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ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (PRUNUS CERASUS): EFFECT OF GUM ON XYLEM FUNCTION AND INFLUENCE OF TEMPERATURE

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ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (*PRUNUS CERASUS*): EFFECT OF GUM ON XYLEM FUNCTION AND INFLUENCE OF TEMPERATURE

By

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ABSTRACT

ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (PRUNUS CERASUS): EFFECT OF GUM ON XYLEM FUNCTION AND INFLUENCE OF TEMPERATURE

By

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The induction of gummosis over large portions of the tree and the occurence of shoot die-back has been a limitation in the use of ethephon, (2-chloroethyl)phosphonic acid, for the promotion of cherry fruit abscission as a harvest aid. This is especially true with high temperatures. The goals of this research were to determine the effects of ethephon-induced gummosis on xylem function and water status of sour cherry shoots (Prunus cerasus L. cv Montmorency), and how induced gummosis might be minimized while obtaining a consistent fruit-loosening response.

Vigorous, uniform trees were sprayed with ethephon at concentrations (0 to 69.2 mM) sufficient to induce gummosis. One-year-old control and gummed shoots and shoots that died back subsequent to the development of severe gummosis were sampled. Flow rates were measured by forcing deionized, distilled water through excised shoot segments over a range of pressure gradients (0 to 0.5 MPa m^{-1}). Hydraulic conductance was calculated from the slope of pressure graident vs flow rate. Number and radii of

functional and occluded vessels were determined from cross-sections after passing a 0.2% (w/v) safranin solution through excised shoot segments. Water potentials of internode and leaf tissue were determined with a thermocouple hygrometer. As the hydraulic conductance decreased, the number of conducting vessels and leaf and internode water potentials also decreased, while the water-extractable gum from ground shoot samples and the amount of gum flushed from the xylem of excised shoot segments increased. Hydraulic conductance was predicted by the Hagen-Poiseuille equation from the measured radii of functional vessels and assuming the viscosity of water was unaltered as it passed through the shoot segment. The discrepancy between measured and predicted hydraulic conductance increased as shoot gum content increased, suggesting an increase in vessel sap viscosity. This is consistent with the fact that gum was flushed from the vessels of excised shoot segments. The viscosity of aqueous solutions increased dramatically with an increase in gum concentration under conditions of Newtonian flow. The discrepancy between measured and predicted hydraulic conductance could be accounted for by estimated gum concentrations of less than 1%. Thus, gum interferes with xylem flow both by increasing sap viscosity and by occluding vessels, blocking further flow. Flow of gum solutions through a glass capillary became pseudoplastic at pressure gradients above 1.8 MPa m^{-1}.

William Charles Olien

The effect of temperature on the action of ethylene and the subsequent development of the sour cherry fruit-pedicel abscission layer in fruit explants was studied with and without exogenous ethylene. Fruit explants were collected during Stage III of fruit development. Decrease in fruit removal force was determined after incubation at temperatures between 15 and 35°C. There was no interaction between temperature and ethylene concentration, indicating that ethylene action was not affected by temperature. The subsequent development of the abscission layer was affected to a small extent by temperature (Q_{10} 20 to 30°C of 1.20). Thus, a fruit-loosening compound, whose activity was much less dependent on temperature than ethephon, would be expected to give more predictable results under varying field temperatures. Six potential fruit-loosening compounds were applied to sour cherry trees at equimolar concentrations and compared for time-course of ethylene generation, effect of temperature on ethylene generation, and reduction in fruit removal force. CGA 15281 was found to have good activity which was relatively unaffected by change in temperature. ER 3952, which was not an ethylene generator, also gave a good fruit-loosening response. The mechanism of action of this compound is unknown.

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INTRODUCTION

Mechanical harvest of cherries has become an economic necessity (Larsen 1969). With the development of shake-and-catch harvesters, the promotion of fruit abscission is essential to obtain maximum fruit removal with minimal damage to the fruit and tree. The development of the ethylene-generating compound ethephon, (2-chloroethyl) phosphonic acid, met this need (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Bukovac et al. 1971, Looney and McMechan 1970). Application of high concentrations of ethephon on cherry causes lenticel proliferation, loss of leaves, and gummosis (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Looney and McMechan 1970, Wilde and Edgerton 1975). However, the marked effect of temperature on the rate of ethylene generation from ethephon was not fully appreciated initially. The apparent energy of activation for ethephon degradation is approximately 30 kcal $mole^{-1}$ (Biddle et al. 1976, Gregory and Higgins 1974, Olien and Bukovac 1978). Ethephon-induced phytotoxicity with copious exudation of gum and pronounced shoot die-back has been associated with high field temperatures after ethephon application (Bukovac and Kesner 1973, Wilde and Edgerton 1975). Ethephon is not used for cherry harvest in California

for this reason (Micke et al. 1975). Some gummosis appears to be associated with ethephon use under a wide range of conditions. The effects of gummosis on tree function must be better understood to maximize tree performance and yield.

