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A series of experiments was designed to investigate differential flood-tolerance and the phenomenon of flood-induced stomatal closure of several fruit tree species. The first two experiments were conducted with potted plants submerged outdoors 5-10 cm above the soil line. Plant morphology, growth, leaf conductance (cL), and soil oxygen diffusion rate (ODR) were monitored. Differential waterlogging tolerance of major fruit tree rootstocks tested from high to low was as follows: Pyrus betulaefolia > Pyrus calleryana = Cydonia oblonga cv. Provence BA 29 > Pyrus communis (Bartlett seedling) > Pyrus communis cv. Old Home X Farmingdale 97 (OH X F 97) = Malus domestica cv. Malling Merton 106 > Prunus persica (Halford or Lovell seedling).

Grafting 'Bartlett' scions on the 4 major pear rootstocks listed above did affect plant performance in terms of growth and cL but did not alter overall survivability. Stomatal closure for each species was associated with a specific ODR. Limitations of cL as a screening tool are discussed in the thesis.

Solution culture experiments conducted in the greenhouse revealed that anaerobic-induced stomatal closure was not a function of reduced leaf water potential but was related to a reduction in root hydraulic

conductivity (Lp). Two lines of evidence suggest that increased root resistance to water flow was predominantly in the longitudinal and not the radial direction. Firstly, $10^{-4}$ M abscisic acid (ABA) applied to intact OH X F 97 roots in solution culture enhanced Lp of plants previously exposed to aerobic but not anaerobic conditions. Secondly, excising feeder roots of anaerobically treated roots, thereby exposing xylem tissue directly to the nutrient solution, did not revive Lp to rates observed for intact aerobically treated roots. A basipetal progression of xylem plugging occurred with increasing duration of anaerobiosis.

The promotion of volume flux (Jv) observed for aerobically treated OH X F 97 was studied. All 3 concentrations of ABA tested enhanced Jv within 10-20 minutes with the effect leveling off after 1½ hours. Analysis of the xylem fluid revealed a slight change in osmotic potential and solute flux, far smaller than the driving force required to significantly alter Jv. It is concluded that only changes in Lp can account for this phenomenon.

# EFFECTS OF FLOODING ON PEACH, APPLE, QUINCE AND SEVERAL PEAR SPECIES

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# EFFECTS OF FLOODING ON PEACH, APPLE, QUINCE AND SEVERAL PEAR SPECIES

## INTRODUCTION

Water is usually considered the most important factor limiting agriculture on a world-wide basis (199). Most of the early research in plant water relations was concerned with drought-stress or water-use-efficiency; however, recently much attention has been focused on stresses imposed by excess water. Virtually all adverse plant responses to flooding can be traced to a 10,000 fold reduction in soil oxygen diffusion rate (ODR) when gas filled soil pore spaces are replaced by water (89, 206). Plant tolerance to a waterlogged condition is determined by numerous anatomical, morphological and biochemical adaptations. Mesophytic plant species display quite a variation in their ability to survive inundation. Often times sufficient latitude exists within a genus or, in the case of fruit trees, within graft compatible limits to allow for the selection of plant species with flood-tolerant characteristics.

Excess soil moisture whether due to precipitation, over-irrigation or inadequate drainage is a problem in many regions of the world. The Rogue River Valley, a pear growing region in Southwest Oregon, contains impermeable clay soils with extremely high bulk densities. To compound the problem of poor drainage, flood-irrigation is frequently employed in the summer. Young trees, particularly interplants tend to be especially prone to flooding damage. Pear rootstocks are generally acknowledged to be the most flood-tolerant of fruit tree species (190); however, differential tolerance of Cydonia oblonga

Mill. and Pyrus species has not yet been rigorously evaluated.

Recently, flood-induced stomatal closure has been the subject of many a research endeavor. The difference in the stomatal behavior of drought-stressed and flood-stressed plants has sparked considerable interest and controversy (25). Most of the literature thus far has dealt with flood-susceptible herbaceous species such as tomato (Lycopersicon esculentum Mill.)

The objectives of this study are to determine differential flooding sensitivities of several fruit tree species and to study the phenomenon of flood-induced stomatal closure.

# REVIEW OF LITERATURE

## Effect of Flooding on Water Uptake, Transpiration and Photosynthesis

Root anaerobiosis or root submergence usually results in a rapid decline of the roots' capacity to absorb and conduct water (25, 92, 107, 122, 123). This phenonemon has been demonstrated by measuring rates of root exudation under applied suction for detopped tobacco (Nicotiana tabacum L.)(123) and tomato (Lycopersicon esculentum Mill.) (22, 15, 107) and by determining rates of $H_2O^{18}$ uptake by corn (Zea mays L.) roots (91).

Slatyer (199) suggested that water uptake, although largely passive, depends on a sufficient level of root permeability. The kinetics of tritiated water movement through living and dead carrot (Daucus carota L.) tissue led Glinka and Rheinhold (85) to conclude that water movement across cell membranes is rate limiting. Van Overbeek (220) hypothesized that there exists two mechanisms for water uptake: an osmotic component insensitive to cyanide and an active cyanide sensitive component.

Rigorous experimentation on water uptake through individual root hairs of oats (Avena sativa L.) have shown water absorption to be correlated with root metabolism (1, 19). Cailloux (29) demonstrated that water absorption through root hairs is possible only when the cytoplasm but not when the vacuole is in contact with the outer plasmalemma. Potassium cyanide when applied to roots at low concentration ($2 \times 10^{-6}$ to $2 \times 10^{-5}$ M) increased water uptake of oat root hairs. Although low levels of KCN enhance water uptake, high concen-

trations ($2 \times 10^{-4}$ to $2 \times 10^{-3}$ M) of KCN severely restrict water uptake which is consistent with the known effects of low and high concentrations of KCN on respiration (204). Similarly, respiratory uncouplers when applied to corn (11, 176) and barley (173, 176) roots have been reported to reduce root hydraulic conductivity.

Since root resistance represents the largest resistance in the soil-plant-atmosphere-continuum (19, 23), increased root resistance has been implicated as a possible explanation for decreased leaf conductance upon waterlogging many plant species (25, 121, 167, 184). A flood-induced decline in transpiration has been demonstrated for apple (Malus domestica Borkh.) trees (37, 38), pecan (Carya illinoisensis (Wang.) Koch) seedlings (137), tomato (107, 122), tobacco and sunflower (Helianthus annuus L.)(122), black willow (Salix nigra Marsh.)(167), and numerous forest tree species (121, 162, 167, 184).

Kramer and Jackson (124), working with tobacco plants, were the first to show decreased root hydraulic conductivity occurs shortly after soil submergence. The conducting ability of tomato roots was reported to decrease with short flooding durations (25, 122); however, with longer exposures there is some evidence to show that permeability to water may actually increase (25, 122, 123). Kramer and Jackson (123) speculated that the initial decline in root conductivity is associated with xylem plugging, but with longer exposures they observed stem collapse which may explain subsequent increases. A question which has not yet been adequately explored is whether a flood-induced decline in transpiration is a consequence of decreased membrane permeability in the radial direction or xylem plugging in the longitudinal direction (48, 202).

Pereira and Kozlowski (167) found that one of the earlier responses of woody plants to flooding is stomatal closure followed by root growth inhibition, alteration in stem and root morphology, adventitious root formation, and leaf senescence. Alternately, Wenkert et al. (224) working with corn found that root and stem growth inhibition preceded stomatal closure. Harris and van Bavel (93) concluded a curtailment of root respiration is the earliest and most sensitive factor responsible for growth inhibition in anaerobic soils; however, both transpiration and root respiration were not measured simultaneously in any of the above reports (93, 167, 224).

The most conspicuous difference in water relations between waterlogged and waterstressed plants is that the stomata of the former do not close as a result of reduced leaf water potential (25, 107, 167, 202). Pereira and Kozlowski (167) demonstrated a decline in leaf conductance after several days of flooding 5 woody species, but did not detect a decline in leaf water potential for the entire 37 day period of experimentation. Sojka and Stolzy (202) reported that reductions in soil $O_2$ correlate well with a decline in stomatal conductance but not with leaf water potential for a number of herbaceous species. These results are not surprising from a morphological standpoint since hypocotyl swelling, epinasty, and adventitious rooting known to occur with flooding, are processes requiring turgor (25, 114). Recently, the cause of stomatal closure in waterlogged plants has been an area of considerable interest (25). (Considerations of the effects of plant hormones and nutrient status on stomatal closure are discussed in their respective sections). Although evidence does not implicate water stress as a cause of flooding symptoms, prolonged flooding

durations may induce a leaf water deficit. For instance, decreased leaf water potentials were reported after 22 and 150 days of submergence for Sitka spruce (Picea sitchensis (Bong.) Carr)(48) and common alder (Alnus glutinosa (L.) Gaertn.)(81), respectively.

Stomatal closure of waterlogged trees obviously has some survival value, most likely, the protection against leaf dehydration. Regehr et al. (184) pointed out that flooded cottonwoods (Populus deltoides (Batr.) Marsh.) maintain a favorable leaf water balance by stomatal closure thus compensating for reduced water uptake. Stomata of green ash (Fraxinus pennsylvanica Marsh.) were reported to exhibit an adaptation to flooding after 6-10 days which is accompanied by the formation of adventitious roots (121, 196). After approximately 1 month of submergence, preflooding levels of leaf conductance occur within a week of flooding termination for green ash (121, 196), 3 weeks for several Quercus species (162); however, preflooding levels of leaf conductance are not attained after 3 weeks for loblolly pine (Pinus taeda L.), flowering dogwood (Cornus florida L.), and Eastern redcedar (Cedrus atlantica (Manetti) Carriere)(162). Bald cypress (Taxodium distichum L. Rich.) was the only of numerous plant species tested not to undergo a decline in transpiration with flooding (162).

A necessary but undesireable consequence of stomatal closure is a decline in photosynthesis (182). Flooding has been shown to reduce the photosynthetic rate of 'Stayman Winesap' apple trees (37), pecan seedling (137), 4 Citrus species (171), and eastern cottonwood (184). Decreased photosynthesis closely paralleled the reduction in transpiration with flooded Citrus species (171) and eastern cottonwood (184). Regehr et al. (184) concluded that the association between leaf

conductance and photosynthesis was sufficiently close to allow one to obtain information on photosynthesis by using a diffusion porometer.

Since mycorrhizae have been associated with a decreased root resistance of waterstressed soybean (Glycine max (L.) Merrill)(193), the effects of flooding on mycorrhizae is a pertinent topic. Coutts and Philipson (51), working with flooded Sitka spruce and lodgepole pine (Pinus contorta L.), found mycorrhizae to be limited to the aerated soil horizons. Similarly, mycorrhizae of lodgepole pine penetrated agar to a depth of rhizosphere oxidation (95). Waterlogging durations of 2 and 4 weeks for Douglas-fir (Pseudotsuga mensiesii (Mirb.) Carriere) and radiata pine (Pinus radiata (D.) Don) are sufficient to depress $^{32}P$ uptake by roots with mycorrhizae (78).

## Effect of Flooding on Plant Growth

Terrestrial plants, with the exception of hydrophytic species, manifest decreased root, shoot, and leaf growth with diminished $O_2$ levels in the root zone. The degree of waterlogging damage is a function of species tolerance, the stage of plant development, and temperature (48, 190, 191). Actively growing root tips are more susceptible than the region behind the tip (48, 49, 50, 51). Dormant roots may survive even extended periods of flooding (48, 190, 191). A temperature dependent sensitivity to waterlogging has been demonstrated for peach (Prunus persica (L.) Batsch), plum (Prunus cerasifera Ehrh.) and apricot (Prunus armeniaca L.)(189, 191); walnut (Juglans) species (35); and Sitka spruce and lodgepole pine (8, 49, 50), with damage being more severe at higher temperatures.

Much research has been conducted correlating soil oxygen diffusion rate (ODR) and plant growth. An ODR below 20 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ reduces or stops root elongation of sunflower (209), cotton (Gossypium arboreum L.)(134), tomato (133), and Newport bluegrass (Poa pratensis L.)(134). Optimum root growth is achieved above 40 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ and an ODR between 40 and 20 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ can inhibit root growth to some degree (207). Waddington and Baker (222) reported that rates below 10 X $10^{-10}$ g $cm^{-2}$ $min^{-1}$ are required to inhibit root growth of Kentucky bluegrass (Poa pratensis L.). In general, soil ODR of 40 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ is optimum for good shoot growth (133, 134, 135, 207). Top growth of corn is increased above 20 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ (17); however, a similar study with corn failed to detect increases in top growth or yield above 10 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ (207).

## Effect of Flooding on Root Respiration

A switch from predominantly aerobic to anaerobic respiration of roots and soil organisms occurs with soil flooding because of a low diffusion rate and solubility of $O_2$ in water. The ability to both regulate anaerobic respiration by controlling the Pasteur effect and to maintain the capacity for aerobic respiration has been shown to be a feature of flood-tolerant plant roots (53-59, 149). (The Pasteur effect, which is the acceleration of glycolysis with low $O_2$, is largely controlled by the properties of the allosteric enzyme, phosphofructokinase).

Crawford (53), examining root respiration characteristics of seven Senecio species when flooded, found that flood-intolerant species

differ from flood-tolerant species in that anaerobic respiration and ethanol production greatly increase and top growth decreases with the former. No consistent differences, however are apparent in aerobic rates. Lambers and Steingrover (127), working with a flood-tolerant and a flood-intolerant Senecio species, emphasized that there exists a need to distinguish between maintenance respiration and growth respiration, the latter occurring with active uptake, translocation, and root growth.

Carpenter and Mitchell (32) assessed the value of root respiration as a flood tolerance indicator for bald cypress, red (Acer rubrum L.) and sugar maple (A. saccharum Marsh.). Although 8 days of flooding reduces respiration rates of all 3 species, flood-intolerant red maple manifests the greatest decline at both 0.5 and 21% $O_2$. The reduction in root respiration of red maple at 0.5% $O_2$ is not a function of inadequate substrate since sucrose 2% (v/v) does not significantly enhance respiration. In contrast to the work of Crawford (53, 54), respiratory quotients were not indicative of a greater increase in glycolytic activity in flood-intolerant red maple compared to flood-intolerant bald cypress or sugar maple. An attempt to distinguish differential inhibition of the cyanide-sensitive and cyanide-insensitive respiratory pathway of plant roots failed to provide evidence that the degree of flood tolerance is due to the presence or absence of a particular respiratory pathway (33).

Rowe and Beardsell (190) suggested that anaerobic respiration cannot meet the energy requirements of roots indefinitely; however, Hook et al. (99) concluded that anaerobic respiration may be adequate for prolonged survival but inadequate for the species to flourish.

## Effect of Flooding on Plant Metabolism

Flooding has been shown to induce ethanol accumulation in roots of tomato (77), winter wheat (Triticum aestivum L.) and rye (Secale cereale L.)(16), Sitka spruce (58), corn (230), and several flood intolerant Senecio species (53, 59, 149). Although the authors have suggested that ethanol accumulation is toxic none established that endogenous ethanol concentrations reach toxic levels. Plant species demonstrating only moderate ethanol accumulation when flooded include lodgepole pine (58); a marsh grass (Spartina alterniflora Loisel) (151); flooded tolerant Senecio species (53); and pear (Pyrus communis L.), peach, and plum (189). Rowe (189) observed rates of ethanol production of detached peach, plum, and pear roots are rapid with the onset of anaerobiosis but fall to almost zero after 4 hours, even when $CO_2$ and ethanol are flushed from the system. He also showed that pear roots catabolize ethanol when reexposed to $O_2$. The ability to metabolize ethanol has been demonstrated by different tissues of many plant species (46, 116, 189). Production of but not necessarily accumulation of ethanol may serve as an indicator of the degree of anaerobiosis. Beletskaya (16) found that 0.3% acetaldehyde, in fact, is much more toxic to germinating winter wheat and rye seeds than is an equivalent amount of ethanol.

The activity of alcohol dehydrogenase, assessed by the Km for acetaldehyde or the oxidation of NADH, has been used by several investigators as an indicator of flood tolerance (76, 115, 149, 151, 230, 231). McMannmon and Crawford (149), examining 19 flood-tolerant and flood-intolerant species, reported that flooding induces large

increases in alcohol dehydrogenase for only the intolerant species. Increased alcohol dehydrogenase activity in flooded roots has been observed after 4 hours for corn (230) and after 12 hours for wheat and rye (16). Francis et al. (76) observed a dramatic increase in alcohol dehydrogenase activity of all Trifolium subterraneum L. subspecies in both flooded sand and anaerobic solution culture although the increase is relatively less with the tolerant yannicum subspecies. As expected, anaerobic solution culture increased alcohol dehydrogenase activity relatively more than flooded sand culture after 3 days.

In contrast to the good correlation between the induction of alcohol dehydrogenase activity and flooding susceptiblilty noted above, Wignarajah et al. (231) found that flooding induces a higher alcohol dehyrogenase activity for flood-tolerant cultivars of barley (Hordeum vulgare L.) even though root and shoot growth is inhibited much more with flood susceptible cultivars. Alcohol dehydrogenase activity of barley roots is highest at 3 to 13% $O_2$, lower with pure $N_2$, and lowest with 20% $O_2$ (231). Similarly, intermediate concentrations of 8-13% $O_2$ leads to the greatest alcohol dehydrogenase activity of corn roots (230). Mendehlson et al. (151) determined that alcohol dehydrogenase levels are highest at intermediate levels of $O_2$ for Spartina marsh grass. Therefore, the induction of alcohol dehydrogenase activity with flooding is not necessarily a reliable indicator of flood tolerance or the degree of $O_2$ stress. For example, a flood-tolerant species with larger aerenchyma or more surface rooting may contain both higher $O_2$ and greater alcohol dehydrogenase activity.

McManmon and Crawford (149) proposed that flooding tolerance is based upon the control of glycolysis through the inductive properties

of alcohol dehydrogenase and a shunt away from ethanol to malate synthesis dependent upon the existence or absence of malic enzyme. (Malic enzyme converts malate to pyruvate). According to this theory (149), intolerant plant species can either convert phosphoenol pyruvate to pyruvate directly or via a pathway involving oxaloacetate and malate as the intermediates. An undesireable consequence of flood-tolerant species not possessing the indirect pathway mentioned above is a lower than potential rate of ATP formation. McManmon and Crawford (149) compared 4 flood-intolerant and tolerant species and confirmed the existence of malic enzyme only in the former. Malate dehydrogenase, pyruvate kinase, and phosphoenol carboxylase are also key enzymes involved in the proposed pathway; however, both flood-tolerant and flood-intolerant plant species possess these enzymes (149).

Crawford (56) reported a higher (100:1) malate to ethanol ratio for waterlogged birch (Betula pubescens Ehrh.) compared to those growing on well drained sites. He proposed that the utilization of starch with the production of malate allows the root to respire anaerobically to produce a non-toxic end product of glycolysis and to transfer the $O_2$ debt of the root to the aerated shoot (56, 57). The ability of some flood-tolerant plants to exhibit increased nitrate reductase activity upon flooding facilitates proton disposal in the form of amino acid synthesis, and allows for the continuation of glycolytic activity (55, 56, 79). There is some evidence that $O_2$ derived from fertilizer supplied $NO_3^-$ may serve as electron acceptors (9, 118). Arnon (9) reported increased root respiration when $NO_3^-$ is supplied instead of $NH_3$ in nutrient solutions.

Experiments with both herbaceous (60) and woody plant roots (56,

57, 79, 205) have shown that dark fixation of $^{14}CO_2$ may be incorporated into various organic and amino acids, most notably malic, aspartic, and glutamic acid. Anaerobiosis affects dark $^{14}CO_2$ fixation more in roots of flood-intolerant than in flood-tolerant plant species (56, 57, 60, 79).

A corollary to the theory that flood-tolerant plants exhibit a shunt from ethanol to malate production is that malate and ethanol concentration should be inversely correlated; however, this is not always true (115, 151). Concentrations of ethanol and malate are found to parallel each other in roots of black gum (Nyssa sylvatica Marsh.) (115), and malate concentration varies despite constant ethanol levels in roots of Spartina marsh grass growing in soils of different redox potentials (151).

Consistent with depressed levels of respiration, one might expect flooded roots to contain lower levels of ATP; however, root ATP levels are not often measured. Mendehlson et al. (151) found that ATP and the energy charge ratio (i.e., ATP + ½ADP : ATP + ADP + AMP) in roots of Spartina marsh grass is highest at a soil redox potential where alcohol dehydrogenase activity is also highest. Waterlogging roots of flood-tolerant and intolerant plant species results in a higher NADPH : NADP ratio for only flood-intolerant species (149). Elevated adenylate kinase activity of corn root, particularly levels within adventitious roots, has been reported to increase with flooding (188).

Adventitious roots are a region of high metabolic activity as evidenced by a study involving leaf applied $^{14}C$ sucrose to flooded corn plants. Both accumulation and metabolic interconversion occur preferentially in adventitious roots and the basal region of the

stem with intermediate flooding duration; however, with prolonged flooding durations, maximum radioactivity is found progressively higher up the stem (158).

## Effect of Flooding on Ethylene Production

The occurrence of ethylene in a waterlogged soil at concentrations sufficient to be capable of inhibiting root expansion was first reported by Smith and Russell (201). Ethylene in waterlogged plants is apparently due to accelerated production in the plant (25, 104, 112), decreased diffusion out of the plant (25, 103, 104, 111, 112), and absorption of microbially produced ethylene (86, 103, 200, 201).

Ethylene concentration in flooded soils has been shown to accumulate to 0.4 ppm in a Levington compost (103), 0.8 ppm in a sandy loam (63), and 4.5 ppm in a compost peat mixture (104). Smith and Russell (201) reported soil ethylene levels exceeding 10 ppm in clay and peat soils. Typically, soil ethylene levels begin to increase within 10 hours of soil submergence and reach a peak after 4-7 days (63, 103, 104, 200, 201). Ethylene concentrations as low as 0.05 ppm are capable of inducing leaf epinasty of tomato (103). Ethylene concentrations below 1 ppm stimulate and concentrations above 1 ppm inhibit root elongation of mustard (Brassica juncea (L.) Czernia), rice (Oryza sativa L.), and tomato (119). Jackson and Campbell (103) found that when soil ethylene exceeds 2 ppm, sufficient ethylene is translocated to promote leaf epinasty of tomato. However, later work by the same authors (104) suggests that soil ethylene may be only contributory to epinasty promotion since plants exhibit the response when grown in soil

producing virtually no ethylene. Similarly, Kawase (112) found ethylene accumulation to continue with anaerobiosis despite surrounding the root with plastic wrap.

Evidence now exists to support the contention that flooding results in high endogenous levels of ethylene in roots, stems, and leaves of tomato (24, 104), sunflower (11, 223), broadbean (Vicia faba L.)(67), corn (65), several Eucalyptus species (44), sugar maple (148), and white willow (Salix alba L.)(40). Plant responses to flooding which have been attributed to ethylene include leaf chlorosis, leaf epinasty, stem hypertropy, stem height reduction, aerenchyma formation, and adventitious root production (25, 64, 65, 67, 103-107, 111-114, 167). The factor responsible for inducing shoot damage with soil flooding has been shown to eminate from the root, since with root removal shoots do not show the anaerobic stress symptoms (107, 122).

The explanation that anaerobically produced ethylene in flooded roots affects plant tops was somewhat of a paradox since ethylene synthesis has been shown to require $O_2$ (27). Jackson and Campbell (104) suggested that an ethylene precursor such as methionine or tryptophan may be produced by the root and cause shoot damage. Bradford and Yang (24) identified the ethylene precursor as 1-aminocyclopropane-1-carboxylic acid (ACC) and found that ACC requires $O_2$ to be enzymatically converted to ethylene. They collected ACC in the xylem sap of flooded tomato plants and found that ACC always precedes both leaf epinasty and elevated ethylene levels in the stem. Research is continuing into the elucidation of the pathway in regard to specific enzymes and intermediates involved and factors affecting ethylene biosynthesis (24, 25, 120).

## Effect of Flooding on Abscisic Acid and Other Hormones

Recently, some attention has been focused on the relationship between plant responses to flooding and abscisic acid (ABA)(25, 35, 67, 134, 198, 234). Wright and Hiron (234) found leaves of flooded bean plants contain five times more ABA than control plants. Several investigators suggest that ABA accumulation in leaves of flooded plants is the result of a water deficit (67, 198, 234). The major argument against ABA being responsible for flood-induced stomatal closure is that closure precedes a reduction in leaf water potential or a loss of leaf turgor (25, 107, 167, 202). Catlin et al. (35) reported little relationship between increases in ABA content and flood tolerance of two Juglans species and Chinese wingnut (Pteracarya stenoptera D. C.). Moreover, since only one of two Juglans species exhibited increased ABA levels before gross morphological symptoms were expressed, it is unlikely that ABA can serve as a useful tool for screening flood tolerance of at least these species.

Abscisic acid when applied in solution can have variable effects on root hydraulic conductivity and ion transport of roots. Exogenously applied ABA has been reported to increase root hydraulic conductivity of bean (73, 110), corn (45), sunflower (82, 84), and carrot (85); to decrease that of soybean (73, 147), and to have no effect on that of sunflower (82, 84), barley (52), and corn (52). Much of the above variability is due to different pressure and time selections (73, 147, 174, 175). For instance, most of the above reports utilized applied pressures of less than 1 bar (45, 52, 82, 84, 85, 174, 175), permitting interaction between osmotic and pressure driving forces which

then become exceedlingly difficult to separate (71, 72, 146, 147).

There is a need to distinguish between exudation rate (Jv) and hydraulic conductivity ($L_p$) which are related as follows:

$$JV = L_p (\Delta P - \sigma \Delta \pi)$$

where $\Delta P$ = hydrostatic pressures, $\sigma$ = reflection coefficient, and $\Delta \pi$ = osmotic driving force (71). Pitman and Welfare (175), working with barley roots, found exudation rates but not hydraulic conductivity to decrease with the addition of ABA. Decreased exudation is a function of reduced ion uptake, $K^+$ in particular, but also $Mg^{+2}$, $Ca^{+2}$, $Na^+$, and $PO_4^{-3}$ (175). Addition of ABA to root medium has been shown to inhibit ion transport into the xylem of the roots of corn and barley (52), sunflower (68), and soybean (147). Markhart et al. (147) reported that the ABA effect on ion uptake is correlated with changes in membrane viscosity as evidenced by breaks in the Arrhenius plot.

Whether ABA is responsible for the observed decreased stomatal conductance or hydraulic conductivity with plant flooding has not been determined. It is known that ABA buildup is related to a decline in turgor (172). Clearly, since stomatal closure of flooded plants is not due to increased water stress (25, 107, 167, 202), the stimulus for ABA release or formation must be something other than a decline in turgor. The dramatic decline in $K^+$ transport with ABA treated plants is a possible explanation for flood-induced stomatal closure (156, 166, 202).

Alternatively, stomatal closure may be due to a deficiency of cytokinins or gibberellins emanating from the roots, although direct evidence for this is admittedly meager (28, 105, 185, 186). Burrows and Carr (28) showed that flooded roots of sunflower plants lose

their capacity to produce cytokinins after 4 days. Reid and Crozier (185) also found that gibberellin content of the roots, shoots, and xylem sap of tomato plants decline upon flooding. Transpiration rates of flooded tomato plants have been reported to return to near preflooding levels with exposure to benzyladenine (105, 180) or gibberellins (105). Reduced cytokinin synthesis can decrease indoleacetic acid (IAA) oxidase and thus lead to increased auxin levels (131). The shoots of waterlogged sunflower plants were reported to contain 3 times the auxin levels of control plants, moreover, auxin applications relieved flood-induced leaf epinasty (170). Thus, an understanding of the hormonal relationships in flooded plants awaits future research.

## Effect of Flooding on Plant Anatomy

Submerging the roots of mesophytes usually results in reduced growth and eventual death. Some plant species when flooded produce new roots better adapted to an anaerobic environment (6, 7, 39, 40, 50, 51, 73, 97, 136, 196, 231). Forest trees subjected to perennial flooding sometimes survive by surface rooting (40, 80, 81, 97). Upon submergence, many herbaceous (103-107, 111-114, 236) and woody plant species (7, 50, 80, 81, 167, 196) produce adventitious roots. Armstrong and Boatman (7) reported that adventitious roots of various sedges grow horizontally within a saturated horizon along a tolerable redox plane.

Several investigators have proposed that adventitious rooting is an adaptation to flooding (44, 65, 97, 114, 148, 196, 236), but others

noting a poor correlation between flood tolerance and adventitious rooting judge adventitious rooting to be a flooding induced stress symptom (81, 92). Since adventitious rooting also occurs upon submerging flood-intolerant plants such as tomato and sunflower, it does not by any means imply tolerance.

The most frequently inferred mechanism for plant waterlogging tolerance is the development of aerenchyma. Aerenchyma may be produced either in a lysigenous or schizogenous manner (69). Lysigenous aerenchyma production may be a result of decreased cell integrity and turgidity brought about by anaerobic conditions (150). More recently, several studies have shown lysigenous aerenchyma formation to be a function of increased cellulase activity triggered by ethylene (111, 113). Schizogenous aerenchyma forming as cells separate along pectinaceous middle lamella, sometimes merely as a function of differential cell growth (69), appears to be related to $Ca^{+2}$ and pectinase levels (87).

Wetland herbaceous species (47) and even agricultural crops such as rice in particular, but also corn and barley, develop roots with a high porosity when flooded (236). Yamasaki (235) indicated that wetland herbaceous species are more likely to possess cortex cells packed in a columnar rather than an oblique configuration since the former has twice the calculated intercellular air space.

Drew et al. (64) have demonstrated extensive breakdown of the midcortex of corn to occur with anaerobiosis; however the stele, endodermis, epidermis, and the inner and outer layer of cortical cells remained intact. When reexposed to air, intact root segments with aerenchyma absorb and translocate $Rb^{+}$ at rates similar to those obser-

ved for aerobic roots (64). Aerenchyma has been reported to occur in the cortex of black willow stems (143); however, definitive observations in other tree species are lacking (49). Extensive studies have shown that aerenchyma is unlikely to exist in the xylem of tree species (49).

Both secondary tissue outside the xylem and secondary xylem itself may serve as potential gaseous pathways connecting root and stem tissue. The cambium is considered to present a barrier to gaseous exchange (124). Hook and Brown (96) presented micrographs showing small intercellular spaces in the ray initials; however, these may be artifacts of fixation (99). Indirect evidence of radial permeability of $O_2$ through stem tissue has been obtained by experiments utilizing applied pressure (96, 140, 141, 195). Hook and Brown (96) found that although capable of gaseous transport flood-intolerant plant species conduct less in the radial direction than tolerant species.

Longitudinal permeability outside the xylem has been inferred by experiments with oak bark strips (140) and by longitudinally and tangentially connected air spaces of woody dicotyledons (69). Girdling stem tissue of green ash and black gum (96) somewhat reduces rhizosphere oxidation and ringing of bogbean (Menyanthes trifoliata L.) rhizomes depresses $O_2$ movement to the roots (47).

A significant percentage of the vessels or tracheids of secondary xylem is occupied by gas in both angiosperms and gymnosperms and therefore, little resistance to longitudinal movement of gas exists in the secondary xylem (49). In gymnosperms, water in late-wood tracheids of each growth ring becomes replaced by gas soon after differentiation, apparently because bordered pits do not close as in

early wood (140). Gas filled intercellular spaces of medullary rays of spruce have been observed, but intercellular spaces cease at the cambium (237). Back (10) observed pits connecting medullary rays and ray parenchyma in xylem of spruce and pine and speculated that they specifically function to aerate parenchyma cells.

