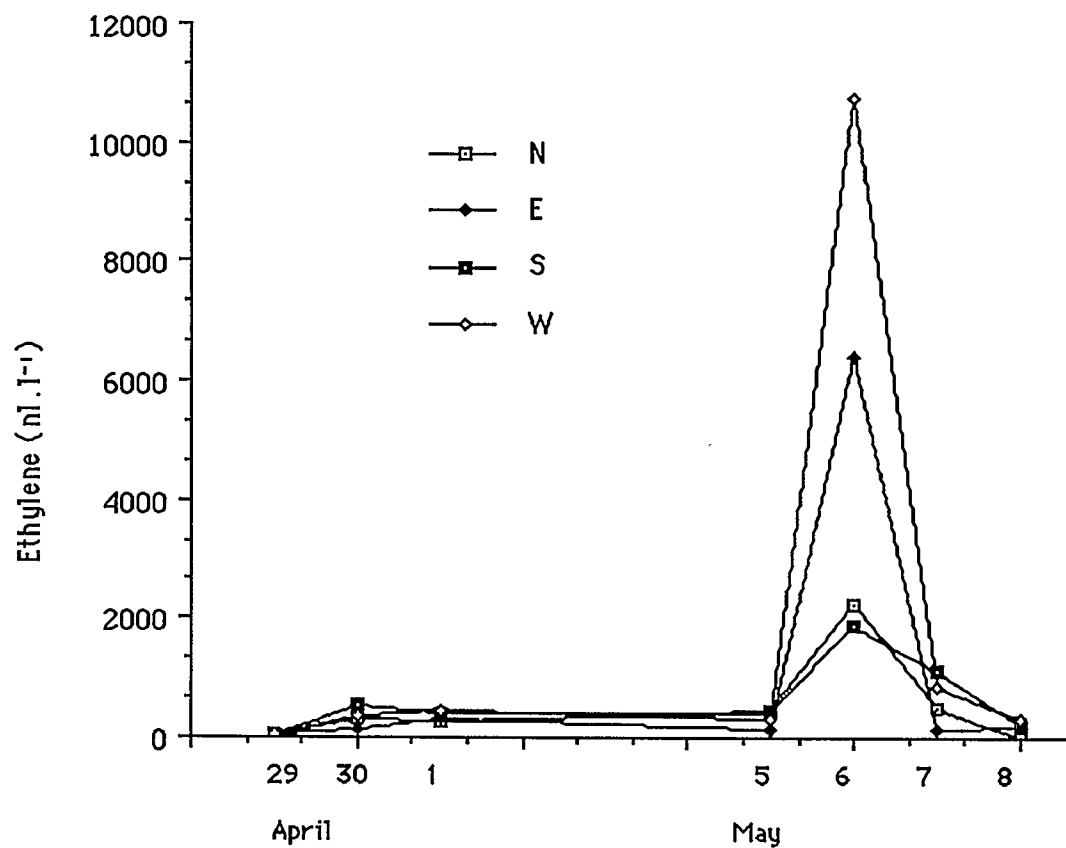


Figure 20. Effect of compass direction on ethylene production in outer xylem of main trunk in poplar.

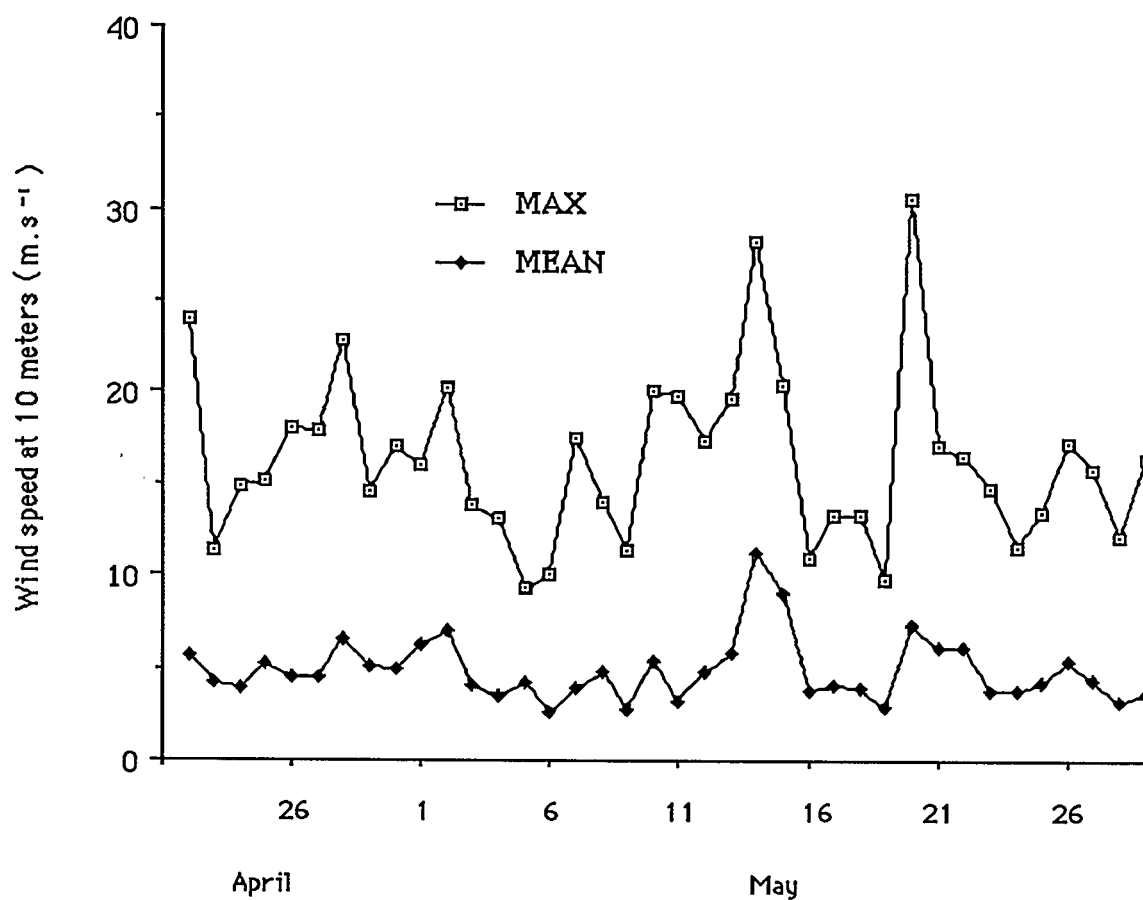


Figure 21. Daily mean and maximum wind speeds at the University of Calgary weather station - May, 1986.

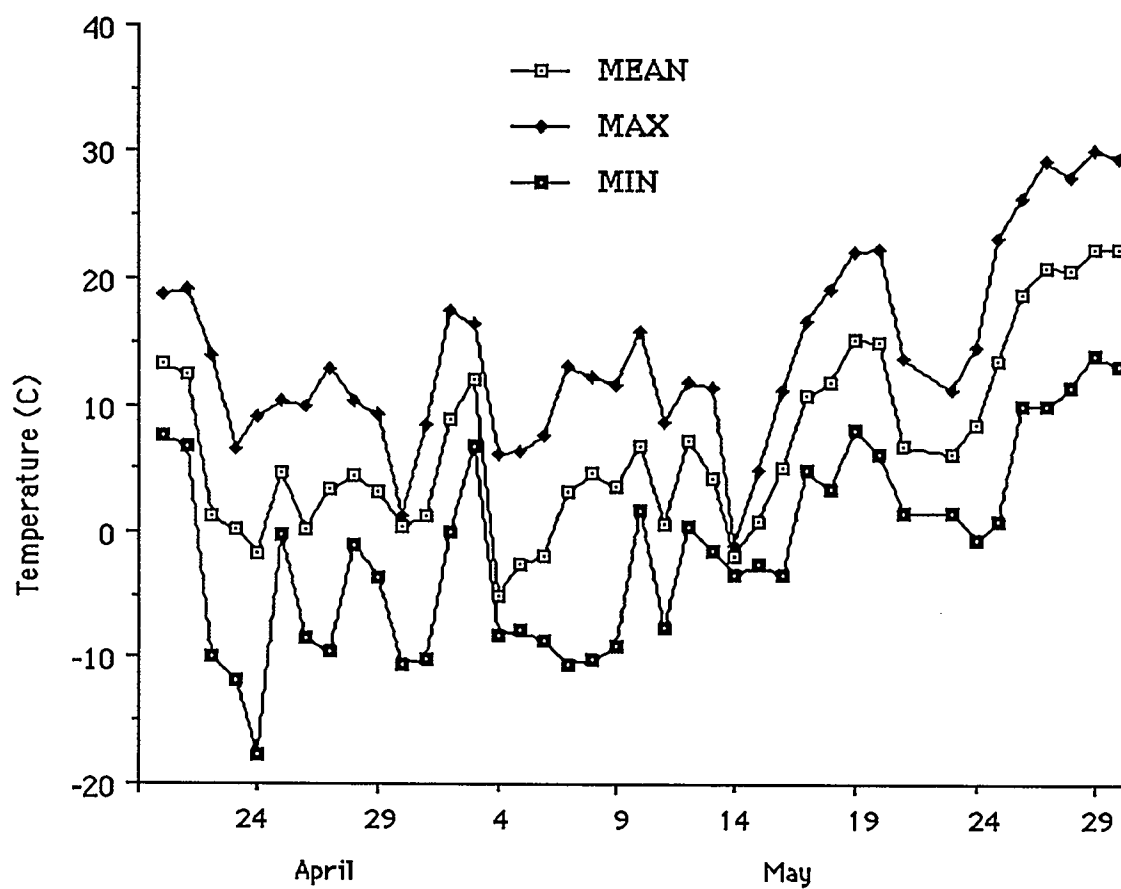


Figure 22. Daily temperatures at the University of Calgary weather station - May, 1986.

ethylene production in apple shoots, but was unable to differentiate between ethylene that resulted from gravistimulation, and that from bending. Jaffe (1985) states that flexing has effected thigmomorphogenesis in various species: however, the references that he lists all involve various combinations of flexing, gravistimulation, and wind. It is difficult to test the effect of flexing exclusive of these, but it is not correct to say that in these experiments the flexing was necessarily responsible for the thigmomorphogenesis. Flexing and thigmostimulation are often lumped together, or even sometimes considered as interchangeable terms (eg Jaffe & Biro, 1979).

Drought, gravistimulation and ethylene

Introduction

Water deficit stress has been reported to cause increased ethylene production in broad bean (El Beltagy & Hall, 1974), wheat (Wright, 1977, 1979; Apelbaum & Yang, 1981; McKeon et al, 1982), cotton (Jordan et al, 1972; McMichael et al, 1972), orange leaves (Ben Yohoshua et al, 1974), and tomato (Jackson, 1987). In contrast, no significant increases in ethylene were reported in drought stressed pea (Rajagopal & Andersen, 1980;

Miyamoto & Kamisaka, 1987) or sunflower (Hubick et al, 1986). Aharoni (1978) surveyed a number of species, and concluded that plants that have abscising leaves (bean, pepper, kohlrabi, castor bean, and orange trees) show a continuous rise in ethylene when droughted, while those with non-abscising leaves (lettuce, sugar beet, tobacco, squash, and cabbage) show only a transient rise in ethylene. It was hypothesized that among these somewhat complex relations between drought and ethylene, there might well be several further complicating factors, namely the effect on ethylene production of gravistimulation, and of variation in ethylene production among various plant organs and locations. In many (though not all) species, water stress eventually leads to leaf and stem wilting - this causes a reorientation of stem and leaves and thus results in gravistimulation. Therefore, it was thought to be useful to measure the ethylene production under different combinations of water stress and gravistimulation, so as to determine the relative contributions of each. The other potentially complicating factor mentioned above, namely differential responses in ethylene production among different plant parts, is an aspect of whole plant physiology that has received little attention in the literature. The usual procedure is to either take an average (or composite) plant sample, or to limit the field of interest to one specific location in the plant (the first fully developed leaf, for example). Since the question being asked here involves the response of the whole plant to a stress condition, it was decided to survey many different parts of the plant individually, in order to address this point. Finally, it might be mentioned parenthetically that there is a relation between drought and wind: when the transpiration rate is increased by persistent winds, mesophytes risk suffering some degree of water stress.

Materials and Methods

Plant Material. Plants were grown in Terragreen and watered automatically once per hour for 60 seconds with half-strength Hoagland's solution (Hoagland & Arnon, 1950). They were kept in a growth chamber, with a photoperiod of 16 hours. Gravistimulation was brought about either by bending the stem downwards, or by tilting the pot and plant together. Water stress was induced by withholding water until the first signs of leaf wilt.

Results

Figure 23 shows that drought in sunflower caused an increase in ethylene production by leaves, but no effect in stems. Gravistimulation caused no change in leaf ethylene, but increased the rate of ethylene production by stems, especially in the lower half. Figure 24 is a variation of the experiment in (A), which demonstrates the same result in stems only in a different way. Non-vertical stems produced more ethylene than vertical stems, whether the change in orientation was due to physical bending, or to wilting.

The distribution of ethylene production in droughted sunflower can be seen for plants with two kinds of stem orientation in Figures 25 and 26. Comparing these with the distribution given for a normal plant in Figure 1, it can be seen that higher ethylene production is characteristic of the lower

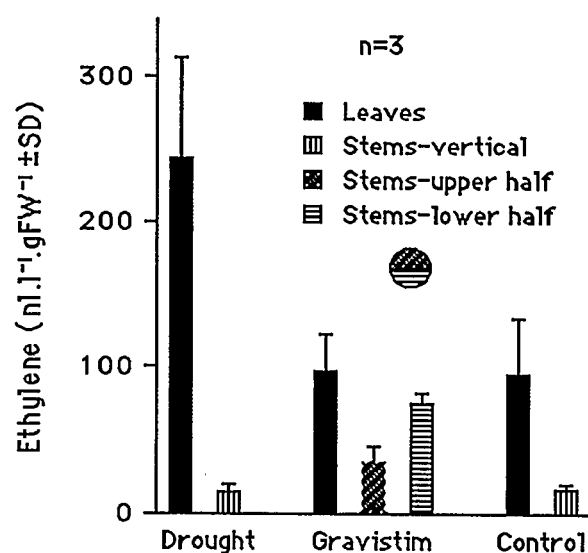


Figure 23. Effect of drought and of gravistimulation on ethylene production in sunflower. All plants were supported by stakes; gravistimulation was by placing the pots at 45 degrees for 2.5 hours. Samples were taken from the first internode (stems) and the first true leaf.

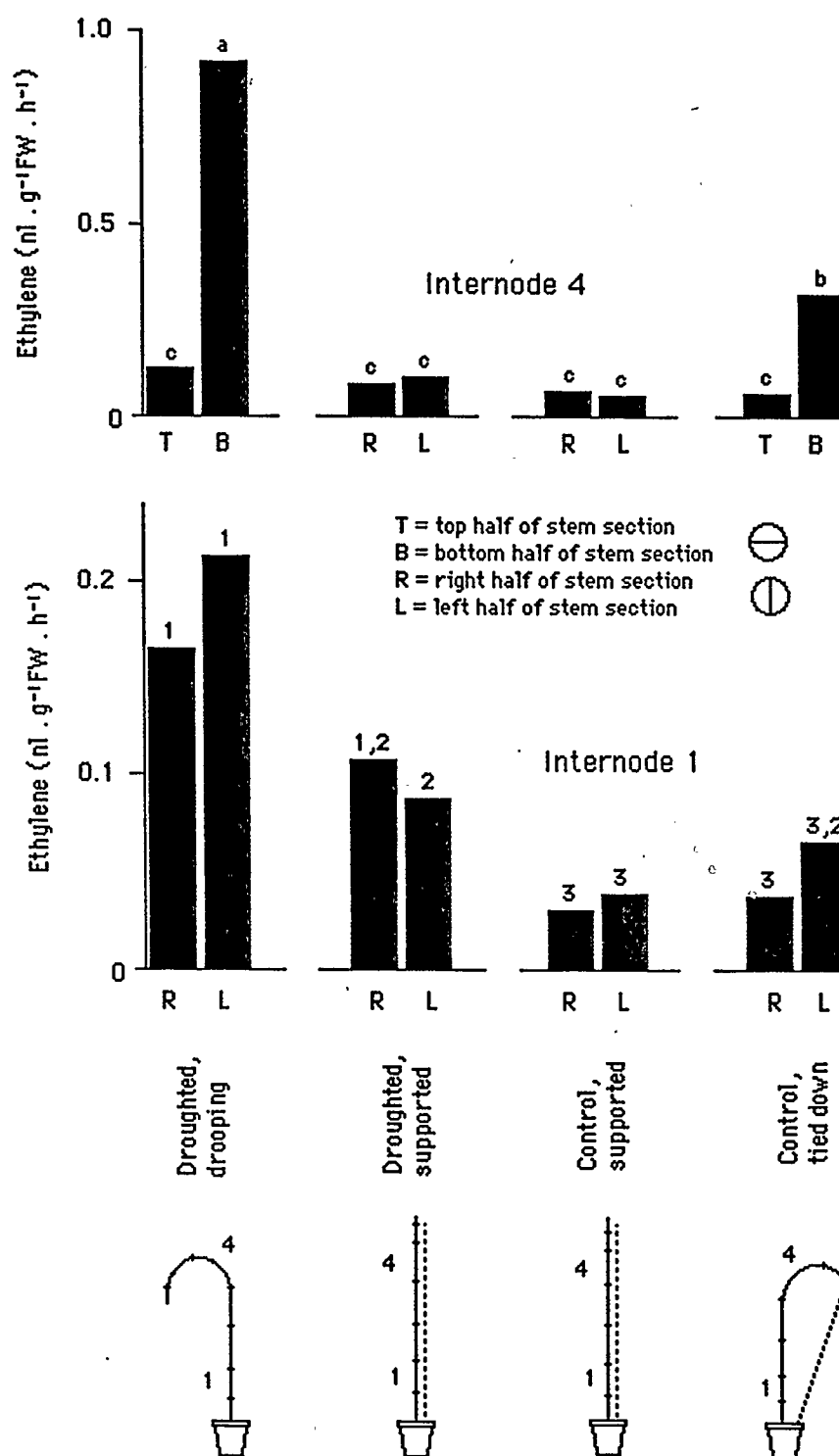


Figure 24. Effect of drought and of gravistimulation on ethylene production in sunflower. Droughting was by withholding water for 1 day; the plant on the right was tied down gradually to keep pace with the gradually drooping plant on the left. (Internodes are counted from the base of the plant.)

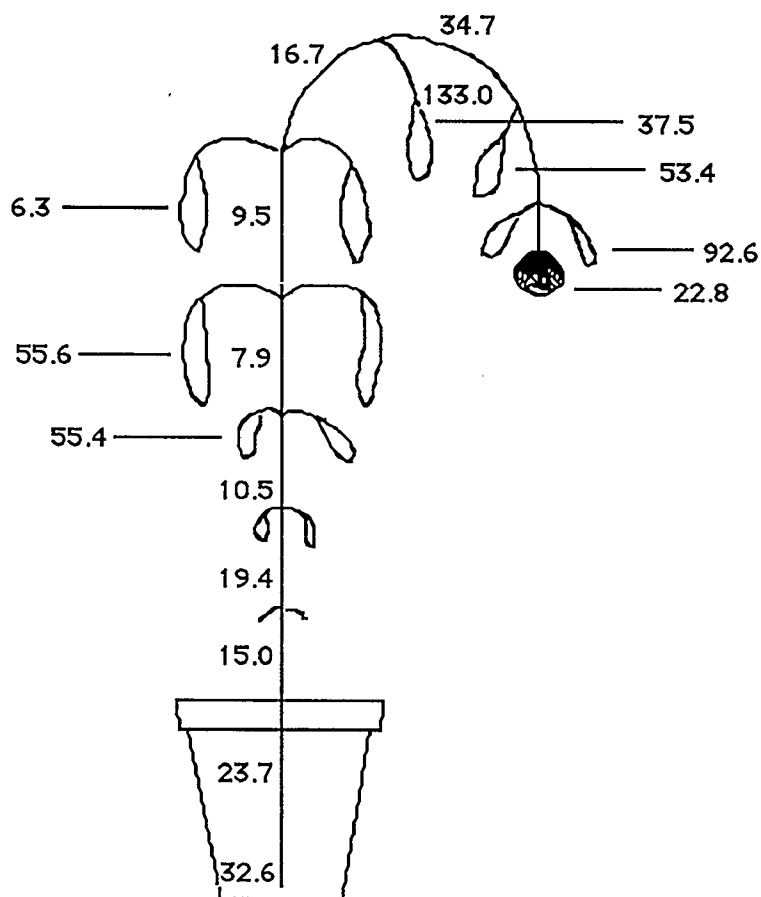


Figure 25. Ethylene production in a droughted sunflower plant. (Ethylene in nl/l/gFW in 20 min.)