Gum formation is a naturally occurring response of many plants to stress, wounding, or attack by pathogens (Agrios 1969, Butler 1911, Esau 1965, Higgins 1919, Smith and Montgomery 1959, Talboys 1968). The term "gummosis" is generally used to describe the extrusion of gum from the plant and can be associated with the formation of lacunas (gum ducts). This process is especially common to species of Prunus, Citrus, and Acacia (Rawlins and Takahashi 1952, Schneider 1973, Smith and Montgomery 1959). Lacunas are formed by cell lysis, and may occur in the region of immature secondary xylem and in the periderm (Bradley et al. 1969, Bukovac 1979, Stosser 1978a, b, c, Wilde and Edgerton 1975). Among the residues of the lysed cells are toxic phenolic compounds, which are enzymatically oxidized to pigmented polyphenols (Talboys 1968). Most of the phenolic compounds in plant cells are bound to carbohydrates (Pridham 1965). In addition to these residues, lacunas are commonly filled with gum, a high molecular weight, water soluble polysaccharide (Smith and Montgomery 1959, Stosser 1978a, b). Prunus gums are arabinogalactans (Smith and Montgomery 1959).

Gum accumulation may result in pressures sufficient to drive the gum, and other entrapped materials, through

the periderm to form gum balls on the exterior of the tree (Bukovac 1979, Rawlins and Takahashi 1952, Schneider 1973, Stosser 1978a). These gum balls range in size from minute to several cm in diameter and in color from clear to yellow or dark brown, depending on the phenolic content (Talboys 1968). Gum has also been found in the lumen of xylem vessels (Stosser 1978a).

Gummosis is part of an overall defense mechanism of the tree which may be localized or may involve the entire tree, depending on the extent of injury or other stimulus (Shigo and Hillis 1973, Talboys 1968). On wounding, the damaged area is quickly sealed off by gum, vascular tissue in the area is occluded by gum and/or tyloses, cell lysis may occur around the injury, and toxic phenolic compounds are released (Agrios 1969, Rawlins and Takahashi 1952, Shigo and Hillis 1973, Talboys 1968). Subsequently, a meristematic region forms around the wound, producing thick walled cells which further isolate the wound from the plant body. Lignification and suberization may also occur (Lipetz 1970, Shigo and Hillis 1973). Gum secretion at wounds and into xylem vessels serves to protect the tree both from water loss and from invasion of pathogens (Agrios 1969, Butler 1911, Eames and MacDaniels 1947, Esau 1965, Higgins 1919, Schneider 1973, Smith and Montgomery 1959). This function of gum in plants has been compared to the clotting of blood in animals to form a scab as a response to wounding (Smith and Montgomery 1959). Gum stopped the

diffusion of applied poisons through the tree, so long as the tissue was not entirely killed by the compounds applied (Higgins 1919). Living tissue is required for gum synthesis (Higgins 1919, Butler 1911, Stosser 1978a, Talboys 1968). The capsular polysaccharides of the pheumococcus bacteria are similar to the gum exudate of cherry and serve a similar function in protecting bacteria (Jirgensons 1958, Smith and Montgomery 1959). Gums can be digested by various micro-organisms (Smith and Montgomery 1959) and thus may function only as a physical barrier to infection. Spores of wilt pathogens germinated when entrapped in a gel matrix occluding vessels (VanderMolen et al. 1977).

Gums may have other functions in plants as well. Arabinoxylan gums of winter cereals can inhibit the growth of ice crystals through critical meristematic tissues and are therefore important to the winter hardiness of these species (Olien 1965, 1967, 1978, Olien et al. 1968). The exudation of polysaccharide root cap slime may aid in the advance of the root tip through the soil matrix (Esau 1965).