The main pathway of gas transport in the roots is assumed to be in the cortex since intercellular spaces are visible (49). Although cortical aerenchyma is presumed to be more extensive with poor aeration, Hook et al. (91) did not detect visible differences in intercellular spaces of black gum roots grown in oxygenated and deoxygenated solution culture. Some evidence exists to show intercellular spaces in the stele of primary pine roots; however, they are eventually closed with growth of secondary xylem (49, 50). A search of the literature revealed that the only reported instance of aerenchyma in woody roots occurs in mangrove species (195). Pneumatophores of bald cypress were assumed for many years to function as entry points for $O_2$; however, work by Kramer et al. (125) could confirm no such function.

Grable (87) pointed out that the existence of intercellular spaces alone does not necessarily imply gaseous permeability, especially if the submerged tissue is in a hydrated state. Ohmura and Howell (161), measuring respiration of excised corn, soybean, and barley roots, reported a 50% respiratory inhibition when water, far less than that required to submerge the tissue, is added. Some of the respiratory inhibition was certainly due to an increased diffusive path length; however, blotting the tissue dry did not fully revive rates. Coutts and Armstrong (49) propose that hydrostatic tensions in the xylem may keep intercellular spaces free from water, but this obviously would

depend on the location of intercellular spaces, xylem structure, and the degree of plant water stress.

The tortuous path of $O_2$ through woody tissue has led investigators to conclude that significant quantities of $O_2$ supplied to flooded roots of woody species can only be through lenticels (98, 149, 190). Experimental support for lenticels functioning as the main entry points for $O_2$ has been obtained by noting a cessation of rhizosphere oxidation once lenticels are covered (4, 98). The formation of hytrophied lenticels has been reported on submerged stems of numerous woody species; however, since they occur below the waterline, gaseous absorption of $O_2$ is unlikely until the water recedes (49, 167, 196). Chirkova and Guttman (39) found lenticels on the stem of white willow to emit more ethylene and other metabolic by-products than those on the stem of cottonwood. Lenticels occurring on the stem and upper roots of pine are hydrophobic and remain capable of gaseous absorption after the water level recedes (49). Lenticels do not provide a continuous interconnected gas network capable of providing $O_2$ to all parts of the root since interior wood of trunks and roots of forest trees is largely anaerobic (58, 74).

One of the main functions of internal $O_2$ transport of plants is to oxidize the rhizosphere, thereby preventing the buildup of soil toxins in that vicinity (3-8). Such oxidation may occur enzymatically, by microbes, or directly by molecular $O_2$ (3). The extent of rhizosphere oxidation is a function of a root's respiratory demand and its internal porosity (49). Rhizosphere oxidation, which is assessed by the color change of reduced dyes, has been observed for roots of flooded lodgepole pine and Sitka spruce (50, 51, 169), black willow

(97, 136), black gum and green ash (97); but not for American sweetgum (Liquidamber styraciflua L.), sycamore (Platanus occidentalis L.), or tulip tree (Liriodendron tulipifera L.)(97); or for 4 Citrus species (171). Most of the above authors neglected to mention that the dye reduction method does not conclusively demonstrate $O_2$ transport since oxidation of dyes such as alpha-naphthylamine can be oxidized without the involvement of $O_2$ (136). The polargraphic method used to determine $O_2$ diffusion through the root is contingent upon the degree of oxidation reduction taking place on a thin cylinder of platinum foil surrounding a root (3). Although a more quantitative technique than the dye method, $O_2$ is still not directly determined, and the measurement depends on $O_2$ diffusion outside the root. The polargraphic method just described, has shown rhizosphere oxidation to occur for rice roots (5), several Pinus species (8), and 10 species of bog plants (7).

The most direct approach to evaluate $O_2$ transport through a plant has been demonstrated with $^{15}O$ for many herbaceous species with kinetics similar to gaseous diffusion through continuous gas spaces (13, 70, 90). Rates up to 36 $m\,hr^{-1}$ have been reported for rice (13). It must be stressed that $O_2$ transport does not necessarily confer flood tolerance since flood susceptible species such as onion (Allium cepa L.), pea (Pisum sativum L.), and lettuce (Lactuca sativa L.) have also been reported to transport $O_2$ to the root (90). The question whether aerenchyma or other anatomical responses to flooding serve as an adaptation or are simply a symptom of damage depends on the duration of flooding. Most likely, anatomical responses to flooding exhibited by even flood-intolerant species temporarily ease the deleterious effects of anoxia (114).

One significant aspect of internal $O_2$ transfer which thus far has not been adequately addressed is the maximum distance $O_2$ may diffuse through woody species. The polargraphic technique indicates a maximum distance of 4 and 6 cm for roots of lodgepole and black pine, respectively (8). Philipson and Coutt (169), utilizing the dye method on detached roots, detected rhizosphere oxidation of lodgepole pine with root lengths greater than 10 cm in certain experiments, but it has not been established that rates of $O_2$ diffusion meet respiratory needs. Theoretical evaluations of $O_2$ flux, root porosity and permeability, root respiration, and soil aeration (94, 139) are a step in the right direction but are based on numerous assumptions.

In summary, the bulk of the evidence suggests that while internal $O_2$ diffusion of woody species may be of importance to adventitious roots, it appears doubtful whether it plays a significant role in aerating deeper roots. Rowe and Beardsell (190) considered it unlikely that internal $O_2$ transport contributes to the waterlogging tolerance of quince (Cydonia oblonga Mill.) and pear.

## Effect of Flooding on Soil Chemistry

The most important difference in chemistry between a waterlogged and a well drained soil is that a waterlogged soil is predominantly in a reduced state (179). A sudden decline in $O_2$ follows soil submergence since $O_2$ diffusion is 10,000 times slower in water than in gas filled pores (89, 206). Upon soil flooding, the once abundant aerobic organisms use up the $O_2$ present in the soil, become quiescent or die, and the facultative and obligate anaerobes flourish (214, 215).

Reduction of the soil is largely a consequence of anaerobic respiration (214, 215). Primarily bacteria oxidize organic matter and utilize oxidized soil components as their electron acceptors. As a result, the reduced counterparts of $NO_3^-$, $SO_4^{-2}$, $Mn^{+4}$, $Fe^{+3}$, and $CO_2$; $NH_4^+$, $H_2S$, $Mn^{+2}$, $Fe^{+2}$, and $CH_4$ predominate (179, 213, 218).

Aerobic organisms completely oxidize organic matter and regenerate $NAD^+$ by utilizing $O_2$ as the terminal electron acceptor. Under anaerobic conditions facultative and obligate anaerobes use $NO_3^-$, $Mn^{+4}$, $Fe^{+3}$, $SO_4^{-2}$, $CO_2$, $N_2$, $H^+$, and products of organic matter as electron acceptors thereby reducing $NO_3^-$ to $N_2$, $Mn^{+4}$ to $Mn^{+2}$, $Fe^{+3}$ to $Fe^{+2}$, $SO_4^{-2}$ to $H_2S$, $CO_2$ to $CH_4$, $N_2$ to $NH_3$, and $H^+$ to $H_2$. The above, as well as various bacterial excretory by-products serve to reduce soil components and lower the soil redox potential (20). The rate and degree of soil reduction is dependent upon soil pH, temperature, organic matter content, and the species of electron acceptors. Reduction of a submerged soil proceeds in a sequence determined by the thermodynamics of redox systems (179, 213, 218). Roughly the order of reduction is $O_2 > NO_3^- > Mn^{+4} > Fe^{+3} > SO_4^{-2}$. A sequence of microbial succession also follows -- aerobes, facultative anaerobes, strict anaerobes -- after soil submergence (215).

Redox potentials (Eh) for flooded soils are usually between 0.2 and -0.4 volts and those of well drained soils between 0.8 and 0.3 volts (179); however, because values for flooded and nonflooded soils may overlap, redox potentials do not indicate soil $O_2$ quantitatively (190). Despite limitations, Eh of a given soil type may correlate with plant species destribution (165).

Lemon and Erickson (132) first described a technique to measure

soil oxygen diffusion rates (ODR) by the platinum microelectrode technique. Oxygen diffusion rate is measured in proportion to the amount of oxidation-reduction reactions occurring on the platinum tip of a microelectrode. Both the theory of (206, 211) and plant responses to ODR (207) have been reviewed. The method is not adequate to measure ODR in unsaturated soils since hydraulic continuity must exist between the soil particles, the platinum surface, and the calomel electrode. The preference of the platinum microelectrode technique over measurement of Eh is that it is quantitative (132, 133, 144, 206, 211). The advantage of measurements of ODR over percent $O_2$ is that it provides an indication of a plant's accessibility to $O_2$ independent of soil type (132, 133). For example, transitions in plant communities growing on wet sites are associated with ODR between 25 and 5 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ (177).

Oxygen is the first element in the soil to be reduced and becomes undetectable by many methods within a few days after soil submergence (214, 217). Oxygen may also be low in nonflooded compact soils. For instance, Smith and Dowdell (200) reported that $O_2$ in heavy clay soil remains below 10% v/v throughout the winter and spring before rising to atmospheric levels in the summer. Nitrate reduction proceeds when the $O_2$ concentration has dropped to a very low level. Similarly, the presence of $NO_3^-$ retards the reduction of elements with a less positive Eh such as $Mn^{+4}$, $Fe^{+3}$, and $SO_4^{-2}$ (217).

The overall effect of submergence on soil pH is to increase the pH of acid soils and to depress the pH of sodic and calcareous soils (178). Upon submergence pH declines for several days and reaches a minimum then increases to a stable value of 6.5 to 7.2 in a few weeks regard-

less of soil pH (178, 179). The only exception to this rule is acid soils containing little Fe and organic matter may attain pH values lower than 6.5 (179). Low temperature and/or $NO_3^-$ retards the increase in pH (179). The temporary initial decline in pH after submergence is due to the accumulation of $CO_2$ by aerobic bacteria since $CO_2$ depresses the pH of even acid soils (160). The subsequent increase in soil pH is due to soil reduction. Since most soils contain more Fe oxide hydrates than any other oxidant, the increase in pH is believed to be primarily due to the reduction of $Fe^{+3}$ (179).

Soil $CO_2$ produced after submergence forms carbonic acid, bicarbonates, and insoluble bicarbonates and the excess accumulates as gas (41, 153). A decline in $CO_2$ actually occurs after 1-4 weeks of soil submergence because of its escape, leaching, removal as insoluble carbonates, and utilization as a terminal electron acceptor (41). Organic acids, particularly formic, acetic, and propionic acids, occur in waterlogged soils roughly in proportion to its organic matter content (154, 194). Organic acids and other organic compounds are eventually converted to $CH_4$ (178, 179) which is an end product of anaerobic respiration. Carbon dioxide and organic acids persist longer and $CH_4$ production is diminished in soils of low temperatures (41).

The mineralization of organic nitrogen in submerged soils stops at $NH_3$ because little $O_2$ is present to allow $NO_2^{-2}$ or $NO_3^-$ formation (179). When a well drained soil is submerged, $NO_3^-$ undergoes two transformations: assimilation and reduction into cellular material or utilization as an alternative to $O_2$ as an electron acceptor. Most $NO_3^-$ disappears in a few days as a result of the latter process, and much

of it is eventually lost as $N_2$ gas (179). Drew and Sisworo (63) noted a 50 fold decrease in $NO_3^-$ and a 2 fold increase in $NH_3$ after 15 days of soil submergence. Denitrification is slower in anaerobic soils low in organic matter since denitrifying organisms require $NH_3$ as sources of C and $H^+$ for growth. Alternate wetting and drying increases the denitrification loss, although continuous submergence minimizes it and even may lead to a net accumulation under certain conditions (164).

Iron undergoes a drastic increase in solubility upon conversion of $Fe^{+3}$ to $Fe^{+2}$ in submerged soils. Iron is the greatest single factor responsible for stabilizing a decline in redox potential and pH (20). An investigation into waterlogging sensitivity of Ericea species implicate Fe toxicity as a possible cause (109). Manganese also increases in availability and is present in waterlogged soils as water soluble $Mn^{+2}$, $MnHCO_3^+$, and as organic complexes. Drew and Sisworo (63) reported available Mn to increase 100 fold after 15 days of soil flooding. Like $Fe^{+2}$, $Mn^{+2}$ undergoes reoxidation upon diffusion to oxygenated interfaces. Perumal (168) concluded that flooding may induce Mn toxicity in rice.

In anaerobic soils, $SO_4^{-2}$ and the S containing amino acids are degraded to $H_2S$, thiols, $NH_3$, and fatty acids at an Eh of -0.150V (212). If the soil contains sufficient amounts of $Fe^{+2}$, injurious levels of $H_2S$ can be avoided by precipitation to FeS (179). Phosphorus generally becomes more available when the soil is flooded (142). The release of $PO_4^{-3}$ upon flooding is due to its release from Fe or Al in acid soils or its release from calcium phosphates in alkaline soil (142). The former release $PO_4^{-3}$ as the pH increases while

calcium phosphates liberate $PO_4^{-3}$ as pH decreases. The net result of soil submergence on micronutrients is to increase the availablilty of Co, Cu, and Mo but not Zn (159).

## Effect of Flooding on Leaf Nutrient Levels

Brouwer (26), reviewing numerous experiments, concluded that ion uptake and ion transport into the xylem is under metabolic control. Active ion uptake, being against a concentration gradient, requires energy.

Of particular interest is the work of Arnon (9), where he shows increased root respiration rates of barley when $NO_3^-$ is substituted for $NH_3$ in unaerated solution cultures. Kessler (118) concluded that $O_2$ derived from $NO_3^-$ could account for a very small percentage of normal $O_2$ consumption, but even the addition of small amounts of $O_2$ may be beneficial under anaerobic conditions.

Hosner and Leaf (102), flooding 14 bottomland species, found that nutrient accumulation (mg/seedling) correlates with dry weight, although growth is more affected than either parameter. All species tested except willow, black gum, green ash, and cottonwood were adversely affected by saturation (102). Root growth of tomato, tobacco, and soybean was reported to cease at 0.5% $O_2$; however, top growth and ion accumulation continues at this $O_2$ level (100). Not all elements are affected by flooding to the same degree. For example, the reduction in the absolute quantities of the major elements for wheat, corn, and rice on a dry weight basis are as follows: K > N > P > Ca > Mg (36). Leaf $K^+$ concentration of corn (221), tomato and barley (129)

plants are depressed to at least one half by flooding. Leaf analysis of flooded tomato, tobacco and soybean plants (100), and sweet orange (Citrus sinensis (L.) Osbeek) seedlings (126) showed decreased macronutrient and micronutrient nutrient levels with the exception of $Na^+$, indicating a loss of membrane selectivity. Reduced mineral uptake of apple (157) and peach (117) roots upon anaerobiosis has also been reported. Studies with sweet orange seedlings (208) and sunflower plants (134) indicate ODR below 30 X $10^{-8}$ g $cm^{-2}$ $min^{-1}$ results in decreased leaf macronutrient levels accompanied by an increase in $Na^+$.

The evidence discussed above showing a marked decline in $K^+$ uptake and accumulation with soil flooding (36, 63, 100, 126, 129, 221) is pertinent in regard to flooding induced stomatal closure. The involvement of $K^+$ in stomatal regulation is well documented (182). It has been speculated that curtailed $K^+$ uptake or translocation may be involved with flood-induced stomatal closure (24, 202). Micronutrient toxicity has also been shown to elicit stomatal closure of bean without decreasing leaf water potential (183). Although micronutrient availability increases upon flooding (159, 179), investigators have consistently demonstrated micronutrient deficiencies to occur in plant tops (100, 126).

## Effect of Flooding on Excess $CO_2$ Supply to Roots

The high solubility and concentration of $CO_2$ in flooded soils necessitates its consideration in evaluating plant responses to waterlogging. For example, flooding a sandy loam for a 2 week duration alters $O_2$ and $CO_2$ from 20% to 1% and from 0.34% to 3.4% (v/v), respect-

ively (63). Carbon dioxide concentration reaches a peak after 1-4 weeks of soil submergence (41). The distinction between the deleterious effects of excess $CO_2$ or insufficient $O_2$ on plants deserves particular attention. Childs (38) applied different concentrations of $O_2$ and $CO_2$ to the roots of apple and found $CO_2$ to have no effect on transpiration or photosynthesis. Williamson (232) demonstrated that flooding injury of tobacco plants is due to low $O_2$ and not high $CO_2$ since a mixture of 1% $O_2$ and 20% $CO_2$ and a mixture of 1% $O_2$ and 0% $CO_2$ reduces growth in a similar manner, but the $N_2$ treatment nearly causes plant death after 48 hours. Hook and Brown (97) exposed black gum and sweet gum to 1% $O_2$ in combination with 2%, 10%, and 31% $CO_2$ and reported that root growth of sweetgum is inhibited in 1% $O_2$ at all $CO_2$ concentrations; however, all concentrations except 31% stimulate root and shoot growth of black gum. Investigations into the effects of high $CO_2$ on nutrient uptake have produced inconsistent and often insignificant results (88, 126, 157). Rowe and Beardsell (190) concluded that more dramatic nutritional effects result from low $O_2$ than high $CO_2$.

High $CO_2$ may even have a beneficial effect on plant growth due to fixation and utilization by roots and foliage (55, 56, 79, 205). Stemmet et al. (205) demonstrated that $^{14}CO_2$ applied in solution to roots is fixed in both roots and shoots. Similarly, Crawford (57) showed anaerobically applied $^{14}CO_2$ in solution to excised root pieces of several tree species is recovered in the form of amino and organic acids, especially malic acid. He concluded that $CO_2$ by combining with pyruvate serves to prevent ethanol accumulation, releases the electron acceptor $NAD^+$ thereby allowing for continued glycolytic

activity, and also provides $CO_2$ for leaf photosynthesis (55, 56).

Reviews comparing the deleterious effects of excess $CO_2$ or insufficient $O_2$ in a waterlogged soil all conclude that excess $CO_2$ is probably a minor source of damage relative to insufficient $O_2$ (87, 190, 192).

## Effect of Flooding on Soil and Plant Toxins

Hydrogen sulfide is the most extensively studied phytotoxic substance of waterlogged soils. Hydrogen sulphide at low concentrations may inhibit root growth, root respiration, and nutrient uptake (190). The reduction of $SO_4^{-2}$ is brought about by obligate anaerobic bacteria of the genus Desulfovibrio, which uses $SO_4^{-2}$ as a terminal electron acceptor (179). Culbert and Ford (61) demonstrated the extremely rapid flooding injury of Citrus species is directly related to $H_2S$ formation in the flooded sandy soil. Although very little work has been attempted to assess the contribution $H_2S$ imparts to flooding sensitivity, Sanderson and Armstrong (194) concluded that neither $H_2S$, ethylene, $Fe^{+2}$, nor $Mn^{+2}$ accumulate to toxic levels for Sitka spruce growing in a waterlogged clay soil.

Members of the genus Prunus are known to contain varying amounts of cyanogenic compounds (189, 191). Rowe and Catlin (191) showed that peach, apricot, and plum roots release large amounts of HCN after short periods of flooding. They also found phytotoxic amounts of HCN evolve when these roots are placed under anaerobic stress. Flooding damage due to $CN^-$ release is, itself, probably preceded by anaerobic-induced membrane alterations (191). Since the cyanogenic glycoside

content within the roots of Prunus species is correlated with flooding tolerance, it potentially may serve as a screening tool for members of this genus (191).

Although walnut species do not possess significant levels of cyanogenic compounds, Catlin et al. (35) proposed that a loss of membrane integrity induced by anaerobiosis leads to a loss of phenolic compounds from the vacuole. They also suggested that the phenolic substances, lost by cells in the root, enter the transpiration stream and damage aerial plant parts as well.

## Effect of Flooding on Susceptibility to Pathogens

Flooding is often associated with an increased susceptibility of plants to pathogens; however, the question as to whether pathogens are the primary cause of or a secondary response to decline has not often been determined. The host-parasite relationship in waterlogged soils is dependent upon the relative ability of the host and parasite to survive and proliferate with anoxia.

Phytophthora species are the most commonly implicated pathogens associated with waterlogging damage. Phytophthora species are not greatly affected by flooding and in fact, require high water levels for production and dispersal of zoospores (208). Evidence exists showing susceptibility of Citrus to P. citrophthora (208), avocado to P. cinnamomi (62), peach to P. cinnamomi (152), apple to P. cactorum (187), and pear to both P. cinnamomi and P. cactorum (30).

Stolzy et al. (208) reported little relationship between soil aeration and infection of Citrus roots by Phytophthora species.

Culbert and Ford (61) later demonstrated differential flooding tolerance of Citrus is directly related to its ability to withstand $H_2S$. Avocado (Persea americana Mill.) seedlings grown in culture solutions survive well at $O_2$ concentrations between 0.5 and 7.2 ppm; however, innoculations with P. cinnamomi result in eventual death at all $O_2$ levels (62). Pathogenicity is greatest at high $O_2$ levels. Remy and Bidabe (187), evaluating different apple rootstocks for flooding tolerance, found little correlation between waterlogging susceptibility and P. cactorum susceptibility. Although Pythium species have been suggested as a cause of decline of fruit trees on wet soils (15), Mircetich and Keil (152) indicated that they play a secondary role to Phytophthora in peach roots. Rowe and Catlin (191) have since demonstrated that waterlogging sensitivity among Prunus species is correlated with cyanogenic glycoside release during anaerobosis. Cameron (30) reported that Pyrus calleryana and Pyrus communis cvs. Old Home and Old Home X Farmingdale are less affected by Phytophthora than Bartlett or Winter Nelis seedlings of Pyrus communis L. grown in nutrient solution.

Although little is known concerning host-pathogen interrelationships in flooded soils, the evidence appears to indicate that pathogens are not usually the primary cause of waterlogging damage of horticultural species (190).

# SURVIVAL, GROWTH AND LEAF CONDUCTANCE OF WILLOW AND SEVERAL FRUIT TREE SPECIES: PEAR, QUINCE, APPLE AND PEACH UNDER FLOODED SOIL CONDITIONS

Additional index words. Soil oxygen diffusion rate, Pyrus betulaefolia, Pyrus calleryana, Pyrus communis, Pyrus pyrifolia, Pyrus ussuriensis, Cydonia oblonga, Malus domestica, Prunus persica, Salix discolor.

Abstract. Potted seedlings and cuttings of various fruit tree species were submerged to 5-10 cm above the soil level for prolonged periods. Three of the 4 most often used commercial pear rootstock species: Pyrus betulaefolia Bunge (Bet), Pyrus calleryana Decne (Call), and Cydonia oblonga Mill. cv. Provence BA 29 (Prov Q BA 29) were extremely flood-tolerant with Bet exhibiting 100% survival after 20 consecutive months of flooding. Within the fourth major pear rootstock species, Bartlett seedlings of Pyrus communis L. (Bart) were slightly less tolerant than Prov Q BA 29 but Pyrus communis L. cv. Old Home x Farmingdale 97 (OH x F 97) was relatively flood susceptible. Pyrus pyrifolia Burm. and Nak. (Pyri), Pyrus ussuriensis Max. (Ussuri) and Malus domestica Borkh. cv. Malling Merton 106 (MM 106 apple) were of similar tolerance to OH x F 97 while Prunus persica (L.) Batsch cv. Halford (Halford peach) was extremely flood-susceptible. Salix discolor Muhl. (pussy willow) was the only species to exhibit an increase in growth with inundation. One month of fall flooding was sufficient to reduce subsequent growth rates for all fruit tree species tested the following year. Flooding pear rootstocks with a

P. communis cv. Bartlett scion did influence growth rates and leaf conductance (cL) compared to the ungrafted rootstock but did not alter overall survivability.

Flooding promoted adventitious root formation of Prov Q BA 29, MM 106 apple and willow. All species tested developed hypertrophied lenticels with soil flooding. Obvious morphological symptoms of flooding exhibited by leaves of ungrafted rootstocks such as a build-up of anthocyanin pigmentation were not displayed by 'Bartlett' scions on the same rootstock. Thus, visual detection of waterlogging damage of pear trees in the field would likely be very difficult.

A significant decline in cL upon flooding was associated with a particular soil oxygen diffusion rate (ODR). A reduced stomatal aperture was associated with an ODR of 30 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ for seedlings of Halford peach and an ODR of 15 x 10 g $cm^{-2}$ $min^{-1}$ for Bet. Measurements of cL were of some value in assessing flood tolerance. Defoliation occurring after 40 days for OH x F 97, although directly related to the flood susceptible nature of the roots is also partially a function of poor stomatal control.

## Introduction

Flooding has been shown to adversely affect numerous processes of mesophytic plant species due to $O_2$ displacement and depletion by roots and soil microbes (24, 35). Morphological symptoms are similar to plants experiencing a water deficit in that flooding induces stomatal closure, shoot dessication, and death (10, 11, 30). Pereira and Kozlowski (27) found that one of the first responses to flooding is stomatal closure, followed by an inhibition of root growth, alterations in stem and root morphology, adventitious root formation and leaf senescence. Diurnal patterns of leaf conductance are similar to control plants although absolute values are reduced upon flooding (6, 23, 27, 32). In contrast to stomatal behavior during drought stress, flood-induced stomatal closure does not appear to be a function of reduced leaf water potential (6, 20, 27, 33).

Variations in leaf conductance are important in terms of photosynthesis since stomatal aperture is the limiting factor at high light intensities (28, 29). Separate studies concerning the tolerance of peach and other Prunus species (5, 31) and apple (8) imply that the former are more susceptible to soil anoxia (30). Quince and Pyrus species are regarded as being the most flood-tolerant of all fruit tree species (5, 30, 38), although there is disagreement as to the tolerance of quince (5, 40). A knowledge of relative waterlogging tolerance would be of value in selecting rootstocks for use in extremely compact soils or in areas with inadequate drainage. Young trees, particularly interplants, appear to be especially vulnerable to waterlogged soil conditions.

This experiment was undertaken to determine differential flooding sensitivity of quince, apple, peach, willow and several Pyrus species.

## Materials and Methods

Six-month-old seedlings of Bet, Call, Bart, Pyri, and Ussuri; 2½-year-old seedlings of Halford peach; and 1½-year-old clonal cuttings of Prov Q BA 29 were potted in 1:1:1 mixtures of unsterilized soil, sand, and peat in 4-liter pots. (The sources of plant material can be found in App. A). Thirty plants were randomly selected for the following 3 treatments: (1) 1 month submergence, (2) 20 months submergence, and (3) control maintained near field capacity by frequent irrigations and by surrounding pots with sawdust. The plants were actively growing although not extremely vigorous. Plants of the 2 flooding treatments were submerged outdoors September 8, 1980, 5-10 cm above the soil in 1.5 x 1.5 x 0.75 m plastic bins.

A second experiment initiated during spring 1981 consisted of 2- to 3-year-old seedlings of Bet, Call, and Halford peach; 1-year-old clonal cuttings of OH x F 97, Prov Q BA 29 and MM 106 apple; and 1-year-old rooted cuttings of willow. In addition, during the previous winter, P. communis cv. Bartlett OP 9 scions were grafted onto pear rootstocks mentioned above. Prior to bud break, plants were potted in 8-liter pots containing 1:1:1 mixtures of unsterilized soil, sand, and peat. Fifty days later, June 3, 20 plants of each rootstock and grafted combination were randomly separated into a flooded and a control treatment. Individuals of the flooded

treatment were submerged continuously for 1 year. Specifics of the control and flooded treatment were as mentioned earlier. (Submergence for grafted individuals was at or slightly below the graft union.)

Soil oxygen diffusion rates (ODR) were measured with a Jensen Model A oxygen diffusion ratemeter via the platinum microelectrode technique (24, 35). Measurements were taken with 25-gauge platinum electrodes after a 5-minute equilibration period at an applied voltage of -0.65 volts. Ten to 20 measurements were taken on each sampling date at a depth of 10-20 cm below the soil surface. Soil temperatures were recorded in the same zone with thermometers (App. B).

Leaf conductance (cL) was monitored with a ventilated diffusion porometer (37). The time required for a 4-7 μA deflection was recorded unless the time lapse was greater than 25 seconds, in which case a 4-6 μA or a 4-5 μA deflection was noted. The porometer was calibrated at 20°, 22.5°, 25°, 30°, and 34°C with a drilled acrylic plate with known resistances several times during the course of the experiment. Measurements of cL were conducted midday during periods conducive to maximum stress on 3 recently matured, fully expanded leaves with 3-4 replications per treatment for each species. Abaxial conductance was at least an order of amgnitude greater than adaxial conductance, therefore only abaxial conductance was monitored.

Roots of flooded and control plants were evaluated for the presence of Phytophthora by utilizing a specialized media. The media consisted of 20 ppm Piramycin added to a 2% corn meal agar before autoclaving and 200 ppm Vanomycin and 50 ppm Hymexizole were

added after autoclaving. Evaluations for the presence of Phytophthora were made periodically by qualified Plant Pathologists.

## Results and Discussion

### Morphological Responses

The sequence of alterations in foliage morphology of flooded Pyrus rootstocks was generally as follows: a buildup of red pigmentation in the leaves, a reduction in the production of and/or the size of new leaves, stem dieback; and for Pyri, Ussuri and OH x F 97 leaf wilting and browning followed by defoliation. Leaves of Prov Q BA 29 on the other hand, became pale green and chlorotic showing no red pigmentation at all. A minimum of 18 days of flooding was required to initiate alterations in leaf morphology of Prov Q BA 29 and Pyrus species. The appearance of Bet was not noticeably affected until after 1 month of soil submergence. A reduction in subsequent leaf size but not wilting or defoliation was observed for Bet rootstocks even after 20 months of flooding.

Generally, foliage abnormalities were more quickly attained with spring as compared to fall flooding. Flooding induced particularly high anthocyanin pigmentation in leaves of ungrafted Call yet very little occurred in leaves of 'Bartlett' scions on any pear rootstock until a reduction in growth rate was achieved. This phenomenon is likely to make the detection of flooding damage of 'Bartlett' trees in the field very difficult.

Halford peach, MM 106 apple and, to some degree, Prov Q BA 29

rootstocks responded to soil flooding by an acropetal progression of leaf chlorosis and leaf abscission. Drew and Sisworo (12) explained this phenomenon on the basis of decreased nutrient uptake by anaerobic roots and remobilization of N, P and K from the older to the younger leaves of barley (Hordeum vulgaris L.). In the present study, Halford peach was by far the most susceptible to inundation consistent with previous reports (30, 31). Defoliation occurred after 15, 40 and 70 days of flooding for Halford peach, OH x F 97 and MM 106 apple, respectively. About 50% of MM 106 apple and OH x F 97 leafed out the following season but at much reduced levels. Both species experienced massive stem dieback and, in the case of grafted or ungrafted OH x F 97 dieback, was too near the stem base.

Hypertrophied lenticels were particularly abundant on submerged trunks of Bet and Call but occurred to some degree for all tree fruit species after 1 to 3 weeks of submergence. Several investigators have proposed that if significant quantities of $O_2$ are transported from the shoot to the root, the shortest pathlength and the most likely entry point would be the lenticels because of a high internal resistance to gas movement (2, 3, 16, 17, 22, 25, 32). Gaseous absorption of $O_2$ through hypertrophied lenticels is not possible until the water level recedes (2, 3, 9, 17). Alternatively, hypertrophied lenticels may serve as a potential exit for damaging metabolic by-products before reaching aerial plant parts (9).

Willow, Prov Q BA 29 and MM 106 apple developed adventitious roots after 14, 18 and 30 days of soil flooding, respectively. None

cf the Pyrus species manifested this response regardless of the degree of flood tolerance. Several researchers have suggested that adventitious rooting is a stress symptom (15, 27), but others a plant adaptation to flooding (22, 32). Since this and other studies (14, 15, 21, 22, 27) have shown plants of a wide range of tolerance to produce adventitious roots in response to flooding, adventitious rooting does not confer tolerance. Thus, although the production of adventitious roots may temporarily ease the deleterious effects of anoxia, it should not be considered a criterion by which to judge waterlogging tolerance. Adventitious root formation has been shown to be stimulated by ethylene (19). (Results concerning levels of ethylene and 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor are addressed elsewhere (Thesis papers 2, 3; Apps. H, I, J).)