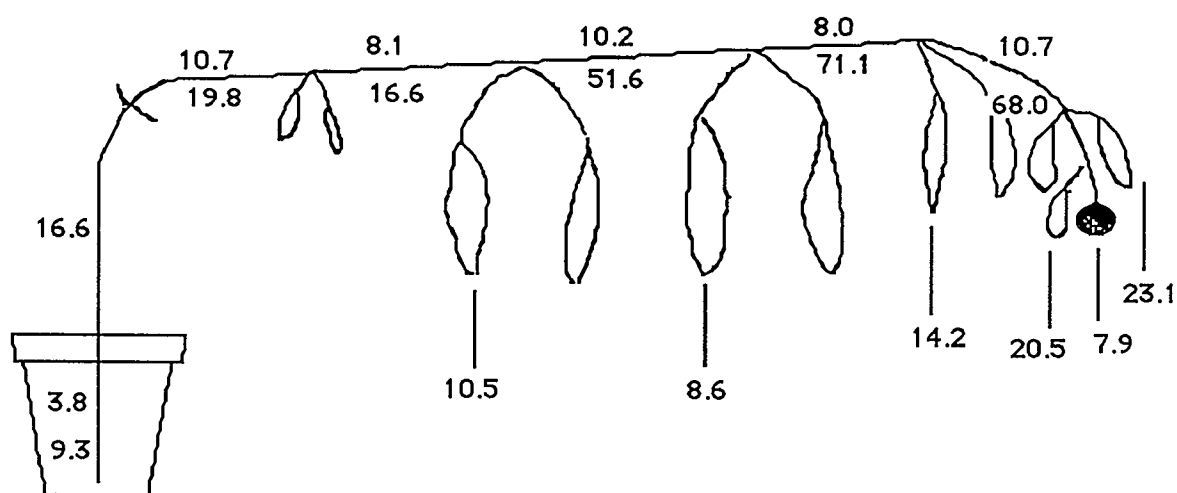


Figure 26. A second droughted sunflower plant, this time one that had been gravistimulated by allowing the stem to fall down under its own weight 10 days prior to the start of droughting. The plant grew upright during days 1 to 80, then horizontally from day 80 to day 90, when drought was induced by withholding water for 1 day. (See Figure 9 for ethylene levels in a similar but non-droughted plant.)

half of horizontal stems, and to some extent in the leaves.

Hubick et al (1986) also found little effect of drought on ethylene production in 17-day-old sunflower plants. The effect of gravistimulation on ethylene production in wilted sunflower is obviously an important consideration in the interpretation of experiments involving drought and ethylene measurement. Is the situation similar in other species? Figure 27 below suggests that in tomato, wilting will cause dramatic increases in the ethylene produced by the lower half of the stem, as it did in sunflower.

It was suggested above that an apparent trend can be seen in the pattern of ethylene production of plants of various growth habits: those plants that are self-supporting, and need to have the capacity to regain an upright position, or to make adjustments in the supporting tissues when a stem develops a lean, also seem to be the species that produce ethylene in response to gravistimulation. What is the situation in a climbing plant, where there does not appear to be a need to be responsive to changes in orientation, nor any need to develop short sturdy stems in places that are exposed to the wind? In climbers, the function of the xylem tissue is mainly to conduct sap, and while a certain degree of sturdiness is still essential, the important point here is that climbers need not be so responsive to changing or variable load conditions on their stems. Figure 28 shows the rates of ethylene production in pea, under conditions of drought and gravistimulation. Gravistimulation had no apparent effect on ethylene production; drought caused a moderate increase in both leaf and stem ethylene. Going on the principle that ethylene is a general signal of stress in plants, one

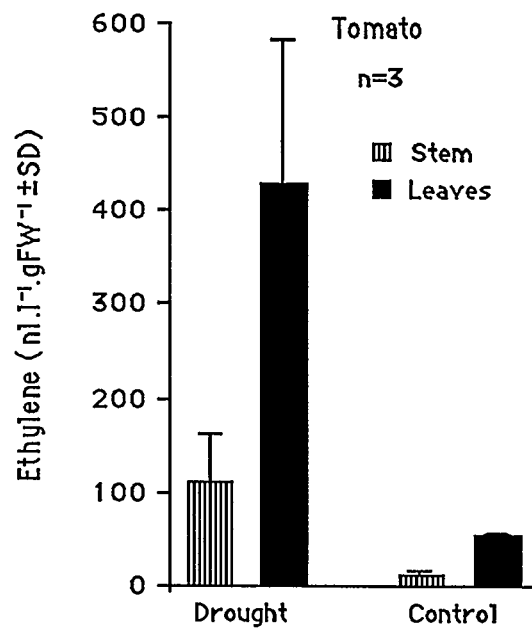


Figure 27. Effect of drought on ethylene production in tomato. Plants were 43 days old; stems were supported so that there was no gravistimulation.

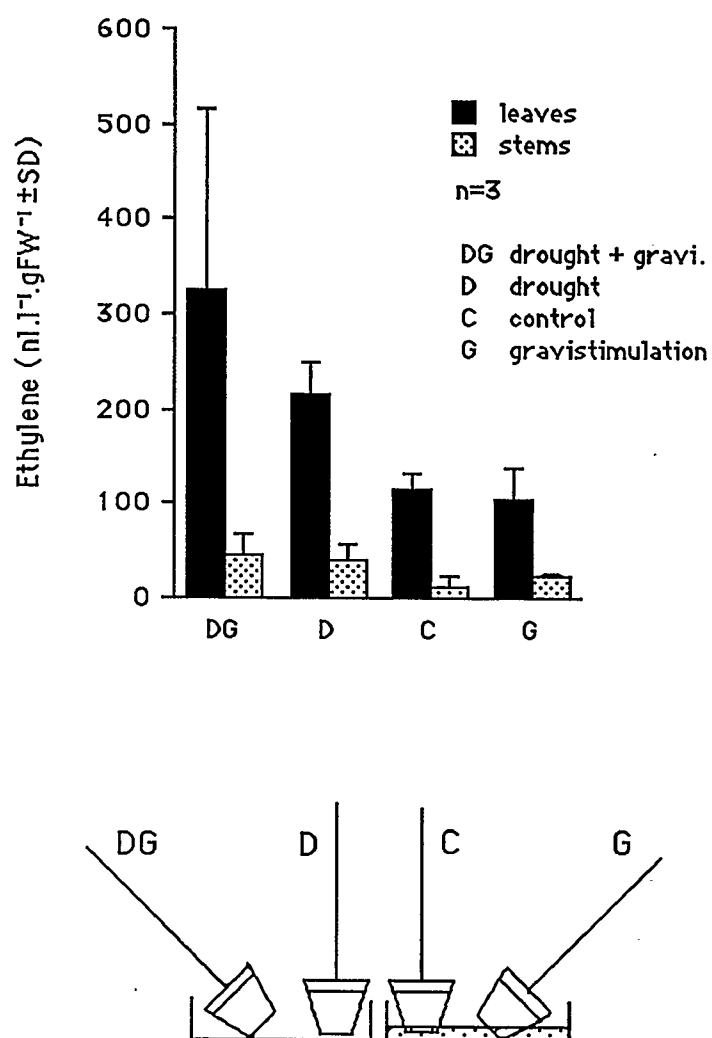


Figure 28. Effect of drought and of gravistimulation on ethylene production in pea.

could summarize the situation in pea by saying that gravistimulation of mature stems or leaves is not stressful to this species, whereas drought does create stress in both stems and leaves. Miyamoto and Kamisaka (1987) also found that neither osmotic stress caused by mannitol nor drought stress stimulated ethylene production in cuttings from 5-day-old pea plants.

It was seen in the previous section on gravistimulation that broadbean did respond to gravistimulation by slightly increasing ethylene production, but the increase was not most pronounced on the lower side of the stem, as it was in sunflower and tomato. Experiments involving both drought and gravistimulation in broadbean supported this finding, as shown in Figure 29. Stem ethylene did increase slightly in gravistimulated stems, but if anything, the increase was greater on the upper side of the stem in this species. Ethylene production in leaves of droughted Broadbean plants revealed a pattern shown in Figure 30A: namely, the uppermost leaves produced less ethylene, while the lower leaves produced more. This result might be interpreted in two ways. First, one could argue that the droughted broadbean plant reduces the supply of water to the lowermost leaves first, so that these show the initial signs of stress. The other interpretation is that all leaves feel the effects of water stress equally, but that the lower leaves are at a more advanced stage of maturity, and that therefore their response is to produce higher ethylene levels. In either case, the effect is that the greatest amounts of ethylene, and therefore the greatest degree of slowing down in the biochemical machinery of the cells, occur in the lowest and most expendable leaves. The pattern of ethylene production in the stems of droughted broadbean (Figure 30B) was slightly different, but again the

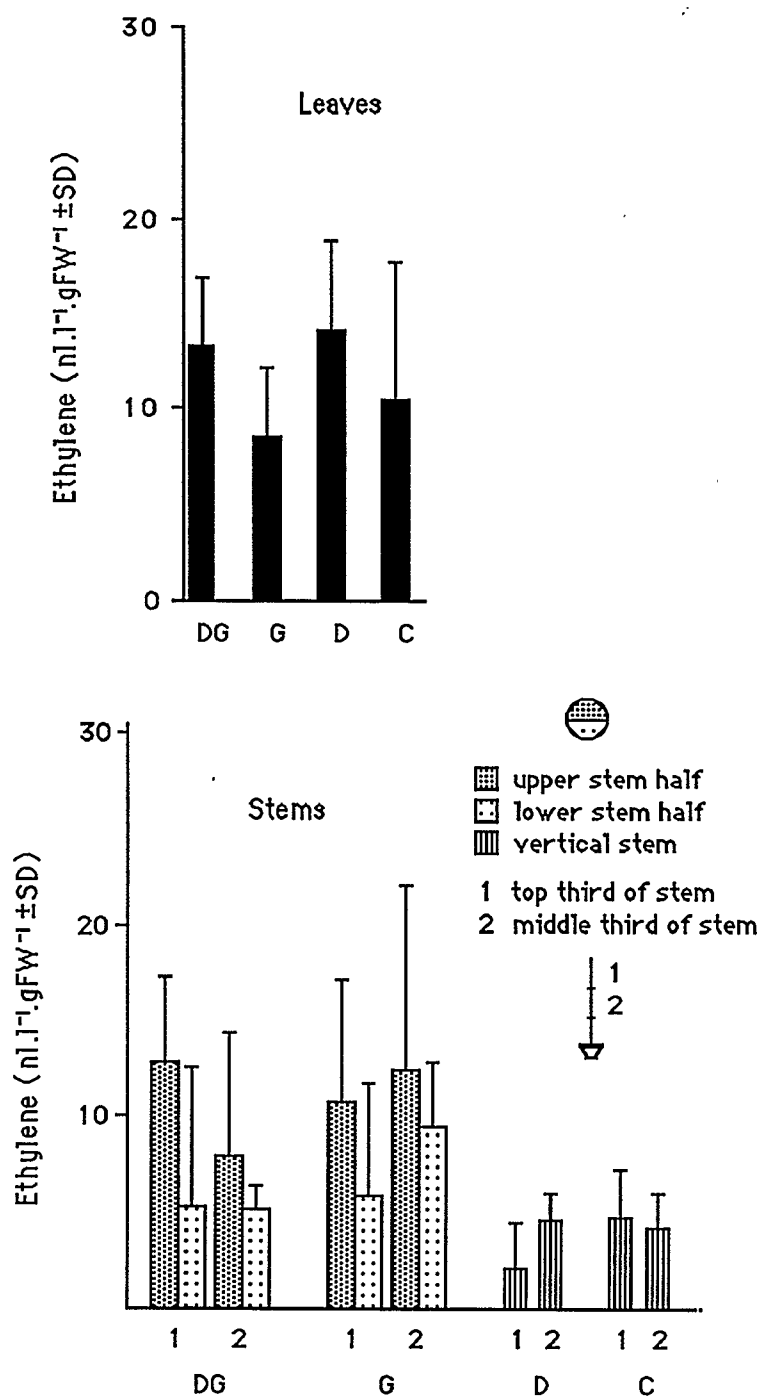


Figure 29. Effect of drought and of gravistimulation on ethylene production in broadbean.

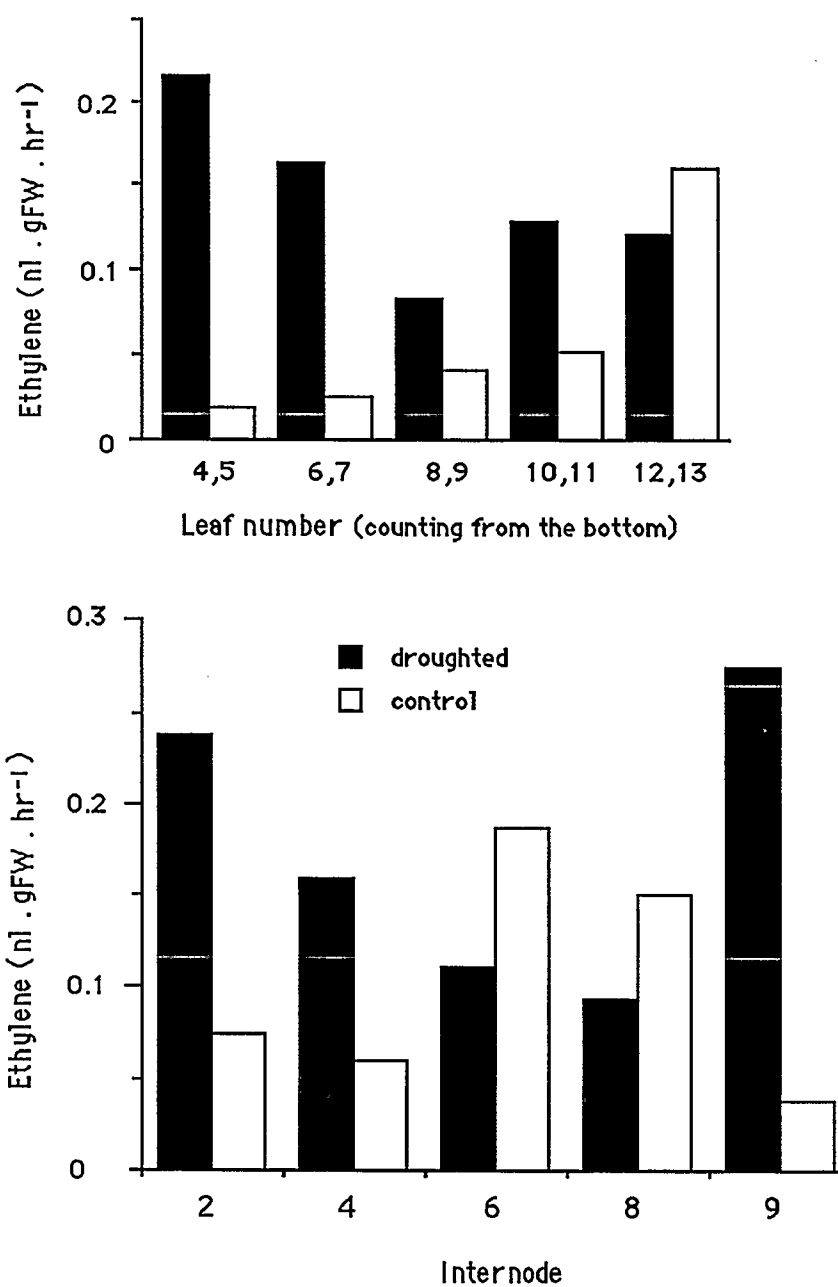


Figure 30. A) Effect of drought on ethylene production in broadbean leaves. B) Effect of drought on ethylene production in broadbean stems.

greatest increases for the droughted plant occurred in the lowest internodes. The results shown in Figure 30 indicate that in a droughted broadbean plant the highest ethylene levels are near the base. Here once again (as we found also in gravistimulated plants) the region of greatest ethylene production is not always the apex. This goes counter to some commonly held ideas about ethylene distribution, and raises further questions. For one thing, one would be curious to know whether the machinery for ethylene production, especially the precursors SAM (S-adenosyl methionine) and ACC, is distributed uniformly throughout a normally growing plant. Another matter worthy of investigation is the distribution of auxin in these instances of non-apical ethylene production. Auxin and ethylene so often appear to be linked in their actions: are there instances in which the two act separately? One would not expect to see increased auxin at the base of a droughted plant, but of course this must be tested to be sure. One final observation that came out of the experiment involving droughted broadbean plants was that in this species, very little wilting occurs either in the leaves or the stem of a water stressed individual. Unlike sunflower and tomato, whose leaves and stems droop quite readily, the leaves of broadbean in my experience curl up in response to drought, but the petioles remain at essentially the same angle as before. The stem of a mature broadbean plant droops somewhat when droughted, but it retains its upright habit to a surprising degree.

El Beltagy and Hall (1974) did not state which portions of their 85-day-old broadbean plants they used, only that they extracted gas from "the internal air spaces of stems and leaves". In my experiments, broadbean plants of this age had from 13 to 32 internodes, and were up to 1 m in height. Beyer

(1975) suggests that increased levels of ethylene in the leaf blade reduce the amount of auxin transported out of this region, indirectly causing abscission of the petiole from the stem. Droughted broadbean leaves were never seen to abscise, nor even to show signs of epinasty or drooping. They just shrivelled up in their original positions. Thus these data are not completely in agreement with the suggestion of Aharoni (1978) that drought-induced ethylene is characteristic only of plants with abscising leaves.

Figure 31 shows the results of a similar experiment in oats. Here drought caused several-fold increases in ethylene production, while gravistimulation had little effect. The plants were 21 days old. At time zero, the soil was washed from the roots of all four treatment classes. The plants were attached to a metal grid so as to maintain the desired orientation. This precluded wilting in any plant. Non-droughted plants were kept in a hydrated condition by means of paper towels that covered the roots from two sides, and a continuous water drip onto the paper towels. The plants were harvested after 6 hours. Since whole plants were used in these experiments, and since the gravitropic response of monocotyledonous plants is mainly restricted to the node pulvini, it is perhaps not surprising that no ethylene increase was detectable in the gravistimulated plants, especially since Kaufmann et al (1985) found that gravistimulated oat plants produced an ethylene peak after about 24 hours.

The rate of ethylene production from excised wheat leaves increases when they are subjected to water stress (Wright, 1977). McKeon et al (1982) found that water stressed wheat leaves synthesized more ethylene, as a result of increased synthesis of ACC and an increased activity of the

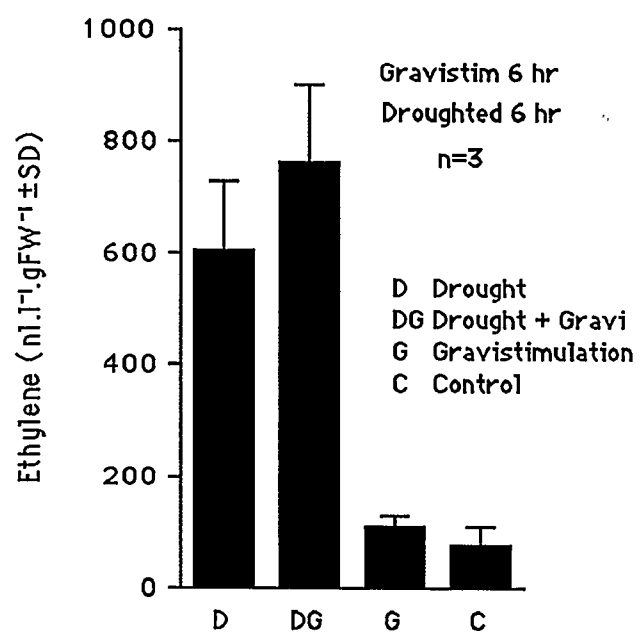


Figure 31. Effect of drought and of gravistimulation on ethylene production in leaves and stems of oats.

ethylene-forming enzyme (EFE). Hoffman et al (1983) reported that MACC (1-(malonylamino)cyclopropane-1-carboxylic acid) levels in droughted wheat leaves increased. They point out that MACC, the major metabolite of the ethylene precursor ACC is a good indicator of the stress history of leaves.

Chrominski et al (1988) found that osmotic stress stimulated the conversion of ACC to ethylene in the halophyte, Allenrolfea occidentalis. They used 1 cm excised sections of the succulent stems, and incubated these for 24 hours. The tissue was therefore wounded, flooded, and gravistimulated (in addition to being stressed osmotically), but there was nevertheless a clear difference between ethylene production by experimental and control sections.