Plant gums are not randomly polymerized end products of cell lysis, but are genotypically specific, high molecular weight polysaccharides composed of a number of different sugar monomers (Aspinall 1969, Smith and Montgomery 1959). The composition of many plant gums are listed by Smith and Montgomery (1959). Dea (1970) was able to distinguish between five varieties of Prunus avium by the

chemical characteristics of the gums. Apart from their importance to the survival of plants, gums are also important commercial commodities, being used as adhesives, emulsifiers, and as additives to foods, pharmaceuticals, cosmetics, drilling fluids, etc. (Smith and Montgomery 1959, Whistler 1959). Sour cherry gum has been collected and used commercially in Europe for centuries, but has now largely been replaced by gum arabic (Howes 1949).

Plant gums are sometimes grouped with pectic substances because of their ease of extraction with water (Aspinall 1969, Esau 1965, Gortner and Gortner 1949). When concentrated, gum polymers easily form gel matricies which swell in water (Butler 1911, Esau 1965), and this could be a mechanism providing the pressure necessary for gum exudation from the shoot observed with gummosis. Thus, the extent that exudation of gum from the shoot reflects internal gum content is dependent on the water status of the tree as well as the amount of gum present. Butler (1911) has emphasized the importance of water in the development of gummosis. The high matric potential of gums in the water relations of the plant has also been exploited by plants in arid regions as a means of retaining water (Gortner and Gortner 1949, McBain 1959, Meidner and Sheriff 1976, Smith and Montgomery 1959).

Gum polysaccharides have been divided into gums and mucilages (Smith and Montgomery 1959). Gums form sols in water with sufficient dilution while mucilages only form expanded gel structures. This distinction is based on the

commercial use of gums and has no clear chemical basis (Smith and Montgomery 1959). Smith and Montgomery (1959) have, instead, grouped these polysaccharides into acidic and neutral gums. No naturally occurring basic gums have been found. The acidic gums occur as their neutral salts (Gortner and Gortner 1949).

Sour cherry gum is an acidic arabinogalactan (Smith and Montgomery 1959). Structurally, the gum is similar to the hemicellulose arabinogalactan extracted from larch wood (Aspinall 1969, Keegstra et al. 1973, Timell 1965) and the pectic arabinogalactan of sycamore primary cell walls (Keegstra et al. 1973). Galactose is linked β 1, 3 to form a linear backbone with frequent arabinosyl side chains. Small amounts of galactose, glucuronic acid, mannose, and rhamnose are also present in side chains (Aspinall 1969, Keegstra et al. 1973, Smith and Montgomery 1959, Timel 1965). The gum may contain xylose as well (Gortner and Gortner 1949). The glucuronic acid residues are present as end groups and are linked to mannose (Smith and Montgomery 1959). The molecular weight of gum from four different sweet cherry (Prunus avium L.) varieties ranged from 0.15 to 1.75 x 10^6 (Dea 1970).

Gum is often assumed to form from the remains of cell walls as cells are lysed in the formation of lucunas (Eames and McDaniels 1947, Esau 1965, Jane 1970, Schneider 1973). In light of current knowledge of gum chemistry (Aspinall 1969, Smith and Montgomery 1959) and the biochemistry of polysaccharide synthesis and secretion from plant cells

(Aspinall 1970, Darvill et al. (in press), Delmer 1977, Albersheim 1976, Paull and Jones 1975, Robinson 1977) this is very unlikely. Gum biosynthesis undoubtedly involves nucleotide sugars as precursors (Turner and Turner 1975), uridine diphosphate being most commonly associated with polysaccharide polymerization (Darvill et al. (in press)). Both glycolipids and glycoproteins may play a role in this process (Aspinall 1970, Darvill et al. (in press), Delmer 1977). Repeating units may be built up as glycolipids, which are then transferred to a growing polysaccaride polymer. Precursors may be transported as glycoproteins within membranes. It is uncertain whether synthesis begins in the endoplasmic reticulum or in the Golgi dictyosomes (Chrispeels 1967, Darvil et al. (in press), Robinson 1977). In any event, once initiated, synthesis appears to proceed in the Golgi dictyosomes (Darvill et al. (in press), Delmer 1977, Millinhauer 1974, Paull and Jones 1975, Robinson 1977). Pulse-chase experiments designed to follow the secretion of maize root-cap slime showed that the labeled fucose-containing polysaccharide migrated from the dictyosomes to the exterior of the cell in 20 to 30 min (Paull and Jones 1975). Dictyosome vesicles fused with the plasmalemma and evaginated, releasing the polysaccharide. The secreted material accumulated between the plasmalemma and the cell wall, indicating that there is some resistance offered by the cell wall to the release of the polysaccharide.