Growth and Survival

One month of fall flooding was sufficient to reduce growth the following season (Fig. 1). Thus, similar to drought conditions, short-term flooding will have a residual effect on subsequent growth. All pear rootstocks except Bart manifested such a response (Fig. 1). P. communis is not considered to be a particularly flood-tolerant pear rootstock (1, 5), and we believe the variance was due to low growth rates for the control. Results of 20 continuous months of flooding indicated that pear rootstocks grew somewhat in height for the first month but negligibly thereafter (Fig. 1). Growth rate reductions proved to be an insensitive method of assessing flood tolerance since all species exhibited curtailed growth when inundated.

Bet was the most, and Pyri and Ussuri were the least flood-tolerant pear rootstocks involved in the first study as evidenced by 100%, 30% and 30% survival, respectively, when flooded 20 consecutive months beginning in the fall (Table 1). Pear rootstocks subsisted for many months in a state characterized by stem dieback and the absence of all but a few small leaves, making an evaluation of death difficult in certain cases. The criteria used for denoting plant death was complete defoliation and an entire blackening of the stem.

Of the pear rootstocks flooded in the spring for 12 months, Bet, Call and Prov Q BA 29 were the most and OH x F 97 was the least tolerant (Table 2). Willow responded most favorably to inundation by manifesting increased growth when flooded. The only striking difference in growth between flooded rootstocks and their grafted counterparts was observed for Call with grafted individuals being superior (Fig. 2). Waterlogging susceptibility, however, is determined by the root and not the shoot (Table 2), in agreement with the work of Rowe and Catlin (31). For instance, OH x F 97 rootstocks, whether grafted with 'Bartlett' or not, were the most susceptible of the 4 major pear rootstocks tested. The transport of $O_2$ from the shoot to the root has been demonstrated for limited distances in several herbaceous and woody species (2, 3, 16), but apparently does not appreciably affect flood tolerance of Citrus (11) or Prunus (31) species. Anatomical observations of cortical tissue of roots and stems of Bet and OH x F 97, whether grown under aerobic or anaerobic conditions did not show any evidence of

aerenchyma (Jose Montano, pers. comm.).

Few researchers involved with waterlogging tolerance have submerged plants for more than one month (14). Baker (4) reported all bottomland hardwood species tested suffered some fatalities after 1 month of flooding. All species tested except green ash (Fraxinus pennsylvanica Marsh.) died back to the root collar. In another study, all bottomland species except black willow (Salix nigra Marsh.) were killed within 1 month of inundation (18). Of the commercial pear rootstocks tested, only OH x F 97 died back to the root collar. When other clones of OH x F were subsequently evaluated, their appearance after 2 months of flooding was similar to OH x F 97 and worse than Bart (App. O). Since Bet did not suffer 1 fatality with 20 months of flooding (Tables 1, 2), this species ranks among the most flood-tolerant of mesophytic tree species.

## Leaf Conductance

A decline in leaf conductance (cL) correlated fairly well with waterlogging susceptibility (Figs. 3, 4). A shorter duration of soil flooding was required to elicit stomatal closure of flood-susceptible Pyrus rootstocks and Halford peach compared to flood-tolerant Bet. In terms of ODR, a significant decline in cL was associated with an ODR of 30, 20 and 15 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ for Halford peach, Ussuri and Bet, respectively (Figs. 3, 4). Soil temperatures measured at midday averaged 20°C during cL measurements in the fall (App. B). An ODR of 20 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ has been correlated with stomatal closure of wheat (Triticum aestivum L.) (34),

and root growth inhibition of numerous herbaceous species (24, 35). Values of ODR of 5 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ recorded after 30 to 40 days of submergence were similar to those measured in a pear orchard with standing water for several months (App. C).

No difference in cL was apparent between control and flooded Prov Q BA 29 after 30 days of fall flooding (Fig. 3) due to low rates for the control, perhaps because of oncoming senescence. The next summer, cL of all species was significantly reduced by flooding except Bet, where only a numerical decline was apparent (Fig. 3). The following year, reductions in transpiration per leaf and per plant were certainly much greater than depicted (Fig. 1), since a plant responses to flooding is a reduction in leaf size and/or leaf number.

To investigate how fruit tree flooding sensitivity is affected by temperature and the stage of development, another experiment was initiated in the spring. Values of ODR roughly resembled the time-course depicted earlier (Fig. 5). Significant reductions in cL were apparent within 5 and 20 days for flooded Halford peach and MM 106 apple rootstocks, respectively, but not until after 30 days for pear rootstocks (Figs. 6-9). Soil temperature averaged 16-23°C during May and June, September and October, and 25-30°C during July and August (App. B). Numerous studies have shown flooding sensitivity to be extremely dependent on temperature and the stage of plant development, with damage being more severe with higher temperature or an active growth stage (5, 8, 10, 30, 31, 33). In the present study, vigorously growing plants flooded in the spring (Figs. 6-9)

took longer to manifest a reduction in cL than plants flooded in the fall (Figs. 3-4) presumably because of the overriding effects of reduced environmental stress during June and/or an increased resistance to flooding by larger plants.

Grafted or ungrafted Bet or Prov Q BA 29, when waterlogged, maintained relatively high levels of cL throughout the summer (Figs. 6-7). After 30 days of soil flooding, leaf stomata of Call rootstocks were almost completely closed, but this was not the case for stomata of 'Bartlett' scions grafted on Call (Fig. 6), suggesting a greater flood-induced sensitivity of stomatal aperture for Call leaves and/or a rootstock-scion interaction. Another example of differential behavior when grafted was observed with OH x F 97 (Fig. 7). Although all individuals of OH x F 97 responded to flooding by complete defoliation, the ungrafted rootstock abscised its leaves a week earlier than 'Bartlett'/OH x F 97 (Fig. 7). This probably relates to poor stomatal control for leaves of OH x F 97, but this itself is secondary to some flood-susceptible characteristic of OH x F 97 roots. The short period of time between a significant decline in cL and complete defoliation necessitates frequent cL measurements, if porometry is to be employed as an indicator of flood tolerance.

Flood tolerance has been associated with maintenance of sufficient leaf turgor without severely restricting stomatal aperture for photosynthesis (23, 27, 28, 29, 32). Another school of thought suggests that stomatal closure may be an adaptation to flooding by preventing nutrient and hormonal imbalances and soil toxins from

reaching the plant top (10). Since reductions in transpiration and photosynthesis were found to parallel one another for flooded 'Stayman Winesap' apple (8), pecan (Carya illinoisesis Wangenh.) (26), cottonwood (Populus deltoides Marsh.) (29) and 4 Citrus species (28), flood-induced stomatal closure does have undesirable consequences.

In the present study, survival data of flooded fruit trees suggest that species which exhibit early and sustained stomatal closure are generally adversely affected more than those exhibiting partial or delayed stomatal closure. Flooded willow generally maintained a greater cL than the control (Fig. 9), which undoubtedly contributed to its improved growth rate when inundated. Our data suggest that early or sustained flood-induced stomatal closure is symptomatic of plant stress. On the other hand, it is conceivable that a plant may avoid damage from short-term flooding by temporary stomatal closure.

Results from this study indicate that Bet is the most flood-tolerant pear rootstock; however, it is also regarded as the most drought-tolerant of all commercial pear rootstocks (1). Similarly, rough lemon (Citrus jambhiri Lush.) has been reported to be the most drought-tolerant (7, 36) and the most flood-tolerant (11, 13, 28) commercial Citrus rootstock based on studies with potted tree and field experiments. The flood-tolerant nature of Bet or Call in this study is consistent with field observations (38); however, the flood tolerance observed for Prov Q BA 29 quince is at issue with one report (39), and in agreement with another (5). The particular

study in disagreement involved 'Bartlett' on Old Home/quince A (OH/Q A) with OH serving as an interstock (39). The present study showed that Prov Q BA 29 either ungrafted or with 'Bartlett' scions survived extended periods of inundation, while OH x F 97 whether grafted or ungrafted was flood-susceptible. When another selection of quince (EMLA C) was assessed stomatal closure occurred earlier (Figs. 7, 8) but, more importantly, survival was similar to Prov Q BA 29 (Apps. E, G).

We were not able to isolate Phytophthora from roots of flooded or nonflooded fruit trees. Pythium was found and judged as a secondary infection. The study which determined 'Bartlett' on OH/Q A to be flood-intolerant apparently did not include a test for Phytophthora (39). Thus, the variance may be explained by the presence of Phytophthora or to the use of an OH interstock.

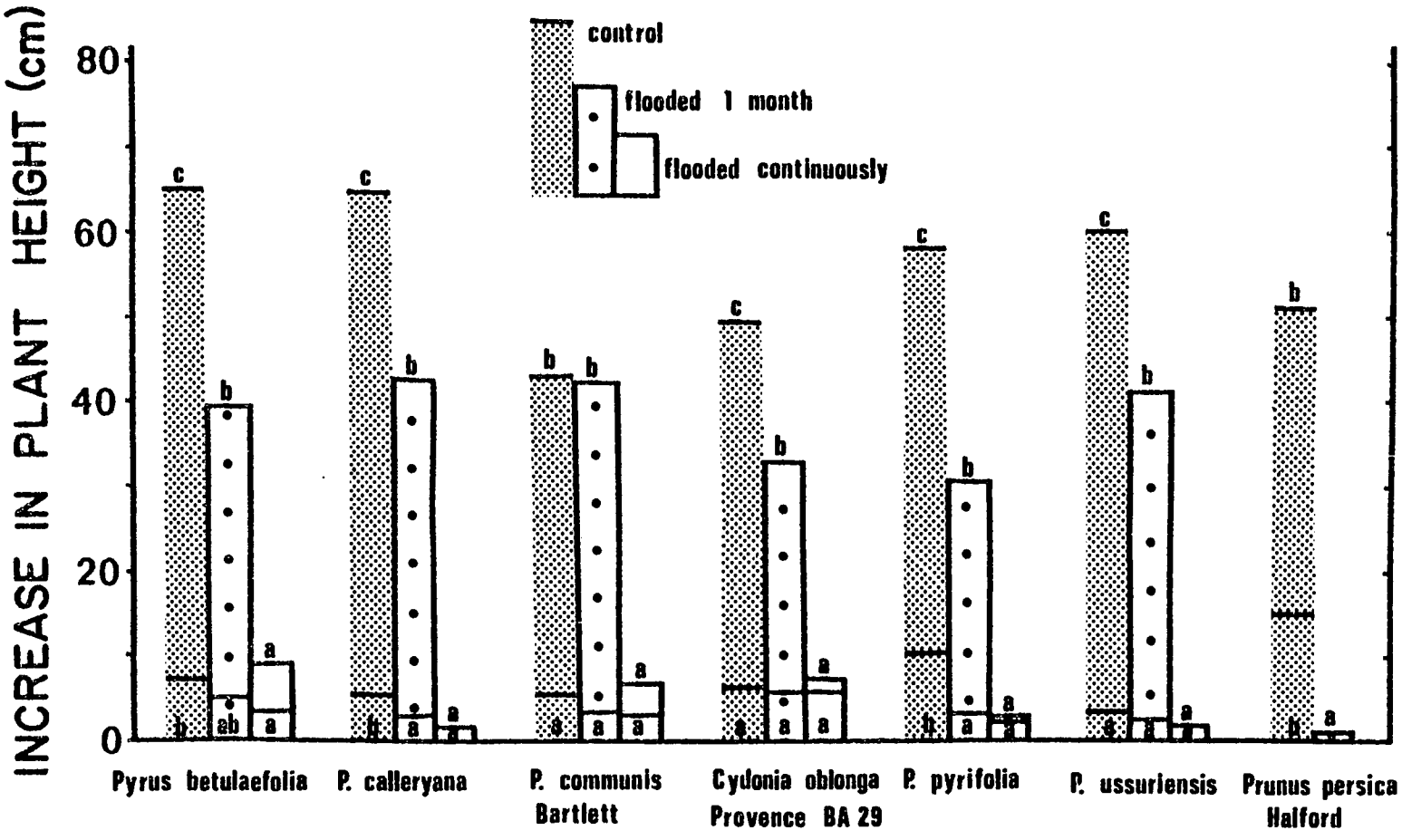


Figure 1. Paper 1 The effect of 1 and 20 continuous months of flooding on growth of ungrafted Pyrus, Prunus and Cydonia rootstocks. Soil flooding was initiated Sept. 8, 1980, and an increase in plant height was determined after 1 month (lower series of horizontal lines), and after 20 months (upper series of horizontal lines). Significance determined for each species on each sampling date separately by Duncan's new multiple range test, 5% level.

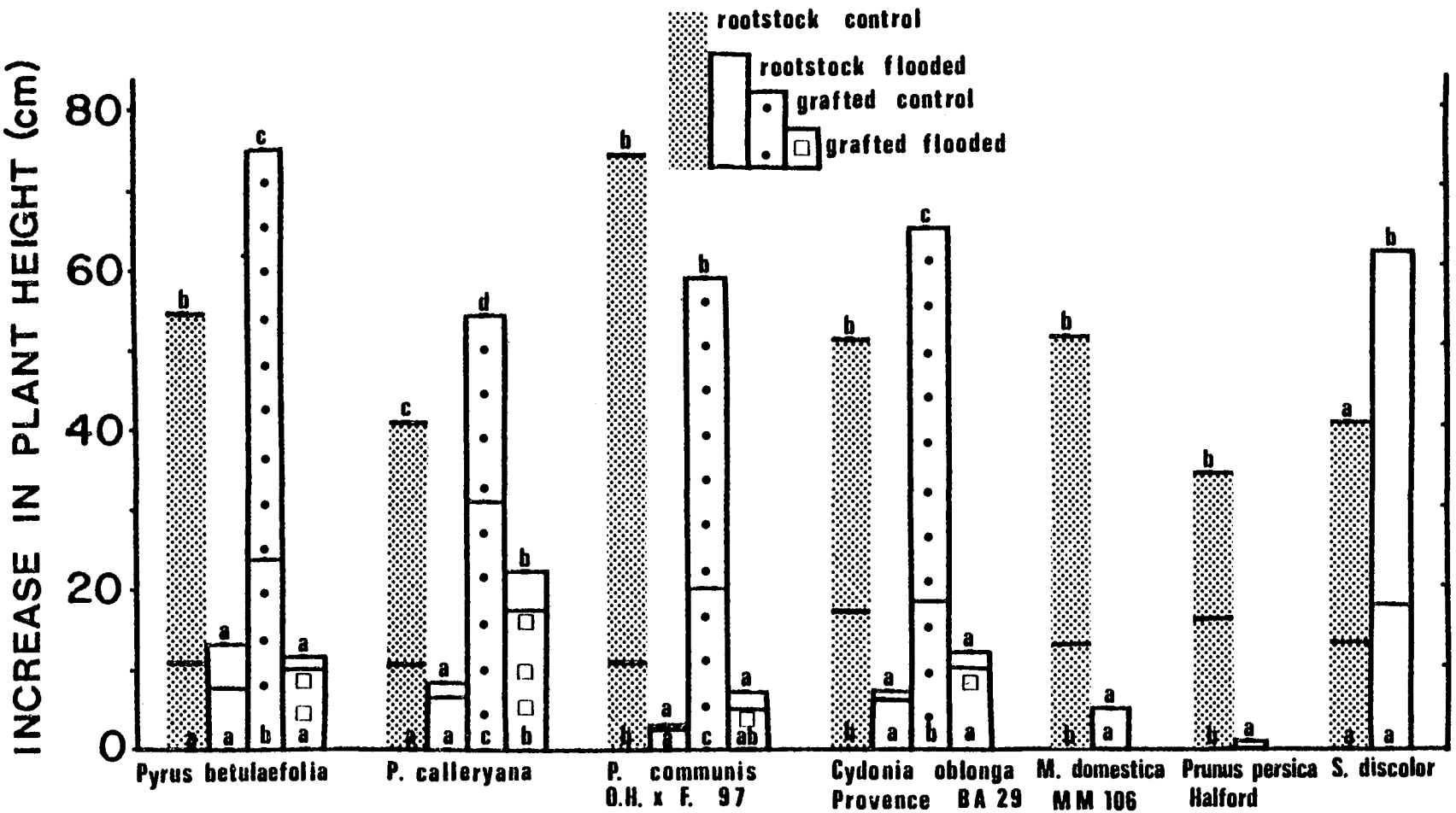


Figure 2. Paper 1 The effect of 1 year of continuous flooding on growth of Pyrus and Cydonia rootstocks with and without a grafted P. communis cv. Bartlett scion, and on ungrafted Malus, Prunus, and Salix. Soil flooding was initiated June 3, 1981, and an increase in plant height was determined after 1 month (lower series of horizontal lines) and after 1 year (upper series of horizontal lines). Significance determined for each species on each sampling date separately by Duncan's new multiple range test, 5% level.

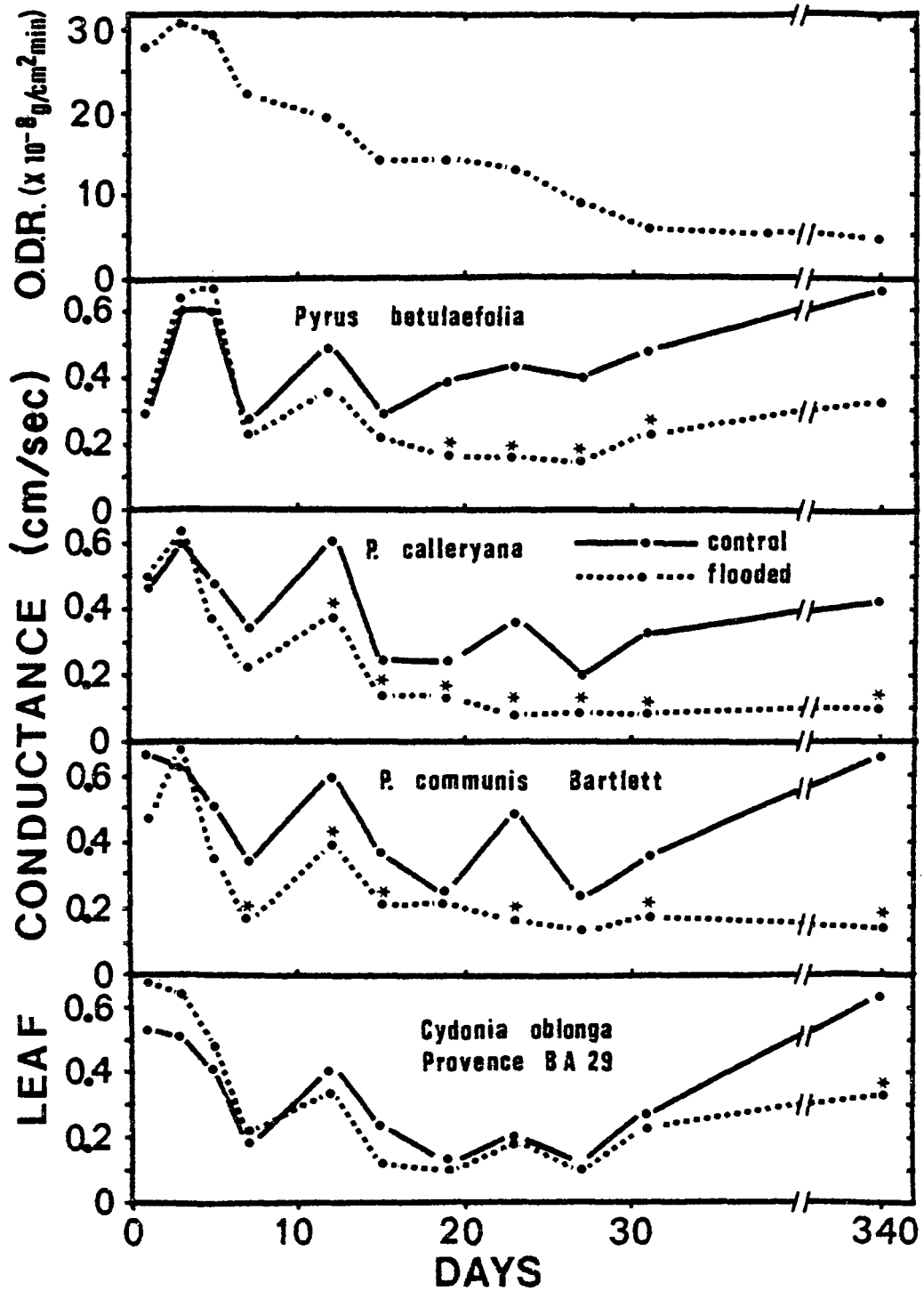


Figure 3. Paper 1 Soil oxygen diffusion rate (ODR) in the flooded soil and leaf conductance of control (unflooded) and flooded ungrafted Pyrus and Cydonia rootstocks. Soil flooding was initiated Sept. 8, 1980. Significant differences (*) between treatments were determined by LSD, 5% level.

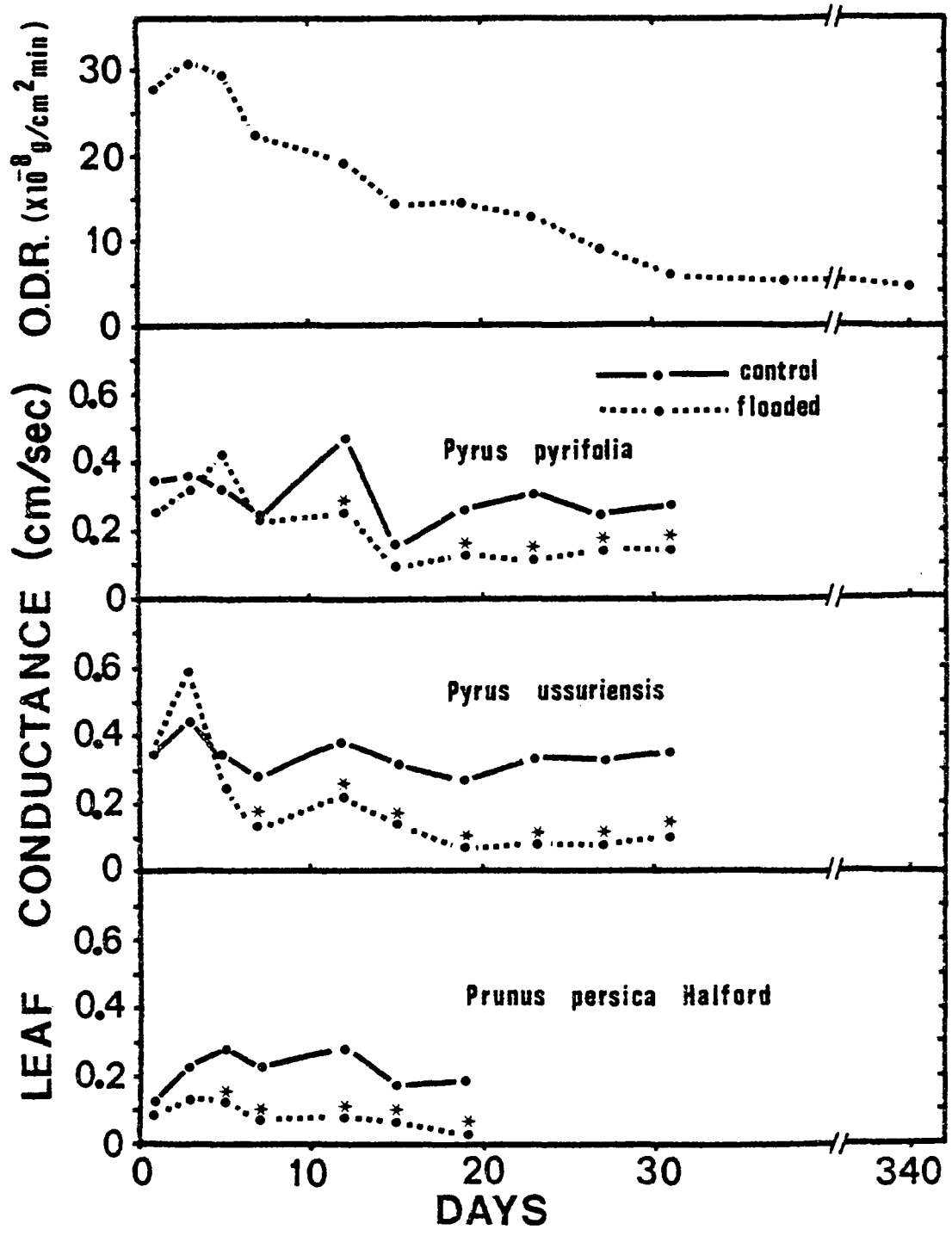


Figure 4. Paper 1 Soil oxygen diffusion rate (ODR) in the flooded soil and leaf conductance of control (unflooded) and flooded ungrafted Pyrus and Prunus rootstocks. Soil flooding was initiated Sept. 8, 1980. Significant differences (*) between treatments determined by LSD, 5% level.

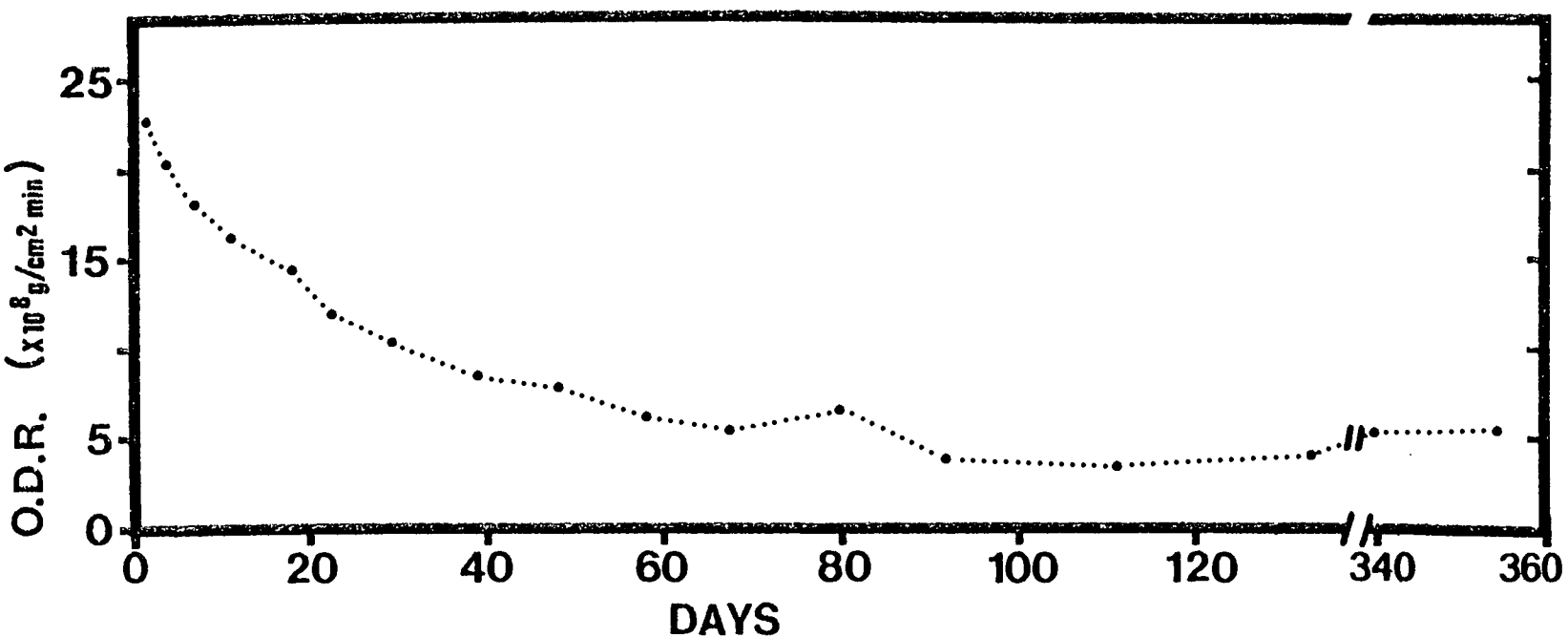


Figure 5. Soil oxygen diffusion rate (ODR) as a function of time after soil submergence. Soil flooding was initiated June 3, 1981.

Paper 1

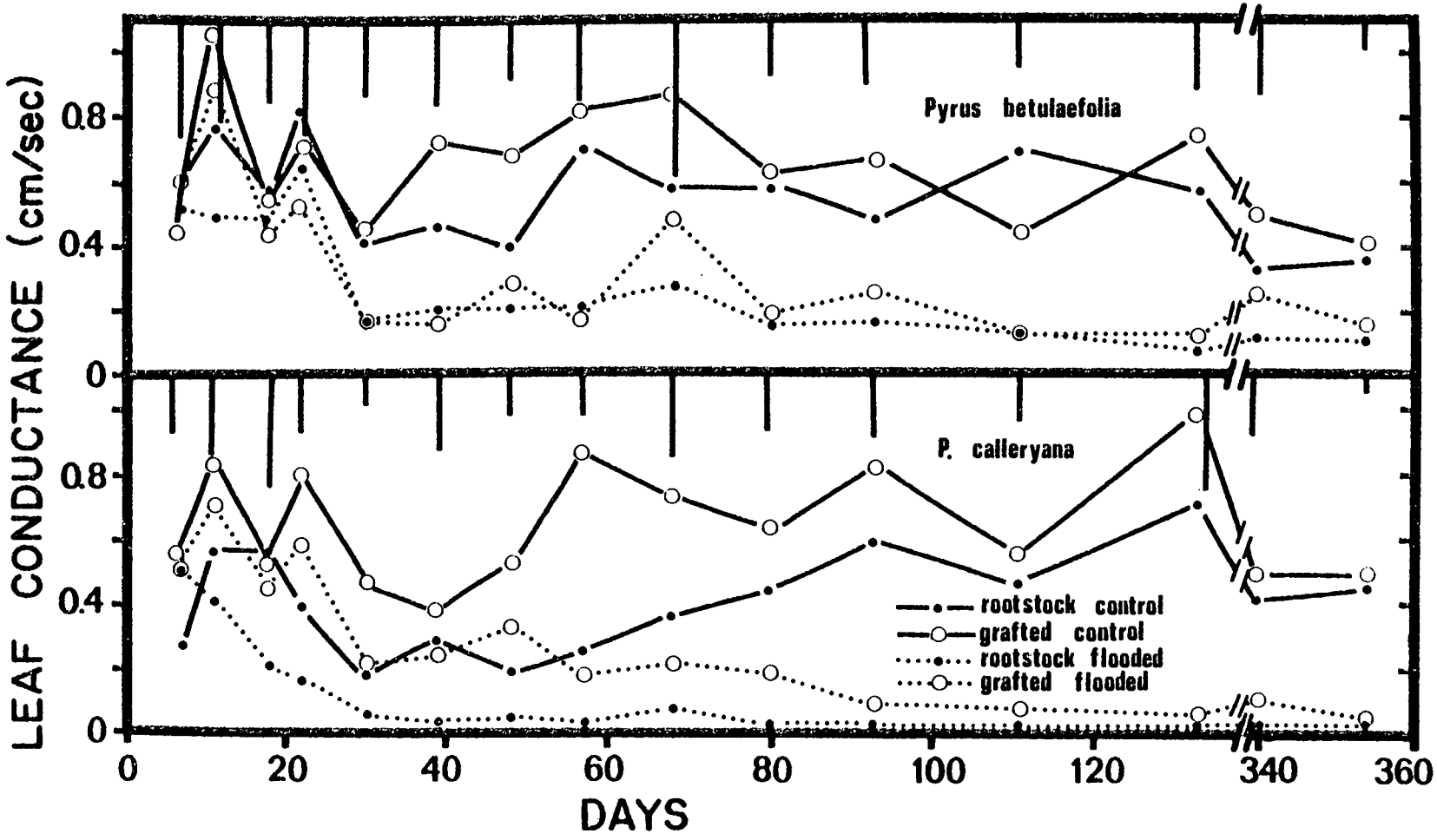


Figure 6. Paper 1 The effect of 1 year of continuous flooding on leaf conductance of P. betulaefolia and P. calleryana rootstocks with and without a grafted P. communis cv. Bartlett scion. Soil flooding was initiated June 3, 1981. Confidence intervals above each sampling date determined by HSD, 5% level.