Vaadia (1976) discusses the roles of cytokinins and abscisic acid in plant response to water stress. The results of the experiments described above suggest that ethylene may also play a limited role in the response to drought for leaves of sunflower, broadbean, oats, pea, and tomato, and for the stems of all these species to a lesser degree. Aspinall (1980) points out that it is possible that the response is indirect, since applied ABA will also induce ethylene production.

To sum up once again therefore the findings of this section, the influence of wilting-induced gravistimulation on ethylene production is of importance in some species, and in other species it is of no concern. The responsive

species in this respect are apparently those plants which are free standing; climbing plants do not vary their rates of stem ethylene production when mature portions of the plant are gravistimulated. This is something that might be kept in mind when designing experiments to measure the production of ethylene in water stressed plants.

e. Thigmostimulation and ethylene

It was argued in the general introduction above that thigmostimulation by wind is unlikely to be the signal by which a plant takes in the information necessary to adjust its shape to the yearly, daily, and hourly changes in the requirements for strength in all its parts. Nevertheless, the extensive work by Pickard (1971), Jaffe (1973, 1976, 1979), Hiraki and Ota (1975), and Mitchell (1977) in this area has clearly shown that thigmostimulation (a disturbance to the plant pertaining to touch) of stem epidermal tissues results in decreased axial growth, increased radial growth, and increased ethylene production. Therefore this is a potential component of the effect of wind on plant growth, and is considered below. Furthermore, in the light of the experiments described above on gravistimulation, bending, and drought, it begins to appear that there is a pattern of different levels of ethylene production between free-standing species and those which are climbers and do not depend on their own stems for support. The climbers, like pea, bean, and cucumber, do not respond much or at all to gravistimulation by changing their levels of ethylene production; self-supporting plants like sunflower and

tomato on the other hand are responsive - they produce more ethylene when gravistimulated. Thigmostimulation therefore, is one more way of testing this idea of a general responsiveness in free-standing plants. Or, stated another way, one might suggest that the plants that produce ethylene are those that need to.

The experiments on thigmostimulation were done in a similar way to that described in the various experiments by Jaffe, with several differences. Jaffe chose to use mainly very young plants, and usually concentrated his treatment of "mechanical perturbation" to the youngest internodes. I used his technique of rubbing the internode between the thumb and forefinger twenty times on each occasion. My hypothesis was that a plant should be responsive to the changing forces of wind and gravity throughout its growing period (which in most plants extends through the greater part of its life), and so I chose plants that were somewhat older, and the "mechanical perturbation" was likewise applied to older stems - usually the hypocotyl or first few internodes. On the basis of the results of ethylene measurements in the experiments on gravistimulation and drought, I suspected that certain species would be more responsive to thigmostimulation as well: self-supporting plants would be more likely to respond to disturbance of passing animals, wind, and the like. Thus sunflower and tomato should produce ethylene when rubbed on an internode, while climbers like pea, cucumber, and bean should not. The experiments bore out this supposition, as shown in Figures 32 and 33.

Jaffe (1973) likewise found no increase in ethylene production in thigmostimulated Alaska pea. The results from the experiments using bean

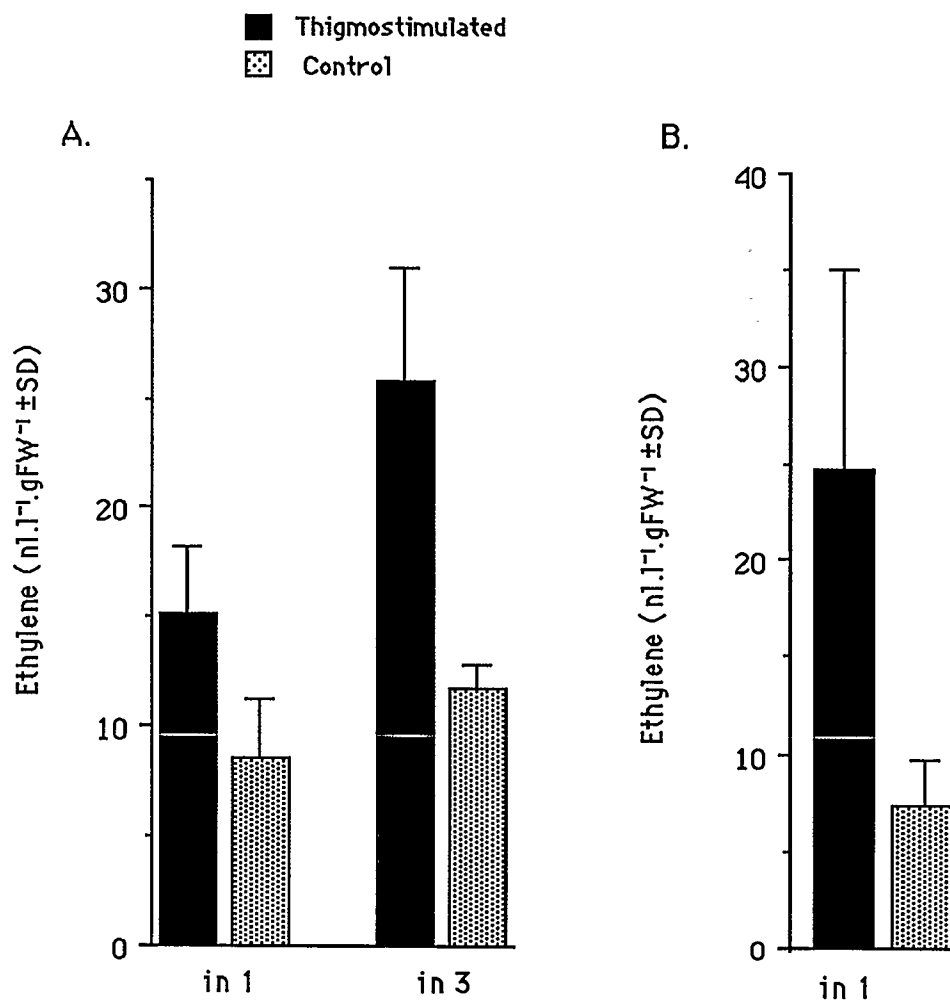


Figure 32. Effect of thigmostimulation on ethylene production in self-supporting plants: A) sunflower. B) tomato. (in1 is the first internode above the cotyledons).

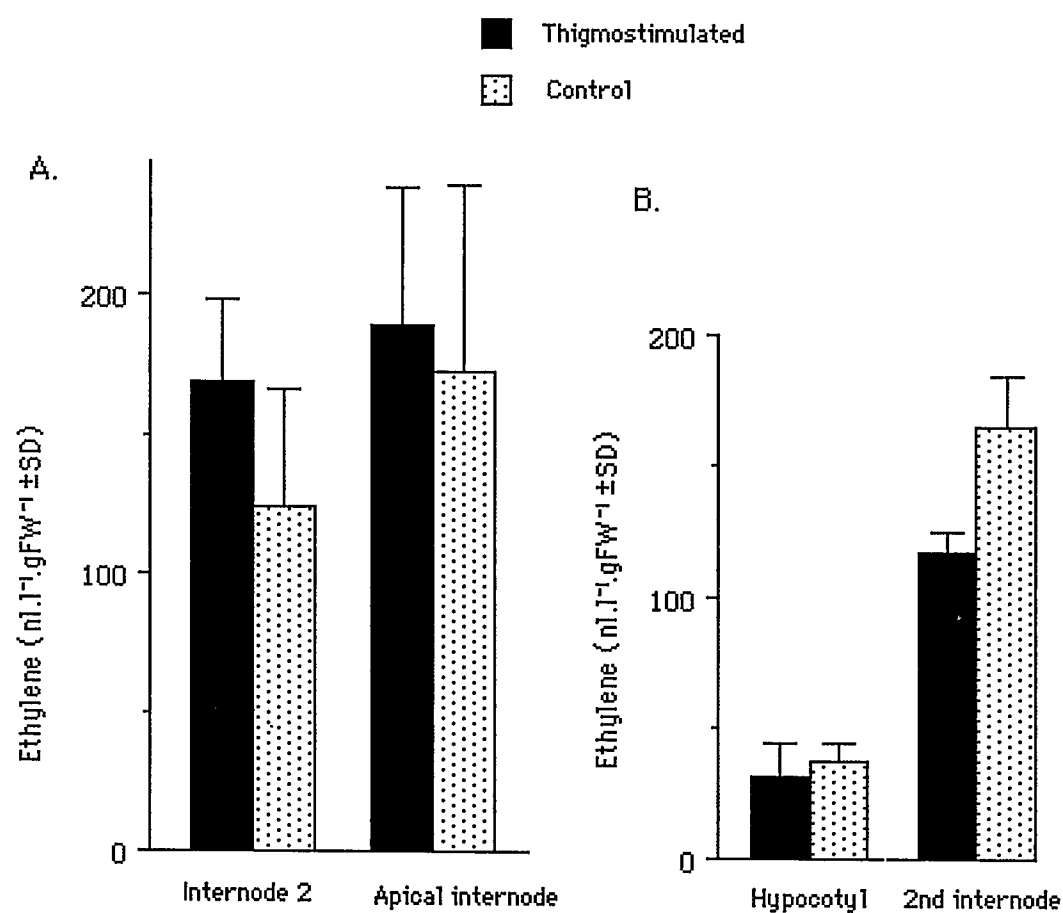


Figure 33. Effect of thigmostimulation on ethylene production in climbing plants: A) pea; B) bean.

plants were consistent with the findings of the other kinds of ethylene experiments done in this project, as already explained, but they were somewhat surprising in that the extensive work of Jaffe has utilized this species very often, and cites it as one of the more responsive plants to this treatment. The differences in method between mine and those of Jaffe (together with most other workers in this area) have already been described: Jaffe usually uses younger plants, and concentrates on the very youngest portions of the elongating stems. Prasad and Cline (1985) reported increased ethylene production in Pharbitis nil when the apical internode was rubbed. Similarly, Boyer (1967) reported reduced growth in Bryonia dioica (a climber) when the apical internodes were regularly rubbed. In one experiment however (Jaffe et al, 1985), 16-day-old bean plants (P. vulgaris var. Cherokee Wax) were used, exactly the same age as those in Figure 33, and his results are very different. Biro et al (1980) observe that bean plants show wide varietal differences in response to mechanical stimulation. The Cherokee Wax variety of bean is a bush type plant, whereas the variety I used (Tender Green) is a climber. I did this experiment five times. If I might be allowed to return once again to this theme of responsiveness, and the apparent tendency of plants to produce ethylene only in those places and under those circumstances when it is needed, it might be argued that in a climbing variety of bean only the stem tips need be responsive to the various signals from their environment, and that if these signals have been interpreted correctly throughout the life of the bean plant, then the older portions of the stem will have become entwined around some supporting structure. In this state, the older stems need not respond to the brushing of passing animals, or bending movements caused

by the wind: in fact, it would be wasteful of energy for these older stems to respond, and wasteful even to maintain a system capable of responding. In a bush bean like that used by Jaffe, the plant is self-supporting, and should be expected to respond to perturbations to its stems, and of course that is what it did.

In considering the responsiveness of free standing or climbing types of plants to gravity or to touch, it becomes important to specify the age of the particular organ in question. For a climber, the young growing portion of the stem must seek new supports to attach itself to. These will normally be above or beyond the previous growth; therefore, it is important that the growing apex be responsive to gravity. Likewise, touch is important to the elongating apex in twiners, or to the tendrils if these are present. Jaffe (1970) has shown that tendrils coiling due to mechanical stimulation produced three times as much ethylene during the first hour as did resting tendrils. Therefore it would be incorrect to say that climbers are never responsive to gravity and touch; only that in the mature stems of climbing plants the responsiveness is seen only in the growing tissues, and becomes lost in the older parts of the plant.

Overview and some general comments on chapter two.

The experiments described above have explored several matters. The first section described the distribution of ethylene-producing tissues in

mature individuals of plants from species having several kinds of growth habit. These were herbs (both self-supporting and climbing), and two tree species. Having studied the normal levels of ethylene production in mature plants, the next four sections tried to separate the effects of wind into individual components, and to see what effect if any each of these had on ethylene production. These components were gravistimulation, bending, and thigmostimulation. There was also a section on drought, in which the relationships of ethylene, gravistimulation and drought were examined in a number of species.

Gravistimulation and thigmostimulation caused increased ethylene in some parts of some species, while bending was not shown to cause changes in ethylene production. Drought had a somewhat different pattern of ethylene production in these experiments, but in the case of the other treatments, there emerged a pattern of responsiveness: self-supporting plants like sunflower and tomato were responsive, and produced increased stem ethylene when stressed, whereas climbers like pea, cucumber and bean were less responsive, and did not in general produce more stem ethylene when stressed. All this was in keeping with the original hypothesis that supportive tissues should be strengthened in those places where it was necessary, and with the hypothesis that ethylene might be one of the messengers in the system of perception and response. A final series of experiments was then initiated to do the obvious experiment for an investigation of ethylene and wind: fans were set up in front of one group of plants, and the rate of ethylene production in these was compared to that in another group of control plants. These experiments were repeated six times

in sunflower, a species which was anticipated to be among the most responsive. The result was that no difference could be detected between rates of ethylene production in windy versus control plants. Some plants were grown in continuous wind, while in others the fans were started once the plants were growing in the third internode. The movements of the leaves and stems that were produced in the wind-treated plants were vigorous, but not so severe as to cause physical damage. The plants waved to and fro in about the same manner as one normally sees in a flower bed when a moderate wind is blowing. Rates of ethylene production were measured from samples taken at times of one, six, and twenty-four hours after the start of the fans. Fans were placed in two different positions (above and to the side of the plants). In all cases, no clear difference emerged between the ethylene production of wind grown and control plants. Plants grown in continuous wind were smaller than control plants, as also reported by Whitehead (1962) and Fluckiger et al (1978), but the rates of ethylene production were no different. This unexpected finding in no way detracts from the significance of the findings of the previous five sections of this chapter, but it raises some questions to which I have no answers. One explanation that comes to mind most readily is that all of the individual treatments investigated do produce increases in ethylene when isolated and exaggerated, but that when these stresses occur in the diminished form that results from windy conditions, there is no change in ethylene production. Another possible explanation is that the same kinds of responses do occur in windy conditions, but that the differences in ethylene levels are much more subtle (i.e. small but continuous higher levels of ethylene), and were not detected by the techniques that were used. Also, wind might produce many small and additive effects of ethylene. The matter will have to remain

unresolved for the present.

To conclude this chapter then, perhaps the most noteworthy finding is that plants appear to produce ethylene when it is useful for them to do so. This may mean in some cases that the machinery for ethylene production is lacking, and in other cases that the control systems over its synthesis operate differently according to the location and the circumstances. This may be merely stating the obvious, but it is intuitively sensible that in the economy of the plant, the greater the specificity and degree of appropriateness of the plant's response, the more competitive it will be. Fluctuations of ethylene production reflect the rate of respiration in plants as measured by oxygen uptake (Saltveit et al, 1978; Warman & Solomos, 1988). Imaseki et al (1968) found that ethylene was involved in such other wound-induced changes as elevation of the activities of peroxidase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase, and mitochondria. Thus not only is ethylene associated with reduced growth, this reduced growth is also more expensive than the alternative situation: namely, lower ethylene, increased growth, and reduced respiration. Furthermore, it is perhaps unusual that the rate of production of a hormone by a tissue should be similar to its presumed degree of activity in that location. One thinks of the auxins and cytokinins, where the regions of greatest productivity are not necessarily the areas in which their effects are most pronounced. It may be too that this pattern of ethylene production is a reflection of the unique place of the gaseous ethylene among the plant hormones.

There is another aspect to this that should be briefly considered here. That is, a plant may be constructed so as to respond only to those signals that are of use to it at a particular time, but can it also dampen out the system of perception (and thus reduce this cost) in those situations where it has not enough resources to carry out the response? One thinks of branch angle - this is said to be an inherent characteristic of a species, and yet it is only the younger, more vigorous branches that can maintain the ideal angle. The lowest and oldest branches can often be seen to have "given up" - they bend right down towards the ground, and then only the terminal twigs have enough vigor to curl upwards. In Figure 6, a mature sunflower was not able to respond to gravistimulation (by bending upwards) except at the upper internodes, and yet there was still an ethylene gradient in the older and unresponsive portion of the stem. To a certain extent this may be a question of lignification (loss of flexibility), but it may also involve the supply of photosynthates available to a tissue. We may ask of a certain plant tissue: "Does it want to respond (to gravity, bending, etc.)?" secondly "Is it possible for it to respond?", and finally, "Does it have the resources to respond?"

Yang and Pratt (1978) suggest that any condition deviating from the normal environment of the intact healthy plant may lead to an increase in ethylene ("stress" ethylene); the results reported here lead one to believe that the role of ethylene may be broader than this. That is to say, the "normal environment of the intact healthy plant" also needs to be perceived by that plant: the degree to which gravity, bending, drought, and thigmostimulation (and probably other signals as well) act on every cell throughout the plant during the eternally changing but still "normal" conditions, and, according to

the hypothesis advanced here, in particular on lateral meristematic cells. Woodrow et al (1988) have reported that application of Ethephon to the leaves of tomato plants caused a shift in carbon partitioning from the upper to the lower parts of the plant. This is consistent with the idea that ethylene is one of the messengers by which a plant determines the degree of disturbance that it may expect during its life: in a situation where there is much disturbance, the best strategy is to stay small and stout; under more peaceable conditions, it may take the greater risk associated with developing a tall thin growth habit. The growth habit of an individual plant may be regarded as the cumulative sum of the direct inhibitory effects of all the various stresses to which it was exposed. Alternatively, its growth habit may be regarded as having resulted from a more general kind of sensing of these stresses, resulting in diffuse effects (like shifts in carbon partitioning) whose end result is the same, namely to create a short stout plant. On the basis of the experiments reported here, it may be suggested that if ethylene is involved as a transducer in this process, the second mechanism (a more diffuse effect) is the more likely of the two.

CHAPTER THREE - XYLEM FLOW

Introduction

The question of whether bending of plant stems results in increased resistance to the flow of xylem sap is one that can be put to experimental test without much difficulty. The results of these experiments are given below, and the answer appears to be affirmative. However, the graphs that are obtained by measuring the flow rates in excised stems are almost never straight lines - they show characteristic increases and decreases that are unrelated to any bending imposed on the stems. Many people have measured xylem flow rate in excised stems previously, and the way in which the fluctuations of flow are regarded by these people varies considerably. Some workers (eg Petty, 1978) design systems which claim to eliminate the variations, without establishing the mechanisms that are responsible. Others have addressed the question, at least in part, and attempted to find reasons for the varying patterns of flow. Among the latter group are workers with an interest in helping the cut flower industry: it is well known that a cut flower lasts a much shorter length of time than one left to mature on the intact plant. I decided to explore these increases and decreases in flow rate, since the experimental set-up is so simple (a cut stem, some tubing, and a graduated pipette, as described below), and the experiments on bending did not themselves immediately suggest further investigations along this line. The results are presented in three sections: Bending, Increasing Flow Rate, and Decreasing Flow Rate.

Methods and Materials

The method in all cases is essentially that of Zimmermann (1978). A piece of stem was cut from a bush or tree growing on the university grounds, and brought inside where it was placed under water. There both ends were cut off with a new razor blade, so that the final length of the section was 10 cm. The diameter of the excised sections ranged between 0.5 and 0.7 cm. A short piece of Tygon tubing was then placed over the basal end of the section, and the tubing filled with 10 mM NaCl solution. The other end of the tubing was then attached to a series of two three-way valves, and finally to a 0.01 ml glass pipette. The whole apparatus was supported in a vertical position, and attached to a reservoir of 10mM NaCl, which was placed slightly above the glass pipette. The procedure for measuring flow rate through the section of wood was then a matter of adjusting the fluid level in the pipette to zero, and starting a timer. After a period of two minutes (more or less in some cases depending on flow rate), the volume of fluid lost through the stem was read off from the glass pipette. The system was then opened up to the reservoir, and at intervals of several minutes or hours (depending on the experiment) the flow rate was measured again. Species used were Cornus canadensis (dogwood), Betula pendula (birch), Caragana arborescens (caragana), Syringa vulgaris (lilac), Salix sp. (willow), Picea glauca (White Spruce), and Populus balsamifera (poplar).

Bending

This experiment included measurements of flow rates in bent sections of 6 species, as shown in the figures below. Bending was by clamping the stem in a plexiglass device, and then creating an angle of the desired magnitude and tightening up the clamps.

The experiments were begun in January 1987. At this time, the outside temperature was well below freezing, which simplified the protocol for the initial experiments, since there was no danger of breaking the water columns that exist in the xylem vessels during warmer seasons (cf Lybeck, 1960). The frozen branch segments were simply cut from the tree or bush, brought indoors, and thawed out under water. Subsequent experiments at all times of the year suggested strongly that breaking even an unfrozen water column had no obvious effect on the characteristics of water flow in excised stem segments. This matter is taken up again later in this chapter, with evidence to support that contention.