Lacuna formation in the immature secondary xylem of cherry wood begins with unlignified initial cells located

between the medullary rays (Stosser 1978a, Rawlins and Takahashi 1952). The initial cells are similar to meristematic cells and the initial cells of abscission zones (Stosser 1978a, Esau 1965). These cells are very metabolically active with dense cytoplasm, a high demand for sucrose, glucose, and amino acids, high protein synthesis, and high enzymatic activity (Stosser, 1978a, b, c). Nucleoli are present and many cells are multinucleate (Stosser 1978a). Stosser (1978a) followed gum synthesis from the interior of these cells to the extrusion of gum into the cell wall. Only then did the cell lyse, beginning the formation of a lacuna. A meristematic zone is evident around the young lacuna and the process of intense synthesis, followed by cell lysis, continues as the lacuna enlarges (Stosser 1978a). This is similar to the epithelial layer of schizogenously formed ducts in lacticifers and gymnosperms, except that epithelial cells do not lyse (Esau 1965). Gum and cell debris accumulate in the lacuna. After feeding ^{14}C-glucose and sucrose, labeled gum was found both in lacunas and as exuded from the shoot as gum balls (Stosser 1978b). Gum synthesis was most active at the time of active vegetative growth (Stosser 1978a). The amount of gum produced was greater than could have been accounted for by the amount of cell wall material hydrolyzed during cell lysis. The contents of the lacuna consists of cherry gum and hydrolysates of both cell wall and protoplasm, including phenols with antibiotic activity (Talboys 1968). Phenolic content is greater in cells of the periderm (Esau

1965) and lacuna formed in this region would be expected to produce a more pigmented gum exudate. Light and dark colored gum balls of the same age were often seen next to each other on the same shoot in the studies of gummosis in sour cherry which I conducted and report in this thesis.

Gum synthesis and cell lysis do not necessarily occur together. Stosser noted xylem vessels of cherry which were plugged with gum in regions of the shoot where lacuna were absent (Stosser 1978a). Gum is synthesized in the formation of heartwood and cell lysis is not evident during this process (Shigo and Hillis 1973). Gums of many plants are formed without cell lysis, such as root-cap slime exudates (Paull and Jones 1975) and the polysaccharide inhibitors of ice crystal growth in cereals (Olien 1965). Cereal arabinoxylan gums have been considered to be a water-soluble fraction of the cell wall (Bass et al. 1953).

During heartwood formation, or as a result of injury, xylem vessels are plugged by gum or tyloses, depending on the species (Nelson 1978). Whether the vessels are plugged predominantly by gum or tyloses is related to the size of the pits connecting vessels and ray cells, gum being more prominent in species with small pits (Talboys 1968). Occlusion of vessels prevents loss of water and the movement of micro-organisms in the transpiration stream (Agrios 1969, Dimond 1970, Esau 1965, Smith and Montgomery 1959). The release of gum into intercellular spaces and the lumen of vessels is the initial step in protecting leaf scars of olive from bacterial infection after leaf drop (Hewitt 1938).

In addition to these beneficial effects of gum, there is also an interference with the transpiration stream. Lateral transport between vessels is possible, however (Dimond 1966, 1970, Zimmerman and Brown 1971). The pits themselves can filter out some micro-organisms.

Vessels are not necessarily entirely occluded by the presence of gum. Gum and other materials can occur as a coating on the vessel wall, blocking pit membranes and narrowing the conducting cross-sectional area of the vessel (Daniell and Crosby 1968, Dimond 1970, Hillis 1976). Lateral movement of the xylem sap is thus blocked. Any occlusion that subsequently occurs in the vessel due to an air embolism or plugging by gum would make the vessel nonfunctional over the affected length. Wall coatings might make complete occlusion more likely to occur.

Aside from plugging or coating the vessel lumen, gums also have a profound effect on solution viscosity, and thus on the flow of sap through the vessels. In ideal Newtonian solutions, as envisioned by Einstein (Gortner and Gortner 1949), the solute molecules are spheres, each acting independently with the solvent. The rate of shear is proportional to the stress applied for such a solution, and the proportionality constant is the viscosity coefficient.