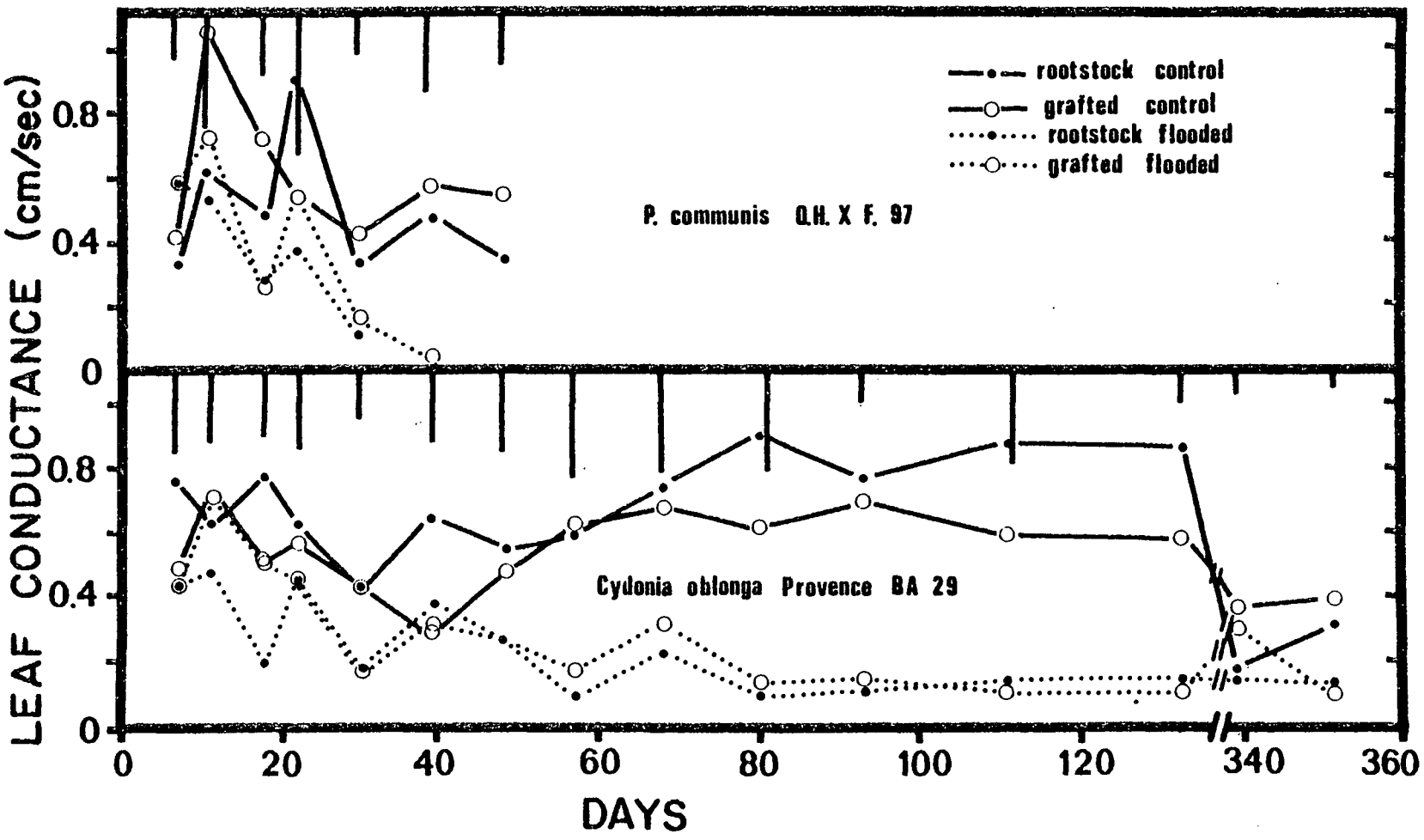


Figure 7. Paper 1 The effect of 1 year of continuous flooding on leaf conductance of P. communis cv. OH X F 97 and Cydonia oblonga cv. Provence BA 29 rootstocks with and without a grafted P. communis cv. Bartlett scion. Soil flooding was initiated June 3, 1981. Confidence intervals above each sampling date determined by HSD, 5% level.

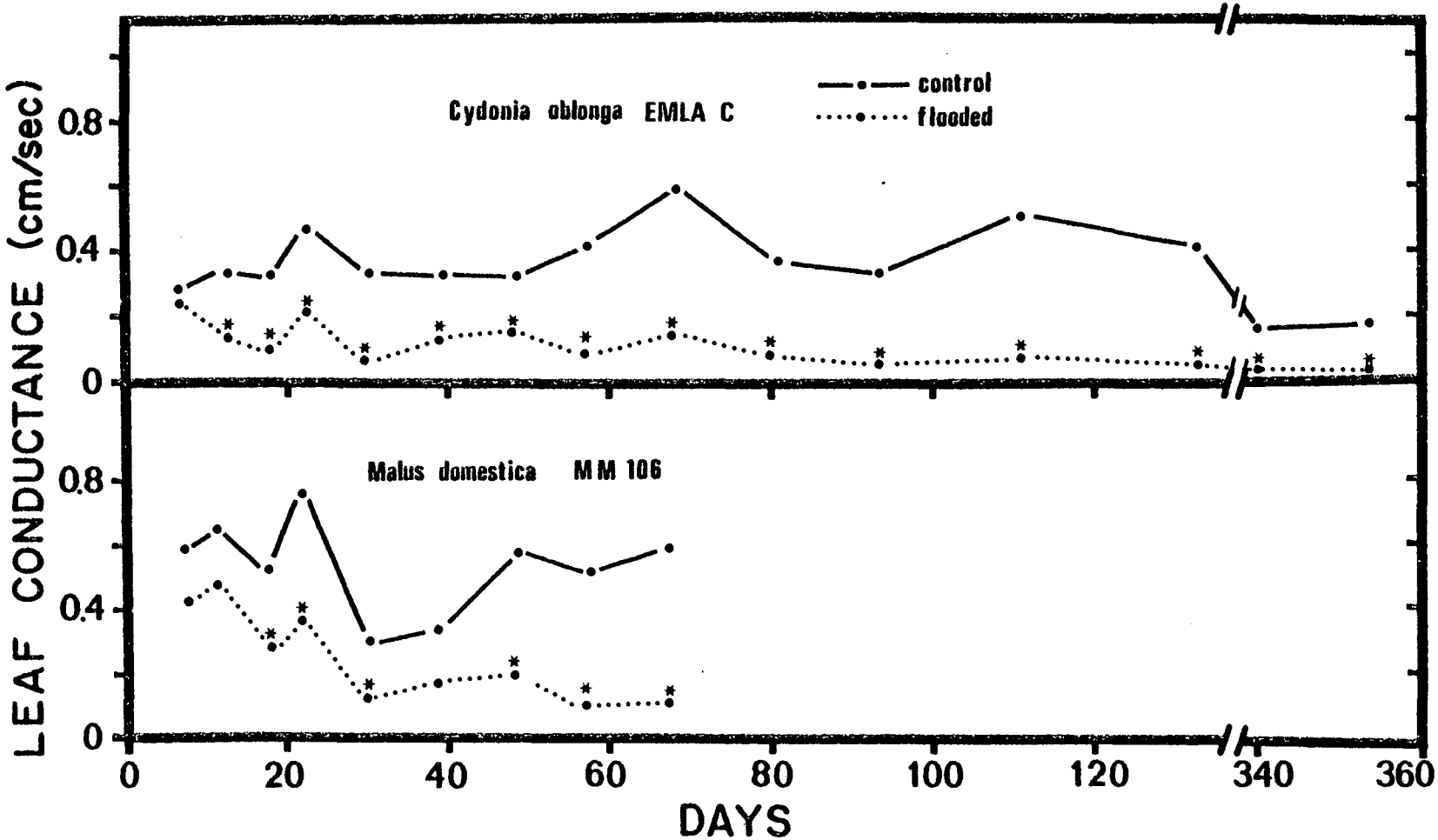


Figure 8. The effect of 1 year of continuous flooding on leaf conductance of ungrafted Cydonia oblonga cv. EMLA C and Malus domestica cv. MM 106 rootstocks. Soil flooding was initiated June 3, 1981. Significant differences (*) between treatments were determined by LSD, 5% level.
Paper 1

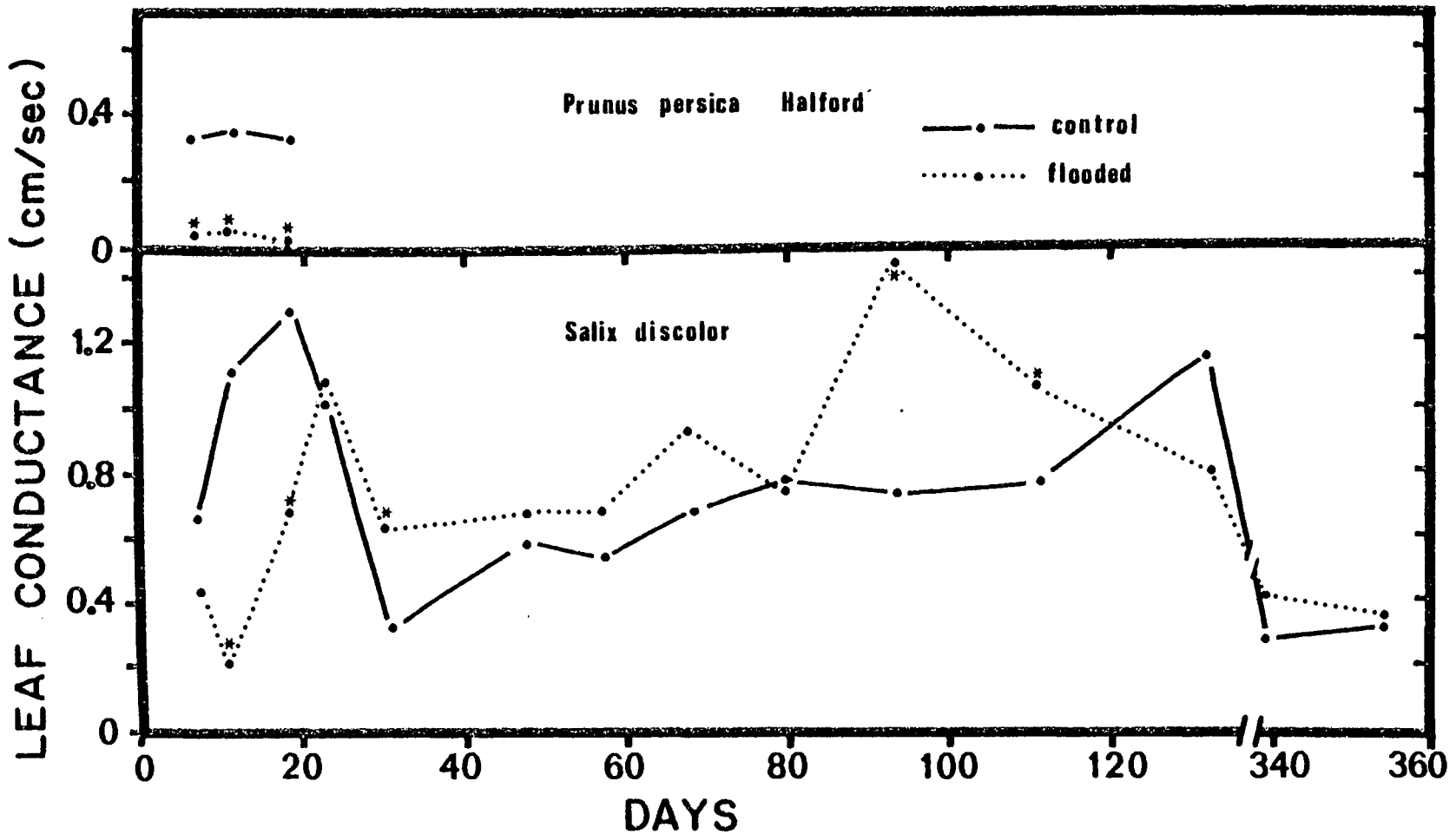


Figure 9. The effect of 1 year of continuous flooding on leaf conductance of ungrafted Prunus persica cv. Halford and Salix discolor. Soil flooding was initiated June 3, 1981. Significant differences (*) between treatments were determined by LSD, 5% level.

Paper 1

Table 1. Paper 1 Percent plant survival of ungrafted Pyrus, Cydonia and Prunus rootstocks subjected to 1 and 20 months of continuous flooding beginning Sept. 8, 1980. Evaluations of plant survival were after 1, 8, and 20 months of treatment.

| | % Survival | | |
|---|---|---|---|
| Species | 1 Month | 8 Months | 20 Months |
| Pyrus betulaefolia | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 100 | 100 |
| Pyrus calleryana | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 90 | 60 |
| Pyrus communis | | | |
| Bartlett | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 90 | 70 |
| Cydonia oblonga | | | |
| Provence BA 29 | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 90 | 70 |
| Pyrus pyrifolia | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 70 | 30 |
| Pyrus ussuriensis | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 70 | 30 |
| Prunus persica | | | |
| Halford | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 0 | 0 | 0 |

Table 2. Paper 1 Percent plant survival of *Pyrus* and *Cydonia* rootstocks with and without a grafted *P. communis* cv. Bartlett scion and of ungrafted *Malus*, *Prunus* and *Salix* species subjected to 1 year of continuous flooding. Soil flooding was initiated June 3, 1981 and evaluations were after 1, 2 and 12 months of treatment.

| | % Survival | | |
|---|---|---|---|
| Species | 1 Month | 2 Months | 12 Months |
| *Pyrus betulaefolia* | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 100 | 100 |
| Grafted control | 100 | 100 | 100 |
| Grafted flooded | 100 | 100 | 100 |
| *Pyrus calleryana* | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 100 | 90 |
| Grafted control | 100 | 100 | 100 |
| Grafted flooded | 100 | 100 | 90 |
| *Pyrus communis* OH X F 97 | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 60 | 40 |
| Grafted control | 100 | 100 | 100 |
| Grafted flooded | 100 | 60 | 50 |
| *Cydonia oblonga* Provence BA 29 | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 100 | 100 |
| Grafted control | 100 | 100 | 100 |
| Grafted flooded | 100 | 100 | 90 |
| *Malus domestica* MM 106 | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 90 | 50 |
| *Prunus persica* Halford | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 0 | 0 | 0 |
| *Salix discolor* | | | |
| Control | 100 | 100 | 100 |
| Flooded | 100 | 100 | 100 |

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# EFFECT OF SOIL FLOODING AND ROOT ANAEROBIOSIS ON ROOT RESPIRATION AND LEAF CONDUCTANCE OF QUINCE, PEACH, WILLOW AND SEVERAL PEAR SPECIES

Additional index words. Soil oxygen diffusion rate, ethylene, Pyrus betulaefolia, Pyrus calleryana, Pyrus communis, Cydonia oblonga, Prunus persica, Salix discolor.

Abstract. Potted plants were submerged in water 5-10 cm above the soil surface during the fall 1980. Feeder roots $\leq$ 1 mm diameter were separated from the soil, wrapped in moist filter paper and placed in sealed vials. Rates of $CO_2$ evolution were recorded after incubation at 22°C. Excised root pieces of all pear rootstock species tested maintained at least 70%, and 50% respiratory capacity when returned to air after 15 and 30 days of soil flooding, respectively. Associated values of soil oxygen diffusion rate were approximately 15 and $9 \times 10^{-8}$ g $cm^{-2}$ $min^{-1}$ on day 15 and 30, respectively.

Two additional studies were conducted in the greenhouse with plants grown in solution culture. Root anaerobiosis was achieved by supplying the nutrient solutions with $N_2$ gas. Plant roots of the aerobic treatment were provided with air from a compressor. Root respiration and leaf conductance (cL) measurements after various durations of anaerobiosis indicated that cL was generally a more sensitive and consistent indicator of anaerobic stress than maintenance of root respiration. Upon exposure to low (0.5%) $O_2$, respiration rates of pear roots were not greatly inhibited compared to rates in air. Of the pear rootstocks Pyrus betulaefolia Bunge (Bet)

and Cydonia oblonga Mill. cv. Provence BA29 (Prov Q BA 29) were least affected, Pyrus calleryana Decne. (Call) was moderately affected and Pyrus communis L. cv. Old Home x Farmingdale 97 (OH x F 97) was most affected by root anaerobiosis in terms of root appearance, root respiration and cL. Bartlett seedlings of P. communis L. (Bart) were incorporated in only the first study where root respiration in flooded soils was monitored; where it was judged to be moderately tolerant compared to other pear rootstocks. Salix discolor Muhl. (pussy willow) and Prunus persica (L.) Batsch (Halford or Lovell seedlings) were consistently more and less tolerant to anaerobiosis, respectively, than pear rootstocks. Although this work implies some correlation between root respiration and cL, further work is required to delineate the relationship.

## Introduction

Several hypotheses have been forwarded to explain flood tolerance of plants, including an ability for longitudinal transport of $O_2$ from the shoot to the root (1, 5, 12, 13, 15, 17), biochemical modifications (6, 7, 8), production of surface or adventitious roots (13, 15, 24), a reduced production of autotoxic substances produced in the root (23) and tolerance to anaerobic substances produced in the soil (9).

Stomatal closure, one of the earlier plant responses to flooding (19, 24), often occurs within several days after submergence (19, 24, 26). A reduction in root respiration has also been considered to be one of the first plant responses to anaerobiosis (11). Although flooding invariably reduces the transpiration rate of all except hydrophytic species, the exact underlying cause is unknown (19, 24, 26). The requirement of $O_2$ for active nutrient uptake is well documented (3). More recent evidence suggests that the rate of water uptake is linked somehow to rates of root respiration (4, 20).

In terms of root respiration, flood tolerance may be due to a metabolic control over glycolysis (regulation of the Pasteur effect) (6, 7, 8) and an ability to maintain a capacity for aerobic respiration (5, 17) after periods of $O_2$ deprivation. In a study with pear roots, Rowe (21) found a control over the Pasteur effect as evidenced by a short-term increase followed by a reduction in ethanol accumulation after several hours of anaerobiosis.

The objectives are to study the effect of root anaerobiosis on leaf stomatal closure; and to assess the relative merits of a

technique to measure stomatal closure and a reduction in root respiration rates as flood tolerance indicators.

## Materials and Methods

### First Study

The first of three experiments was conducted in a growth chamber with 1-year-old seedlings of Bet, Call, and Bart; 1-year-old clonal cuttings of Prov Q BA 29; and 2-year-old seedlings of Halford peach. All plants were grown outdoors in a 1:1:1 mixture unsterilized soil, sand and peat, until being transferred to the growth chamber October 1, 1979. Plants were exposed to growth chamber conditions of 25°C temperature, 40-60% relative humidity, 14-hour day length and a light intensity of 400 $\mu Em^{-2}sec^{-1}$ 2 months prior to treatment imposition. After random separation into a control and a flooded treatment, plants of the latter group were submerged 4-8 cm above the soil line in large plastic bins. Soil oxygen diffusion rates (ODR) were measured as described elsewhere (16, 24, 26, Thesis paper 1).

Root respiration rates were evaluated on 3 samples per plant with 3-4 replications per species for the flooded and control treatment. Roots less than 2 mm in diameter (feeder roots) were separated from the soil, wrapped in moist filter paper and placed in 38 cc vials covered with a rubber serum stopper. Following several hours of incubation at 22°C, a 1 cc volume of gas from the vials was injected into a Carle Model 331 thermal conductivity gas chromatograph equipped with a molecular sieve 5A and Porapak R column in series. Bacterial respiration was found to be negligible by noting no effect

of pretreatment with 1% chlorox and 0.05 M sodium phosphate buffer. Preliminary experiments also indicated that the rate of $CO_2$ evolution was approximately linear from 0-5 hours after excision (App. K).

Second Study

The second study was conducted with plants grown in solution culture because of difficulties encountered separating roots from the soil in the previous experiment. Two-year-old seedlings of Bet, Call, and Lovell peach; 2-year-old rooted cuttings of OH x F 97, and Prov Q BA 29; and 2-year-old rooted cuttings of pussy willow were included in this experiment. Plants, after being grown in sand for a season, were transferred to the greenhouse and placed in 6 liters of half-strength nutrient solution as modified by Johnson et al. (14). Air temperature in the greenhouse varied between 20° and 27°C and water temperature between 22° and 26°C. A 16-hour photoperiod was achieved with supplemental fluorescent lighting maintained active shoot growth. The study was conducted during the fall of 1980 and, although growth continued, plants involved in this and the other two studies were not as vigorous as plants in the spring.

Nutrient solutions surrounding the roots were enclosed by 2 plastic bags which were firmly attached to the trunk, one inside the other. Air supplied by a compressor was bubbled continuously through air stones via plastic tubing which entered the nutrient solution adjacent to the stem base. All plants were acclimatized for 3 weeks prior to randomly separating plants into 2 treatments: the plants of the control treatment continued to be treated as above

and those of the anaerobic treatment were supplied with $N_2$ gas for 8 continuous hours a day (9:00 a.m. - 5:00 p.m.). Additional nutrient solution was added as needed to maintain the 6-liter capacity. Respiration rates of feeder roots were determined as previously described. In addition, on days root respiration was measured, leaf conductance (cL) was determined with a ventilated diffusion porometer according to the method of Turner and Parlange (28) as discussed previously in the thesis (Thesis paper 1).

Third Study

The third experiment comparing root respiration and cL was performed during March and April 1981 in a similar manner to the second. The major differences being: $N_2$ gas was supplied for 20 minutes of every hour controlled by a time clock hooked to a solenoid valve. P. communis cv. Bartlett scions were grafted on Bet, Call, OH x F 97, and Prov Q BA 29 rootstocks, cL was more extensively monitored, and root respiration rates of excised root pieces were determined in the presence of 21% and 0.5% $O_2$ for both the aerobic and anaerobic treatments. A 0.5% $O_2$ level was obtained by flushing the vials twice one-half hour apart with $N_2$ gas. This procedure resulted in a final $O_2$ concentration of 0.5% ± 0.2% $O_2$.

## Results and Discussion

First Study

Root respiration rates declined for all rootstocks after 15 days of soil submergence but significantly for all Call and Halford

peach (Fig. 1). Bet did not exhibit a significant reduction, even on day 30. Associated values of ODR were 35, 15, and 10 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ on day 1, 15, and 30, respectively (Fig. 2). Generally, root growth is inhibited by values of ODR below 20 x $10^{-8}$ g $cm^{-2}$ $min^{-1}$ (25). The effect of soil flooding on plant morphology of pear rootstocks was generally as discussed elsewhere in this thesis (Thesis paper 1).

The ability of pear rootstocks to maintain a high respiratory capacity after prolonged periods in standing water is not surprising since pear rootstocks are commonly acknowledged to be the most flood-tolerant horticultural fruit tree (22). The waterlogging susceptibility of Prunus species, including peach, has been shown to be correlated with cyanogenic glycoside content of the root, although HCN release iteself is thought to be secondary to anaerobic-induced alterations in membrane integrity and compartmentalization (21, 23).

Second Study

The relatively high tolerance of pear rootstocks in flooded soil initiated two other studies conducted in solution culture. The morphological plant responses in anaerobic solution culture are described elsewhere (Thesis paper 3). Although $O_2$ was certainly curtailed at a faster rate in anaerobic solution culture than would occur in a flooded soil, even 21 days of continuous anaerobiosis was insufficient to significantly reduce the respiratory capacity of Bet, Call, Prov Q BA 29, and willow (Fig. 3). In fact, willow responded to 21 days of anaerobiosis by increasing its respiratory

rate when exposed to air (Fig. 3). In contrast, cL was inhibited to some degree for all plant species at this time (Fig. 3). A significant decline in cL occurred after 7 days of anaerobiosis for OH x F 97, yet a reduced root respiratory capacity only on day 21. However, this does not imply that root respiration rates after 7 days in anaerobic solution culture were not reduced. OH x F 97 has been shown to be more susceptible to waterlogged soil conditions than other pear rootstocks tested (Thesis paper 1). The same field studies (Thesis paper 1) and results from Figures 1 and 3 indicate the tolerance of Bet to anaerobic conditions. As a rootstock, Bet produces a large and vigorous pear tree (29, 31) and it is conceivable that characteristics imparting high vigor also contribute to flood tolerance. However, since control rates of root respiration are similar to other Pyrus species, it is doubtful that high rates of root respiration are indicative of flood tolerance, which is in agreement with prior studies (5, 6, 7).

Recently, much attention has been focused on the role of ethylene in determining plant responses to waterlogging (15). Ethylene was evolved from roots of flooded or unflooded Pyrus species only when vials were immediately sealed after root excision (Apps. H, I). This short-term burst of ethylene (ceasing 30-45 minutes after root excision) probably is due to wounding (16). The absence of leaf epinasty, or other obvious ethylene-induced responses, casts doubt on the significance of ethylene with Pyrus species upon flooding.

Several studies have implicated $O_2$ transport from the shoot to the root as a factor responsible for maintenance of aerobic root

respiration in an anaerobic environment (5, 17). Members of the genus Salix possess wood capable of transporting $O_2$ to the root for limited distances as evidenced by rhizosphere oxidation (18). A preliminary study of aerobic and anaerobic treated roots of Bet and OH x F 97 did not reveal any evidence of aerenchyma (J. Montano, pers. comm.). The limited shoot and root lengths through which $O_2$ may diffuse in an intact plant (1, 12) led Rowe and Beardsell (22) to conclude longitudinal air transport does not contribute to the waterlogging tolerance of quince and pear.

Third Study

In order to investigate root respiration in the presence of very low $O_2$, roots were incubated in both 0.5% and 21% $O_2$ (Fig. 4). When incubated in 21% $O_2$ the respiration rate of aerobically treated roots of all pear rootstocks, except Bet on day 12 and 26 and Prov Q BA 29 on day 26 was considerably reduced compared to anaerobically treated roots. Roots of all pear rootstocks evolved relatively high rates of $CO_2$ when incubated in 0.5% $O_2$. Generally, respiratory rates for anaerobically treated plants were more affected by low (0.5%) $O_2$ than anaerobically treated plants (Fig. 4). Respiration rates for anaerobically treated roots incubated in 21% $O_2$ were generally greater than or equal to rates of aerobically treated roots incubated in 0.5% on day 12, but the reverse was true for all rootstocks except Bet on day 26. The inconsistency between day 12 and 26 is probably related to relatively high respiration rates of aerobically treated roots incubated in 0.5% $O_2$ on day 26 and the

reason for this is unknown.

Rowe (21) found that 9 hours of anaerobiosis at 27°C reduced subsequent $O_2$ uptake of peach and plum (Prunus cerasifera Ehrh.) but not Bart roots. Alcoholic fermentation began when $O_2$ was reduced to 5% for peach and 1% for plum and pear, indicating these latter species possess terminal oxidases with a high Km for $O_2$ (21). The high respiratory capacity at 0.5% $O_2$ suggesting a high affinity for $O_2$ (Fig. 4), is in agreement with this report. (The present study reports rates of $CO_2$ evolution and does not distinguish between aerobic and anaerobic respiration.) Ethanol is produced and accumulated to high levels for some flood susceptible species (6, 7, 8, 10). Pear, peach, and plum roots have been reported to maintain control over the Pasteur effect while exposed to anaerobiosis and also catabolize ethanol when returned to air (20). Thus, although there is no evidence that flood tolerance of pear rootstocks is due to $O_2$ transport from the shoot to the root, it appears that tolerance of pear roots to low $O_2$ may be partially due to regulation of the Pasteur effect and a high affinity for $O_2$. Consistent with the earlier observations of curtailed growth but prolonged survival of pear rootstocks with soil flooding (Thesis paper 1), it has been suggested that anaerobic respiration may be satisfactory for prolonged survival but inadequate for the species to flourish (23).

Leaf conductance (cL) of 'Bartlett' scions on all pear rootstocks were reduced by anaerobiosis (Fig. 5). Call and OH x F 97 were significantly affected on day 12, and Bet and Prov Q BA 29 on day 21. On day 26, differences in cL were lessened because of

cloudy humid weather. Caution must be used in evaluating differential flood tolerance by methods which measure cL since the degree of stomatal aperature is dependent on solar intensity and other atmospheric factors in addition to plant factors. The water conducting capacity of pear roots is known to decline in response to root anaerobiosis (Thesis paper 3). Therefore, a greater reduction in cL may be expected for the anaerobic treatment on days of high vapor pressure deficit between the leaf and the atmosphere.

It has been implied that root respiration (5, 6, 7) and stomatal closure (19, 24) may serve as screening methods for assessing flood tolerance. Results from the experiment (Figs. 3, 5) and earlier field experiments (Thesis paper 1) indicate that inhibition of cL and root respiration are of some usefulness. The procedure for determining root respiration is relatively time-consuming as roots must be separated from the soil, roots must be weighed then incubated for an appropriate time interval. A technique which measures a reduction in water uptake or water loss such as diffusion porometry is more practical and expedient. However, one must be cognizant of atmospheric factors affecting such measurements.

Of the 4 pear rootstocks tested, Bet has consistently performed the best and OH x F 97 the worst under anaerobic conditions. Call and Prov Q BA 29 appear to be slightly less flood-tolerant than Bet. While pear rootstocks are flood-tolerant relative to other fruit trees, they are distinguishable from willow in that they just survive prolonged flooding yet willow flourishes with inundation (Thesis paper 1). Quince has not been recommended for poorly drained

soils (30). This and earlier studies (2, Thesis papers 1, 3) do not support the contention on the basis of survival, cL or root respiration.

More work is required to elucidate the influence of root respiration on water uptake or stomatal behavior. It would be particularly useful to measure both aerobic and anaerobic respiration by evaluating $O_2$ uptake and $CO_2$ evolution. This work suggests some relationship between the intensity of root respiration and cL, but a quantative mathematical approach awaits further research.