Figure 34 shows that the rate of flow through the straight section of dogwood stem was nearly twice the rate through the bent section in this experiment. The degree of bending was approximately 25 degrees each time. It should be noted that the two lines represent flow rates through the same stem section, and over the same time period. The section was alternately bent and straightened out to obtain the two sets of data points. Sometimes two readings were taken in each position to ensure that the set

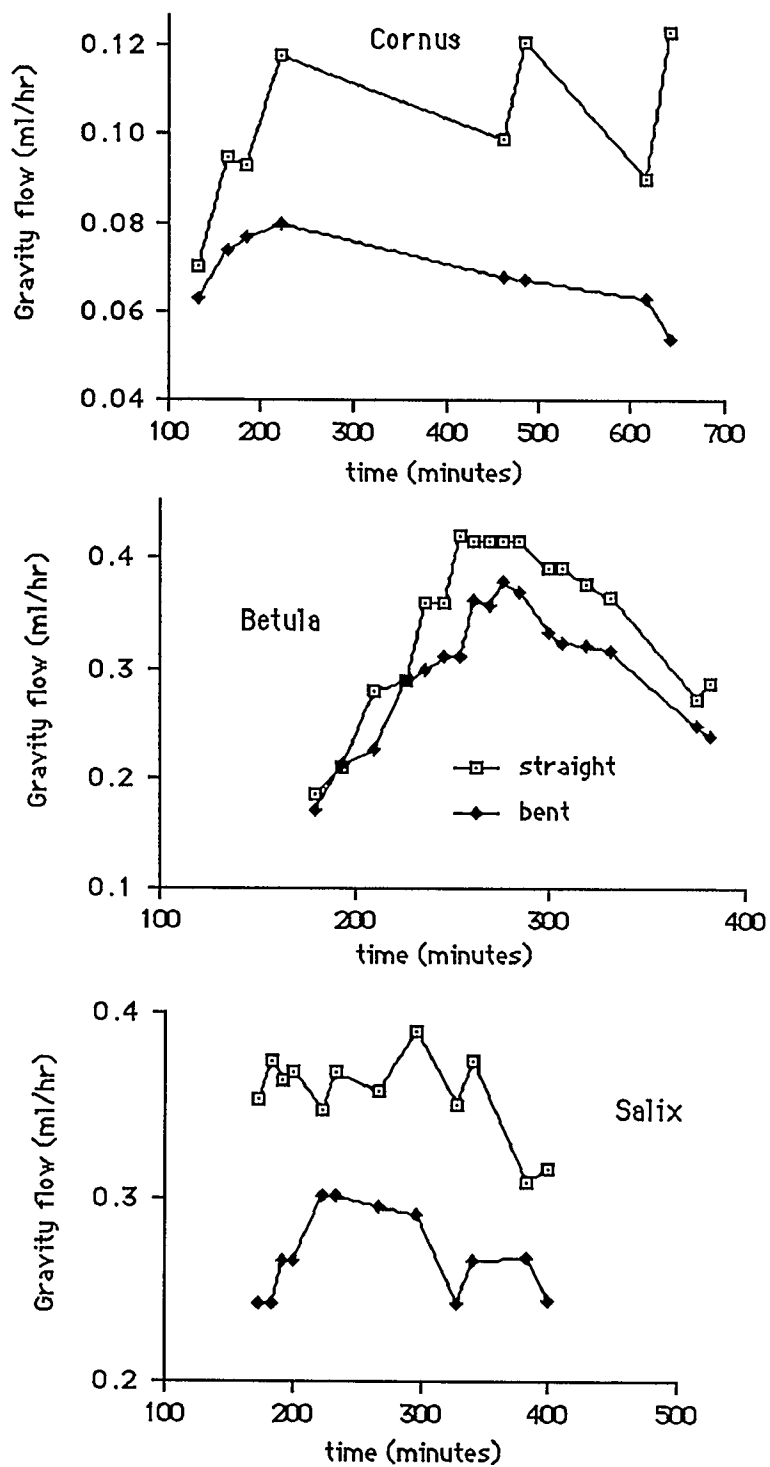


Figure 34. Effect of bending on rate of water flow through excised stem segments of dogwood, birch and willow.

up was not artificially inducing cyclical variations in flow rate independent of the bending. In birch the difference in flow rates between the two positions was not so great, but the bent stem still showed a consistently lower flow. Likewise willow showed large differences in the flow rate of bent and straight positions.

Figure 35 shows the effect of bending on flow rate in three additional species. In spruce and lilac the effect was less pronounced, but in all three species the rate of flow was consistently lower in the bent sections. It may be objected that the above experiments, in which the amount of bending ranged from 20 to 40 degrees, represent stresses that would not always occur under natural conditions. This is a valid objection; one might respond by suggesting that the differences in flow between bent and unbent stems were often large, and that even very slight reductions in the water supply may have effects on growth (eg Begg & Turner, 1976). Kellogg & Steucek (1977) found that mild shaking caused a marked reduction in leader growth in Douglas fir; they did not suggest possible mechanisms.

Parkhurst & Pearman (1971) point out another potential mechanism by which shaken stems may experience reductions in xylem flow, namely vessel blockage due to cavitation. No evidence for this phenomenon was discovered in the current experiments. This is not surprising though, since with the constant head of water provided by this experimental setup, the chances are very slim that the stem section should approach a condition of water deficit (and thus become susceptible to cavitation).

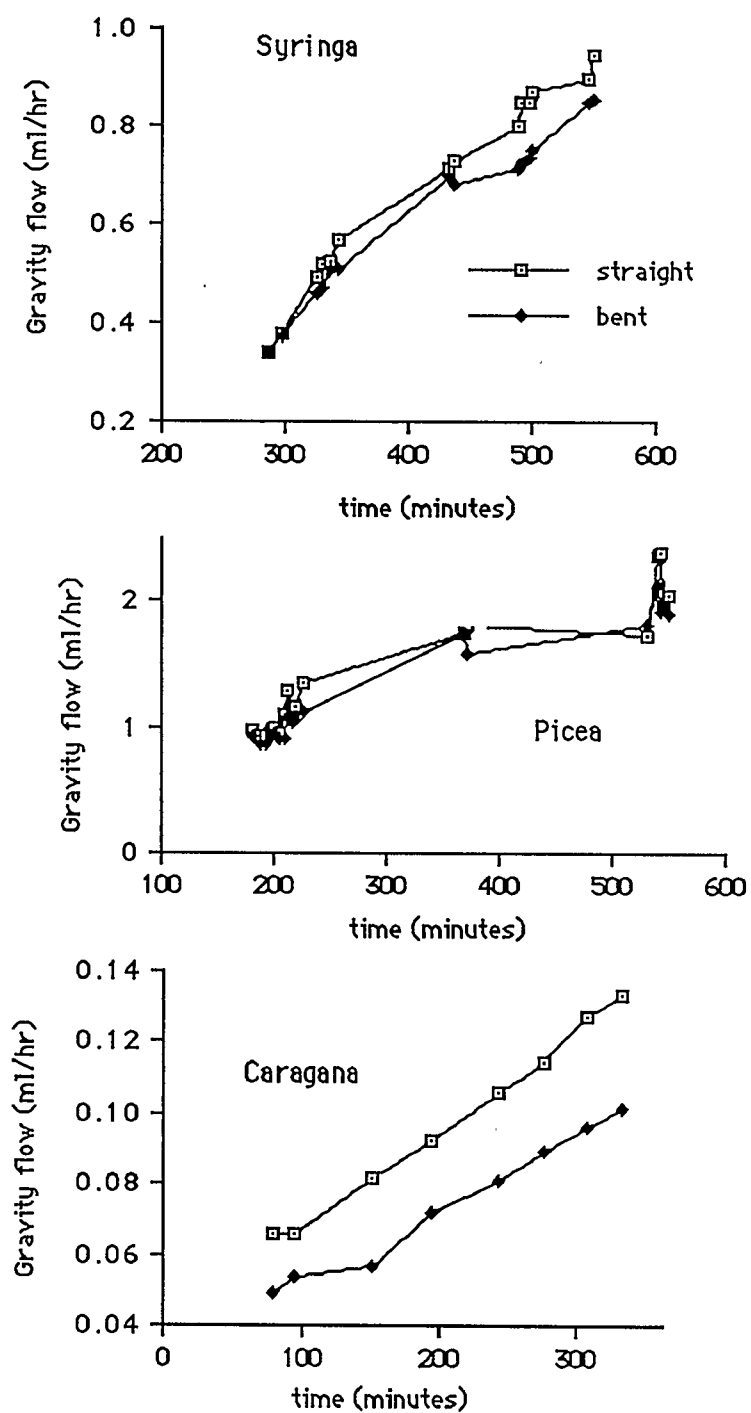


Figure 35. Effect of bending on xylem flow rate in excised stem sections of lilac, white spruce, and caragana.

Neumann and Stein (1983) have argued that the hydraulic architecture of the plant determines the resistance of xylem pathways leading to specific leaves and may thus play a role in the coordination of leaf metabolism and whole plant development. This has been supported by measurements of transpirational losses from different leaf strata in lupin plants (Layzell et al, 1981), of stomatal resistances during the development of bean plants, and leaf-specific conductivities along the axes of various tree species (Zimmermann, 1983). If the system of water distribution in the plant is thus responsive to slight differences in the hydraulic architecture from leaf to leaf and branch to branch, it is not difficult to imagine that these differences could be modulated by wind-induced deformations of the vessels. If maximum xylem flow occurs in calm conditions (when the cross sections of the vessels are closest to being round) then every time a stem flexes there will be a slight loss in the water supply to the leaves, and the more frequent the flexures the more significant will be the deviation of the flow from the maximum.

Increasing flow rates

While many previous workers have concerned themselves with the gradual falling off of flow rates over a period of several hours or days, as discussed below, there has been little investigation into the initial increase in flow rate that is characteristically seen during most times of the year, especially in some species. Having read Zimmerman's (1983) discussion

of embolism and air seeding in xylem vessels, one immediately thinks of air as perhaps being the agent that initially impedes the flow of fluid through the stem section. This goes counter to some fairly prevalent thinking that says a xylem vessel, once filled with air, loses its water column and is incapable of regaining its ability to conduct xylem sap. However, I had earlier on observed an apparent pressure system in the stems of willows and poplars during spring, and therefore was not deterred by the notion that some of the xylem vessels might be empty of water during some parts of the year, and that they might then be refilled at other times. Furthermore, other pressure systems and apparent air-filled vessels during winter have been described previously (eg Merwin & Lyon, 1909; MacDougal & Workin, 1933; Gibbs, 1935; Scholander et al, 1955; and Sperry et al, 1987, 1988). Also, the extensive experiments of Clark & Gibbs (1957) clearly demonstrate annual cycles in the moisture contents of trees, the maximum annual moisture often approaching double the level of the minimum.

If indeed the xylem vessels are completely filled with water only during some parts of the year, and if they contain varying proportions of air during other times, then this should be reflected in the rates of flow through excised stem sections during the year. At times when there are air bubbles in the vessels, perfusion of a stem with water should drive out these bubbles, but during the initial period when these bubbles are being driven out, the flow rate through the stem section should be slower. The results of an experiment shown in Figure 36 support this idea: a large air bubble was

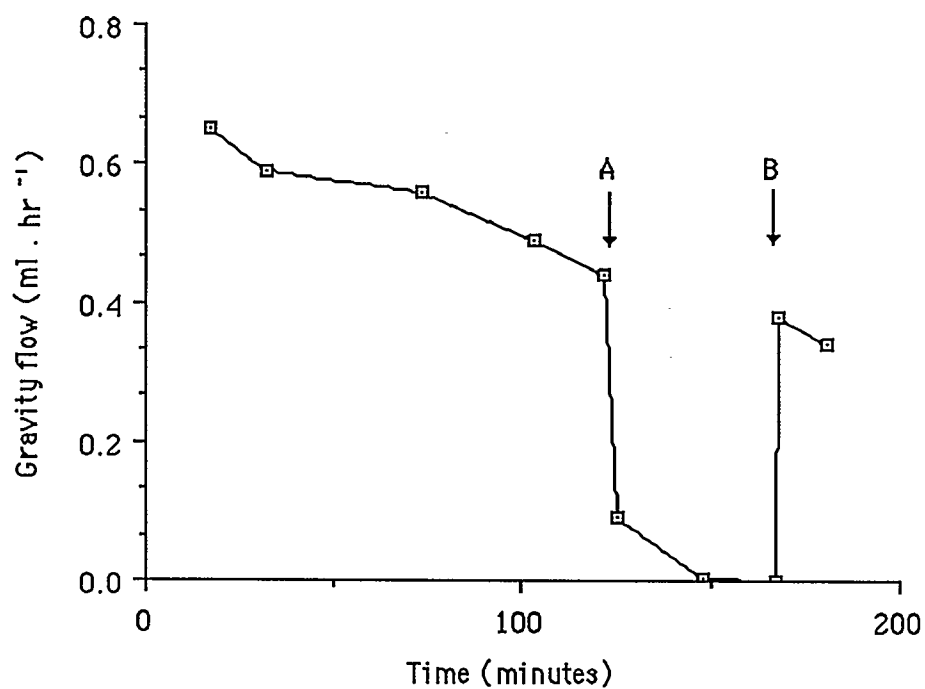


Figure 36. Effect on flow rate in excised stem of dogwood of introducing an air bubble into the water column. The air bubble entered the 10 cm long stem section at A; at B, a rubber bulb was placed over the end of the pipette, and pressure was applied to the top of the water column.

introduced into the water-filled tubing just above the stem section. The rate of water flow through this stem stopped completely once the air bubble had reached the proximal end of the stem. However, the elimination of water flow was not permanent - after a period of 40 minutes, a rubber bulb was placed over the top of the glass pipette, and gently squeezed. This increase in pressure resulted in the resumption of flow through the stem. Evidently the air bubble had passed all the way through the vessels of the stem, and eventually was driven out the distal end. Even the rate at which the flow gradually decreased hour by hour was restored to its original slope after the resumption of flow.

The experiments of Greenidge (1958), in which sap flow patterns were monitored with the use of acid fuchsin dye in conjunction with various kinds of sawcuts into the trees, likewise demonstrated that introducing air into xylem vessels does not irreversibly alter the flow characteristics, so long as the forces driving the sap movement continue to operate. Scholander (1958) found the same thing in grapevines and in Tetracera lianas - introducing gross air embolisms had very little effect on the rate of water uptake.

The results shown in Figure 37 too are consistent with this idea of air in the xylem vessels during some portions of the year. The graph shows the number of minutes required to achieve maximum flow rate in dogwood. Sperry et al (1987) have done similar experiments at various times of the year. Instead of measuring the time required to reach maximum flow rate however, they applied pressures of 175 kPa to the water column to drive out the air (presumably), and then compared flow rates before and after the

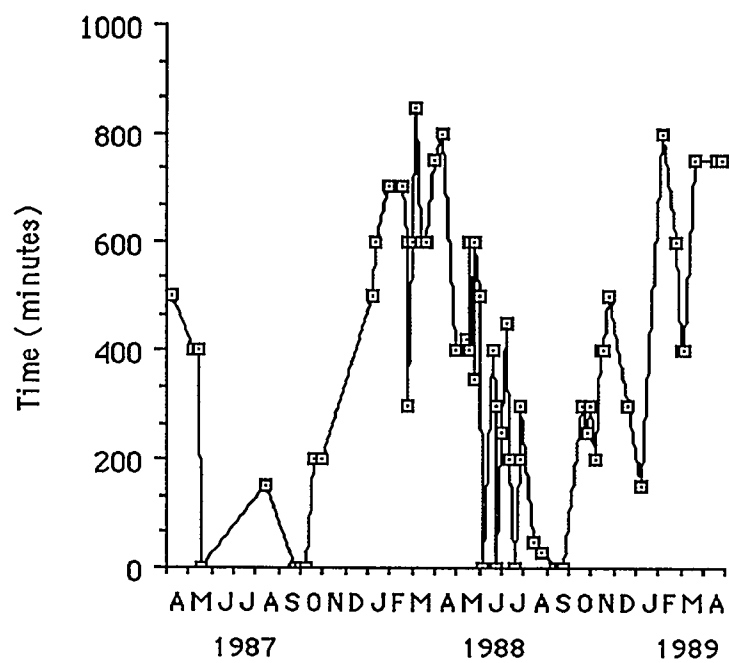


Figure 37. Influence of the season on the time taken to reach maximum flow rate in excised stem sections of dogwood.

treatment. Just to give a few examples of the types of graphs that generated the data in Figure 37, the flow rates for all three species are given in Figure 38 for April 18-21, and for June 7-9, 1988. It seems that in April, there may have still been air bubbles blocking many of the vessels, and that the maximum flow rate was not reached until after about 6 to 12 hours. By June 7, however, there was no longer any phase of increasing flow rate: the rate of flow began to drop off in all three species right from time zero, suggesting that there was little air blockage of the vessels at this time. This is also the time of leaf flush and shoot growth, and it makes very good sense that during this period, the chemical potential of the xylem water should be very high, to provide the turgor pressure that allows for shoot and leaf expansion (something that would be hard to imagine happening through the negative pressures of the transpiration pull alone).

Sperry et al (1987) show similar flow profiles for wild grapevine, describing these as summer and winter patterns.

Figure 39 compares the flow rates (bottom of Figure) in poplar, dogwood and lilac with the gradually increasing weights (top of Figure) of the stem sections. Here again, the results support the idea of air-filled vessels gradually being replenished with water: as long as the weights of the sections are increasing (as long as air is being replaced by water) the flow rate increases as well. Poplar and dogwood seem to fill up with water quickly, as evidenced by both the flow rate and the weights of the stem sections. Lilac, on the other hand, fills up more slowly: maximum flow rate did not occur until about 1500 minutes (about six times longer than with the

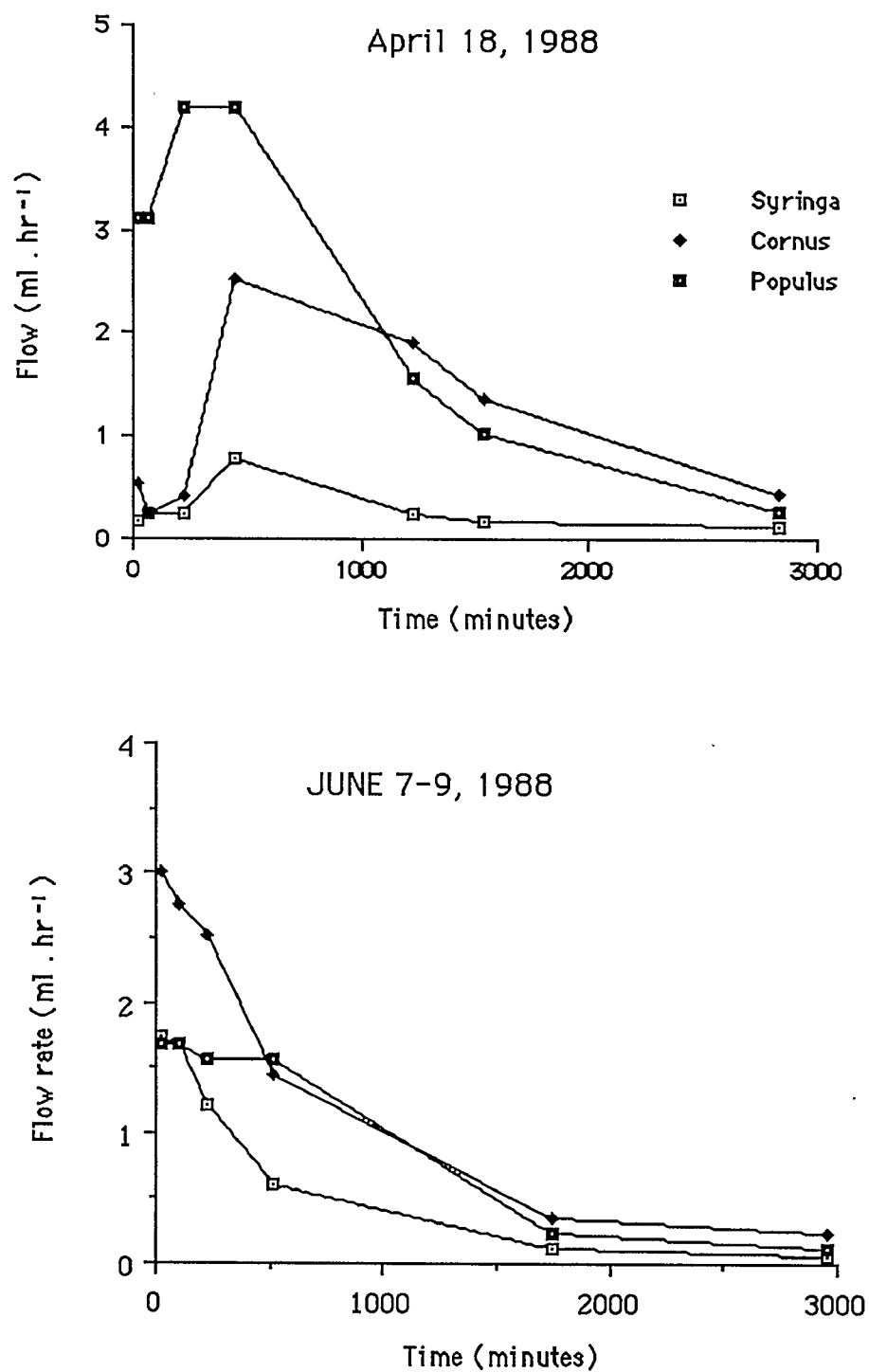


Figure 38. Flow rates for April 18-21 and June 7-9.