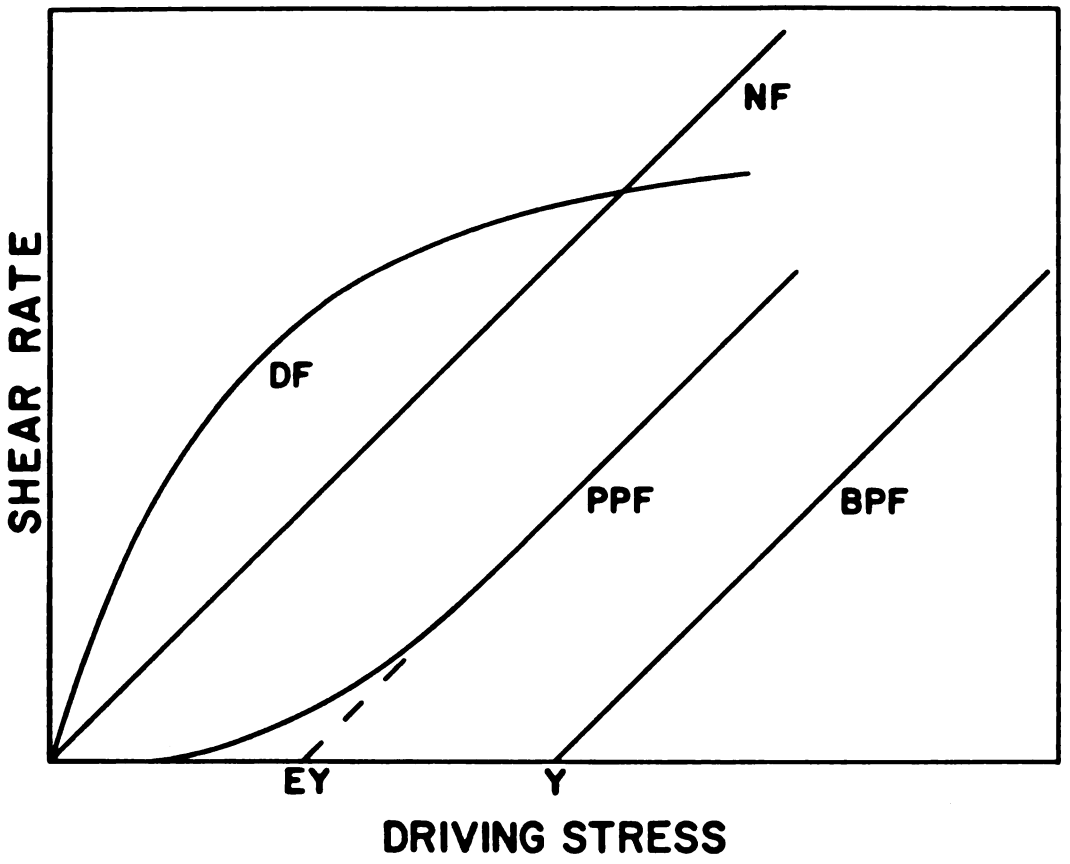
The Hagen-Poiseuille equation (Prandtl and Tietjens 1934) is an empirical description of flow through capillaries. It is assumed that the capillary is straight, smooth walled, and of uniform diameter, that the rate of shear of the fluid is proportional to the stress applied (Newtonian flow), and

that the flow is laminar, with maximum velocity occurring along the central axis of the capillary and no flow occurring at the capillary wall. A derivation of the Hagen-Poiseuille equation from these assumptions is given in Appendix A. Whether flow in the capillary is laminar or turbulent is determined by calculation of the Reynolds number (Handbook of Chemistry and Physics) (Appendix B).

The Hagen-Poiseuille equation is generally used to describe flow dynamics in the xylem (Zimmerman and Brown 1974), even though vessels are not ideal capillaries. The vessel walls are not perfectly smooth and lateral water movement is possible due to the presence of pits. Vessel end walls establish vessel length and resistance to flow from one vessel to the next. These are species specific characteristics and determine how nearly vessels approximate ideal capillaries. Greenidge (1952) reported the average apparent length of Prunus pensylvanica vessels to be 1.3 m.