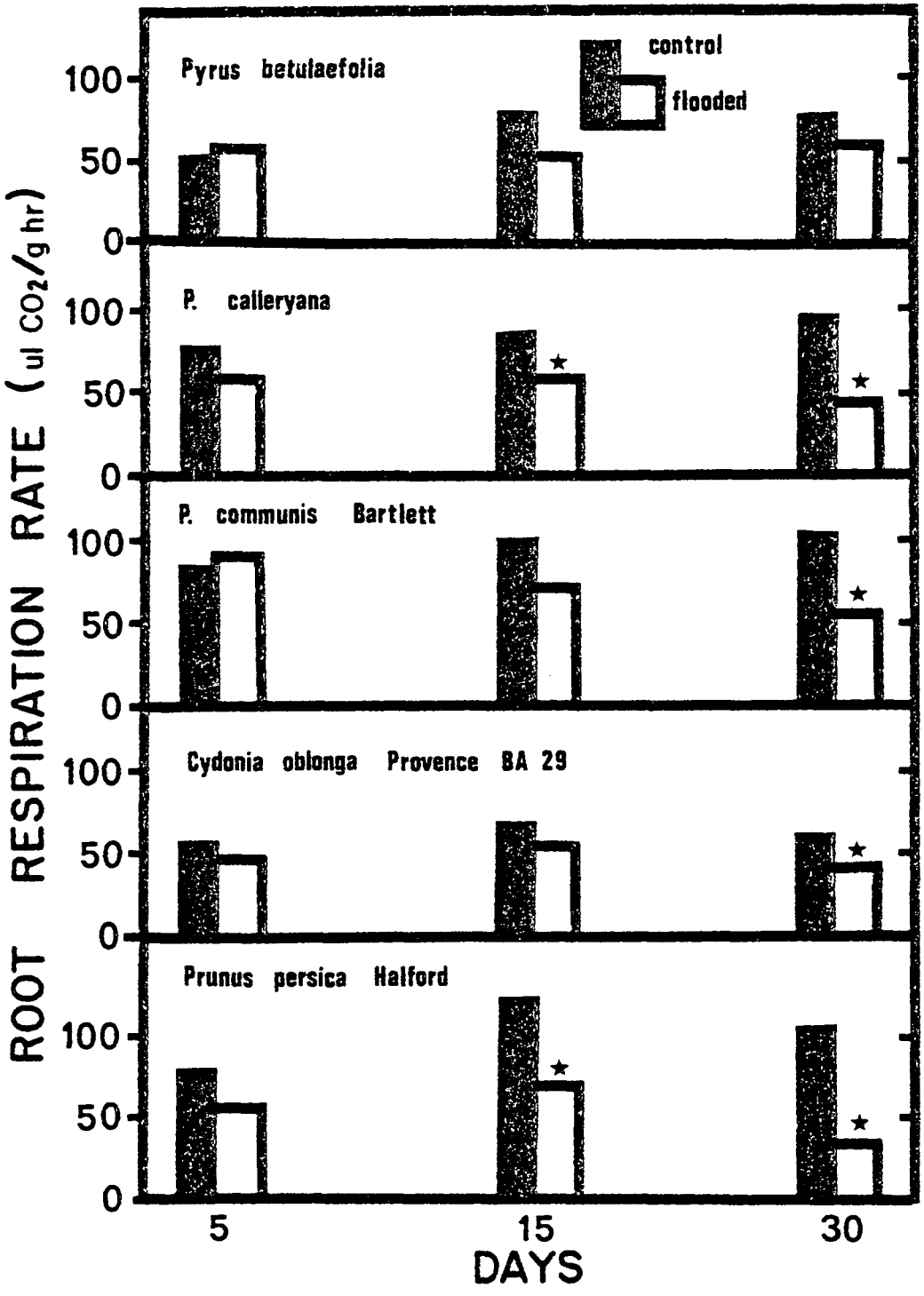


Figure 1. Paper 2 Respiration rates of excised roots of ungrafted Pyrus, Cydonia and Prunus rootstocks as influenced by soil flooding in a growth chamber. Root respiration was determined in 21% $O_2$. Significant differences (*) were calculated for each species on each day separately by LSD, 5% level.

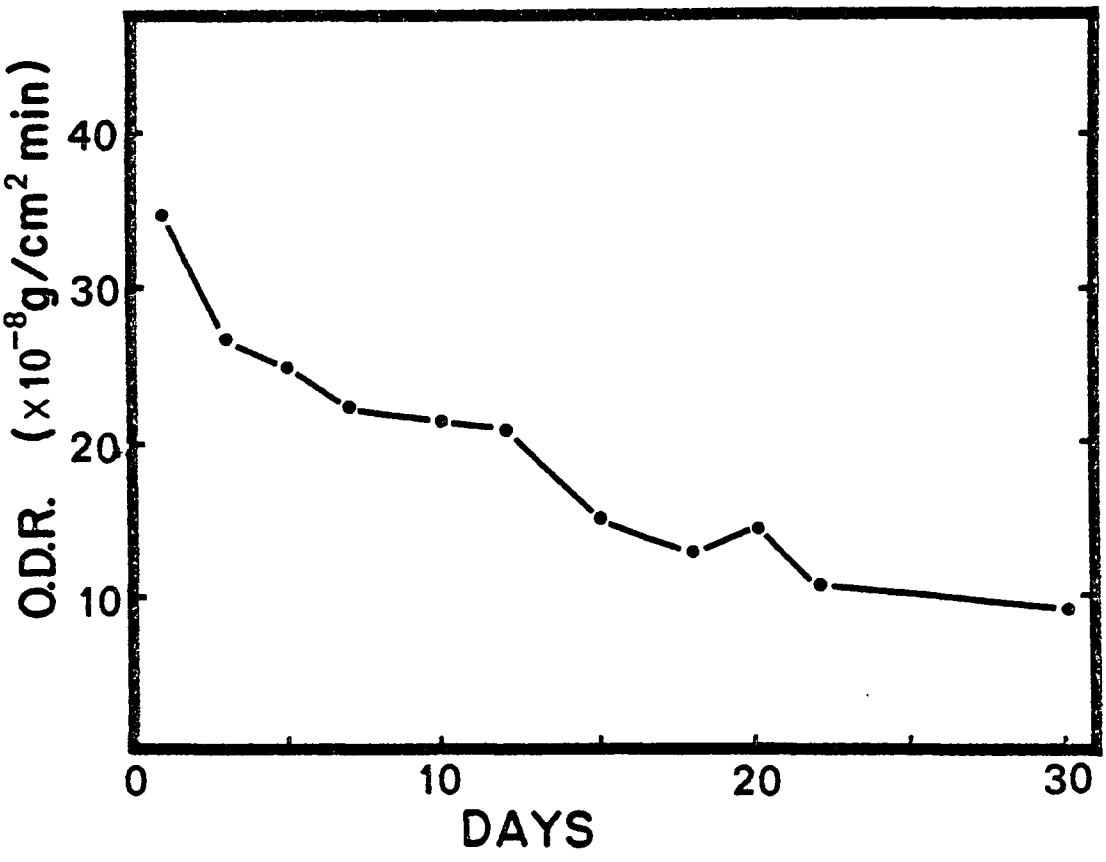


Figure 2. Soil oxygen diffusion rates (ODR) in a flooded soil as a function of time after soil submergence in a growth chamber.
Paper 2

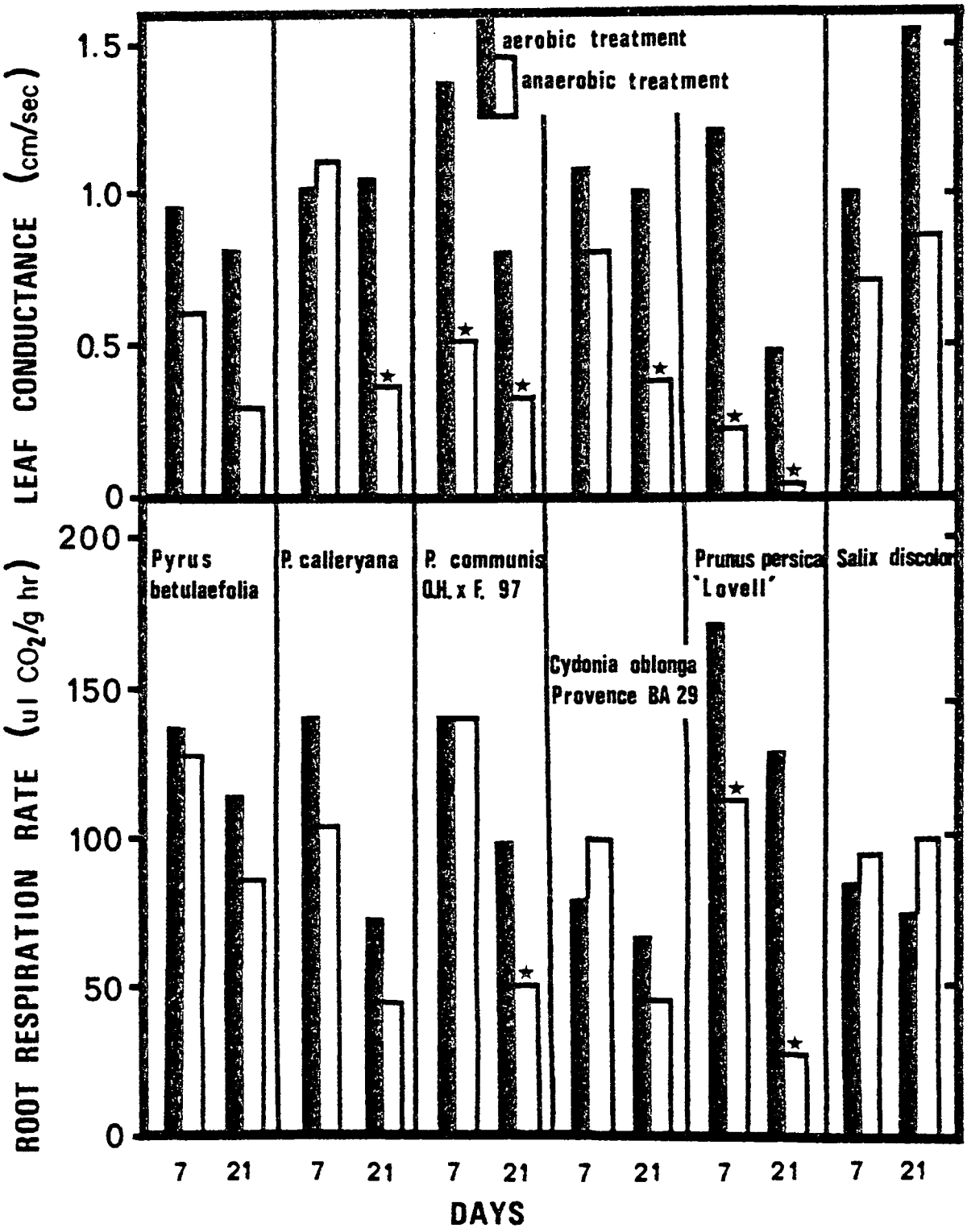


Figure 3. Paper 2 Leaf conductance and root respiration rate of ungrafted Pyrus, Cydonia, Prunus and Salix species 7 and 20 days after treatment imposition. Plants were grown in solution culture in the greenhouse. Root respiration was determined in 21% $O_2$. Significant differences (*) between treatments were determined for each species on each day separately by LSD, 5% level.

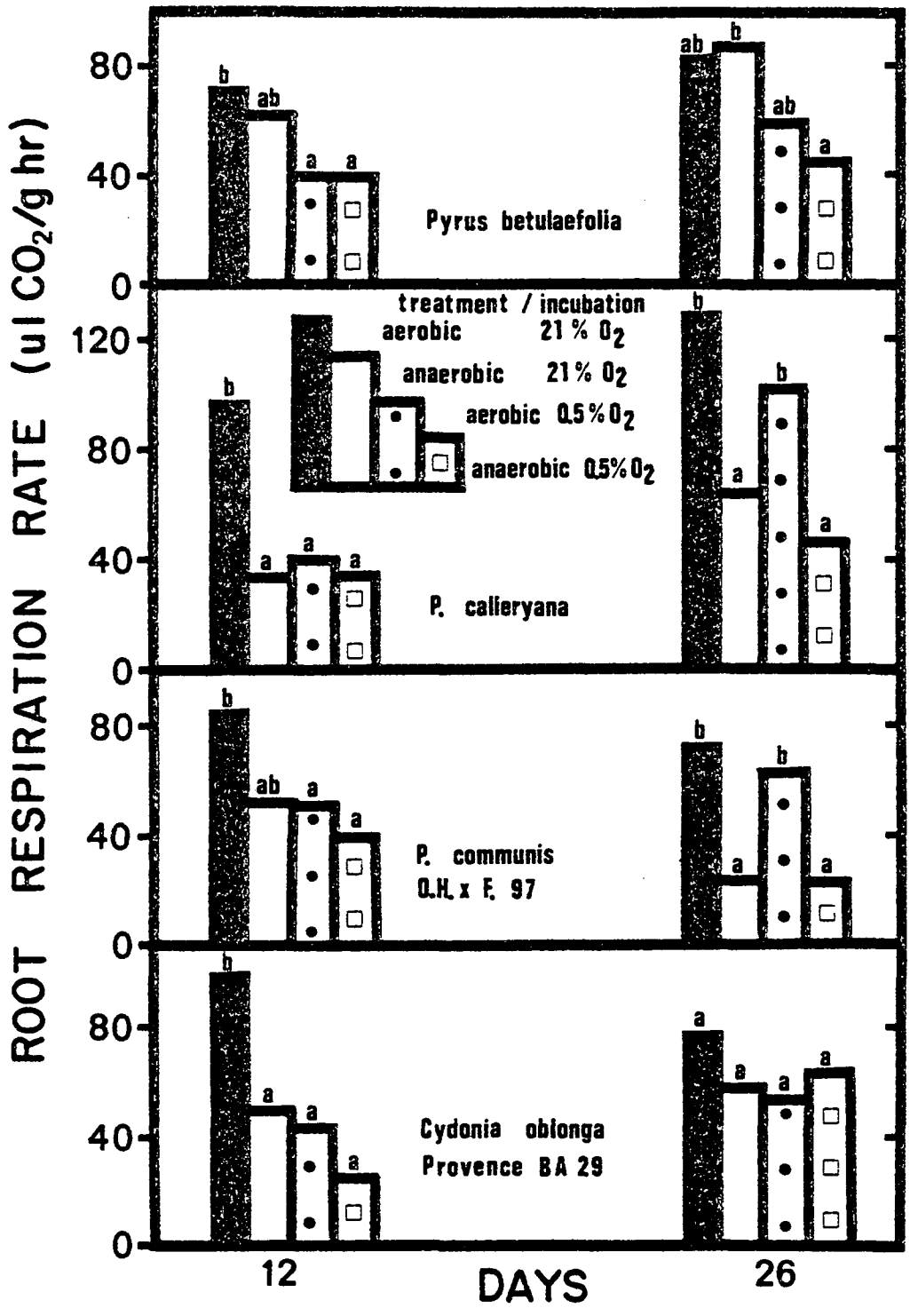


Figure 4. Paper 2 Respiration rates of excised roots of Pyrus and Cydonia rootstocks grafted to P. communis cv. Bartlett scions 12 and 26 days after treatment imposition. Plants were grown in solution culture in the greenhouse. Root respiration was determined in 0.5% and 21% $O_2$. Significant differences between treatments were determined for each species on each day separately by Duncan's new multiple range test, 5% level.

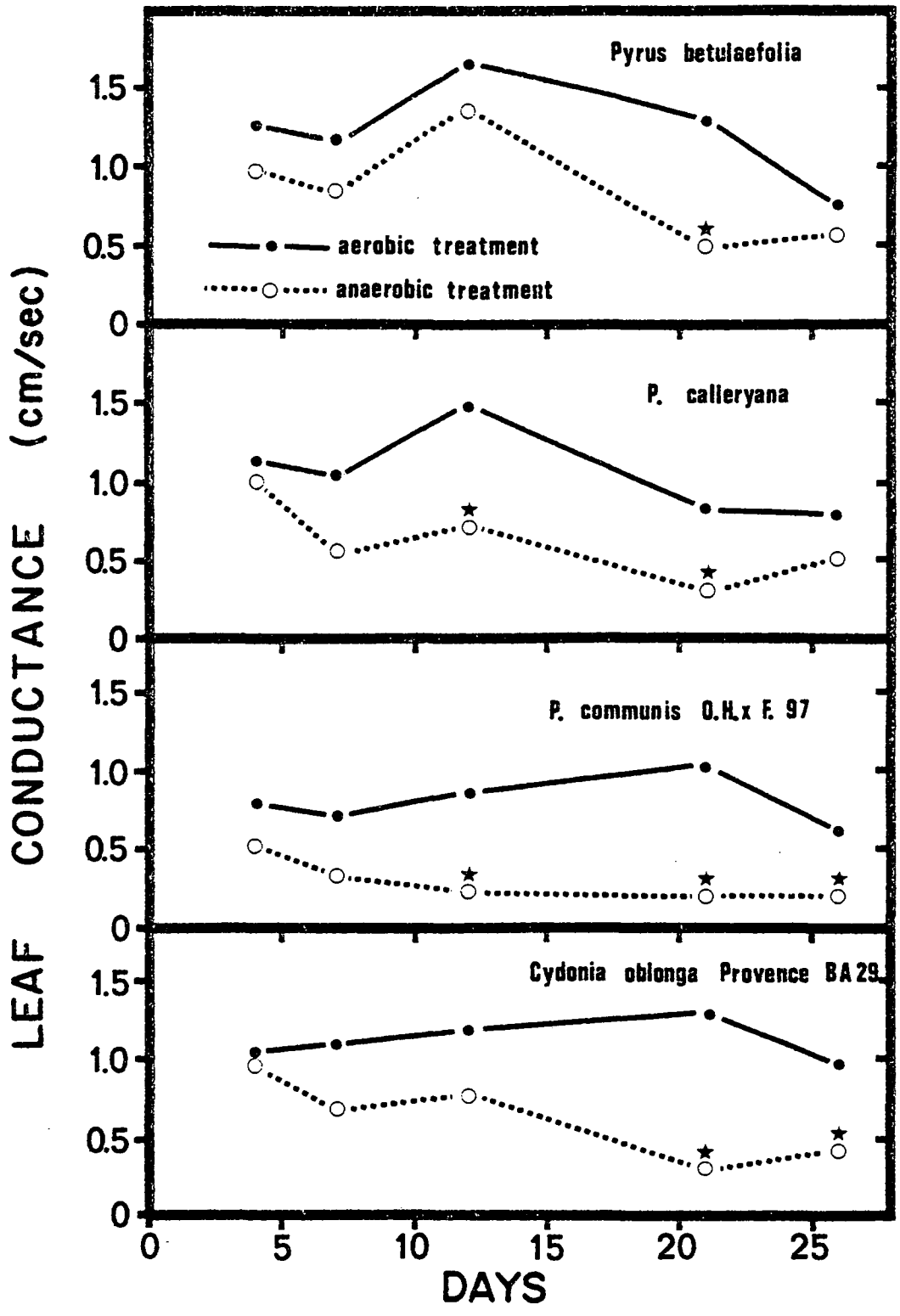


Figure 5. Leaf conductance of P. communis cv. Bartlett scions on Pyrus and Cydonia rootstocks grown in solution culture in the greenhouse. Significant differences (*) were determined for each species on each day separately by LSD, 5% level.

Paper 2

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# EFFECT OF ROOT ANAEROBIOSIS ON THE WATER RELATIONS OS QUINCE, PEACH, WILLOW AND SEVERAL PEAR SPECIES

Additional index words. Leaf conductance, leaf water potential, root osmotic potential, root hydraulic conductivity, abscisic acid, Pyrus betulaefolia, Pyrus calleryana, Pyrus communis, Cydonia oblonga, Prunus persica, Salix discolor.

Abstract. The water relations of aerobically and anaerobically treated plants grown in solution culture were compared in several greenhouse experiments. An anaerobic condition was achieved by bubbling $N_2$ gas around plant roots in a nutrient solution surrounded by 2 plastic bags. Plants of the aerobic treatment were supplied with air from a compressor.

Studies conducted in the fall 1981, incorporating measurements of leaf conductance (cL), leaf water potential ($\Psi$LW) and root osmotic potential ($\Psi R\pi$), revealed that midday stomatal closure occurred prior to alterations in $\Psi$LW or $\Psi R\pi$. In contrast, stomatal closure was accompanied by a decline in root hydraulic conductivity (Lp). Anoxia resulted in a marked decline in $\Psi$LW for Prunus persica (L.) Batsch (Halford peach) and Pyrus communis L. cv. Old Home x Farmingdale 97 (OH x F 97) and eventually led to wilting and defoliation. Wilting was not observed for Pyrus betulaefolia Bunge (Bet), Pyrus calleryana Decne (Call), Cydonia oblonga Mill. cv. Provence BA 29 (Prov Q BA 29), and Salix discolor Muhl. (pussy willow) for the entire 30- to 40-day period of experimentation.

The last series of experiments were performed in the spring of 1982 with plants in an extremely vigorous stage of growth. Susceptibility of OH x F 97 to anaerobiosis was much greater under these circumstances, with a decline in cL and Lp recorded after only 4 days. After 20 days of anaerobiosis, root systems of OH x F 97 completely lost the ability to conduct water with 0.50 MPa applied pressure. Root systems of flood-tolerant Bet maintained a relatively high Lp after being deprived of $O_2$ for 20 days.

To delineate whether the increased root resistance was in the radial or longitudinal direction, $10^{-4}$ M cis-trans abscisic acid (ABA) was added to detopped root systems of OH x F 97 in solution culture after steady-state rates of Lp were established. A consistent 25 to 30% promotion of Lp was observed 1½ hours after the addition of ABA for aerobically treated plants. ABA did not intensify Lp when applied to roots previously deprived of $O_2$ for 4 days. Additional evidence against the limiting resistance being in the symplasm (or in the radial direction) was obtained when Lp of intact OH x F 97 roots, roots with all feeder roots detached, and stems without root systems were compared. Severing feeder roots from anaerobically treated plants only partially revived Lp to rates observed for aerobically treated plants. Resistance to water flow progressed basipetally to eventually encompass the stem itself. These results can only be explained by occlusion of the xylem vessels. It is concluded that stomatal closure offsets an anaerobic reduction in Lp, thus serving an ameliorating effect by preventing a loss in plant turgor or a decline in $\Psi_{LW}$.

## Introduction

Root submergence or root anaerobiosis results in stomatal closure of numerous herbaceous and woody plant species (4, 11, 19, 28, 39). Water flow through root systems treated with respiratory inhibitors (potassium cyanide [KCN], carbonyl cyanide m-chlorophenyl hydrazone [CCCP]), is reduced and it has been suggested that root permeability to water is somehow linked to respiratory metabolism (1, 30). The water conducting ability of tomato (Lycopersicon esculentum Mill.) (4, 19, 21) and tobacco (Nicotiana tabacum L.) (22) was found to decline in response to flooding. A reduced capacity for water flow through roots of herbaceous plants deprived of $O_2$ has been considered to be only a partial explanation for flood-induced stomatal closure (4, 28, 39).

Stomata of flooded plants do not close as a result of a leaf water deficit (4, 28, 39), in contrast to a plant response to drought stress. For instance, Periera and Kozlowski (28), working with several woody angiosperms, found an early decrease in cL but no decrease in $\Psi_{LW}$ for the entire 37 days of flooding. Stomata of waterlogged plants still show diurnal cycling but with a somewhat reduced amplitude (4, 28). Numerous theories may be invoked to explain an anaerobic-induced stomatal closure in the absence of water stress, including a buildup of abscisic acid (14, 34), a decline in the root to shoot supply of K (27, 39), or cytokinins (5, 31) and possibly nutrient imbalances due to a loss of membrane selectivity (33), or a reduced transport of root-supplied gibberellins (35).

Previous studies have shown pear rootstocks to be extremely flood tolerant, with certain species such as Bet exhibiting 100% survival after 20 months of continuous soil flooding (Thesis paper 1). Halford peach (37)(Thesis paper 1) and OH x F 97 (Thesis paper 1) are extremely and moderately susceptible to flooding, respectively.

The objective of this study is to determine the influence of several water status parameters: $\Psi_{LW}$, $\Psi_{R\pi}$, and Lp on flood-induced stomatal closure of various flood-tolerant and intolerant plant species.

## Materials and Methods

Two-year-old seedlings of Bet, Call, and Halford peach; 1-year-old clonal cuttings of OH x F 97; and 1-year-old cuttings of willow were included in two greenhouse studies conducted during late summer and fall of 1980. One-year-old clonal cuttings of Prov Q BA 29 were included in 1 study conducted in the fall of 1980. Plants were grown in sand prior to acclimatization for 3 weeks in 6 liters of half-strength nutrient solution as modified by Johnson et al. (20). Compressed air was supplied continuously via an aquarium-type air-stone. Additional quantities of nutrient solution were added as needed to maintain the 6-liter capacity. Plants were randomly separated into 2 treatments: the aerobic (control) treatment as indicated above and an anaerobic treatment in which $N_2$ gas was supplied instead of air. A time clock associated with a liquid $N_2$ gas pack allowed for a controlled release of $N_2$ gas for 20 minutes at hourly intervals. Two plastic bags, one inside the other,

surrounding the nutrient solution were tightly secured to the stem base and the tubing supplying the gas. Air temperature varied between 20° and 28°C but water temperature held fairly steady at 24 ± 2°C. Growth was maintained with a 16-hour photoperiod provided by supplemental fluorescent lighting.

Leaf conductance (cL), leaf water potential ($\Psi_{LW}$), and root osmotic potential ($\Psi_{R\pi}$) were measured in a second study (October and November). A ventilated diffusion porometer (40) (Thesis paper 1) and a pressure chamber apparatus (3) were employed to determine cL and $\Psi_{LW}$, respectively, on recently matured fully expanded leaves. Both cL and $\Psi_{LW}$ were determined on three leaves per tree and 3 or 4 trees per treatment at midday (between 11:00 a.m. and 3:00 p.m.) for each species.

After cL and $\Psi_{LW}$ were measured, $\Psi_{R\pi}$ was estimated on the same plants in the following manner: approximately 5 grams of feeder roots were excised from the plant, blotted dry, then frozen at -60°C. Several weeks later, root samples were thawed to room temperature and ground thoroughly with a mortar and pestle. The liquid slurry was absorbed on a filter paper disc and placed in a sample chamber of a Wescor HR 33 thermocouple psychrometer. A microvolt reading was converted to $\Psi_{R\pi}$ by the use of KCl standards (6). A minimum of 2 repeatable $\Psi_{R\pi}$ measurements were performed for each plant.

A second experiment initiated October 1980 incorporated measurements of cL and Lp. Lp was determined with the aid of a modified pressure chamber apparatus (15, 25, 32). Plants were severed approximately 15 cm up the stem, and intact root systems were placed

in the chamber filled with nutrient solution. The chamber was sealed with the severed stem protruding through a rubber gasket and compressed air was increased at approximately 0.1 MPa/min to 0.275 MPa. After 2 minutes of exposure to 0.275 MPa pressure, tygon tubing was attached to the severed stem. Exudate volume was measured with a calibrated syringe after a 10-minute collection period. Root systems were then oven-dried and subsequently divided into two classes: those $\leq$ 1 mm diameter and those $\geq$ 1 mm diameter.

Lp ($\mu l\ cm^2\ MPa^{-1}\ min^{-1}$) is related to total exudation rate (Jv) ($\mu l\ cm^{-2}\ min^{-1}$) by the equation:

$$Jv = Lp\ (\Delta P - \sigma\Delta\pi) \qquad \text{Eq. \#1}$$

where $\Delta P$ is equal to the hydrostatic pressure driving force (MPa), $\sigma$ is the dimensionless reflection coefficient ($0 \leq \sigma \leq 1$) and $\Delta\pi$ is the osmotic gradient between the ambient solution and the xylem fluid (15). Since the applied pressure $\Delta P$ is much greater than $\sigma\Delta\pi$ the latter term was not used in the calculation of Lp. In this study Lp was expressed on the basis of g roots on a dry weight basis $\leq$ 1 mm diamter. The temperature of the pressure chamber was maintained at 25 ± 0.5°C with the use of a water bath.

The last study was conducted during the spring of 1982 with Bet seedlings and clonal cuttings of OH x F 97. Plants used in this study were in an extremely vigorous stage of growth. cL and Lp were measured as described previously with several differences. cL was evaluated on 3 leaves per plant and replicated 6 times per treatment. Lp was measured at a pressure of 0.50 MPa and replicated a

minimum of 4 times per treatment. Also, Lp vas calculated on the basis of g root dry weight $\leq$ 1 mm diameter, stem cross-sectional area 15 cm up from the stem base and leaf area. Leaf area was measured with a Licor Model 3100 leaf area meter. In addition, Lp was determined for OH x F 97 with feeder roots detached and for stems in the absence of all roots after various durations of anaerobiosis.

The effect of root-applied abscisic acid (ABA) was monitored for individuals of OH x F 97 involved in the last study. A stock solution of ABA was solubilized in a minimum quantity of 95% ethanol. Compressed air was increased at approximately 0.10 MPa/min to an applied pressure of 0.50 MPa inside the pressure chamber apparatus. After establishing steady-state rates of Lp after 30 minutes, ABA was added to plants in the pressure chamber to obtain a final concentration of $10^{-4}$ M ABA and 0.13% ethanol. Lp was recorded over 10-minute intervals for 130 minutes after the addition of ABA. A control treatment consisted of an application of 0.13% ethanol without ABA.

## Results

### Morphological Observations

The general sequence of morphological responses to anaerobiosis was a browning of the root apices accompanied by a reduction in root and shoot growth. Browning of OH x F 97 and Halford peach roots proceeded in basipetal direction to encompass whole root segments and eventually entire root systems. Results of the first study

conducted in late summer and fall of 1981 will be discussed first.

Tip browning and entire root browning occurred between 2 to 5 days and 5 to 12 days, respectively, for peach and between 6 to 12 days and 10 to 20 days, respectively, for OH x F 97. Roots of Bet and willow maintained a healthier appearance--generally off-white in color after 30 days and tip browning occurred after 20-30 days for certain individuals. Root appearance of Call and Prov Q BA 29 were less affected by anaerobiosis than OH x F 97, but more than Bet. Hypertrophied lenticels and adventitious rooting were more pronounced for the aerobic treatment. Hypertrophied lenticels occurred for all pear rootstocks after 10 to 15 days but not to the degree observed with plants flooded in soil (Thesis paper 1). With 15 to 20 days of anaerobiosis, Prov Q BA 29 and willow produced adventitious roots and then only in very limited quantities relative to the observed situation in flooded soil (Thesis paper 1). Adventitious rooting for anaerobically treated Prov Q BA 29 and willow was restricted to the small space in between the 2 plastic bags separating the anaerobic solution culture from the atmosphere.

Wilting began after 4 to 8 days of anaerobiosis for peach and between 10 and 15 days for OH x F 97. Wilting did not occur for the other species for the entire 30- to 40-day period of experimentation. However, leaves of all species manifested a pale green appearance after varying periods of anaerobiosis.

Plants of the third study (Table 1) were in a more active growth stage. After 4 days of anaerobiosis, root apices of OH x F 97 were brown and after 20 days, root systems were generally completely

brown. Roots of Bet began to show a brown appearance after 20 to 30 days, although some individuals maintained a white appearance after 30 days. Wilting typically began at midday after 5 to 12 days for OH x F 97 and after 20 and 30 days for some of the individuals of Bet.

Water Relations

An anaerobic-induced decline in leaf conductance (cL) preceded changes in either leaf water potential ($\Psi$LW) or root osmotic potential ($\Psi R\pi$), for all species except OH x F 97 and Halford peach (Figs. 1, 2). Nevertheless, both species exhibited a greater numerical decline in cL than the other 2 parameters (Figs. 1, 2), suggesting a similar relationship to that noted above. A concomitant change in $\Psi$LW and root $\Psi R\pi$ (an indicator of cell damage and death) was apparent after 32 days for Bet and Call (Fig. 1). Of the 3 parameters measured for willow, only cL was significantly altered after 32 days of anaerobiosis (Fig. 2).

A much better correlation was obtained when cL was compared to root hydraulic conductivity (Lp), where a parallel decline for both was evident with anaerobiosis (Figs. 3, 4). Preliminary experiments revealed that Jv was constant over a 2-hour period and Jv was linearly related to pressure between 0.14 MPa and 0.80 MPa for all species except willow where a much greater Jv was recorded with increasing $\Delta P$ above 0.50 MPa (App. P). In addition, a preliminary study revealed no diurnal cycling of Lp to occur (App. M).

Another experiment relating cL and Lp was performed in the spring with vigorously growing plants (Table 1). OH x F 97 was much more

susceptible to anaerobiosis when compared to the fall studies. In this spring study the 2 types of P. communis (Bartlett seedlings and OH x F 97 cuttings) responded similarly to anaerobiosis in terms of cL (Table 1, App. N). Only 4 days of anaerobiosis were required to reduce cL and Lp whether expressed on a root weight, stem cross-sectional area, or leaf area basis (Table 1). An applied pressure of 0.50 MPa was not sufficient to move fluid through root systems of OH x F 97 after 20 days of treatment. Roots of flood-tolerant Bet, on the other hand, maintained a capacity to conduct water even after 30 days of anaerobiosis, but at reduced levels after day 20 (Table 1).