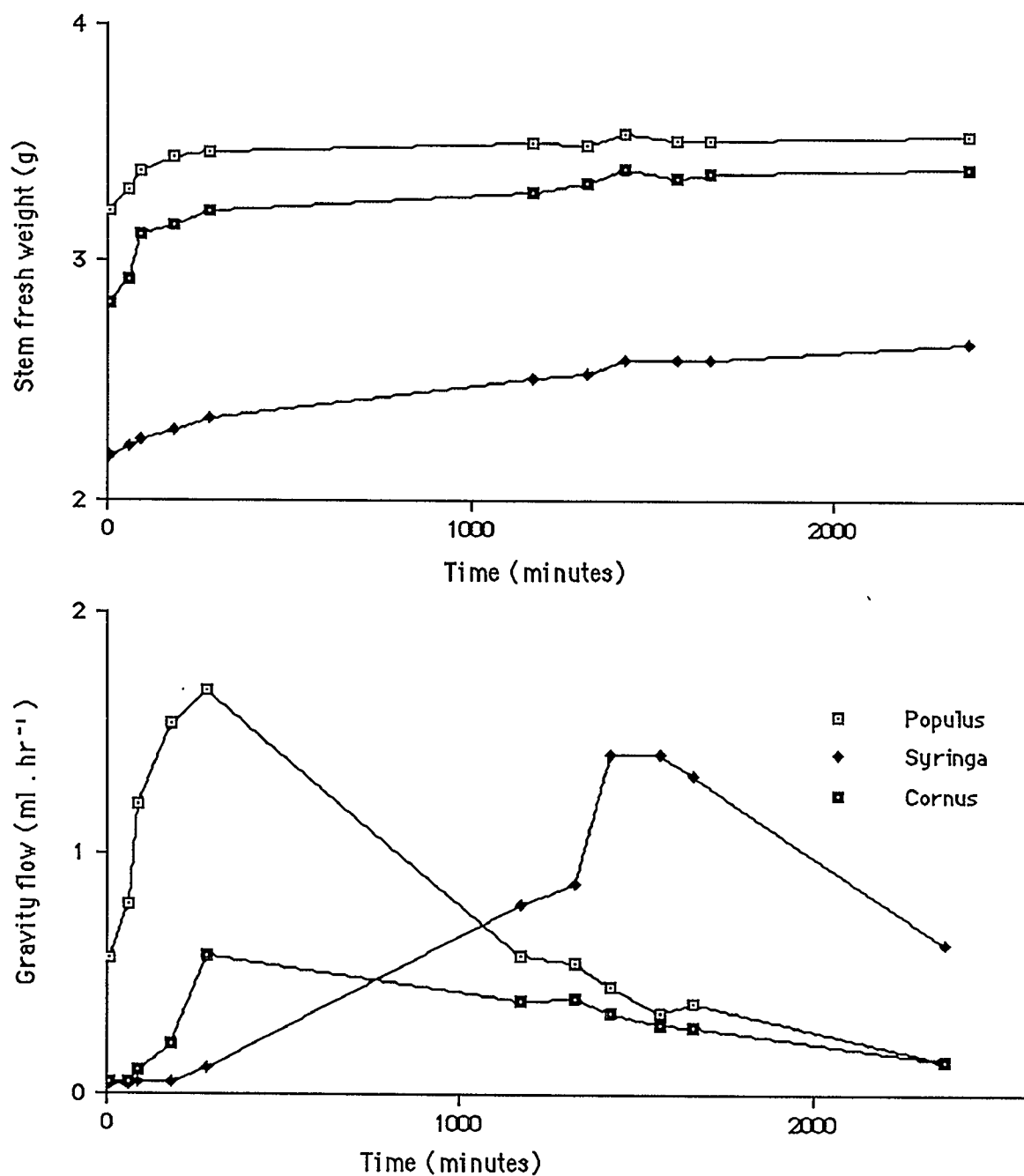


Figure 39. Flow rate in excised stem sections of poplar, lilac, and dogwood as compared to fresh stem weight (Feb. 25, 1988).

other two species), and there was not the marked initial rise in weight that was characteristic of poplar and dogwood.

Figure 40 gives an example of the effects on flow rate of subjecting a stem segment to vacuum (28 inches of mercury) for a period of 5 hours. The rate of flow increased dramatically afterwards, but the rate also fell off very quickly. This rapid fall-off of flow rate was typical of the experiments that involved vacuum treatment. No reason for this behavior immediately suggests itself, and perhaps to clarify the situation a few more experiments would be in order. In particular, an experiment in which the weight of the stem is closely monitored after vacuum treatment would be helpful in deciding whether the fall-off is due to blockage by bacterial or vascular gels, or whether there is a rapid re-seeding of the vessels with air bubbles, or whether the walls of the vessels become swollen and thus reduce the size of the water channels.

The experimental results given in the figures of this section are consistent with the concept of air-filled xylem vessels at certain times of the year. The evidence presented here is indirect, however. A more direct line of investigation into this question would be to actually measure the air in a stem section at intervals throughout the year. This can be done by placing the stem section under water (already deoxygenated) in a large vacuum dessicator, with an inverted beaker (also immersed in the water) placed over the stem. Most of the air in the stem section can be evacuated in this way during a period of about one hour. The dessicator is then disconnected from the vacuum line, opened up, and the volume of the air

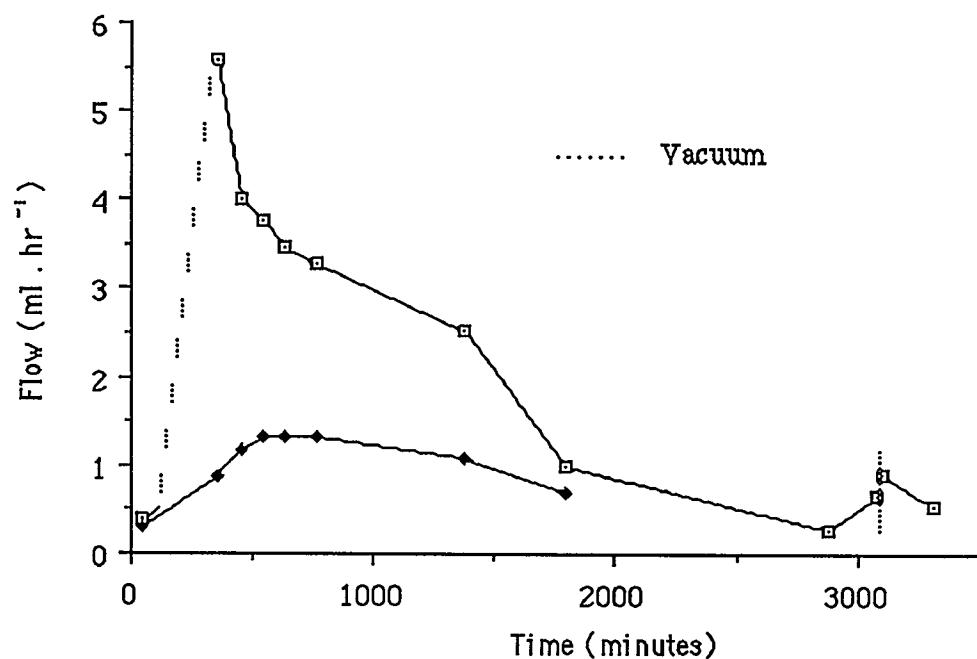


Figure 40. Flow rate in lilac stem after vacuum treatment, together with a control stem section of 10 cm length of lilac. The first vacuum treatment was for 4 hours; the second for 1 hour. In each case vacuum was continued until the rate of air bubble exit from the ends of the stem had slowed to a trickle.

bubble inside the beaker is measured with a syringe. No figures are given here from this method, but it was done a number of times on various species, and the technique is not a difficult one. The direct measurement of the stem air was a tool that was developed toward the end of the study period, and it was therefore not used to obtain supportive data for this study. The method could be used to obtain quite convincing evidence for air seeding of xylem vessels, a worthwhile experiment, since direct evidence of air in vessels has only recently begun to appear (Sperry et al, 1988), despite the fact that it has been observed indirectly for many years (eg Ewart, 1905).

It was mentioned in the general introduction that there appears to be a condition of equilibrium between the symplast and the apoplast (Joly & Zaerr, 1987) which allows a degree of elasticity in the water relations of the xylem. In the light of the present investigation, this communication between interior and exterior of the vessels means that the xylem sap is also in equilibrium with the system of air spaces. This situation results on the one hand in encroachment into the hydraulic domain by air during times of water deficit, but on the other hand provides a mechanism by which under conditions of positive water balance the air pockets can be evacuated. It means also that at times when xylem sap is pressurized, damage to a portion of the xylem vessels has little effect on the water conducting ability of a plant (eg Greenidge, 1958). The pressures have their origin in the osmotic relations of the xylem sap and the cell wall matrix, as explained by Hammel and Scholander (1976). Furthermore, since hydrostatic gradients have not been demonstrated in trees

(Scholander, 1958) it is likely that the water-matrix interaction also has an important bearing on the tensions that move water upwards through the plant. Ewart (1905) showed that resistance to flow is overcome locally from point to point, and not by any enormous tension from above or pressures from below. He points out that a high tension from above leads to blocking of vessels by air; a high pressure from below leads to great loss by lateral exudation from the vessels.

Decreasing flow rates

The literature on this subject is extensive, for the reason as mentioned above that it is of economic importance to florists, whose trade benefits by any treatments that increase the shelf life of cut flowers. The decreasing capacity of a cut stem to take up water has been attributed to a number of possible causes, including microorganisms (Marousky, 1969; Lineberger & Steponkus, 1976; Zagory & Reid, 1986), enzyme action on cell wall material (Burdett, 1970), and air emboli (Dixon, 1987). The experimental results given here do not resolve the matter, but they perhaps add some new pieces of information.

There has been some evidence (Rasmussen & Carpenter, 1974) that the decreasing rate of xylem flow in a cut stem has some component to it that depends on the living cells of the stem. It has also been suggested that the phloem may be the source of exudates (such as callose and sieve tube slime) that contribute to the vascular occlusion. Therefore an

experiment was performed in which the bark was peeled away from one stem, and the pattern of flow rate compared to that of an intact stem segment. The results, shown in Figure 41, suggest that the phloem plays little role in the plugging of the xylem flow.

Another experiment involved cutting a small (0.2 cm) piece from the supply end of a stem being perfused. This was observed to have a dramatic effect on restoring the flow rate to previous maximum levels. As Figure 42 shows, this process can be continued for extended periods, with no apparent falling off of the flow rate, so long as the proximal end is cut off at intervals of one or two days. This experiment also indicated that in this species (lilac), there did not appear to be a directional component to the maximum flow rate, nor to the decrease in flow rate: the stems behaved in approximately the same manner whether they were perfused in the normal (acropetal) direction, or the reverse direction. Over the 500 hours (21 days) that this experiment lasted, there was a slight overall increase in the maximum flow rate each time the proximal end was trimmed. This may be due to the fact that the stem segment was gradually shortened over the course of the experiment, thus reducing the resistance to flow.

Jeje (1988) has found that variations in the moisture content of wood cause changes in the pore size of xylem vessels due to the degree of swelling of the cell walls. Since the water at the proximal end of a perfused stem section is under greater pressure than is the water drop at the distal end, this could explain the results in Figure 42. It does not account for the effect of formaldehyde and sodium hypochlorite in retarding

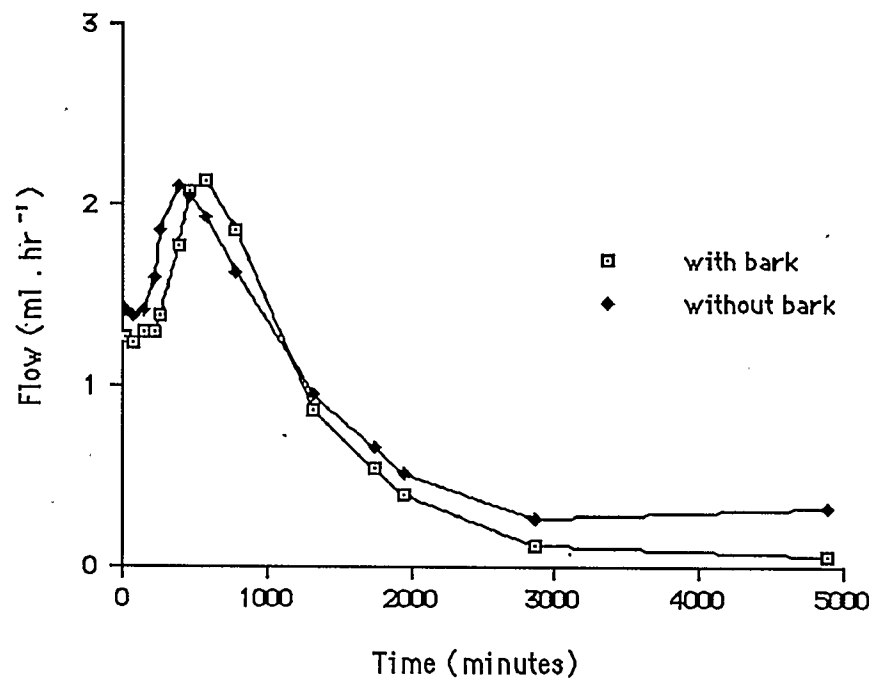


Figure 41. Effect of peeling off the bark on flow rate in poplar. The bark was peeled off the one stem section immediately before setting up the flow experiment. A repetition of the experiment gave a similar trend.

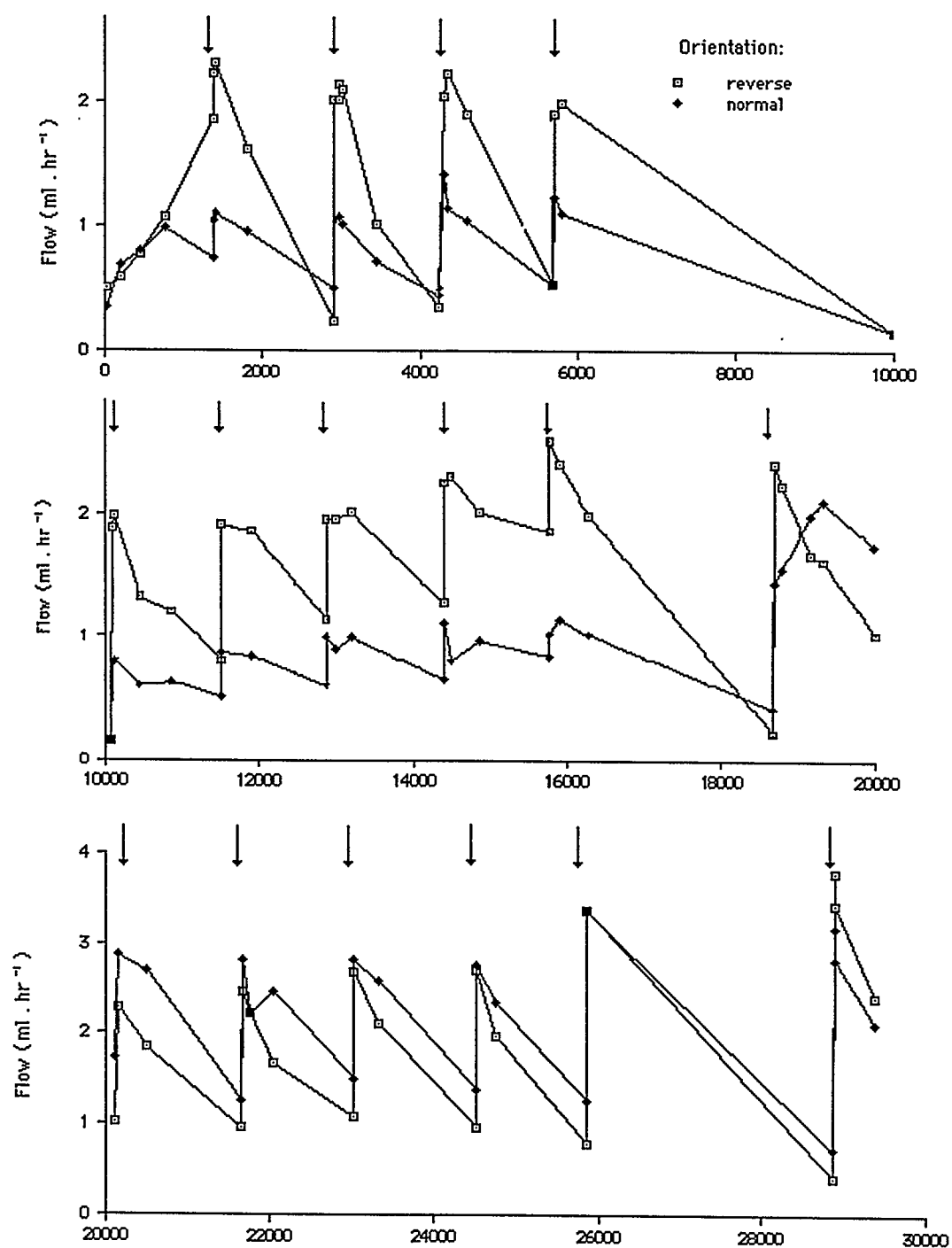


Figure 42. Effect of orientation on flow change resulting from cutting 0.2 cm from the supply end in lilac. The x-axis gives the time in minutes: the experiment lasted for 21 days.

the decrease in flow rate, however.

The results of these first two experiments (Figures 41 & 42) suggest that some substance whose origin is not in the phloem builds up in the proximal (inflow) ends of the cut vessels and gradually plugs them up. Only the inflow end becomes plugged, and the orientation of the stem section makes no difference (that is whether the stem is in the normal or the reverse direction). Electron micrographs were taken of the cut surfaces of several species after 20 hours of perfusion, and compared to scanning micrographs of fresh cut sections, and there was some suggestion that biofilm type bacteria were gradually coating the cut surface. Since flow rate depends inversely on the fourth power of the radius of the vessels, even small amounts of occlusion can quickly reduce the flow. The e.m. pictures of perfused and control surfaces of dogwood are shown in Plates 1 and 2.