Solutions of polymers commonly do not exhibit Newtonian flow due to large molecular size and the tendency to interact with each other. Such solutions are not true sols of independent molecules, but tend to form gels (McBain 1950, Siegel 1952). Non-Newtonian flow can be of several types, and there is a lack of agreement regarding terminology (Dinsdale and Moore 1962, Houwink 1958, Jirgensons 1958, McBain 1950, Scott Blair 1969). A simplified description of the four main categories is given by Dinsdale and Moore (1962) and is summarized in Figure 1. "Plastic flow" implies that the stress applied must be greater than some

Figure 1. Possible types of flow dynamics, modified from Dinsdale and Moore (1962). In considering flow through a capillary, the driving stress is: $\frac{P\ r}{2\ L}$, where P is the hydraulic pressure applied and r and L are the capillary radius and length. Shear rate is the change in flow velocity per change in radius: dv/dr. Line NF represents Newtonian flow as described by the Hagen-Poiseuille equation. In this case, change in shear rate is directly proportional to change in driving stress. Line BPF represents Bingham plastic flow with a yield value of Y. Line PPF represents pseudoplastic flow with an extrapolated yield value of EY. Line DF represents dilatent flow.


NF
DF
PPF
BPF
SHEAR RATE
EY
Y
DRIVING STRESS

threshold value (yield value) for flow to occur at all. In a strict sense (Bingham plastic flow), shear rates are linearly related to the applied stress above the yield value. Curves which deviate positively from linearity, whether a clear yield value occurs or not, are called "pseudoplastic" (Dinsdale and Moore 1962). Others refer to this behavior as "thixotrophic" (McBain 1950). The upper portion of the curve can generally be extrapolated back to a predicted yield value. This is a common behavior for viscous polymer colloids such as cellulose derivatives, rubber, starch paste, and chewing gum, and is often related to the breaking of weak attractive forces between polymer molecules as the applied stress is increased. Negative deviation from linearity is called "dilatant flow" (Dinsdale and Moore 1962) or "shear thickening" (Scott Blair 1969).

"Thixotrophy" refers to the reversible, isothermal sol-gel transformation (Dinsdale and Moore 1962, Houwink 1958, McBain 1950). If a sol is left standing, interactions may occur between the colloid particles which can eventually form a rigid gel. When stress is applied these interactions are broken and, with sufficient stress, the solution will completely revert to a sol. The stress-shear rate curve of this process displays pseudoplastic behavior. A yield value will occur with more stable intermolecular forces. Since there is a time dependence in the formation of the molecular interactions upon release of stress, the stress-shear rate curve will show hysteresis. The amount of

hysteresis will depend on both the number and strength of the molecular interactions and time.

True dilatancy is demonstrated by colloidal solutions in which shear rate cannot be increased above a maximum value as the applied stress is increased (McBain 1950, Scott Blair 1969). For flow to occur through closely packed sand particles, the system must become more open (dilate), resulting in an increase in volume. Flow becomes increasingly difficult with increasing stress as the particles pack instead of moving by each other. Thus, flow will not exceed a maximum limit regardless of the stress applied.

Whether xylem transport is decreased by occlusion of vessels or by increases in sap viscosity, shoot water relations can be disturbed when a sufficient number of vessels are affected (Agrios 1969, Stosser 1978a, Saaltink and Dimond 1964, Talboys 1968, Zimmerman and Brown 1971). Water uptake and shoot water potential decreased when shoot explants were fed solutions of polysaccharides (Sjulin and Beer 1978). Water conductance through stem segments of elm and cotoneaster was reduced by solutions of dextrans or of polysaccharide ooze from fire blight infected pear trees (Sjulin and Beer 1978, VanAllen and Turner 1975). Severe decreases in shoot water potential can cause shoot die-back (Talboys 1968, Syvertsen et al. 1980). Water stress can decrease photosynthesis and tree growth (Hsiao 1973), and at the time of floral initiation reduces the number of flower buds produced (Hall et al. 1977, Kaufmann 1972). Trees can recover from loss of xylem function, however,

with the formation of new xylem vessels (Daniell and Crosby 1968, Talboys 1968). Lacunas in cherry have been found surrounded by normal succeeding annual rings of wood (Bukovac 1979, Stosser 1978b).