After 4 days of treatment, $10^{-4}$ M abscisic acid (ABA) applied to root systems of OH x F 97 in aerated nutrient solutions enhanced Lp by approximately 30% but had no effect when applied to anaerobically treated root systems (Fig. 5). The experiment was replicated a minimum of 3 times for each treatment with comparable results, but because of variable initial root Lp a representative plant was selected for each treatment. Abscisic acid (ABA) at $10^{-4}$ M, the highest concentration tested, had the greatest effect (Thesis paper 4). Rates of Lp for the aerobic treatment were stimulated within 10 to 20 minutes and leveled off approximately 1½ hours after the addition of ABA.

To delineate the site of increased root resistance, Lp of intact root systems of OH x F 97 were compared to root systems with feeder roots detached and to stems in the absence of all roots (Table 2). Feeder roots were not the only site of increased resistance, since their removal only partially revived rates of Lp. Resistance to

flow in the xylem vessels progressed increasingly further up the plant with increasing durations of anaerobiosis.

## Discussion

The relative tolerance to anaerobiosis in this solution culture study is consistent with the results of previous experiments conducted with potted plants flooded in soil (Thesis paper 1), although in the present study plants responded more quickly (Figs. 1-4, Table 1). Bet, Call, Prov Q BA 29, and willow are flood-tolerant species as evidenced by 90% or more survival after 12 months of continuous flooding: OH x F 97 is of intermediate tolerance and peach very susceptible (Thesis paper 1). Investigators comparing the deleterious effects of root submergence in soil versus root submergence in water have usually found more severe damage with the former (21, 36). In the present study, by supplying exclusively $N_2$ gas to the roots and by preventing atmospheric diffusion of $O_2$, the effectiveness of $O_2$ distribution by solution convection currents would be negated and this could explain the apparent discrepancy. One may conclude the adverse effects of a rapid elimination of $O_2$ in solution culture were greater than the combined effects of soil related factors and a slow rate of $O_2$ removal. Temperature and the stage of plant development were similar in the two studies.

Many investigators have reported decreased cL or transpiration (4, 11, 19, 28, 29, 34) and photosynthesis (23, 29, 34) to accompany root submergence, however factor(s) responsible for stomatal closure are unresolved (4, 39). In contrast to stomatal behavior of

drought-stressed plants, results from this (Figs. 1, 2) and other studies (4, 19, 28, 39) suggest that anaerobic-induced stomatal closure is not a function of reduced $\Psi_{LW}$. Prolonged anaerobic durations did result in reduced $\Psi_{LW}$ but well after the onset of stomatal closure. Lovell peach and OH x F 97 were the only species to experience a great deal of water stress (< 3.0 MPa) for the 30-day period of experimentation (Figs. 1, 2). Although Bet and Call are flood-tolerant tree species, they may be distinguished from willow since only the latter species did not experience water stress. Pereira and Kozlowski (28) have demonstrated that 37 days of soil flooding did not reduce $\Psi_{LW}$ of several woody angiosperms, but other long-term experiments have shown a reduction in $\Psi_{LW}$ to eventually occur (11, 17).

Plant responses to a lack of soil $O_2$ can vary markedly depending on temperature, the method of achieving anoxia and the stage of plant development. For instance, dormant and actively growing Sitka spruce (Picea sitchensis [Bong.] Carr.) seedlings responded entirely differently to flooding, the former being much more tolerant (11). A somewhat similar situation was observed in the present study comparing growing but non-vigorous rootstocks in late summer and fall (Figs. 1-4) to vigorously growing rootstocks in the spring (Table 1). Again, vigorously growing plants were affected by a lack of root $O_2$ much more quickly.

$\Psi_{LW}$ and $\Psi_{R\pi}$ were affected approximately at the same time, that is, subsequent to a reduction in cL (Figs. 1, 2). $\Psi_{R\pi}$ was included in this study as an indicator of solute leakage, which is a function

of reduced membrane integrity. (We do not intend to invoke $\Psi R\pi$ as a measure of the driving force of water uptake but only wish to provide an estimate of cell damage or death.) A loss of membrane integrity with low $O_2$ has been considered to be responsible for many adverse plant responses to flooding (4, 37). Several workers (1, 30) have reported rapid changes in root membrane permeability to accompany the addition of respiratory inhibitors, suggesting that anaerobic-induced membrane changes could be one of the earliest effects of root anaerobiosis. The extent of our results on the matter only indicates that partial stomatal closure precedes both a significant loss of solute from the root and cell death. Alterations in membrane integrity would certainly occur prior to psychrometrically detectable reductions in $\Psi R\pi$.

The driving force of water uptake may be defined as $(\Delta P-\sigma\Delta\pi)$ from the flow Equation #1 described earlier. It is clear that $\Delta P$ far outweighs $\sigma\Delta\pi$ even under minimal xylem tensions due to the typically dilute nature of xylem fluid. By measuring Jv of detopped plants exposed to an applied presure in a pressure chamber, only the terms Lp and $\sigma\Delta\pi$ are unknown. Several investigators have suggested a need to distinguish whether reduced root water uptake and transport under anaerobiosis is due to a reduction in Lp or $\Delta\Psi\pi$ (7, 36). Results discussed elsewhere indicate that $\Delta\Psi\pi$ is relatively small, and only changes in Lp can account for this phenomenon (Thesis paper 4). The term Lp is of extreme significance in plant water relations since 1/Lp represents the greatest resistance in the soil plant atmosphere continuum (2).

In the present study, the time course of anaerobically treated roots shows that Lp was closely associated with stomatal closure (Figs. 3, 4; Table 1). The data suggest the possibility of a cause-and-effect relationship between Lp and stomatal closure.

Kramer (21) reported that both transpiration and the ability of tomato roots to conduct water declined with flooding, however, he and others also working with tomato (4, 19) have noted a poor cause-and-effect relationship between Lp and short-term stomatal closure. We have found that Lp measurements of tomato after 4 or more days of anaerobiosis are artifactual because of tissue breakdown and stem collapse. Nevertheless the above reports demonstrating a poor cause-and-effect relationship between Lp and cL of tomato after only a day or two of flooding cannot be overlooked. Considerable differences may be expected between the response of susceptible herbaceous and tolerant woody species to flooding. For instance, short-term reductions in stomatal aperture were absent when cL of flooded pear rootstocks was extensively monitored (Thesis paper 1). Also in the present study, leaf epinasty did not occur for any of the woody species, but did for tomato. In addition, 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, was found in detectable quantities in xylem fluid of anaerobically treated tomato but not in xylem fluid of any fruit tree rootstock (App. J).

A pertinent question may be whether the increased resistance to water movement in the roots with anaerobiosis is predominantly in a radial or longitudinal direction. In the present study, 2 lines of evidence suggest the latter. It may be hypothesized that

elevated levels of ABA with anaerobiosis may influence plant water status by affecting root water uptake and by limiting stomatal aperture. We therefore supplied $10^{-4}$ M ABA to the nutrient solution surrounding intact root systems of OH x F 97. A gradual enhancement of Lp with the addition of ABA occurred almost immediately with a maximum response within 1½ hours (Fig. 5).

Specifically, the endodermis with its associated casparian strip has been implicated to offer the greatest resistance to water uptake (8, 9), and is a possible site for ABA to exert an effect. The absence of an ABA response after 4 days of anaerobiosis suggests that anaerobic reductions of Lp may not be limited by water flow across or through cells in the radial direction (Fig. 5). Alternatively, the ability of cells to respond to ABA is lost with periods of anaerobiosis. Perhaps with shorter durations of anaerobiosis, ABA may play some role in affecting water uptake. It is unlikely, however, that ABA will inhibit Lp to the degree observed in this study since $10^{-4}$ M ABA did not decrease but increased the Lp of aerobic root systems by 25 to 30%. The enhancement was not due to the ethanol solvent since ethanol alone did not have a comparable effect. The literature reveals that ABA may promote (10, 16, 18) or inhibit (12, 16, 24) Lp depending upon plant species, applied pressure, ABA concentration, and duration of exposure. ABA may affect Lp by influencing membrane permeability (18, 24) or perhaps by its effects on plasmodesmata or cytoplasmic streaming (30).

Elevated levels of ABA have been reported in leaves of several flooded plant species (14, 38, 43) but its synthesis was judged to

be in response to increased water stress. The major criticism against the involvement of ABA as the cause of flood-induced stomatal closure in this (Figs. 1, 2) and other studies (4, 11, 19, 28, 39) is that a reduction in cL precedes a reduction in $\Psi_{LW}$. In fact, leaf turgor actually increases with the onset of stomatal closure (4, 19, 28). Thus, if ABA is responsible for stomatal closure, the stimulus for its production must be other than a decline in turgor. Other factors often invoked to explain flood-induced stomatal closure include a reduced supply of K (27, 39) or cytokinins (5, 31) from the root to the shoot. Additional possibilities may include naturally occurring antitranspirants such as farnesol (42) or micronutrient toxicity due to a loss of membrane selectivity (33). The pale green color of anaerobic plants does indicate nutrient imbalances, particularly an N deficiency (13). A preliminary study indicated that leaf K levels were no more reduced than other nutrients (Eric Hanson, pers. comm.). It has been argued that guard cells have a lower affinity for K than mesophyll cells and are thus preferentially affected (27). In the present study, however, the observed reduction in Lp will account for all of the decrease in cL.

Results concerning the effect of applied ABA as well as data with intact and cut root systems support the contention that decreased Lp is primarily in the longitudinal direction (Table 2). This can only be explained by occlusion of the xylem vessels. Occlusion of xylem vessels has been reported to occur in flooded roots of tomato (21), tobacco (22), and Sitka spruce (11). Of the above studies, one by Kramer (21) measured transpiration and Lp concurrently

but he did not observe a cause-and-effect relationship between xylem plugging and reduced transpiration of flooded tomato plants. In the case of Sitka spruce, tests for xylem obstruction were positive but were only performed at the end of the experiment well after the onset of stomatal closure (11).

Xylem plugging has been shown to occur for roots and trunks of pear trees prone to desiccation when infected by pear decline (41). A cause-and-effect relationship has been established for reduced water uptake and xylem plugging in detached peach shoots and, in this case, the occluding material was deemed of pectic origin (26).

In conclusion, a reduction in cL occurred prior to a decline in $\Psi_{LW}$ or an increase in $\Psi_{R\pi}$. Stomatal closure accompanied simultaneous reductions in the roots' water conducting capacity. A reduced stomatal aperture is an adaptive plant response to maintain leaf turgor, compensating for increased resistance to water flow through anaerobic roots. Decreased Lp, due to xylem plugging, progressed basipetally toward the stem with increasing duration of anaerobiosis. Since a concomitant decline in cL and Lp occurred before an increase in plant water stress, more work is required to elucidate the nature of the signal(s) responsible for stomatal closure with root anaerobiosis.

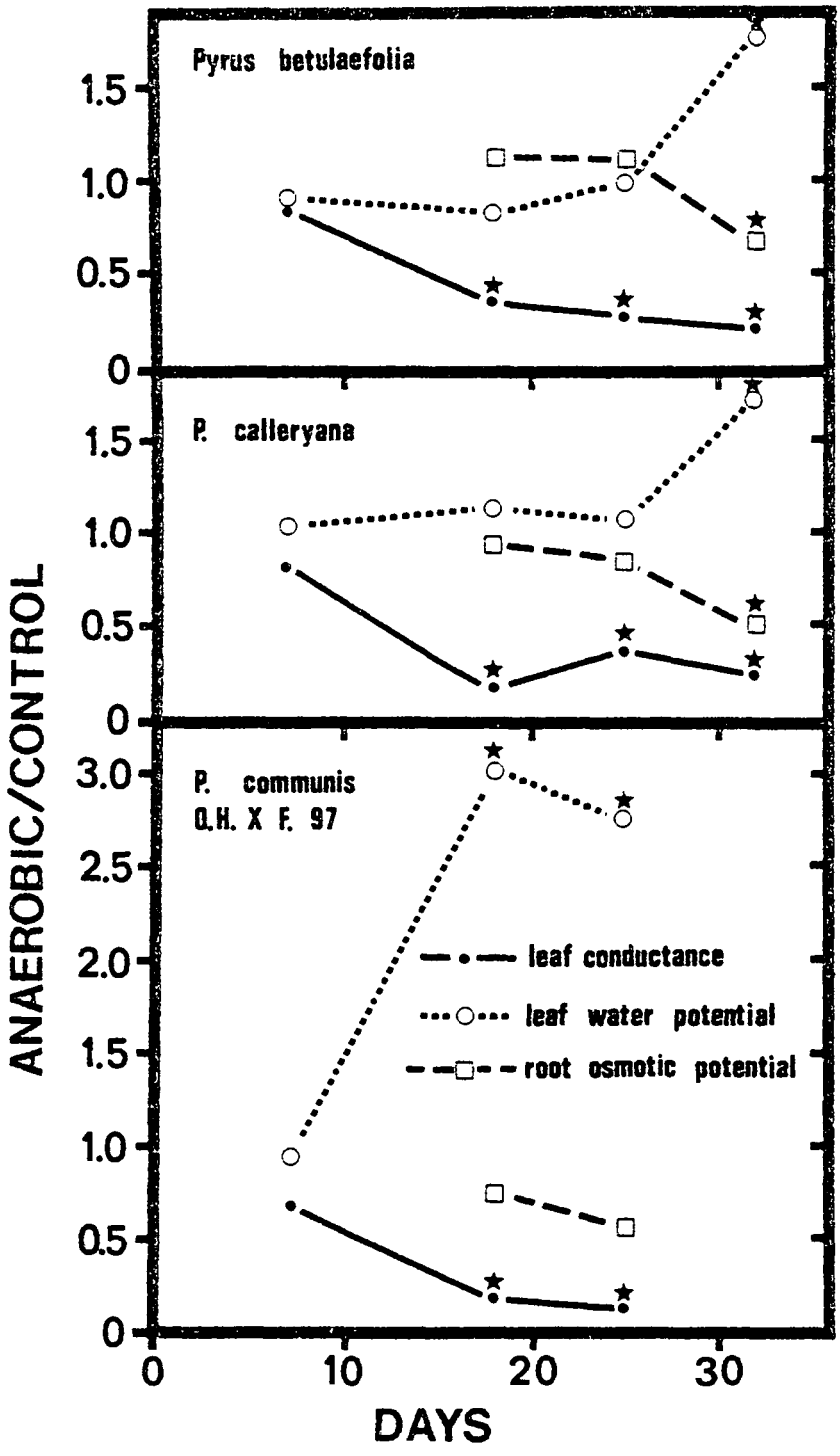


Figure 1. Paper 3 Anaerobic to control (aerobic) ratios of leaf conductance, leaf water potential and root osmotic potential for three ungrafted Pyrus rootstocks. Significant differences (*) between treatments were determined by LSD, 5% level.

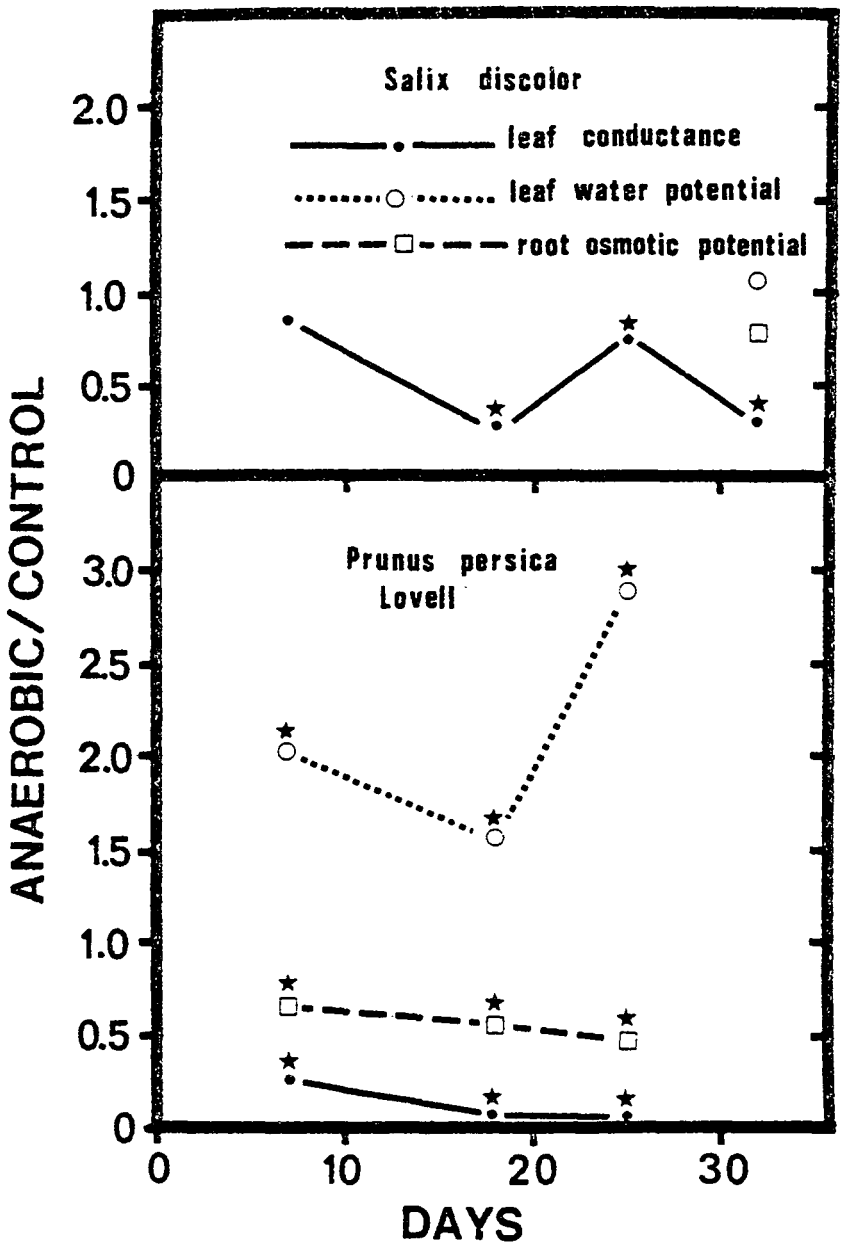


Figure 2. Paper 3 Anaerobic to control (aerobic) ratios of leaf conductance, leaf water potential and root osmotic potential for Salix and Prunus species. Significant differences (*) between treatments were determined by LSD, 5% level.

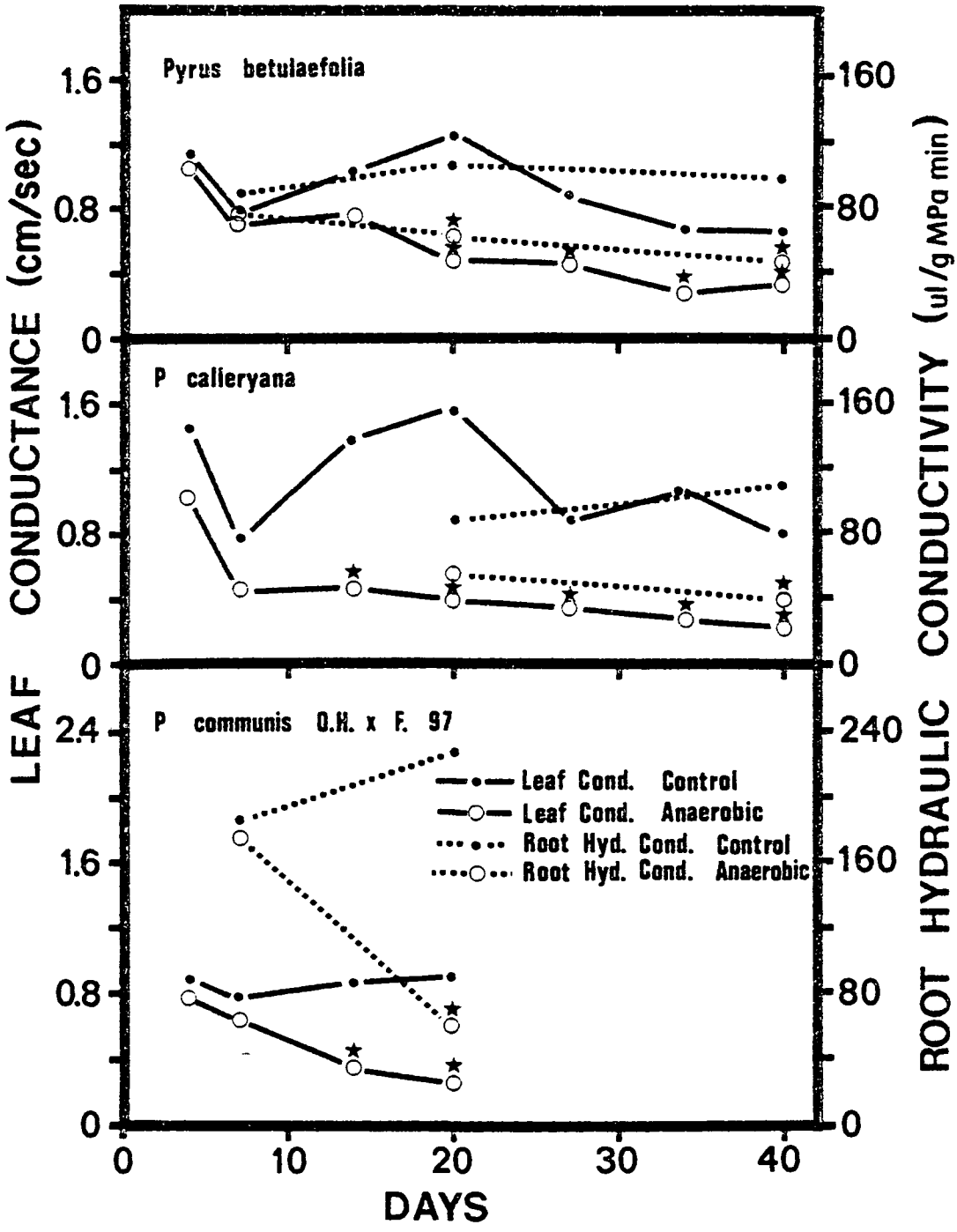


Figure 3. Paper 3 Leaf conductance and root hydraulic conductivity of aerobically (control) and anaerobically treated ungrafted Pyrus rootstocks. Significant differences (*) between treatments were determined by LSD, 5% level.

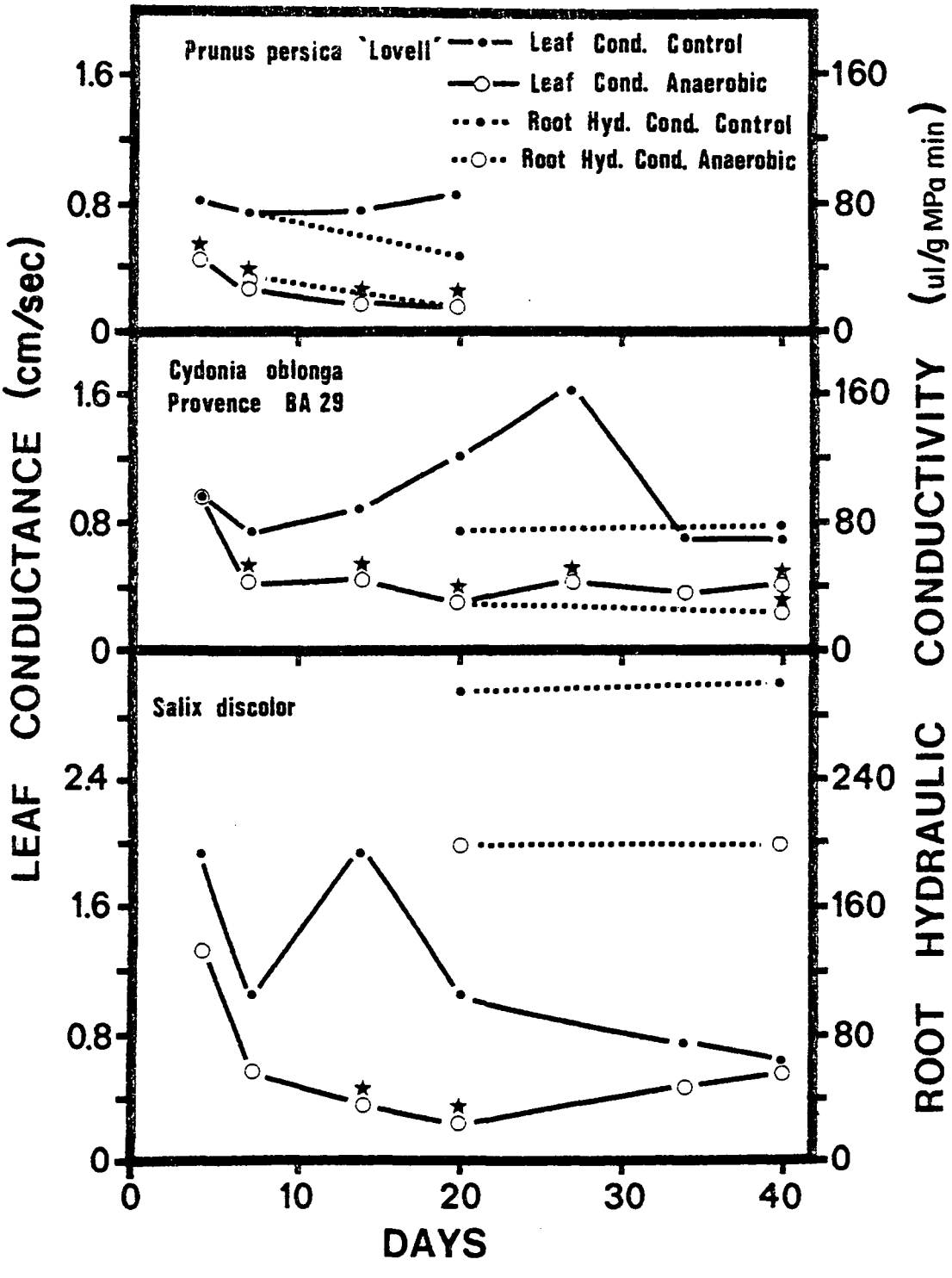


Figure 4. Paper 3 Leaf conductance and root hydraulic conductivity of aerobically (control) and anaerobically treated Prunus, Cydonia and Salix species. Significant differences (*) between treatments were determined by LSD, 5% level.

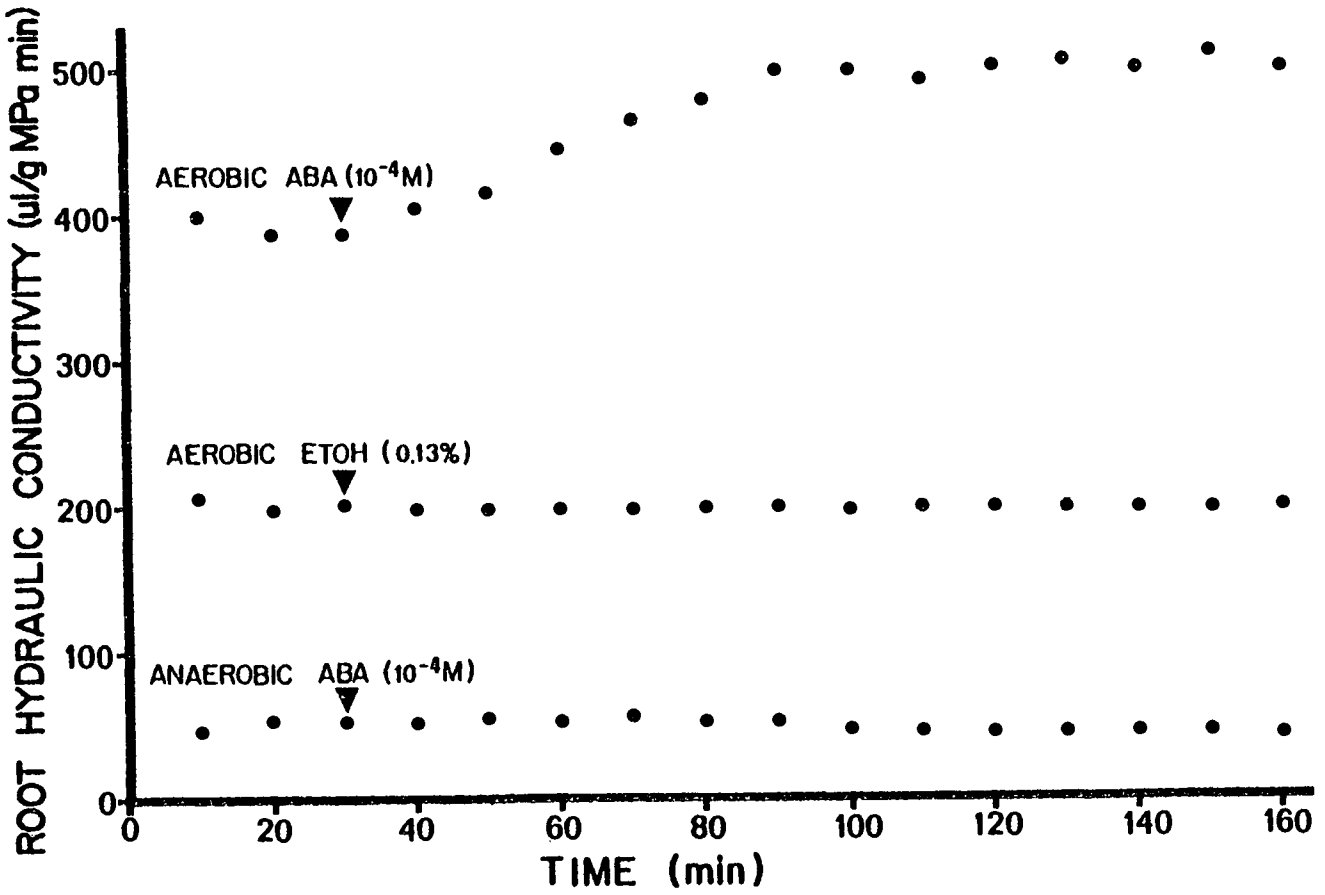


Figure 5. Paper 3 The effect of abscisic acid (ABA) on root hydraulic conductivity of aerobically and anaerobically treated P. communis cv. OH X F 97. Root hydraulic conductivity was monitored for 30 minutes to establish initial steady state rates before ABA or ethanol was added. A representative plant for each treatment was selected due to variable initial steady state rates of root hydraulic conductivity.

Table 1. Paper 3 Leaf conductance (cL) and root hydraulic conductivity (Lp) of aerobically and anaerobically treated *P. betulaefolia* and *P. communis* cv. OH X F 97. Root hydraulic conductivity is expressed on a dry root weight basis (≤ 1 mm), a stem cross-sectional area basis and a leaf area basis.

| | cL (cm/sec) | Lp (root) (µl/g MPa min) | Lp (stem) (µl/cm$^2$ MPa min) | Lp (leaf) (µl/cm$^2$ MPa min) |
|---|---|---|---|---|
| *Pyrus betulaefolia* | | | | |
| Day 4 | | | | |
| aerobic | 0.368 | | | |
| anaerobic | 0.273 | | | |
| Day 9 | | | | |
| aerobic | 0.457 | 589.42 | 11.21 | 0.414 |
| anaerobic | 0.415 | 791.82 | 11.27 | 0.593 |
| Day 20 | | | | |
| aerobic | 0.318 | 785.13 | 13.47 | 0.441 |
| anaerobic | 0.158* | 449.59* | 10.39 | 0.266 |
| Day 30 | | | | |
| aerobic | 0.587 | 689.54 | 11.91 | 0.480 |
| anaerobic | 0.039* | 194.44* | 2.51* | 0.111* |
| *P. communis* OH X F 97 | | | | |
| Day 4 | | | | |
| aerobic | 0.307 | 381.58 | 12.71 | 0.468 |
| anaerobic | 0.138* | 30.38* | 1.02* | 0.036* |
| Day 9 | | | | |
| aerobic | 0.703 | 309.69 | 12.51 | 0.235 |
| anaerobic | 0.041* | 39.53* | 0.787* | 0.025* |
| Day 20 | | | | |
| aerobic | 0.311 | 242.11 | 10.60 | 0.181 |
| anaerobic | 0.026* | 0* | 0* | 0* |
| Day 30 | | | | |
| aerobic | 0.329 | 188.63 | 10.18 | 0.198 |
| anaerobic | 0.009* | 0* | 0* | 0* |

*Treatment means are significantly different on each day by LSD, 5% level.