That microorganisms of a biofilm forming nature may be responsible for a large part of the blockage that occurs is supported by the fact that dilute solutions of formaldehyde, 8-hydroxyquinolone (a bactericide) or of sodium hypochlorite greatly reduced the rate of decrease in flow rate (data not shown). Furthermore, perfusion of a stem section for an extended period resulted in valve-like behavior in the stem. In the fresh stem there is no difference in flow rate no matter which end of the stem is perfused. After about 20 hours, however, flow in the reverse direction becomes from two to five times faster than in the original direction (see Figure 43). A similar kind of flow pattern is observed in stem sections of gymnosperms when the

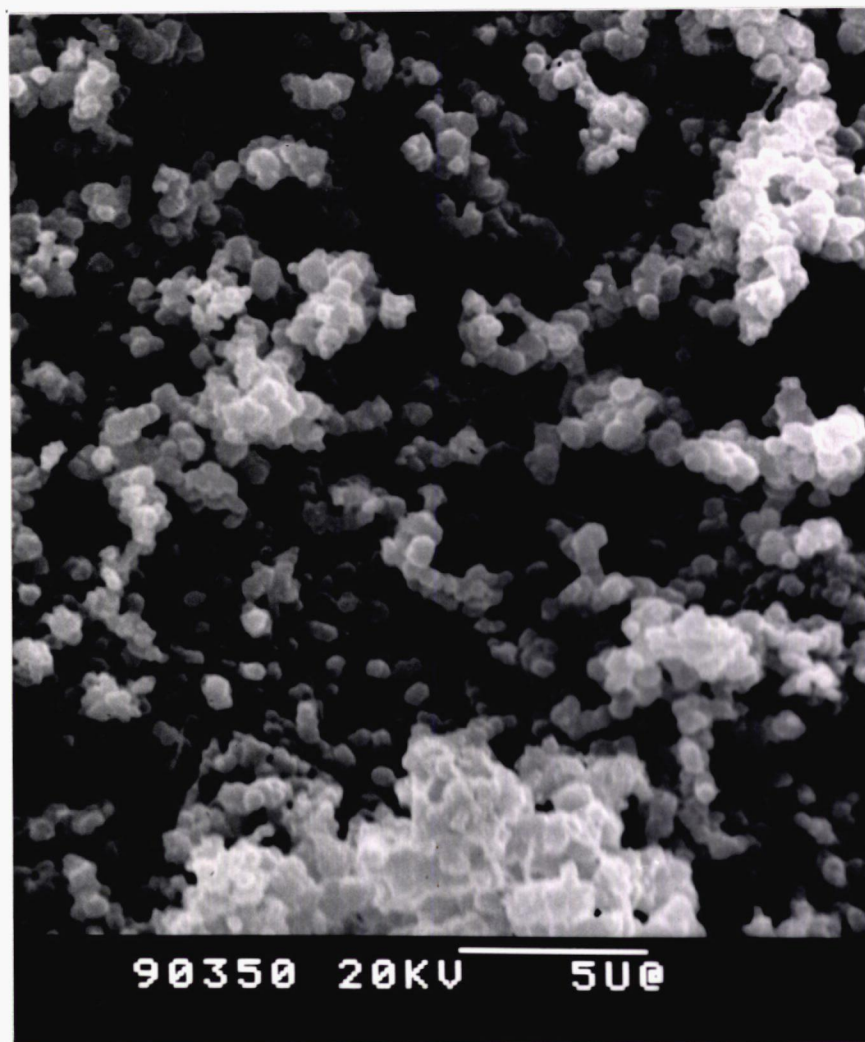


Plate 1. Effect of 20 hour perfusion on the appearance of the transverse cut surface (inflow end) of dogwood stem.

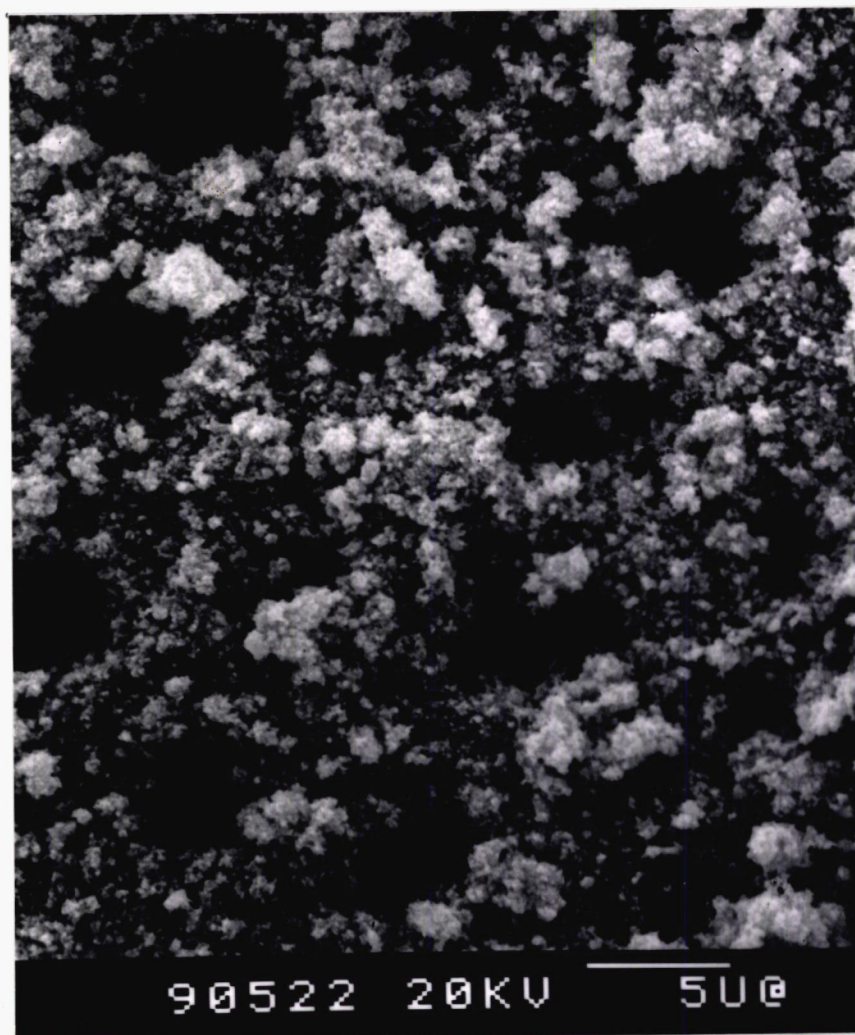


Plate 2. Effect of 1 hour perfusion on the transverse cut surface (inflow end) in dogwood.

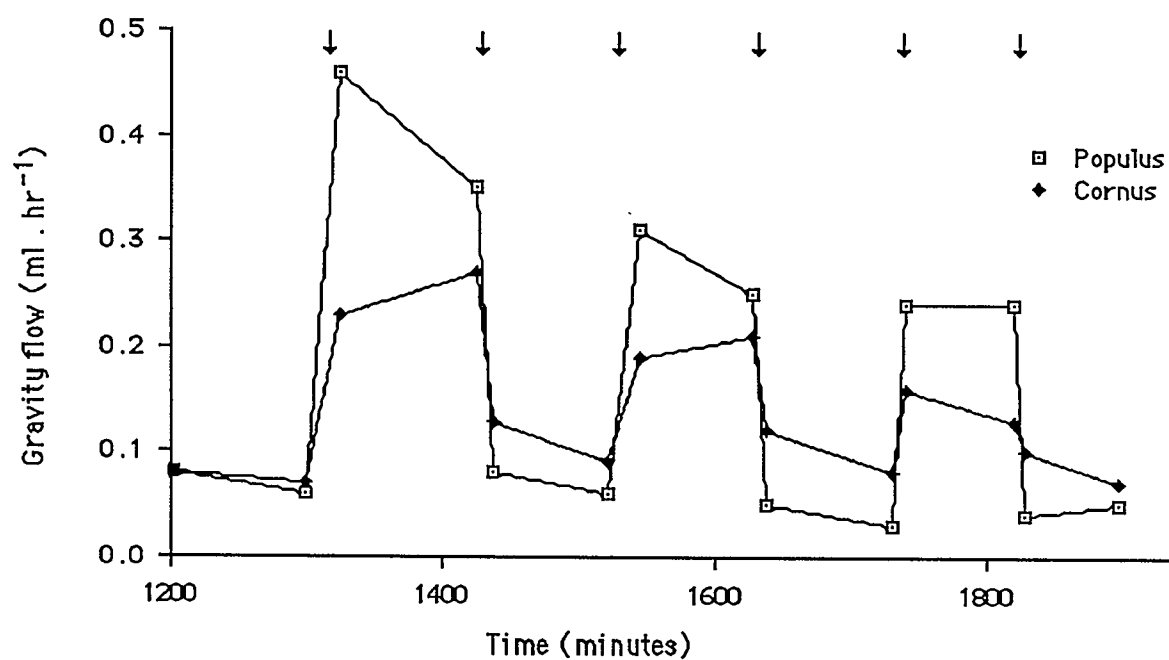


Figure 43. Effect of reversing stem section repeatedly (each arrow represents a reversal of the two stems) on xylem flow after 20 hour perfusion in one direction.

perfusing solution is placed under elevated pressures; in this situation the valve action can be attributed to the tori that surround the pit apertures (Fig. 44). Since angiosperms have no such torus structure, the valve action must be due to gel-like masses that develop on the cut surface. The two situations are illustrated diagrammatically in Figure 44. The gel-like masses may be bacterial in nature, as suggested by Plate 1 above, or they may be vascular gels that form at the plate surfaces as part of the natural defence mechanism of the xylem (eg Beckman, 1969; Vander Molen et al, 1977). Figure 45 illustrates a somewhat different situation: with lilac, there was no obvious valve effect between the two directions of flow; instead, there was a general elevation of flow rate each time after reversing the direction of flow. The reason for this stepwise increase in flow rate probably had nothing to do with microbial growth, but rather with the incomplete evacuation of air bubbles in this species under the experimental conditions used. This hypothesis is supported by the fact that placing the stem under vacuum after several days of perfusion results in a dramatic increase in flow rate, in spite of the fact that many of the vessels are no doubt beginning to plug up with bacteria or other debris after this length of time. Some homeowners who grow lilacs in their gardens follow the practise of beating the cut lilac stems with a hammer before placing them in the vase: it may well be that the reason why this is effective in improving the vase life of lilac is that this procedure somehow eliminates the air bubbles.

To conclude this chapter, let us turn for a moment to the possible implications of the foregoing series of experiments to the problem of cut flower wilting, which was mentioned briefly in the introduction. The results of the experiments described here point to two factors that may influence

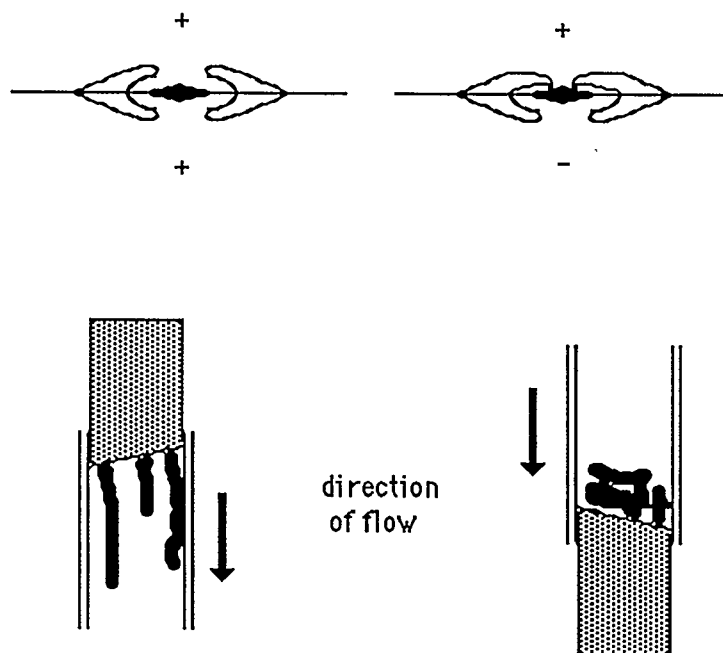


Figure 44. (A) Diagram of a gymnosperm torus under conditions of small pressure differential (left), and of large pressure differential (right). (B) Hypothetical diagram of a xylem vessel partially occluded by microbial growth, in reverse orientation (left) and in the original orientation (right).

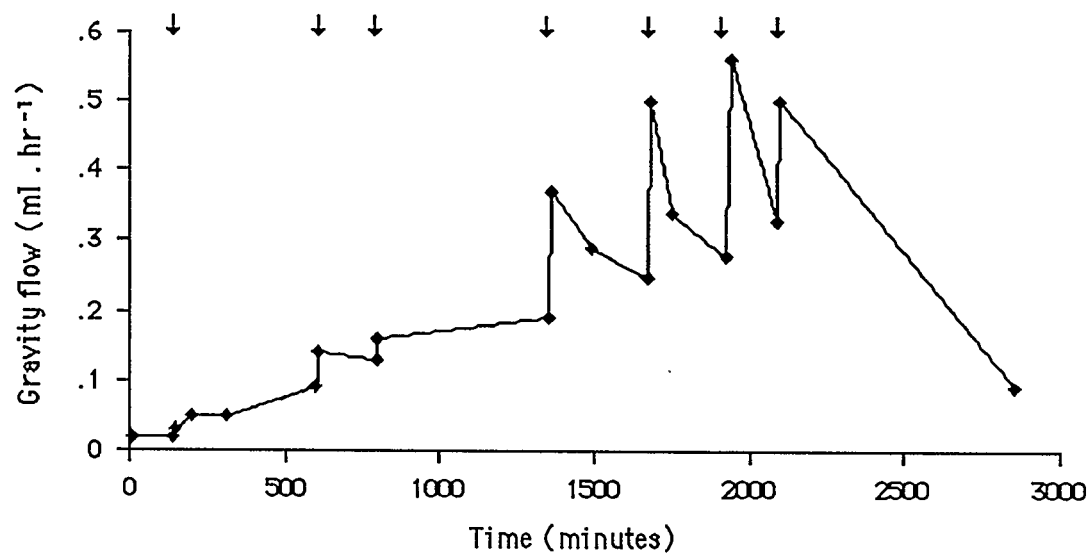


Figure 45. Effect of frequent reversals (each arrow represents one reversal) on xylem flow in lilac.

the water relations of cut flowers. One is the presence of air bubbles in the vessels of a stem; the other is the growth of biofilm type bacteria on the cut surface. In some cases the cut stem may be unable to evacuate its air bubbles quickly enough when suddenly placed in a vase, as described for lilac. The solution to this problem probably lies in the induction of physiological mechanisms to pressurize the xylem, through control of temperature conditions prior to cutting. In other cases there may be blockage by air bubbles as a result of nucleation on the irregularities of the xylem vessel walls. Even on the relatively smooth walls of plastic tubing, small air bubbles soon begin to form when a piece of tubing is filled with water, regardless of whether this water be stagnant or moving. The problem of biofilm bacteria formation can be alleviated by well-known techniques like adding bactericidal compounds to the water, and periodically re-cutting the end of the stem. To these might be added an additional technique: that of simply wiping the ends of the stems several times a day, given the very fragile nature of the biofilm. Data supporting this idea was obtained from several experiments (not shown here).

Summary - Xylem Flow

The first experiments described in this section show that bending can reduce the rate of xylem flow in woody species, although the degree of bending may be more severe than normally occurs in plants. The subsequent two sections describe investigations into the reasons for the fluctuations in flow rate seen in the initial experiments. Evidence was

presented that was consistent with the hypothesis that the water-conducting vessels of the xylem are partially air-filled during some parts of the year. Finally, several experiments concerning decreases in flow rate suggested that most of the occlusion in this kind of experimental set-up occurs in the proximal end of the stem section, and that the occluding material may be either film-forming bacteria, or vascular gels originating in the xylem itself, or both.

CHAPTER FOUR - BRANCH PRUNING

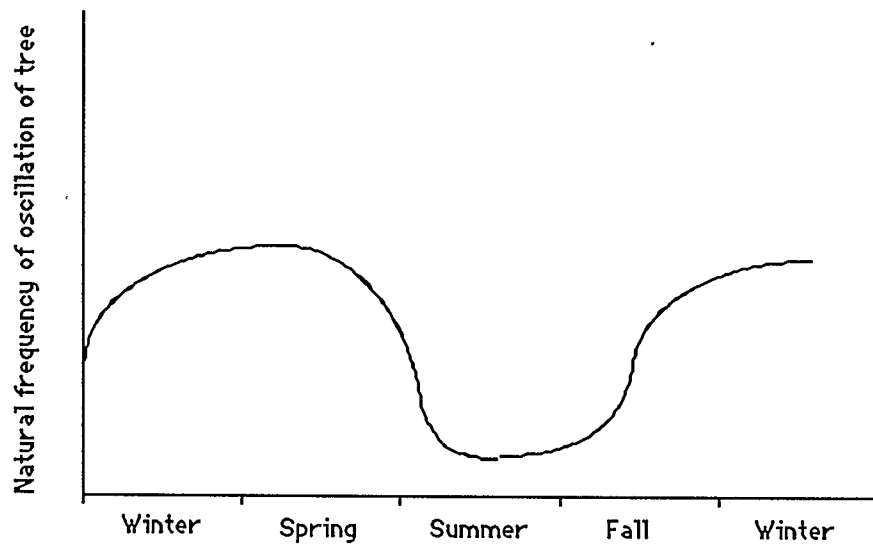
With respect to the plant's role, the pruning of a tree's branches may take place in both passive and active ways. Passive pruning is the loss of branches whose strength is inadequate for a particular situation, the most common causes normally being strong winds and heavy loads of snow or ice. Active pruning is called cladoptosis, or branch abscission, and is the process by which lateral twigs and branches are shed by the formation of well-defined cleavage zones. It will be seen later that the distinction between active and passive is somewhat blurred.

Among Canadian trees, branch abscission occurs in species of Larix, Thuja, Taxodium, Quercus, Ulmus, Acer, and Populus (Millington & Chaney, 1973). The subject has not been studied much: previous work includes brief descriptive papers (Bessey, 1900), anatomical studies (Eames & McDaniels, 1947), and some investigation of the hormonal factors that may be involved (Addicott, 1982). The formation of the abscission zone, like any other process involving cell differentiation, requires the expenditure of energy. That this characteristic has become established in a number of temperate tree species suggests that it confers some advantage to those trees, and that the cost involved in annually forming large numbers of abscission zones, is not as great as would be the cost of retaining those branches. There are at least three potential costs of branch retention. One is that small dead branch stubs are thought to be routes of invasion for wood rotting fungi (Etheridge, 1961). Another has to do with wind resistance, and the contribution of the non-productive twigs towards the sail area of the tree.

McMahon & Kronauer (1976) found that deciduous trees had natural frequencies (the rate of recoil movement exhibited by a trunk that is deflected from its equilibrium position) between two and three times lower in summer than in winter. They point out that the large factor by which the leaves lower the natural frequency of a tree is probably due to the fact that their damping and mass is concentrated at the tip, where the amplitude of oscillation is largest. I would hypothesize, therefore, that the greatest stresses on the bole resulting from wind occur in winter, and that a tree that can reduce the sail area of the leafless crown will achieve a more efficient size-to-strength ratio and therefore a more effective allocation of materials to leaf production and height growth during the summer Fig. 46 is a hypothetical diagram showing how sail area changes with the season. A third possible cost of branch retention has to do with the interception of light. Non-productive or inefficient branches still intercept a share of the light falling on a tree: conceivably, the pruning of inefficient branches may slightly increase the available light for more efficient branches. Theoretically then, there are benefits from active shedding of branches. The graphs that follow characterize the pattern of branch abscission in one Balsam Poplar tree over two years.

Methods

The tree selected for branch abscission study was located on the university campus. There were no other poplar trees closer than 50 meters from this tree. Beneath the tree and around it there was a grass lawn. This



- SAIL AREA :
- a (early winter) - bare tree with many twigs
 - b (mid winter) - bare tree with fewer twigs
 - c (late spring) - expanding leaves
 - d (early summer) - full leaf expansion, plus shoot extension
 - e (mid fall) - branch abscission, followed by leaf abscission

Figure 46. Hypothetical diagram showing the way sail area of a tree changes with the season.

made finding the abscised branches relatively easy. During most of the year, when only a small number of branches were abscised each week, collection was done at weekly intervals, in early morning before grass cutting occurred. During the fall, when large numbers of branches fell per day, the collection was done every morning. Branch and leaf area measurements were done with the use of an ITC Ikegami television camera Model ITC-47, with a delta-T Area Meter and Ikegami monitor Model PM-930. For nutrient analysis, the samples were digested at 360 degrees C in sulphuric acid and hydrogen peroxide (Lowther, 1980), and then analyzed for total nitrogen and potassium concentration (mg/g) on a Technicon II autoanalyzer. Weather records were obtained from the university weather station.

Results

The data relating to branch abscission are presented here in two parts. The first part characterizes the phenomenon in a mature Balsam Poplar tree during 1987-88. This section gives the ages, lengths, and other characteristics of the abscised branches, as well as the frequency of branch fall during the period of greatest activity, namely September to December. The second part of these results relates the shedding of abscised branches, and the pruning of non-abscised (broken off) branches, to the maximum wind speeds as recorded at the University of Calgary weather station.

a. Characterization of Branch Abscission

Figure 47 shows the phenology of abscised branch and leaf fall. Most branches fell before the start of leaf abscission, with the peak branch fall being during the last half of October, and leaf fall during the second week in November. The figure illustrates that for this tree, and in this year the great majority of abscised branches fell during October, and that of the three classes of branches, the peak numbers occurred in the following order: 1. With unabscised green leaves. 2. With unabscised yellow leaves. 3. No unabscised leaves (bare). Leaf fall in contrast occurred later than branch abscission, and within a much shorter span of time - most of the leaves fell in the second week of November.