Table 2. Hydraulic conductivity (Lp) of P. communis cv. OH X F 97 Paper 3 after various periods of anaerobiosis.

| P. communis OH X F 97 | Intact root system | Root system with feeder roots detached | Stem without root system |
|---|---|---|---|
| | Lp(µl/plant MPa min ) | | |
| Aerobic | 143 ± 54[z] | 2573 ± 946 | 10575 ± 4391 |
| Anaerobic (4 days) | 15.6 ± 1.5 | 105.7 ± 9.8 | 4050 ± 1702 |
| Anaerobic (9 days) | 3.5 ± 1.9 | 75.7 ± 27.7 | 2897 ± 984 |
| Anaerobic (20 days) | 0 | 26.0 ± 45.6 | 1336 ± 136 |
| Anaerobic (30 days) | 0 | 0 | 472 ± 92.9 |

[z] ± 1 standard deviation

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# WATER AND ION FLUXES OF ABSCISIC ACID TREATED ROOT SYSTEMS OF PYRUS COMMUNIS

Additional index words. Root hydraulic conductivity, ethanol.

Abstract. Water and ion fluxes of intact root systems immersed in a nutrient solution were determined at various pressures and temperatures. Water flux (Jv) was normalized and expressed as the ratio (Qv) obtained by comparison to initial flow rates of a root system after 30 minutes at a pressure and temperature of 0.50 MPa and 25°C, respectively. Qv was linearly related to pressures between 0.20 and 0.62 MPa, implying a constant root hydraulic conductivity (Lp) within this range, since solute flux (Js) was not affected. Similarly, Qv was linearly related to temperatures between 7° and 35°C; however, larger, rapid temperature changes resulted in a break of the Arrhenius plot of Qv versus the reciprocal of temperature.

Abscisic acid (ABA) from 2 x $10^{-6}$ M to $10^{-4}$ M, applied to root systems increased Qv almost immediately with the effect leveling off after 1½ hours. With a pressure of 0.50 MPa, ABA at $10^{-4}$ M enhanced Qv by 28%. The simulation of Qv was not due to the ethanol solvent since ethanol at 2 concentrations produced the opposite effect. The osmotic potential ($\Psi\pi$) of the xylem fluid was analyzed and was used to calculate total normalized solute flux (Qs). The results suggested that ABA-induced or ethanol-induced changes in water flux were mainly due to changes in Lp and not to changes in ion transport to the xylem.

## Introduction

Although abscisic acid (ABA) has been most often associated with stomatal closure, recent evidence indicates that ABA also influences water permeability and ion transport across the root. Herbaceous plant species have been used exclusively to assess the effect of root applied ABA, often with contradictory results. For instance, root applied ABA has been reported to increase water flux (Jv) through roots of corn (Zea mays) (6), bean (Phaseolus vulgaris) (19), sunflower (Helianthus annuus) (14, 16), tomato (Lycopersicon esculentum) (29); to decrease that of bean (13), soybean (Glycine max) (24), corn (7), and barley (Hordeum vulgare) (7, 27); and to have no effect on sunflower (9).

The stimulation or inhibition of Jv and Js by ABA depends on the conditions in which the plants are grown (26), as well as experimental conditions such as ABA concentration, duration of exposure to ABA and the amount of applied suction or pressure (13, 25). It has been concluded that the effect of ABA on Jv is a result of altered ion transport to the xylem (7, 19, 27, 28) or a change in root hydraulic conductivity (Lp) (1, 14, 15, 17), or both (13, 29).

Most studies have been performed on root systems exposed to atmospheric pressure or up to 0.08 MPa applied suction or pressure (2, 6, 7, 9, 14-17, 19, 27-29). The use of such low hydrostatic pressures precludes a distinction between changes in Lp or changes in the osmotic driving force ($\Delta\Psi\pi$) as the primary cause of alterations in Jv (11). Hydrostatic pressures up to 0.50 MPa have been used

to assess changes in Lp essentially independent of ion flux (11-13, 24, 25). We used this method to determine the effect of hydrostatic pressure, temperature and root applied ABA on water and ion flux through intact root systems of the woody species Pyrus communis.

## Materials and Methods

Two-year-old clonal cuttings of Pyrus communis cv. Old Home X Farmingdale 97 (OH x F 97) were stored outdoors in sawdust bins over the winter. Prior to bud break in April, plants were grown in 6 liters of half-strength nutrient solution (18). Aeration was provided with the aid of an aquarium-type airstone, connected to a compressor by tygon tubing. Additional nutrient solution was added periodically as needed to maintain the 6-liter capacity. Supplemental fluorescent lights provided a 16-hour photoperiod. After 3-4 weeks of acclimatization, plants were actively growing and had fully expanded leaves.

Root exudation rate (Jv) was monitored with the aid of a modified pressure chamber (11, 12, 13, 24, 25). Roots with approximately 10 - 15 cm of stem whose bark was removed to expose the xylem were placed in a pressure chamber filled with fresh nutrient solution. The chamber was sealed with the cut stump protruding through a rubber gasket. Compressed air was increased at approximately 0.10 MPa $min^{-1}$ to the final presure. After 2-3 minutes of exposure to the desired pressure, tygon tubing was attached to the cut stump and the quality of xylem fluid was measured every 10 minutes.

Two hours before measurements, roots were placed in a nutrient

solution with 0.13% ethanol (using 95% ethanol) and subject to 0.50 MPa hydrostatic pressure. Jv was measured successively at 0.20, 0.35, 0.50 and 0.62 MPa in ascending and descending order. Measurements began after 2-4 minutes of equilibration at each pressure. Because each root system had a different initial Jv, Jv was normalized (Qv) in terms of the ratio to rates of 30 minutes of exposure to 0.50 MPa pressure and a temperature of 25°C. Similarly, the effect of temperature on Qv was determined in the following sequences: 25° → 15° → 7° → 35°C and 25° → 35° → 15° → 7°C. Equilibration for 5 minutes at each temperature preceded measurements. Both temperature sequences were determined for the 0.13% ethanol treatments, but only the 25° → 35° → 15° → 7°C sequence was used on plants exposed to a 2-hour treatment of $10^{-4}$ M ABA instead of ethanol alone.

The effects of 3 concentrations of ABA, 2 concentrations of ethanol and distilled water on Jv and Js to the xylem were monitored at a pressure of 0.50 MPa and a temperature of 25°C. Initial rates were established after 3, 10-minute collections of xylem fluid, and then 1 of the 6 treatments was added to the root system. Subsequent changes in Jv were reported as the ratio to pretreatment levels (Qv) (i.e., normalized to a time of 30 minutes). A 2 ml aliquot of ABA dissolved in 95% ethanol was added to the half-strength nutrient solutions to yield net concentrations of $2 \times 10^{-6}$ M, $5 \times 10^{-5}$ M and $10^{-4}$ M ABA. The control was 0.13% ethanol. The 2 other treatments used were 1.33% ethanol and distilled water.

The osmolarity of the xylem fluid was determined with a Wescor 5100C Vapor Pressure Osmometer. Osmotic potentials ($\Psi\pi$) were

calculated from osmolarity according to the Van't Hoff equation. Total normalized solute flux (Qs) was obtained by multiplying osmolarity times Jv for each 10-minute period. Because of variable initial Jv, $\Psi\pi$, or Js, the data were normalized in terms of values recorded after 30 minutes of pressure (i.e., just before the addition of 1 of the 4 treatments).

## Results

Root systems, after being subjected to a 2-hour treatment at 0.50 MPa in a nutrient solution with 0.13% ethanol (control), responded linearly to pressures between 0.20 MPa and 0.62 MPa (Fig. 1). There were no significant differences whether the change in pressure was run in an ascending or descending order, so the data were grouped together. Normalized flow rate (Qv) plotted as a function of applied pressure indicated a constant root hydraulic conductivity (Lp) (i.e., slope of the line). (Significant differences in $\Psi\pi$ of the xylem fluid did not occur with pressure (Appl Q).)

The influence of temperature on Qv was dependent upon the rapidity of temperature change. For instance, the sequence 25° → 15° → 7° → 35°C produced a break in the Arrhenius plot of ln Qv versus the reciprocal of temperature for control root systems (Fig. 2). A linear relationship occurred for the control and $10^{-4}$ M ABA treatment when the temperature change was less drastic (25° → 35° → 15° → 7°C (Fig. 3)). A $Q_{10}$ of approximately 1.5 was apparent for both the ABA and control treatments. Temperature-induced changes in Qv were a function of Lp since differences in $\Psi\pi$ of the xylem fluid did not

occur (App. R).

The addition of ABA to intact root systems increased Qv (Fig. 4). The effect was apparent within 10-20 minutes and leveled off after about 1½ hours. The $10^{-4}$ M ABA treatment stimulated Qv to the greatest extent, although 5 x $10^{-5}$ M and 2 x $10^{-6}$ M ABA were nearly as effective. Qv increased approximately 19%, 21% and 28% at 2 x $10^{-6}$ M, 5 x $10^{-5}$ M and $10^{-4}$ M ABA, respectively. Increased Qv was not a consequence of ethanol, since 0.13% ethanol resulted in slight decrease in Qv in all cases. The reduction caused by 1.33% ethanol ranged from about 14% to 35%.

Analysis of the xylem sap indicated that regardless of the treatment ($10^{-4}$ M ABA, 0.13% ethanol, 1.33% ethanol or distilled water), 0.50 MPa applied to the roots increased $\Psi\pi$ (less negative) of the xylem fluid (Table 1). Different plants manifested quite different absolute $\Psi\pi$. Of the 4 treatments considered, $10^{-4}$ M ABA caused the greatest reduction in normalized $\Psi\pi$, yet total normalized solute flux (Qs) was higher than the other treatments. Conversely, 1.33% ethanol minimized the decrease in normalized $\Psi\pi$ but this treatment resulted in the largest decrease in Qs.

## Discussion

The flow of water across root systems may be described by the following equation:

$$Jv = Lp(\Delta P - \sigma\Delta\pi)$$

where Jv is the total volume flux in $\mu l/cm^2$ min, Lp is hydraulic

conductivity in $\mu l/cm^2$ MPa min, $\Delta P$ and $\Delta\pi$ are the hydrostatic pressure and osmotic driving forces, in MPa, and $\sigma$ is the dimensionless reflection coefficient ($0 \leq \sigma \leq 1$) (11). When $\Delta P$ is controlled as in pressure chamber studies, changes in Jv can be due to changes in Lp or $\sigma\Delta\pi$. With sufficiently high applied pressures ($\geq$ 0.50 MPa) or conditions of transpiration the value of $\Delta P$ far exceeds $\sigma\Delta\pi$ due to the typically dilute nature of xylem sap.

The stimulation in Jv with applications of ABA has been attributed to increased Lp (6, 14, 17), increased $\Delta\Psi\pi$ (19) or both (16, 29). Similarly, reports demonstrating a reduction in Jv conclude that the effect of ABA was due to reduced Lp (13, 25) or reduced $\Delta\Psi\pi$ (7, 27, 28). Besides obvious differences in experiments such as differing growing conditions, ABA concentrations, and duration of exposure to ABA, many of the apparent contradictions may be explained by the interaction of hydrostatic ($\Delta P$) and osmotic driving forces ($\Delta\pi$) with low applied pressures. To distinguish whether alterations in Jv are due to changes in Lp or $\Delta\Psi\pi$, we and others (11-12, 24, 25) have used sufficiently high pressures (0.50 MPa) to insure that $\Delta P$ far outweighs $\Delta\Psi\pi$.

Roots of P. communis manifested a uniform Lp between 0.20 MPa and 0.62 MPa (Fig. 1), in agreement with previous work on herbaceous plant species. The linearity of Qv versus hydrostatic pressure justified the use of any pressure between 0.20 MPa and 0.62 MPa for subsequent studies. A similar rationale could be argued for use of any temperature between 7° and 35°C as long as the change in temperature occurred gradually.

The relatively low $\Psi\pi$ after 10 or 20 minutes of exposure to 0.50 MPa may reflect a loss of solute from cut or injured cells of the stem. ABA reduced solute concentrate in the xylem more than the ethanol or distilled water treatments; however, because of a concomitant increase in Qv (Fig. 4), total normalized solute flux (Qs) was, in fact, higher than the other treatments (Table 1). In contrast, 1.33% ethanol affected normalized $\Psi\pi$ the least of all treatments, but because of a simultaneous decrease in Qv, Qs was reduced more than the other treatments. A short-term (16%) increase in Qs after 20 minutes was limited to the ABA treatment (Table 1). It is known that continued protein synthesis is required for symplastic transport of ions, presumably because of membrane turnover (22). The rapidity of the ABA response reported here indicates that RNA-directed protein synthesis does not mediate the effects of ABA on Lp.

The flow of water and nutrients across the root can be divided into 3 stages: uptake into the root, transport across the root, and release to the xylem (4). There is considerable agreement that ABA, when applied to the roots, somehow affects membrane-found ion carriers. Several studies have shown ABA to inhibit ion transport to the xylem but ion uptake to remain unaffected (2, 7, 26); however, others suggest that both are reduced (9, 28). Specifically, the rate of ion transport may be a function of a release from cortical tissue (1), the inner plasmalemma of the endodermis (8), xylem parenchyma surrounding the vessels (20), and possibly pericycle cells or living xylem vessels (4). One may speculate that ABA and ethanol may exert control over any or all of these regions depending on such factors

as endogenous levels of ABA and tissue sensitivity.

The presence of only a small, transitory increase in Qs (Table 1) leads us to invoke changes in Lp as the cause of increased Qv (Fig. 4). A 500% increase in Qs would be required to enhance Qv to the extent observed here (28%) with an applied pressure of 0.50 MPa--assuming a value of approximately 1 for $\sigma$ (i.e., $0 \leq \sigma \leq 1$). Glinka (16) reached essentially the same conclusion when interpreting changes in Lp to account for increased Jv of sunflower roots exposed to ABA. Since the driving force ($\Delta\Psi\pi$) decreased when Lp increased, one may conclude that ABA appears to exert different effects on water and ion permeability.

Our results with ABA and ethanol, although not contradictory, do not agree entirely with the two reports cited involving herbaceous plant roots subjected to 0.20 MPa to 0.50 MPa applied pressure (13, 25). Markhart et al. (25), working with soybean, found a marked and slight reduction in Qv with exposure to $5 \times 10^{-5}$ M ABA and 0.05% ethanol, respectively, after 4-10 hours. In addition, $\Psi\pi$ of the xylem exudate for both treatments was reported to decrease (more negative) but since Qv also decreased, Js was not altered to a great degree (25). In the present study, an increase in xylem $\Psi\pi$ occurred for all treatments after 0-2 hours. These data agree with those of Markhart et al. (25) where dilution of the xylem sap occurred up to 3 hours after subjecting soybean root systems to applied pressure.

Recently Markhart (23) demonstrated that penetration of the cis-trans ABA into soybean roots required at least 6 hours for

steady-state levels to be achieved and also that ABA entered the transpiration stream. Possibly the ABA-induced elevations in Qv reported here are transitory. Also, Fiscus (13) showed a short-term, 15 - 35% increase in Qv and Qs to occur with the addition of ABA to roots of bean which lasted 1½ hours. Fiscus (13) reasoned that since the enhancement of Qv was most pronounced at low presures, and because Qs of the xylem exudate increased under no applied pressure, an increased $\Delta\Psi\pi$ is the factor responsible for the short-term increase in Qv. Reductions in Qs were reported to accompany the long-term decline in Qv for bean (13) and soybean (25), and, therefore, only changes in Lp could account for this observation.

One must be cautious when interpreting Lp for an entire root system compared to a simple membrane system. Clearly, Lp represents the sum of apoplastic and symplastic pathways of the entire root; young and old. Lateral root initiation, suberization, and a sloughing of the endodermis occurring with the growth and development of woody roots also complicates the issue (10). If we are to assume, as others have, that ABA does not act directly on the apoplastic pathway, then it is also logical to assume that the enhancement of Lp by ABA on the symplastic pathway would be greater than the total increase of 28% reported here.

Discontinuity of the Arrhenius plot of ln Qv versus the reciprocal of temperature with the 0.13% ethanol treatment suggests that Qv may be affected by lipid conformation (Fig. 3). A phase transition in membrane lipids may alter both membrane permeability and enzyme activity (17, 25). The greatest resistances to water movement

are encountered as water moves across cell membranes (3, 17). Because the endodermis is considered to be the only membrane barrier which water and ions must invariably traverse (5, 8, 10), it is often implicated as the site of ABA action (15, 25). Lea and Collins (21), working with egg lecithin bilayer membranes, provided evidence for the existence of membrane channels by noting conductance fluctuations associated with ABA. We (Fig. 3) and others (24) have shown that water flow across roots appears to be determined, at least in part, by lipid conformation. However, because of fundamental uncertainties in the relative importance of the symplastic and apoplastic pathways to water and ion uptake across a root (5, 30), the extent of our data will not allow us to speculate on a specific site of action. Also, we cannot confirm whether ABA enhanced water flow by affecting the membrane directly or by altering cell metabolism which may, in turn, affect the membrane. The rapidity of the response, however, does suggest the former.

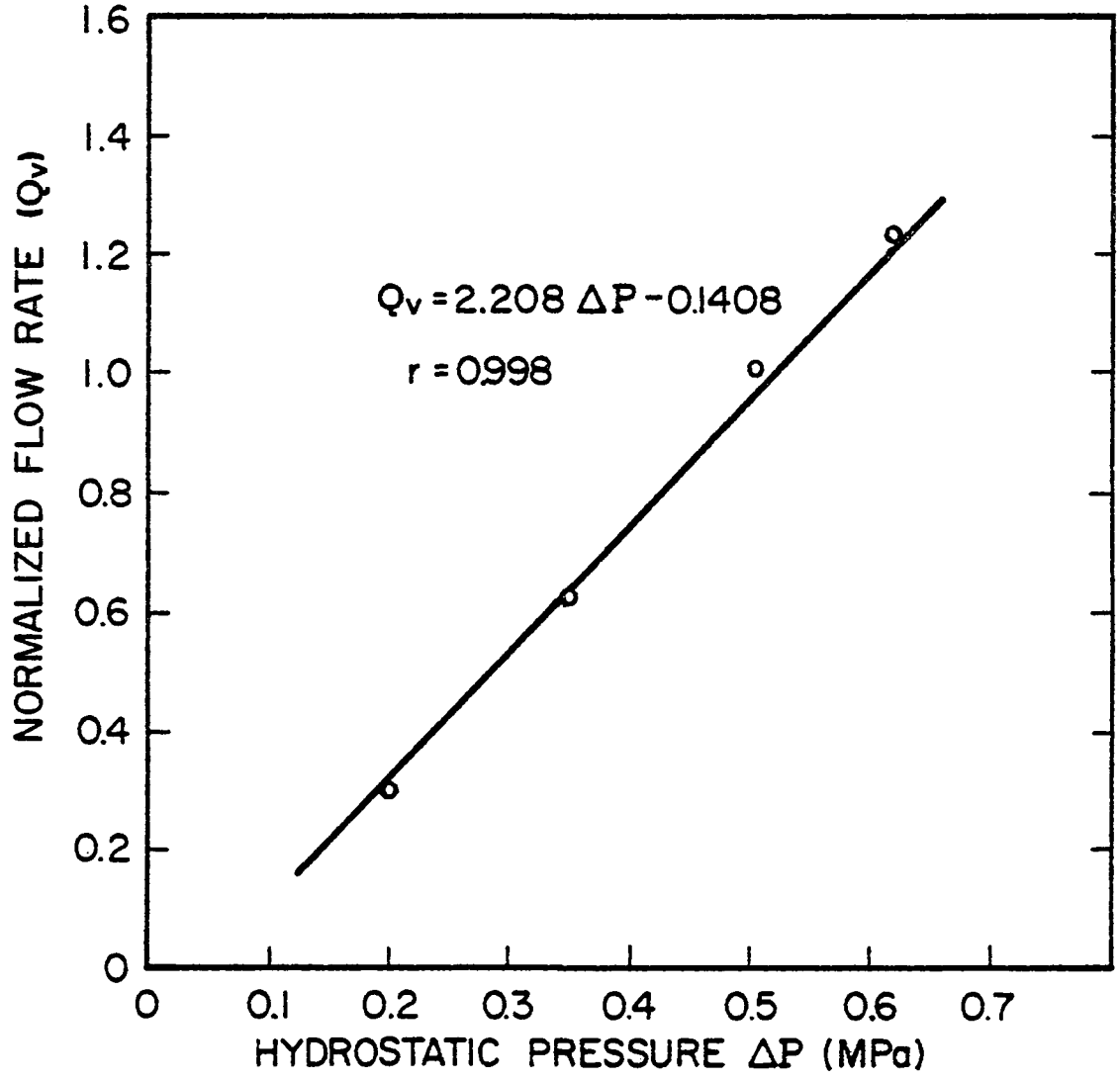


Figure 1. Paper 4 Effect of hydrostatic pressure (ΔP) on normalized flow rate (Qv) of root systems subjected to 0.13% ethanol for 2 hours at 25°C and 0.50 MPa. Qv was normalized to the rate at 0.50 MPa. Root hydraulic conductivity (Lp) is the slope of the line. Each point is the average of 6 root systems.

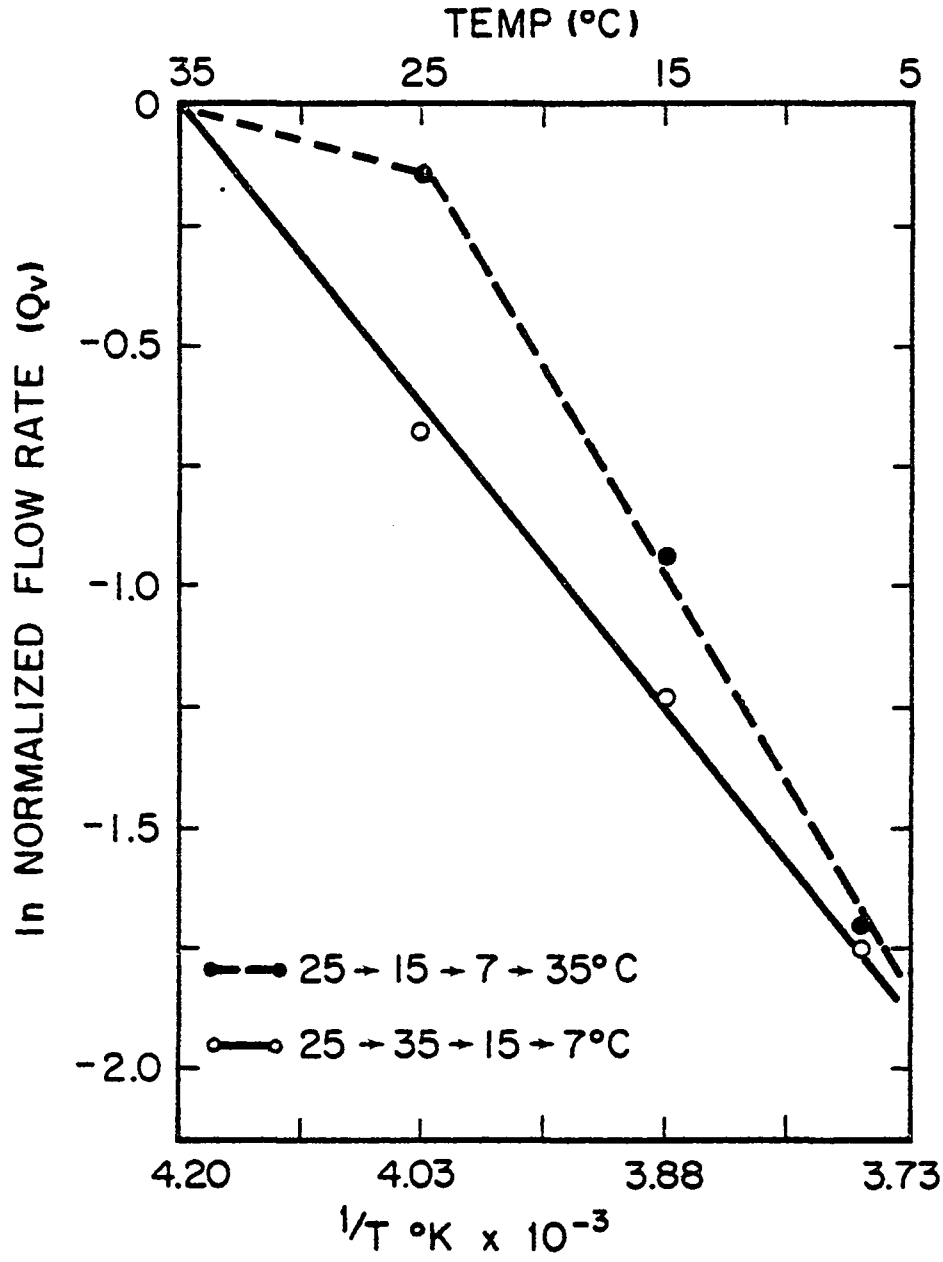


Figure 2. Paper 4 Effect of temperature sequence on the Arrhenius plot of normalized flow rate (Qv) versus the reciprocal temperature for control systems (treated with 0.13% ethanol for 2 hours). Qv was measured at 0.50 MPa and was normalized to rates at 25°C. Each point represents the average of 3 root systems.

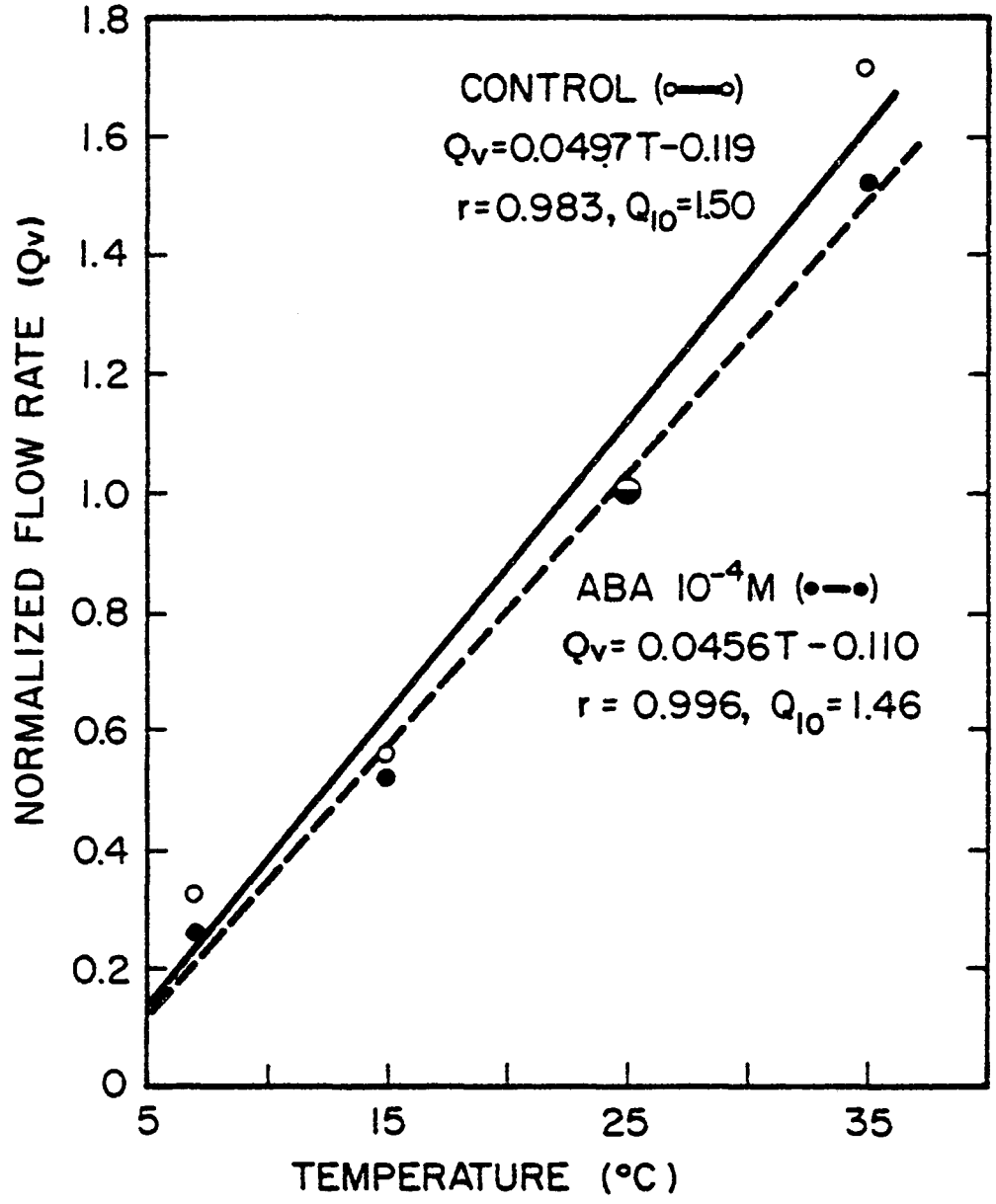


Figure 3. Paper 4 Effect of temperature on normalized flow rate (Qv) of root systems treated with $10^{-4}$ M ABA or 0.13% ethanol (control) for 2 hours at 0.50 MPa. Qv was normalized to the rate of each treatment at 25°C. Each point is the average of 3 root systems.