Figure 48 shows the percent of abscised branches that had non-abscised leaves, secondary branches, and viable-looking (based on size and color) buds. The buds included both those of flowers (large) and of leaves (small). It may seem surprising that a significant number of branches abscise still with green leaves attached, but even when leaves abscise separately, the congruence of abscission and color change is not universal. Busgen (1929) points out that on the one hand many leaves (such as the alder, privet, and others) fall green, and on the other hand leaves which are not falling may also undergo an autumnal change (this is the case in many conifers). Other species like beech and oak tend to retain their withered, dried-up foliage in the winter. Busgen (1929) cites Bordage (date not given) as reporting that peach trees, grown from European seed, become nearly evergreen within a

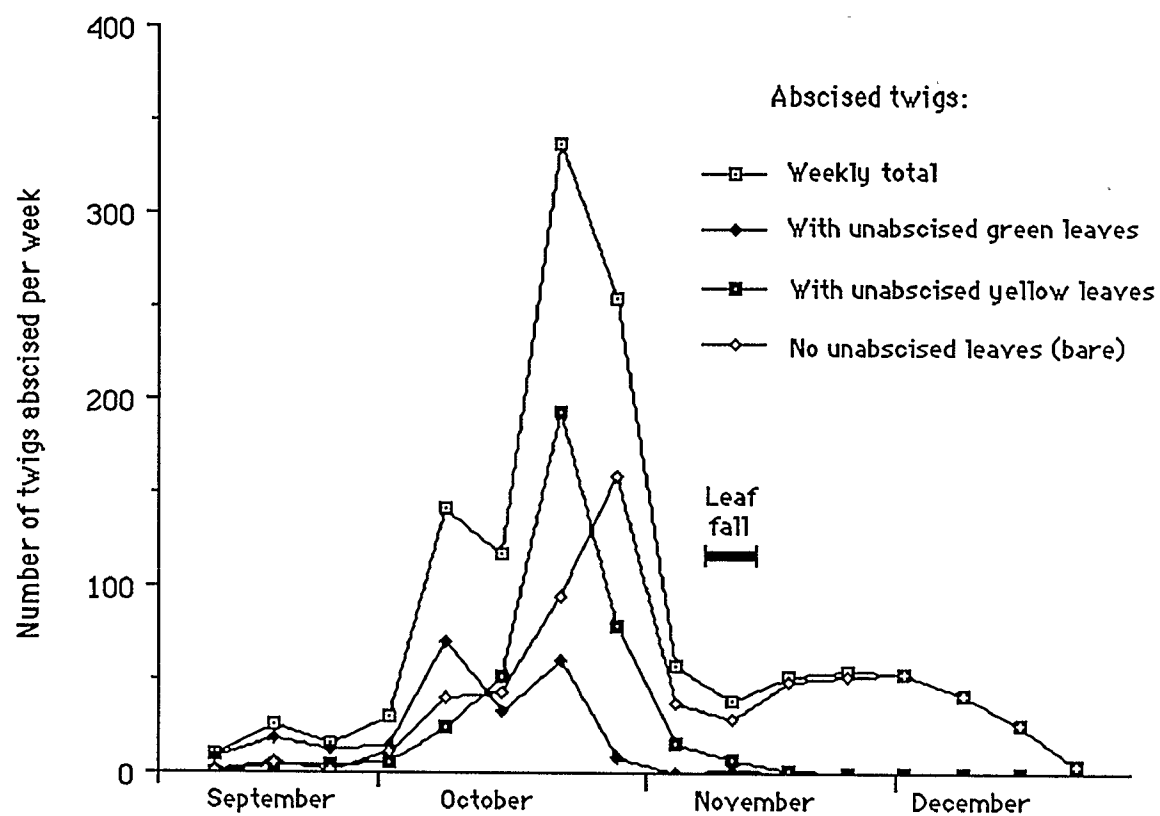


Figure 47. Phenology of branch and leaf abscission from a single poplar tree, September to December, 1987.

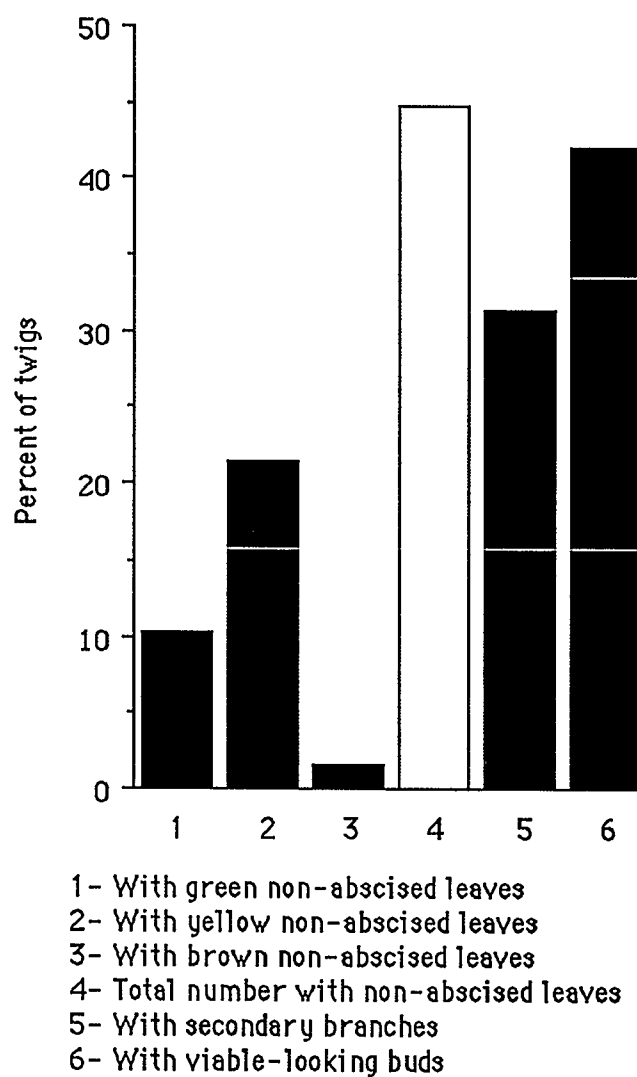


Figure 48. Characteristics of 1415 abscised branches from one poplar tree, (1987).

period of 20 years in the equable climate of Reunion, by standing bare for a shorter time in each successive year. Seedlings of the plants which became evergreen were evergreen even when they were sown in the mountain land where peach trees from European seed shed their foliage periodically. These changes in the pattern of abscission, apparently brought about in response to climate, lead one to speculate that inter-species differences in abscission patterns within the genus Populus, referred to below, may also be determined largely by climate.

Figure 49 shows the ages (A) and lengths (B) of the abscised twigs from the same study tree. It should be pointed out that these characteristics depend on the age and the vigor of the tree. I have observed that very young and very old trees abscise few branches; this is natural since the total number of branches is much smaller in these groups. Likewise trees that have been moved with a tree spade do not begin shedding branches until several years after, when they once again begin to actively grow. Incidentally, these recently moved trees also suffer very little damage in heavy wind storms - this was clear in May, 1988 when heavy winds knocked down numerous branches from trees around the university campus. This supports the notion that the size of the sail area (which in this case relates to the vigor of the current year's growth) determines the force with which the wind acts on the branches. In other words, the trunk and larger branches of the tree are relatively small components of the sail area compared to the leaves. Also, as already pointed out, the moment arms of the leaves are always greater than those of the higher order branches. Therefore, substantial branch pruning occurs only in summers during which a large

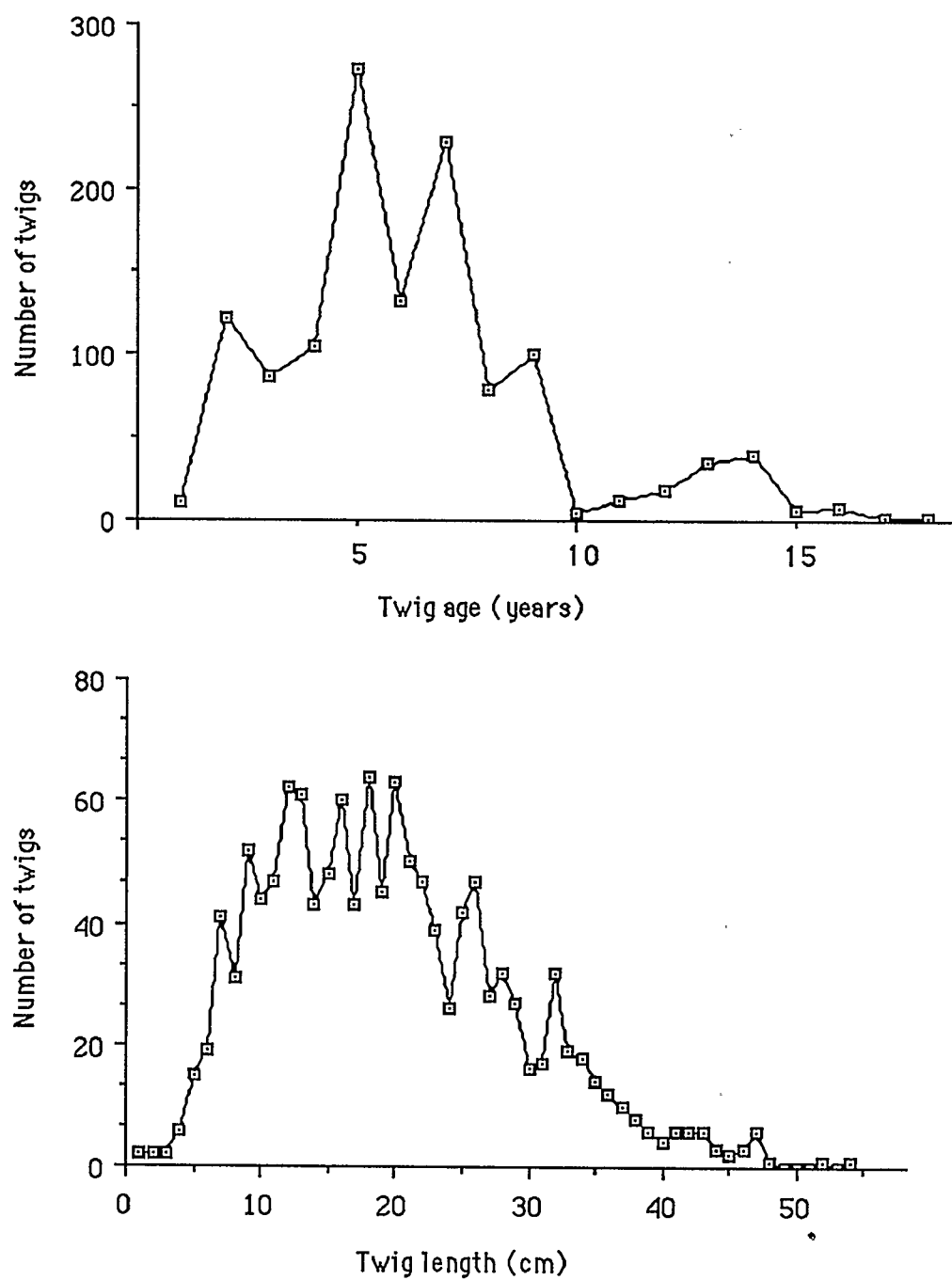


Figure 49. Ages and lengths of abscised branches (1987).

number of leaves have been produced. There is a distinction here between pruning of branches by abscission, which occurs in the fall, and pruning by breakage, which occurs in the summer. This matter is taken up further in section (b) of this chapter.

Other species of poplar have different branch abscission patterns - in Northwest Poplar, for example, the average age of 4030 abscised branches from a single tree in the fall of 1988 was 2.46 years. This no doubt represents a different variation of the strategy of branch abscission seen in Balsam Poplar, perhaps relating to the degree of shade tolerance in the leaves of the two species. In Cottonwood, the average age of abscised branches seems (pers. obs.) to be even older than that of Balsam Poplar.

It may be noticed that there are no abscised branches older than about 18 years, and few above 10 years of age. Branches older than 18 years are broken off, but there is no abscission scar in these branches: this perhaps is another clue in elucidating the abscission process. That is, there are certain limitations either to the number of annual rings or simply to the diameter of the branch, beyond which active abscission can not occur. The age distribution of the abscised branches also gives an indication of the locations in which there is most abscission. The average annual increment of growth for the abscised branches that were collected in this study was about 2 cm. Therefore, the zone in which the greatest number of branches are shed is about 12 cm from the outermost apices. Of course, a tree is not a uniform spherical shape, and the definition of this zone will be diffuse. I hypothesize that the region in which branches commonly begin to suffer

deficits of light will be just inside the outer "shell" formed by the leaves of the growing apices. These apices are highly competitive for light, naturally, and fill in gaps wherever possible. It is only in the gaps that the growing apices cannot reach that substantial amounts of light will penetrate to interior regions of the canopy. The distribution of annual branch abscission within the tree can also be determined more directly, by examining the remaining scars on the intact branches of the tree. Current year scars are a fawn brown color, while those of previous years become nearly black. It would not be too difficult therefore to map out the pattern of branch abscission in an entire tree. This was not done in the present study, although the pattern was mapped out for some large branches of nearby trees that were pruned by university grounds workers.

The effect of tree age on branch abscission was not examined in this study. However, it is clear that neither young saplings nor very old individuals have enough terminal twigs to allow for the abscission of large numbers of branches. A hypothetical graph based on my observations of age versus the number of branches shed is given in Figure 50. Branch abscission first occurs very early on in the life of the sapling: after the first few years of growth, the typical pattern of branch formation becomes established, and this pattern continues through most of the life of the tree. The pattern is that each year many new secondary branches are formed in the second year growth increments (first year growth increments produce only leaves and buds, but no secondary branches). Some of these last for several seasons, others for five or ten years, but the chances that a particular terminal branch will survive more than this are very slim. Year after year, a new flush of terminal branches is formed: its productivity is highest in the

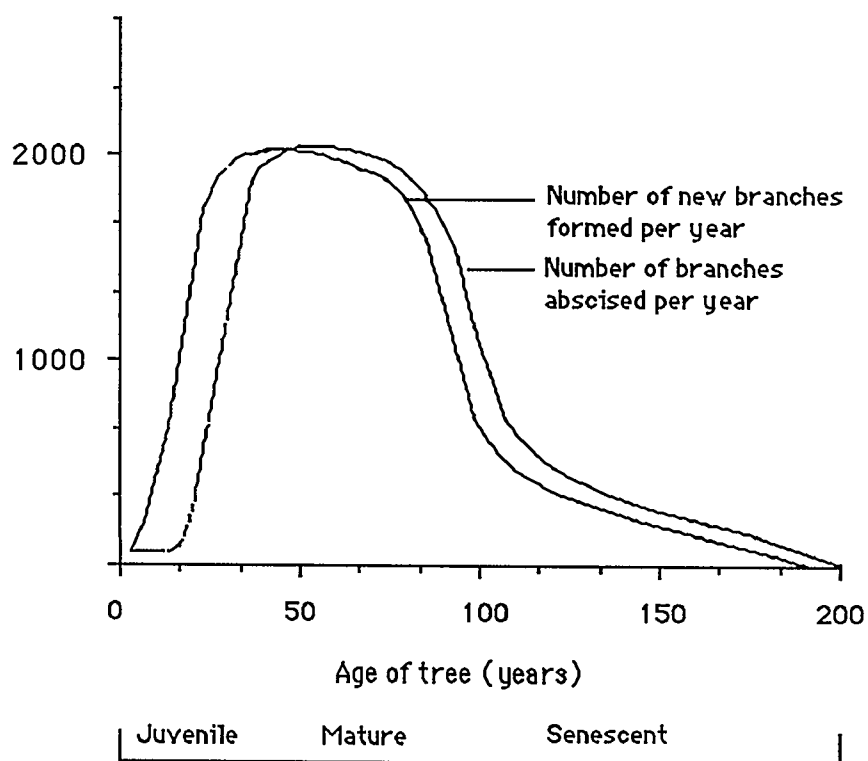


Figure 50. Hypothetical relation between tree age and number of abscised branches per year.

first year, and gradually tapers off after that as it becomes superseded (and overshadowed) by succeeding flushes of branches. This has the effect of maintaining a high density of branches (and of leaves) at the periphery of the canopy, where the light supply is greatest. As the tree increases in size from year to year the annual rate of branch shedding increases until maturity, when branch formation and loss are approximately equal. Once senescence begins, the rate of new branch formation begins to fall off, and for some years branch loss exceeds branch formation. This results in a decline in the number of terminal branches in the canopy: something that is very obvious in the oldest trees: these have often somewhat gnarled trunks, relatively inflexible, with a very meagre number of terminal branches.

Figure 51 gives the nutrient contents of abscised and non-abscised branches. There does not appear to be any difference in the nutrient status of abscised versus non-abscised branches, which is perhaps somewhat surprising. Figure 52 shows the ratio of leaf area to twig length and to fresh weight for abscised twigs versus non-abscised. This time there are differences, in that for abscised twigs the ratios of leaf area to twig length and to fresh twig weight are lower than the ratios for non-abscised twigs. This can be seen even by comparing the two twig types visually: abscised twigs tend to have predominantly terminal leaves. The leaves of all twigs used in this experiment were a solid green color. Figure 47 above showed that leaf fall occurs within a relatively short span of time that takes place well after the abscission of branches with green leaves; therefore, it is unlikely (though possible) that there was some prior leaf abscission in the abscised twigs, to account for the results given in Figure 52.

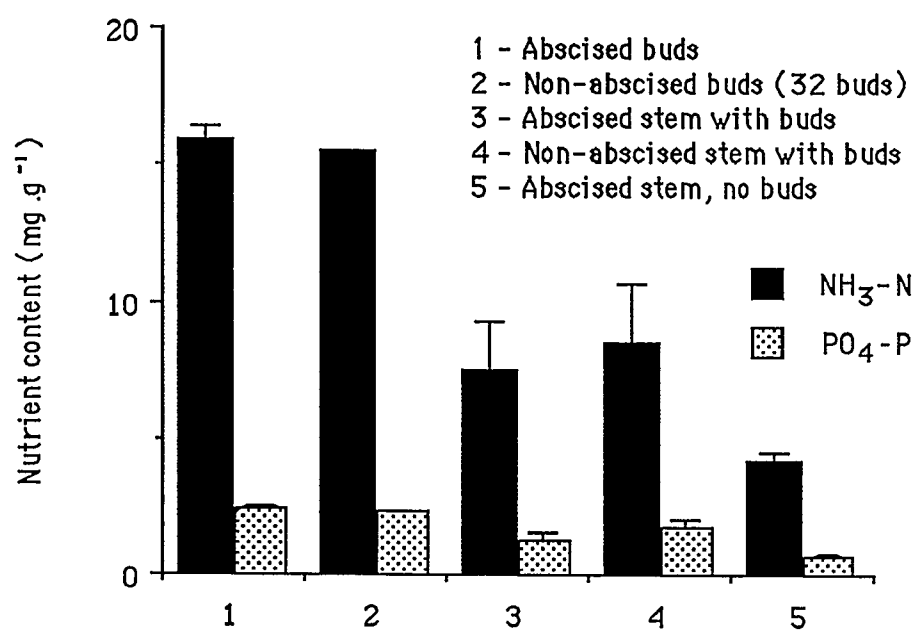


Figure 51. Nutrient content measurements on abscised and non-abscised poplar twigs. Non-abscised twigs were broken off the tree by the experimenter.

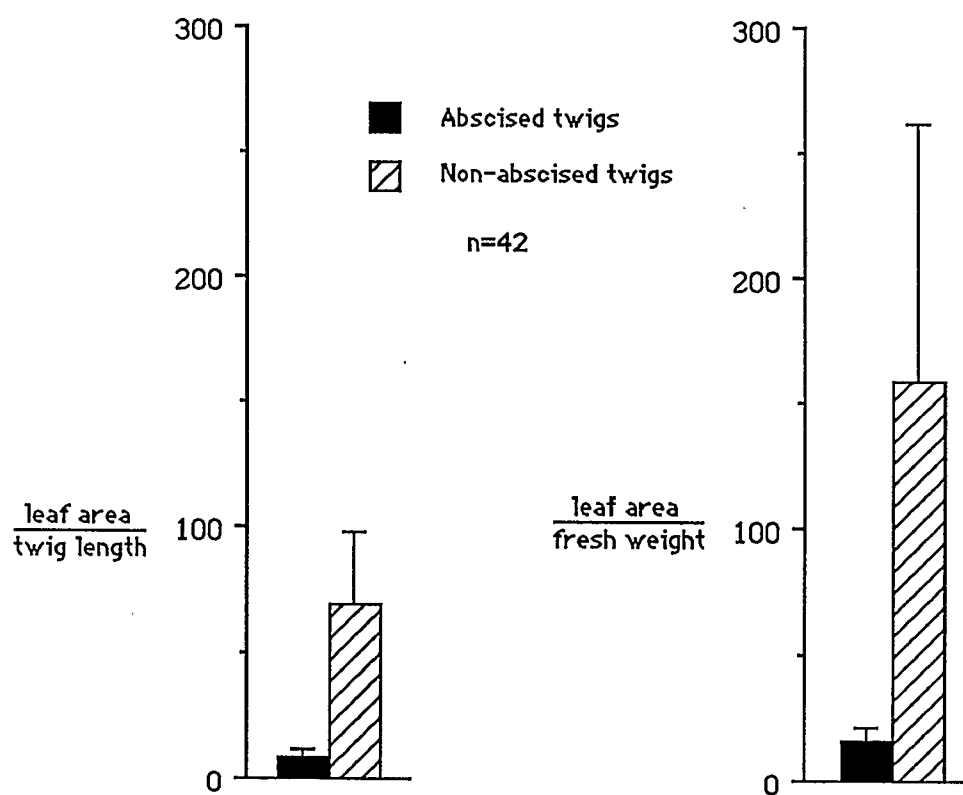


Figure 52. Leaf area, twig length, and twig fresh weight in abscised and non-absceded poplar twigs. The non-absceded twigs were from a large branch that was broken off in a strong wind.

The two experiments described above seem to indicate that there is no progressive loss of vigor in abscising twigs, and that an abscised twig contains similar nutrient levels as a twig that is still on the tree. Rather, the abscission process is the result of a decline in leaf area. Since photosynthates produced by first year poplar leaves are not exported (Ford, 1985) it is not surprising that there are still viable buds on the ends of abscised branches. However, I would hypothesize that when the leaves from portions of the stem that are two or more years old decline to the point where there is no longer any export of photosynthates in the basipetal direction, the formation of an abscission zone begins. Exactly how this comes about is unclear, but two features relating to the branch base area seem to be relevant. One is that the dynamics of fluid flow (probably both xylem and phloem) are very different on the two sides of the branch base junction, once there is no longer any export of photosynthates from the branch. The second is that there is a constriction in the xylem vessel just in this area (Isebrands and Larson, 1977). A very worthwhile experiment relating to this question would be to pinch off the first year leaves from some twigs, and the older leaves from other twigs during early summer. If the idea advanced above is correct, the twigs in the second group should abscise during the autumn.

The finding that nutrient levels of abscised branches are not less than those of intact branches merits a few further comments. Among the kinds of plant organ that can be abscised, it is probably leaf fall that we most commonly associate with this phenomenon. In leaves, it seems that at least

some of the resources are scavenged by the plant before the separation of the petiole (eg Williams, 1955). But this extraction of valuable substances prior to abscission is certainly not typical of all abscising parts. For example, many fruits and seeds are shed by abscission - these structures depart from the plant with very high nitrogen and carbohydrate levels. It appears that branches fall somewhere between leaves and reproductive propagules, with respect to their nutrient contents at the time of abscission. They do not export nutrients, but nor do they accumulate them.

There is another fact that relates to this. Abscised poplar branches may sometimes act as units of vegetative reproduction (Galloway, 1979). This suggests an advantage to retaining some storage nutrient and carbohydrate, although it is doubtful whether this is the primary reason for the characteristic. It seems more likely to me at least that branch abscission is advantageous for the reasons outlined in the introduction, and that the occasional instance in which abscised branches sprout to form new seedlings (mainly along stream beds) is one of the minor components in the complex of characteristics that allow Populus to thrive in the harsh prairie climate. It has also been suggested (Ford, 1986) that leaf abscission allows plants to excrete unwanted substances. There is no reason to believe that branch abscission fulfills such a purpose, but the possibility remains.

b. Relation of branch abscission and pruning to wind speed.

The abscised branches from the one poplar tree that were shed during the period September to December (1987) were weighed individually. The total weight was 2445.2 g. Some estimate of the sail area that these 1415 branches represent was obtained by measuring a single profile area of each abscised branch with an area meter. The sum of all the individual branch areas was 35,045.3 sq. cm. (3.5 sq. m.) The tree is about 15 m in height, and since the region of most vigorous growth is in the upper half of the canopy (see Figure 53), this combined sail area resolves into a force vector which acts on the lower bole over a moment arm of somewhere between 10 and 15 m. This represents a force at the base of the trunk approximated by $4Dh/r^3$ (adapted from Wainwright et al, 1976), and can be regarded as a saving in energy that would otherwise be necessary for radial growth of the trunk. D is the drag that the abscised branches would have exerted had they remained on the tree; h is the resultant height of all the abscised branches, and r is the radius of the trunk at the base. It can be seen from this that very small changes in D and h will significantly affect the requirements for strength (as reflected by r^3). These approximate figures could be made much more precise by mapping the distribution of all the abscised branches in one tree (as described above) or, better still, by cutting off a tree at the base, and then experimentally determining the drag before and after removal of branches from the region of branch abscission.

Figure 54 shows the number of abscised twigs during the 1987 season, together with the maximum daily wind speeds. The data show quite clearly that while strong winds do result in increased numbers of abscised

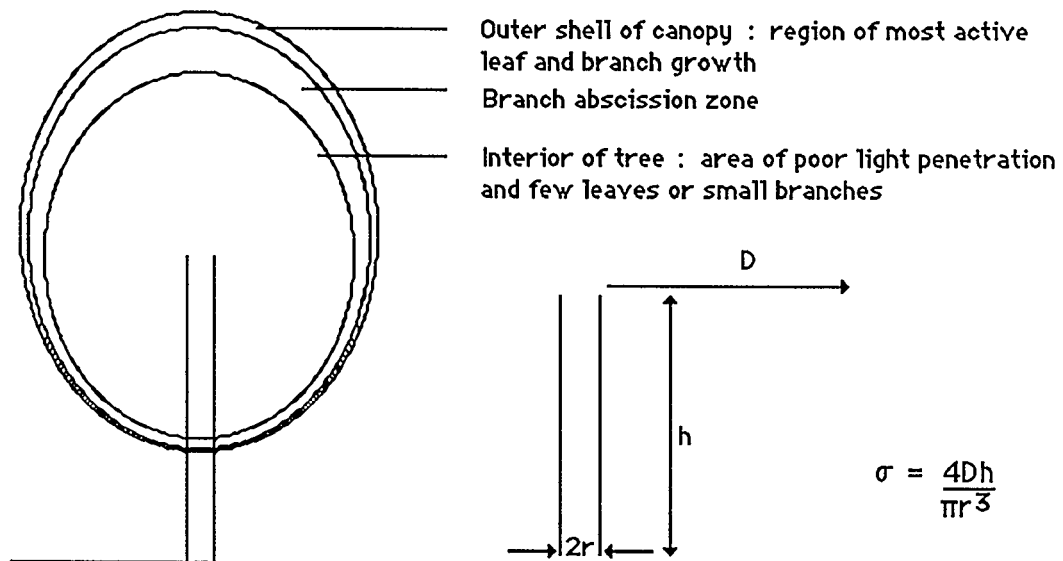


Figure 53. Diagram showing the region of most vigorous growth, and the approximate point through which the combined sail area of the abscised branches act.

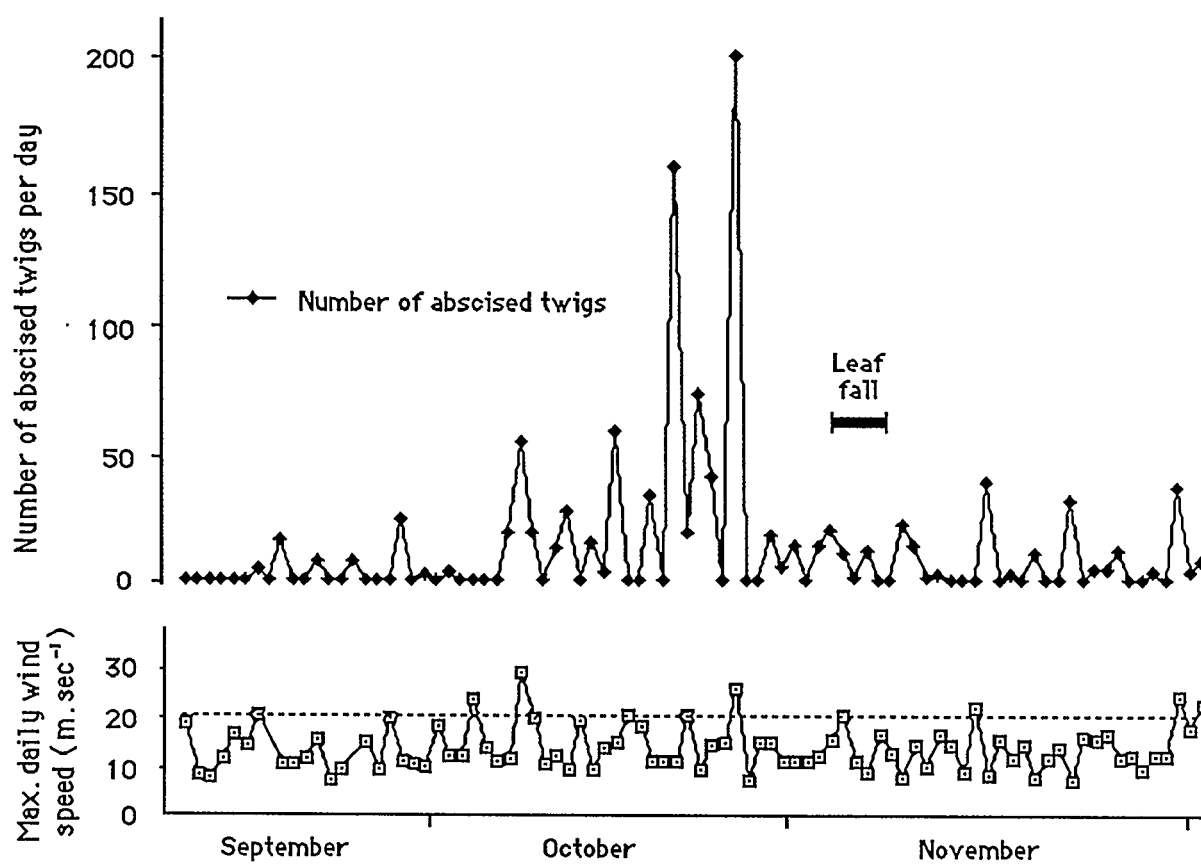


Figure 54. Daily number of abscised twigs as compared to maximum daily wind speeds.

branches, the overall pattern of abscission has more to do with the season than with daily conditions. However, there is an aspect of branch abscission that appears to allow a degree of flexibility in the number of branches shed annually, depending on the magnitude of the wind. Namely, the abscission process is not an all-or-nothing thing. The evidence for this comes from the macroscopic appearance of the abscission zone in various branches, and there is a tremendous variety here in several respects. To consider first the scar at the base of an abscised branch, it can be pointed out that the color of the scar may be yellow-brown (indicating a recent separation from its opposite zone) or it may be blackish and somewhat weathered looking (indicating that the abscission zone formed some time before the branch actually dropped off), but most importantly for the present discussion, the scar may be a combination of these two colors. One side of the scar may be brown or black, indicating that this portion separated the previous year, while the other side has the fresh look of a recent separation. This shows that partial abscission zones are possible. Furthermore, there are several other indications that this is so. If one breaks off several smaller branches from a living poplar tree, some of these will have the radially oriented patterns that are characteristic of the abscission zone, but these patterns often do not extend all across the branch base: often they occupy only the inner (central) areas. Finally, the partial abscission of small branches offers an explanation for the greatly swollen branch bases that are characteristic of some of the small- to medium-sized branches on poplar trees. The explanation would be that in certain branches a partial abscission zone forms in some years, and if the abscission zone fails to develop completely,

or if the winds during that fall and winter are not too severe, these branches remain on the tree. During the following growth season, the partially separated connections at these branch bases heal over, and the branch resumes production for one more year. This may happen a number of years in succession, as suggested by the large size that these branch base swellings sometimes achieve. This phenomenon of partial abscission suggests a degree of flexibility in the amount of pruning that takes place each season through active branch shedding. If winds and snow loads are more severe in some years, then these incipient abscission zones allow the separation of more branches than usual, by building a weak link into the less vigorous branches of the tree, and thus reducing the risk of breakage in the most productive regions.

The non-abscised branches are divided into living and dead branches in Figure 55, and in this case, it is the weight that is recorded. From these graphs, it appears that most wind-caused branch breakage (as opposed to branch abscission) takes place when the new leaves have just emerged in May and June. This suggests still another aspect to the concept of sail area: the tree sends out branches wherever there is sufficient light to support new axial growth, like so many trial balloons. Each time a strong wind comes along, it picks off all those branches whose supporting tissues have insufficient margins of safety. This kind of flexibility of form is much like that provided by the system of partial branch abscission as described above, but the timing is different. Since most of the axial growth in poplar occurs in early summer, that is the season when most of the non-abscised branch pruning should occur, and this was indeed the case. The season of

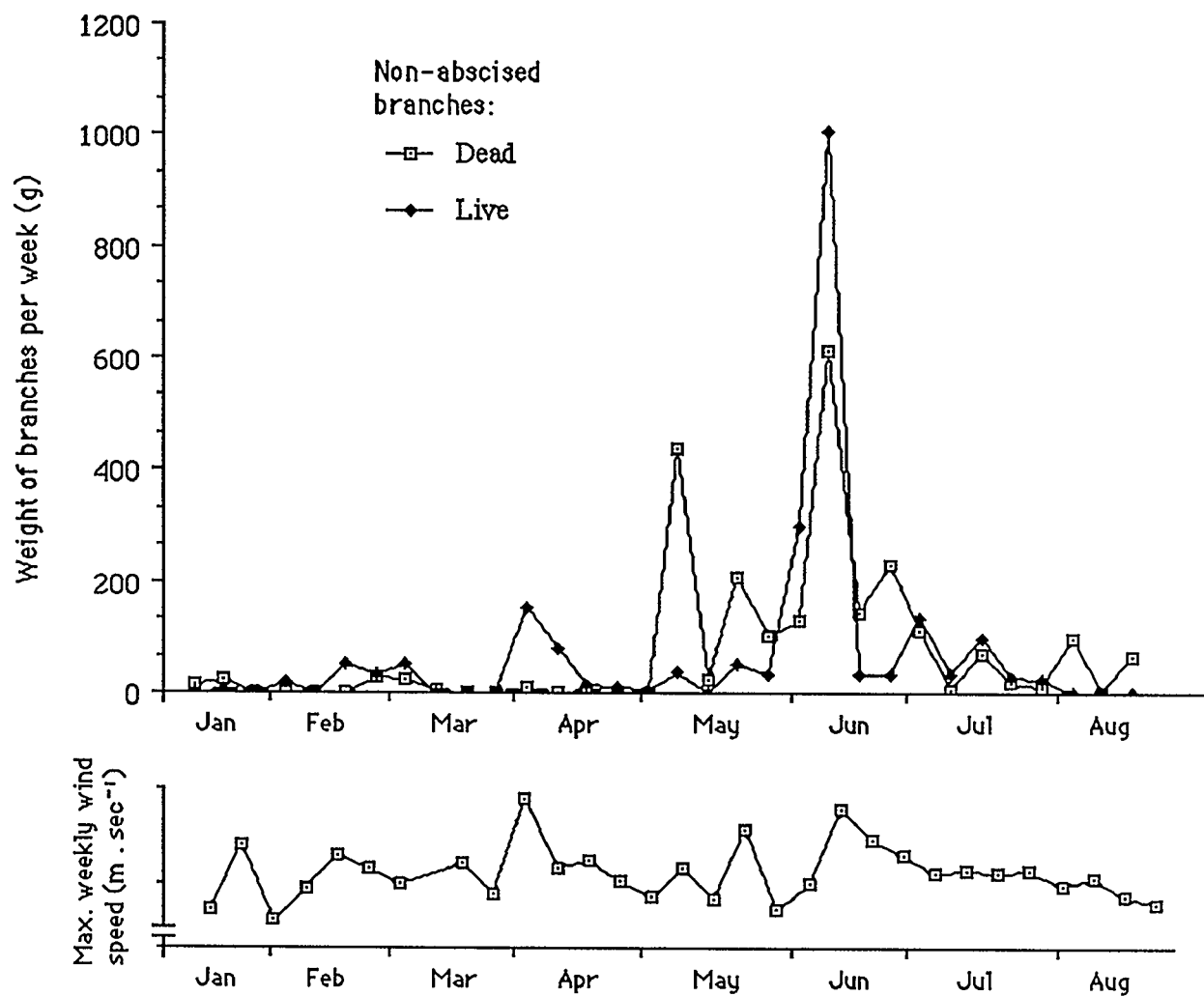


Figure 55. Weight of non-abscising branches broken off one Balsam Poplar during the period January to August, 1988.

abscission, as we have seen, is autumn.

Final comments and summary - branch pruning.

Horn (1971) observes that variable winds call for compromises between the rigid strength of hardwood and the elastic flexibility of softwood. In this chapter we have seen some indications of the brittleness of a hardwood like poplar, and how in this species the annual abscission of substantial numbers of twigs may reduce the likelihood of trunk breakage by decreasing the sail area of the crown during winter. Honda (1971) points out that the crown forms of trees are multifarious and not as similar from individual to individual as the phyllotaxis and shape of flowers, fruits, and leaves. This means that especially in a colonizing species like poplar it is advantageous to have various ways of adjusting to the individual conditions for each tree. Pruning of branches (both abscising and non-abscising) is one agent allowing for such plasticity of form; the formation of epicormic (adventitious) branches is another. King and Loucks (1978) calculated that both the branches and trunks of P. tremuloides are constructed with close to the minimum of wood necessary to withstand gravitational and wind forces, supporting their prediction of lower safety factors for fast-growing, shade-intolerant species.

No mention has been made in this chapter of the role of ethylene in branch abscission. This was investigated only a very little in the present

study, but there are a number of possible experiments of interest. These might involve the application of ethephon to certain branches, as well as the measurement of ethylene production in branches with and without "incipient" abscission zones. Isebrands & Larson (1977) have induced abscission of leaves in Populus deltoides by applying ethephon to the base of the petioles.

Summary: a. Abscised branches. Most branch abscission in Balsam Poplar occurs during October, with an average branch age of about 6 years, and average length of about 15 to 20 cm. About half the abscised branches have viable-looking buds. There does not appear to be any difference in the nutrient content of abscised and non-abscised branches, but abscised branches have a significantly smaller total leaf area. In the proximal sense, wind affects both the daily pattern of branch abscission and the seasonal pattern; in an ultimate sense, wind may in fact be in part responsible for the development of this mechanism in certain fast growing genera, as well as influencing the specific timing of the process within the individual species.

b. Non-abscised branches. These are pruned whenever there are heavy winds. Here too there is a significant seasonal influence: in this case, it is due to the increase in sail area when the new growth has developed in early summer.

CONCLUSIONS

Watson (1984) has pointed out that without wind there would be no precipitation, no erosion, and therefore no plants as we know them. For non-aquatic plants therefore, coping with wind is a part of life, and in the preceding pages the coping process has been considered from several points of view. The question of how a plant adjusts its supporting tissues efficiently to the demands placed on them was the subject of the second chapter. It was hypothesized that ethylene gas might be a part of the process by which plants perceive strains and then respond to these strains by modifying the rates of radial and axial growth. This was tested by applying three kinds of stress that might be a part of the effect of wind: namely gravistimulation, bending, and thigmostimulation. In each case, the production of ethylene was measured. It was found that stems of self-supporting plant species like sunflower and tomato reacted to these stresses in general by producing more stem ethylene. Climbing plants like pea, bean and cucumber on the other hand, were less responsive. This was consistent with the hypothesis that ethylene is a transducer in the response to wind. When fans were used to create windy conditions (in sunflower) however, ethylene production did not appear to be different from that of control plants. The results of the initial experiments that demonstrated "responsive" (self-supporting) and "non-responsive" (climbing) species with respect to ethylene production, could not therefore be extended to the overall response of plants to wind. A section on the relation of ethylene and drought was an offshoot from these investigations, and suggested that in some plants wilting

(and thus gravistimulation) may affect the distribution of ethylene production of a droughted plant. It was pointed out that workers who are investigating the effects of drought on ethylene production should be made aware of this, and advised to design their experiments so as to avoid gravistimulation of plants or plant parts.

The third chapter described investigations into the rate of flow of xylem sap in excised stems. It appeared that bending of a stem caused decreased flow rate, although the degree of bending was more severe than would normally occur through the effect of wind. Further experiments investigated the increase in flow rate that is seen during much of the year when an excised stem is first perfused. Evidence was presented that this increase may be due to the refilling by water of air-filled vessels. The decreasing flow that always occurs later on in such a stem was also investigated - here evidence suggested that the decrease may be due at least in part to obstruction of vessels by biofilm-forming bacteria.

In the fourth chapter the branches of a tree were considered as being a large composite sail, and the investigation explored the apparent strategies of poplar trees that keep this sail structure trimmed to its most efficient form. It seemed that the natural abscission of small branches might be an important mechanism by which a fast-growing tree such as poplar could each year achieve maximum possible height using the available resources. The abscission process was characterized in a number of aspects for this species, and there was some discussion of the events that may play a role in its initiation.

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