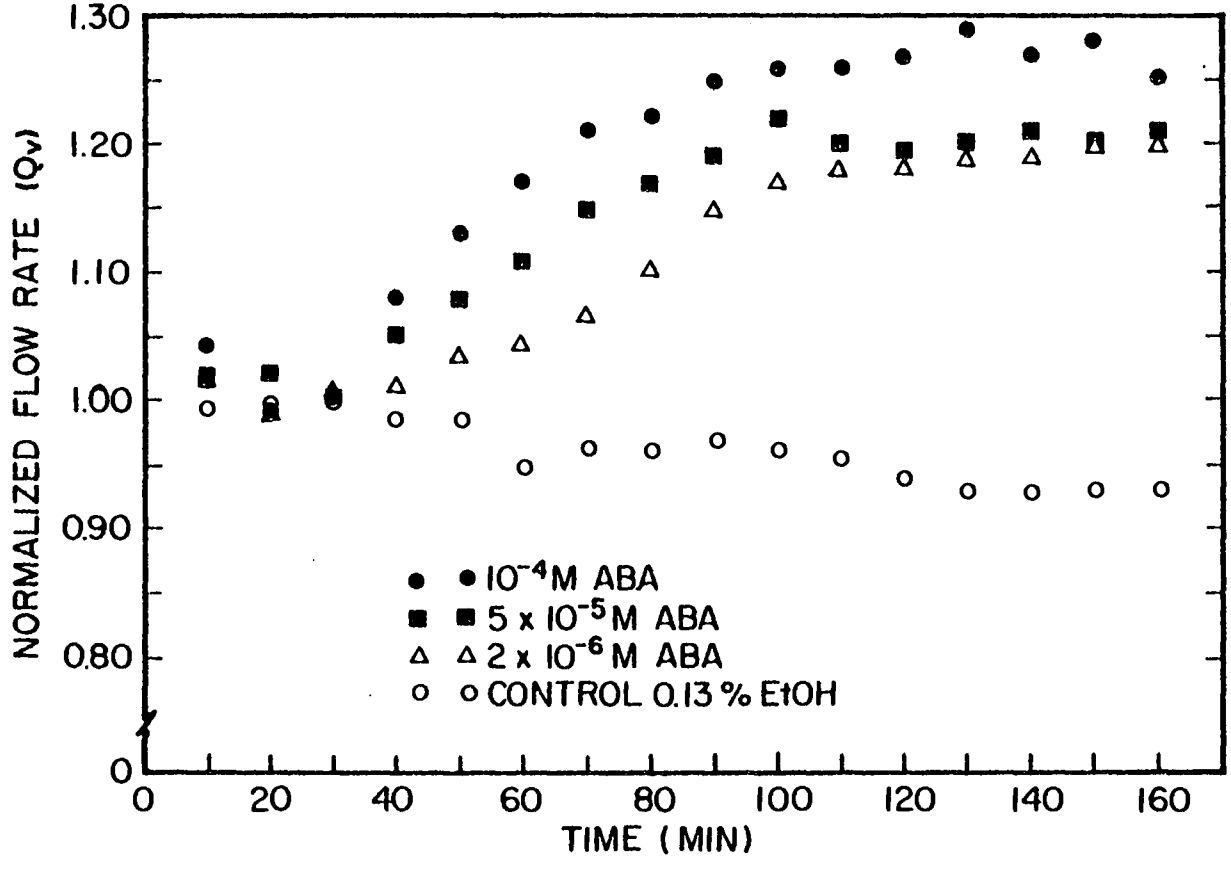


Figure 4. Paper 4 Effect of $10^{-4}$ M ABA, 5 X $10^{-5}$ M ABA, 2 X $10^{-6}$ M ABA and 0.13% ethanol on normalized flow rate (Qv) at a pressure of 0.50 MPa and a temperature of 25°C. Ethanol or the various concentrations of ABA was added at time 30. Qv was normalized according to the rate at 30 min. Each point is the average Qv of 3 root systems.

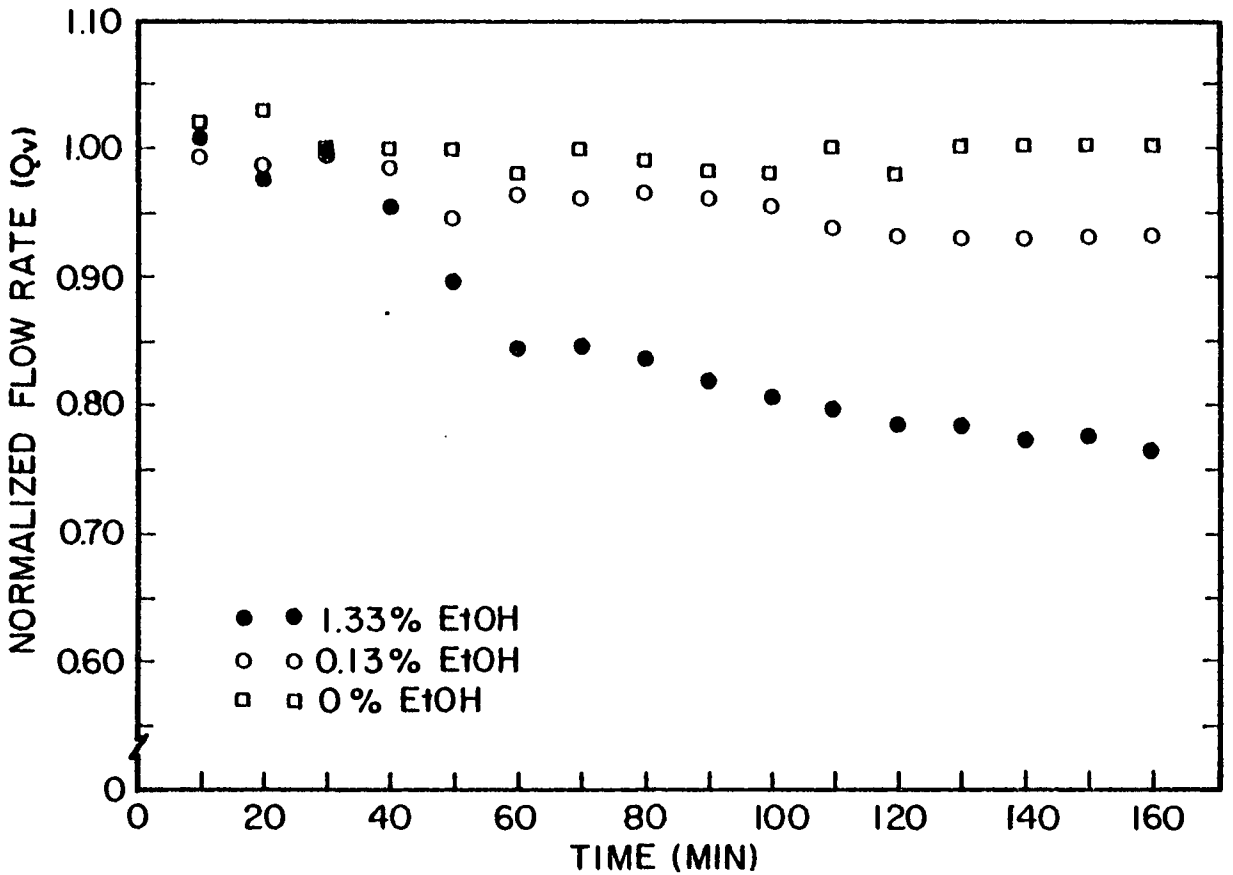


Figure 5. Paper 4 Effect of 1.33% ethanol, 0.13% ethanol and distilled water on normalized flow rate (Qv) at a pressure of 0.50 MPa and a temperature of 25°C. Ethanol or water was added at time 30 min. and Qv was normalized according to rates at this time. Each point is the average Qv of 3 root systems.

Table 1. Paper 4 Absolute osmotic potential ($\Psi\pi$), normalized $\Psi\pi$ and the total normalized solute flux (Qs) in milliosmoles per sample of xylem exudate collected at 10 minute intervals at an applied pressure of 0.50 MPa. After initial Jv was determined (30 min.), one of the following compounds was added: $10^{-4}$ M ABA, 1.33% EtOH, 0.13% EtOH, or distilled water. Normalized values were calculated as the ratio at time (t) to that recorded at time 30.

| | $10^{-4}$ M ABA | | | 1.33% EtOH | | | 0.13% EtOH | | | $H_2O$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | $\Psi\pi$ (KPa) | norm. $\Psi\pi$ | Qs | $\Psi\pi$ (KPa) | norm. $\Psi\pi$ | Qs | $\Psi\pi$ (KPa) | norm. $\Psi\pi$ | Qs | $\Psi\pi$ (KPa) | norm. $\Psi\pi$ | Qs |
| 0[z] | -33.0 | 1.02 | -- | -34.6 | 0.69 | -- | -33.4 | 0.72 | -- | -34.6 | 0.68 | -- |
| 10 | -50.2[y] | 1.57 | 1.57 | -59.3 | 1.10 | 1.11 | -49.4 | 1.19 | 1.19 | -65.6 | 1.39 | 1.41 |
| 20 | -33.8 | 1.05 | 1.04 | -58.3 | 1.06 | 1.02 | -44.5 | 1.02 | 0.99 | -56.9 | 1.19 | 1.23 |
| 30 | -32.1 | 1.00 | 1.00 | -55.1 | 1.00 | 1.00 | -45.7 | 1.00 | 1.00 | -50.7 | 1.00 | 1.00 |
| 40 | -32.9 | 1.03 | 1.11 | -50.1 | 0.91 | 0.86 | -42.0 | 0.99 | 0.98 | -40.8 | 0.84 | 0.84 |
| 50 | -32.1 | 0.95 | 1.16 | -48.6 | 0.89 | 0.77 | -49.4 | 0.89 | 0.88 | -44.5 | 0.92 | 0.92 |
| 60 | -28.8 | 0.90 | 1.11 | -47.8 | 0.88 | 0.70 | -45.7 | 1.08 | 1.06 | -43.3 | 0.90 | 0.88 |
| 70 | -24.7 | 0.75 | 0.96 | -44.5 | 0.81 | 0.66 | -39.5 | 1.08 | 0.83 | -40.8 | 0.84 | 0.84 |
| 80 | -22.2 | 0.70 | 0.88 | -49.4 | 0.91 | 0.71 | -48.2 | 1.09 | 1.06 | -34.6 | 0.72 | 0.71 |
| 90 | -23.9 | 0.75 | 0.97 | -46.1 | 0.85 | 0.63 | -40.7 | 0.92 | 0.90 | -33.3 | 0.69 | 0.68 |
| 100 | -25.5 | 0.77 | 0.99 | -49.4 | 0.90 | 0.67 | -38.3 | 0.89 | 0.85 | -38.3 | 0.78 | 0.76 |
| 110 | -19.8 | 0.61 | 0.80 | -49.4 | 0.91 | 0.67 | -39.5 | 0.90 | 0.88 | -38.3 | 0.79 | 0.79 |
| 120 | -23.0 | 0.73 | 0.93 | -48.6 | 0.90 | 0.66 | -35.8 | 0.79 | 0.75 | -43.2 | 0.91 | 0.86 |
| 130 | -20.6 | 0.65 | 0.84 | -45.7 | 0.85 | 0.64 | -39.5 | 0.96 | 0.88 | -37.0 | 0.76 | 0.76 |
| 140 | -21.4 | 0.68 | 0.86 | -48.6 | 0.90 | 0.68 | -37.1 | 0.90 | 0.84 | -35.8 | 0.75 | 0.72 |
| 150 | -21.4 | 0.67 | 0.83 | -46.9 | 0.88 | 0.66 | -35.8 | 0.82 | 0.76 | -35.8 | 0.73 | 0.71 |
| 160 | -23.9 | 0.72 | 0.92 | -44.5 | 0.88 | 0.68 | -38.3 | 0.89 | 0.83 | -35.8 | 0.73 | 0.71 |

[z] At time 0 the nutrient solution itself was tested.
[y] Each determination is the average of 3 replications.

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# CONCLUSION

Differential waterlogging tolerance of major fruit tree rootstocks tested from high to low was as follows: Pyrus betulaefolia > Pyrus calleryana = Cydonia oblonga cv. Provence BA 29 > Pyrus communis (Bartlett seedling) > Pyrus communis cv. Old Home X Farmingdale 97 (OH X F 97) = Malus domestica cv. Malling Merton 106 > Prunus persica (Halford or Lovell seedling). A decline in leaf conductance (cL) was associated with a species-specific soil oxygen diffusion rate. 'Bartlett' scions when grafted on the 4 pear rootstock species listed above, sometimes performed better than their ungrafted counterparts when flooded in terms of growth and cL, but survivability was not altered. The value of monitoring cL or root respiration rate as screening tools for flood tolerance are discussed in the thesis.

Anaerobic-induced stomatal closure was not a function of reduced leaf water potential or changes in root osmotic potential. In contrast, stomatal closure was related to anaerobic reductions in root hydraulic conductivity (Lp). A reduced stomatal aperture was an adaptative plant response to maintain leaf turgor, thus compensating for increased resistance to water flow through anaerobic roots. However the more flood-tolerant plant species tended to manifest reductions in Lp and cL much later than flood susceptible species. Other adaptations of fruit tree species to ease the deleterious effects of flooding include: a decline in shoot growth and leaf size to better cope with decreased water absorption, and the production of hytrophied lenticels and adventitious roots to allow for increased $O_2$ absorption. (Prunus persica and Pyrus species did not produce adventitious roots).

Root respiration rates of Pyrus and Cydonia in the presence of 0.5% $O_2$ were not greatly reduced compared to rates in 21% $O_2$ indicating roots of these species have a high affinity for $O_2$. Pear rootstocks maintained a high respiratory capacity despite prolonged periods of anoxia. Anatomical investigations of the roots or stems of Pyrus species did not show any evidence of aerenchyma. Therefore it appears that flood-tolerance of Pyrus species is partly due to root respiration characteristics described above but not to $O_2$ transport from the shoot to the root. Consistent with this theory it was noted that pear species merely survived inundation under conditions where Salix discolor (willow) flourished. On the other hand, the ability of willow and other semi-aquatic species to thrive with soil flooding is largely due to their ability to transport $O_2$ from the shoot to the root. Due to the lack knowledge concerning biochemical adaptations of fruit tree species to flooding, a real need exists for progress in this area.

The cause of anaerobic reductions in Lp was investigated. The resistance to water flow in anaerobic roots was predominantly in the longitudinal and not the radial direction. The data presented in the thesis can only be explained in terms of an occlusion of the xylem vessels. Decreased Lp due to xylem plugging progressed basipetally toward the stem with increasing durations of anaerobiosis. Anaerobic-induced stomatal closure, although a function of reduced Lp, occurred before a decline in leaf water potential. More work is required to elucidate the nature of the signal(s) responsible for stomatal closure with root anaerobiosis.

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# APPENDICES

Appendix A. Source of plant material used in the various experiments.

| Species | Paper #1 (Fall Study 1980) | Paper #2 (Study #1) | Papers #1, 2, 3, 4 (All remaining studies when used) |
|---|---|---|---|
| Pyrus betulaefolia Bunge | OSU 3 sdlgs.* | Rt. ctgs. from OSU sdlgs. | OSU 1,2,3,4 sdlgs Fowler Nur. |
| Pyrus calleryana Decne | OSU 6 sdlgs.* | | OSU 8 sdlgs. |
| Pyrus communis L. | | | |
| Bartlett sdlgs. | OSU sdlgs.* | | Fowler Nursery |
| OH X F 97 (clonal) | ----- | ------- | Carleton Nursery |
| Cydonia oblonga Mill. | | | |
| Provence BA 29 | Ore. Rtsks. Inc. | Ore. Rtsks. Inc. | Ore. Rtsks. Inc. |
| EMLA C (clonal) | ----- | ------- | Ore. Rtsks. Inc. |
| Pyrus pyrifolia (Burm.) Nak. | Mikado, Hawaii sdlgs. | ------- | ------ |
| Pyrus ussuriensis Max. | P.I. 305300 sdlgs. | ------- | ------ |
| Pyrus pashia D. Don | Wild sdlgs., India | ------- | ------ |
| Pyrus dimorpophylla Makino | Wild sdlgs., Japan | ------- | ------ |
| Pyrus amydaliformes Vill. | P.I. 349023 sdlgs. | ------- | ------ |
| Pyrus faurei Schneid. | OSU sdlgs.* | ------- | ------ |
| Malus domestica Borkh. | | | |
| Malling Merton 106 (clonal) | ----- | ------- | Ore. Rtsks. Inc. |
| Malling Merton 111 (clonal) | ----- | ------- | Ore. Rtsks. Inc. |
| Prunus persica | | | |
| Halford sdlgs. | Lawyer Nursery | Lawyer Nursery | Lawyer Nursery |
| Lovell sdlgs. | ----- | ------- | undetermined source |
| Salix discolor Muhl. cutting | ----- | ------- | undetermined source |

*open pollinated (in isolation).

Appendix B. Midday soil temperatures at the 15-20 cm depth in pots submerged continuously 5-10 cm above the soil line.

| Fall 1980 Experiment (initiated Sept. 8) | | Spring 1981 Experiment (initiated June 3) | |
|---|---|---|---|
| Days of Flooding | Temp. °C | Days of Flooding | Temp. °C |
| 1 | 22 | 1 | 15 |
| 3 | 22 | 3 | 15 |
| 5 | 20 | 7 | 21 |
| 7 | 23 | 11 | 17 |
| 12 | 21 | 14 | 22 |
| 15 | 21 | 18 | 25 |
| 19 | 21 | 22 | 24 |
| 23 | 21 | 30 | 23 |
| 27 | 19 | 39 | 24 |
| 31 | 15 | 48 | 27 |
| 400 | 27 | 57 | 33 |
| | | 68 | 27 |
| | | 80 | 27 |
| | | 93 | 22 |
| | | 111 | 19 |
| | | 133 | 13 |
| | | 337 | 21 |
| | | 352 | 22 |

Appendix C. Soil oxygen diffusion rates (ODR) and soil temperatures at several locations on the Southern Oregon Experiment Station Rd. Medford, Oregon. Measurements were taken at a depth of 15-20 cm.

| | | ODR ($10^{-8}$ g $cm^{-2}$ $min^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Soil at field capacity | | Soil covered by flowing water | | | Soil covered by stagnant water | |
| Date | Soil Temp. | Site 1 | Site 2 | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 |
| 2/18/81 | 5°C | 11.6[z] | 31.3 | 14.6 | 13.5 | -- | 4.13 | 3.08 |
| 4/30/81 | 16°C | 44.3 | 30.4 | 28.6 | 25.9 | 29.7 | -- | -- |
| 9/3/81 | 24°C | 6.3 | 8.9 | 14.0 | 25.8 | -- | -- | -- |

[z]Means are the average of ten measurements at each site.

Appendix D. Percent plant survival of ungrafted *Pyrus* rootstocks subjected to 1 and 20 months of continuous flooding beginning Sept. 8, 1980. Evaluations of plant survival were after 1, 8 and 20 months of submergence.

| | % Survival | | |
|---|---|---|---|
| Species | 1 Month | 8 Months | 20 Months |
| *Pyrus dimorphophylla* | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 100 | 100 | 100 |
| Flooded continuously | 100 | 100 | 60 |
| *Pyrus pashia* | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 90 | 90 | 90 |
| Flooded continuously | 90 | 70 | 40 |
| *Pyrus amydaliformes* | | | |
| Control | 100 | 100 | 100 |
| Flooded 1 month | 90 | 80 | 80 |
| Flooded continuously | 80 | 30 | 10 |

Appendix E. Percent plant survival of ungrafted *Cydonia*, *Malus* and *Pyrus* rootstocks subjected to 1 year of continuous flooding. Soil flooding was initiated June 3, 1981 and evaluations were after 1, 2, and 12 months of treatment.

| | % Survival | | |
|---|---|---|---|
| Species | 1 Month | 2 Months | 12 Months |
| *Cydonia oblonga* | | | |
| EMLA C | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 100 | 100 |
| *Malus domestica* | | | |
| MM 111 | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 90 | 50 |
| *Pyrus faurei* | | | |
| Rootstock control | 100 | 100 | 100 |
| Rootstock flooded | 100 | 100 | 100 |

Appendix F. The effect of 1 and 20 months of continuous flooding on growth of ungrafted Pyrus species. Soil flooding was initiated Sept. 8, 1980 and an increase in plant height was determined after 1 and 20 months of treatment.

| Species | Increase In Plant Height (cm) | |
|---|---|---|
| | 1 Month | 20 Months |
| Pyrus dimorphophylla | | |
| Control | 9.5b | 64.8c |
| Flooded 1 month | 4.8a | 46.7b |
| Flooded continuously | 3.7a | 4.9a |
| Pyrus pashia | | |
| Control | 9.7b | 51.4c |
| Flooded 1 month | 3.1a | 29.8b |
| Flooded continuously | 4.5a | 0a |
| Pyrus amydaliformes | | |
| Control | 3.6a | 33.4c |
| Flooded 1 month | 2.6a | 20.5b |
| Flooded continuously | 2.5a | 0a |

*Significance determined for each species on each sampling date by Duncan's new multiple range test, 5% level.

Appendix G. The effect of 1 and 12 months of continuous flooding on growth of ungrafted Cydonia, Malus and Pyrus rootstocks. Soil flooding was initiated June 3, 1981 and an increase in plant height was determined after 1 month and 1 year of treatment.

| Species | Increase In Plant Height (cm) | |
|---|---|---|
| | 1 Month | 12 Months |
| Cydonia oblonga | | |
| EMLA C | | |
| Rootstock control | 11.9 | 31.4 |
| Rootstock flooded | 3.3* | 4.7* |
| Malus domestica | | |
| MM 111 | | |
| Rootstock control | 14.1 | 57.1 |
| Rootstock flooded | 5.0* | 0* |
| Pyrus faurei | | |
| Rootstock control | 3.3 | 5.9 |
| Rootstock flooded | 5.5 | 6.8 |

*Significance determined for each species on each sampling date separately by LSD, 5% level.

Appendix H. Ethylene evolution from excised root pieces incubated in air for approximately 5 hours. Measurements were taken after 30 days of soil flooding in the growth chamber.

| Species | Ethylene (ppm/g hr) | |
|---|---|---|
| | Unflooded control | Flooded |
| *P. betulaefolia* | 0.327[z] | 0.764 |
| *P. calleryana* | 0.714 | 0.423 |
| *P. communis* Bartlett | 0.707 | 0.388* |
| *Cydonia oblonga* Province BA 29 | 0.254 | 0.596 |
| *Prunus persica* Halford | 0.236 | 0.301 |

[z]Vials containing the roots were immediately sealed after excision.

*Means significantly different, LSD 5% level.

Appendix I. Ethylene evolution from excised root pieces incubated in air for approximately 5 hours. Measurements were taken after 7 and 21 days of treatment imposition in solution culture.

| Species/treatment | Ethylene (ppm/g hr) Day 7[z] | Day 21[y] |
|---|---|---|
| *P. betulaefolia* | | |
| aerobic | 0.403 | 0.006 |
| anaerobic | 0.382 | 0.008 |
| *P. calleryana* | | |
| aerobic | 0.235 | 0.000 |
| anaerobic | 0.177 | 0.050 |
| *P. communis* OH x F 97 | | |
| aerobic | 0.170 | 0.000 |
| anaerobic | 0.613 | 0.000 |
| *Cydonia oblonga* Provence BA 29 | | |
| aerobic | 0.000 | 0.000 |
| anaerobic | 0.022 | 0.000 |
| *Prunus persica* Halford | | |
| aerobic | 0.387 | 0.000 |
| anaerobic | 0.290 | 0.000 |
| *Salix discolor* | | |
| aerobic | 0.008 | 0.000 |
| anaerobic | 0.519* | 0.344 |

[z]Roots on day 7 were capped in vials immediately after excision.

[y]Roots on day 21 were capped in vials 45 minutes after excision.

*Means significantly different, LSD 5% level.

Appendix J. 1-aminocyclopropane-1-carboxylic acid (ACC) levels in the xylem fluid of various species after 7, 20 and 40 days of exposure to aerobic or anaerobic solution culture treatments.

| Species | ACC (nmol $ml^{-1}$) 7 days | 20 days | 40 days |
|---|---|---|---|
| Pyrus betulaefolia | | | |
| aerobic | 0 [z] | 0 | 0 |
| anaerobic | 0 | 0 | 0 |
| Pyrus calleryana | | | |
| aerobic | - | 0 | 0 |
| anaerobic | - | 0 | 0 |
| Pyrus communis OH X F 97 | | | |
| aerobic | 0 | 0 | 0 |
| anaerobic | 0 | 0 | 0 |
| Cydonia oblonga Provence BA 29 | | | |
| aerobic | - | 0 | 0 |
| anaerobic | - | 0 | 0 |
| Prunus persica Lovell | | | |
| aerobic | 0 | 0 | - |
| anaerobic | 0 | 0 | - |
| Salix discolor | | | |
| aerobic | - | 0 | 0 |
| anaerobic | - | 0.238 | 0.230 |
| Lycopersicon esculentum Rutgers | | | |
| aerobic | 0 | - | - |
| anaerobic | 5.99* | - | - |

*Means are significantly different by LSD, %5 level.
[z]Quantity of xylem fluid used was between 1 and 3 mls for all species except Salix discolor where 3-6 mls was used.

Appendix K. Root respiration rates of excised root pieces of *P. betulaefolia* and *P. communis* cv. OH X F 97 in 0.5% $O_2$ and 21% $O_2$ (air) as a function of time after excission.

| Species/incubation | Δt (hrs) | Respiration Rate (µl $CO_2$ $g^{-1}$ $hr^{-1}$) |
|---|---|---|
| *P. betulaefolia* | | |
| aerobic | 1.25 | 83.35 ± 17.34[z] |
| | 2.83 | 92.79 ± 14.94 |
| | 4.50 | 86.00 ± 20.00 |
| anaerobic | 1.00 | 63.04 ± 24.54 |
| | 2.75 | 53.69 ± 30.19 |
| | 4.00 | 61.59 ± 29.30 |
| *P. communis* cv. OH X F 97 | | |
| aerobic | 1.25 | 125.69 ± 21.74 |
| | 2.83 | 144.52 ± 34.15 |
| | 4.50 | 120.35 ± 32.43 |
| anaerobic | 0.83 | 84.25 ± 12.42 |
| | 2.75 | 61.18 ± 23.20 |
| | 4.00 | 64.85 ± 13.32 |

[z]Mean value of 4 measurements ± 1 standard deviation.

Appendix L. Fractional release of electrolytes of aerobic and anaerobically treated Pyrus, Cydonia, Prunus and Salix roots incubated for 24 hours at 22°C under various treatments. Electroconductivity determined by a Markson conductivity meter.

| Species | Treatment | Incubation | Fractional Leakage Of Electrolytes | |
|---|---|---|---|---|
| | | | $\bar{X}$ | ± SD |
| Pyrus betulaefolia | | | | |
| | aerobic | distilled water | 0.720* | 0.171 |
| | aerobic | 0.05M mannitol | 0.657 | 0.116 |
| | anaerobic | distilled water | 0.698 | 0.185 |
| | anaerobic | deoxygenated distilled water | 0.540 | 0.153 |
| | anaerobic | 0.05M mannitol | 0.551 | 0.153 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.594 | 0.074 |
| Pyrus calleryana | | | | |
| | aerobic | distilled water | 0.519 | 0.113 |
| | aerobic | 0.05M mannitol | 0.508 | 0.117 |
| | anaerobic | distilled water | 0.595 | 0.126 |
| | anaerobic | deoxygenated distilled water | 0.545 | 0.292 |
| | anaerobic | 0.05M mannitol | 0.595 | 0.036 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.632 | 0.063 |
| Pyrus communis OH x F 97 | | | | |
| | aerobic | distilled water | 0.368 | 0.139 |
| | aerobic | 0.05M mannitol | 0.422 | 0.130 |
| | anaerobic | distilled water | 0.282 | 0.153 |
| | anaerobic | deoxygenated distilled water | 0.319 | 0.180 |
| | anaerobic | 0.05M mannitol | 0.371 | 0.182 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.306 | 0.203 |
| Cydonia oblonga Provence BA 29 | | | | |
| | aerobic | distilled water | 0.369 | 0.101 |
| | aerobic | 0.05M mannitol | 0.407 | 0.105 |
| | anaerobic | distilled water | 0.325 | 0.071 |
| | anaerobic | deoxygenated distilled water | 0.346 | 0.024 |
| | anaerobic | 0.05M mannitol | 0.338 | 0.050 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.398 | 0.049 |

*Values represent the mean ± 1 standard deviation.

Appendix L. continued

| Species | Treatment | Incubation | Fractional Leakage Of Electrolytes | |
|---|---|---|---|---|
| | | | $\bar{X}$ | ± SD |
| Prunus persica Lovell | | | | |
| | aerobic | distilled water | 0.603 | 0.261 |
| | aerobic | 0.05M mannitol | 0.542 | 0.184 |
| | anaerobic | distilled water | 0.815 | 0.167 |
| | anaerobic | deoxygenated distilled water | 0.838 | 0.037 |
| | anaerobic | 0.05M mannitol | 0.723 | 0.126 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.861 | 0.120 |
| Salix discolor | | | | |
| | aerobic | distilled water | 0.272 | 0.066 |
| | aerobic | 0.05M mannitol | 0.267 | 0.069 |
| | anaerobic | distilled water | 0.324 | 0.088 |
| | anaerobic | deoxygenated distilled water | 0.516 | 0.068 |
| | anaerobic | 0.05M mannitol | 0.441 | 0.235 |
| | anaerobic | deoxygenated 0.05M mannitol | 0.391 | 0.107 |

Appendix M. Leaf conductance (cL) after various durations of anaerobiosis for Bartlett seedlings of P. communis in solution culture. Experiment conducted during May 1982.

| | cL (cm/sec) | | | |
|---|---|---|---|---|
| | 4 days | 9 days | 20 days | 30 days |
| aerobic | 0.422 | 0.756 | 0.361 | 0.662 |
| anaerobic | 0.215* | 0.090* | 0.204* | 0.009* |

*Means significantly different, LSD 5% level.

Appendix N. Diurnal root hydraulic conductivity (Lp) of *P. communis* cv. OH x F 97 expressed on a dry root basis, leaf area basis, and stem cross-sectional area basis determined at midday and at night.

| | Lp (root basis) (µl/g MPa min) | Lp (leaf basis) (µl/cm$^2$ MPa min) | Lp (stem basis) (µl/cm$^2$ MPa min) |
|---|---|---|---|
| Midday | | | |
| rep 1 | 438.83 | 23.28 | 0.440 |
| rep 2 | 318.18 | 10.70 | 0.263 |
| rep 3 | 369.14 | 12.03 | 0.474 |
| Night | | | |
| rep 1 | 465.44 | 26.27 | 0.585 |
| rep 2 | 487.73 | 22.05 | 0.300 |
| rep 3 | 360.25 | 9.05 | 0.334 |

Appendix O. Observations of foliage morphology as affected by 2 treatments of soil flooding conducted in the spring of 1982: A treatment where plants were submerged and drained at 3 week intervals, and another treatment where plants were flooded continuously for 2 months.

| Species | % of plants with 50% defoliation | |
|---|---|---|
| | Intermittent Flooding | Continuous Flooding |
| *P. betulaefolia* | 0[z] | 0 |
| *P. communis* (Bartlett seedling) | 0 | 0 |
| OH x F 97 | 60 | 0 |
| OH x F 217 | 70 | 0 |
| OH x F 333 | 80 | 50 |

[z]evaluations after 2 months of treatment imposition.

Appendix P. The relationship of exudation rate (Jv) as a function of applied pressure (ΔP).

| ΔP | 0.14 MPa | 0.28 MPa | 0.55 MPa | 0.82 MPa | 1.10 MPa |
|---|---|---|---|---|---|
| Species | Jv/plant (µl/plant MPa min) | | | | |
| P. betulaefolia | 428.6[z] | 428.6 | 418.2 | 512.2 | 527.3 |
| Cydonia oblonga Provence BA 29 | 142.9 | 160.7 | 154.5 | 213.4 | 227.3 |
| Salix discolor | 371.4 | 428.6 | 1000.0 | 1646.3 | 1890.9 |

[z]1 determination at each pressure for each species.

Appendix Q. Solute concentration as a function of applied pressure for xylem fluid of P. communis cv. OH X F 97 roots treated with 0.13 % ethanol for 2 hours.

| | mosmol/kg | | | |
|---|---|---|---|---|
| Pressure | 0.20 MPa | 0.35 MPa | 0.50 MPa | 0.62 MPa |
| | 16.3 ± 2.5[z] | 15.67 ± 3.21 | 16.0 ± 2.6 | 15.7 ± 4.2 |

[z]Mean ± 1 standard deviation.

Appendix R. Solute concentration as a function of temperature for xylem fluid of *P. communis* cv. OH X F 97 roots treated with $10^{-4}$ M ABA or 0.13% ethanol for 2 hours.

| | mosmol/kg | | | |
|---|---|---|---|---|
| Treatment/ Temperature °C | 7° | 15° | 25° | 35° |
| $10^{-4}$ M ABA | 13.0 ± 1.0[z] | 11.33 ± 0.58 | 9.33 ± 1.53 | 11.7 ± 1.15 |
| 0.13% ethanol (control) | 19.0 ± 1.0 | 18.4 ± 8.8 | 20.0 ± 0.82 | 21.0 ± 4.10 |

[z]Mean ± 1 standard deviation