Ethylene synthesis and action are known to be involved in plant responses to stress and wounding, in senescence and heartwood formation (Abeles 1973, Abeles and Abeles 1972, Hillis 1975, Nelson 1978), and have been associated with formation of oxidized polyphenols (Nelson 1978, Hillis 1975). Ethephon increases exudation processes in general, including guttation and the flow of resin and latex (Abeles 1973). Properly timed applications of ethephon increase bud and wood hardiness of dormant sweet cherry (Proebsting and Mills 1976). It would be interesting to determine whether this effect is associated with increased gum synthesis and whether cherry gum has a role in inhibiting the growth of ice crystals similar to arabinoxylan gums in cereals. Branches of peach trees have been induced to gum by treatment with ethylene gas (Martin and Nelson 1969). Naphthaleneacetic acid (NAA) is known to induce endogenous ethylene synthesis (Abeles 1973). NAA used for sprout control of peach increased the amount of gummosis at pruning wounds (Couvillon et al. 1972). Ethephon induces gum synthesis in Eucalyptus (Hissis 1975), and Prunus species (Anderson 1969, Gradley et al. 1969, Buchanan and Biggs 1969, Bukovac 1979, Bukovac et al. 1969, Daniell and Wilkinson 1972, Rom and Scott 1971, Stembridge and Gambrell 1971, Stosser 1978a, Wilde and

Edgerton 1975). Thus, ethylene and ethephon induce the same responses.

Recommended dosages of ethephon for the stimulation of cherry fruit abscission have been established (Anon. 1980) for normal field conditions. The amount of ethephon applied is easily controlled, but field temperatures after ethephon application are not. High temperatures result in much faster rates of ethylene release from ethephon, as previously stated. As a result, the ethylene concentration exceeds the level necessary to promote fruit abscission and gummosis is induced.

A wound must be protected and is generally a localized event. However, when ethephon is applied to the orchard as a harvest aid, gummosis can be induced over large portions of the tree. The intensity of the gummosis reaction, wherever it occurs, is dependent on the amount of ethylene released as well as the sensitivity of the tissue to ethylene (Hall et al. 1977). Severe ethephon-induced gummosis is associated with the death, and thus the loss, of future fruit bearing wood. The ethylene threshold concentration necessary to induce a response is genetically variable (Abeles 1973) and also depends on the physiologic state of the tree (Anderson 1969, Bukovac 1979, Hall et al. 1977). Since wounding and stress increase ethylene production in plants, it is not surprising that cherry trees already wounded or stressed are more apt to develop ethephon induced gummosis than are uninjured trees (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Wilde and Edgerton 1975). In addition, the ethylene thresholds necessary to induce different

ethylene-mediated responses vary. The thresholds for immature fruit abscission and gummosis must be similar in peach because fruit have not been successfully thinned with ethylene-generating compounds without also inducing severe gummosis (Buchanan and Biggs 1969, Daniell and Wilkinson 1972, Martin and Nelson 1969, Rom and Scott 1971, Stembridge and Gambrell 1971). However, abscission of cherry fruit can be promoted during Stage III of development without inducing gummosis of vigorous trees.

The temperature sensitivity of ethephon could be overcome by modifying the conditions affecting ethephon degradation, decreasing the effectiveness of the ethylene released, or by using some other fruit-loosening compound that is less affected by temperature. The rate of ethephon degradation is affected by (a) temperature, (b) pH (Biddle et al. 1976, Olien and Bukovac 1978, Warner and Leopold 1969) and (c) possibly by the formation of ethephon conjugates in tissue (Audley 1979, Edgerton and Hatch 1972, Gilbert et al. 1975). If excessive temperature occurred after ethephon application, the only practical recourse among these three factors would be to lower the temperature by overhead mist irrigation. This, however, would promote fruit pathogens such as brown rot (Agrios 1969, Westwood 1978) and cause cracking of sweet cherries (Westwood 1978). Water vapor pressure and temperature affect the degradation rate of ethephon residues (Klein et al. 1979), but the contribution of ethylene from such residues to the response is unknown. The fungicide chlorothalonil was

reported to have a synergistic effect with ethephon, increasing the rate of ethephon degradation and at the same time reducing the induction of gummosis (Holm and Edgerton 1976). In tests of our own (unpublished data) we found no significant interaction either in incubated solutions or when applied to sour cherry leaves.

The action of ethylene once released can be inhibited by silver ion (Beyer 1976a, b, 1979, Saltveit et al. 1978) and CO_2 (Abeles 1973, Beyer 1979). Silver ions are a health hazzard (Sax 1968), however, and it would be difficult to apply CO_2 in the field. Calcium can prevent ethylene-induced membrane leakage (Poovaiah 1979) and degradation of calcium pectates (Poovaiah and Rasmussem 1973). Calcium reduced ethephon-induced leaf abscission when applied in the ethephon spray (Martin et al. 1980), but application of calcium after ethephon treatment was not evaluated.

The best solution would be to find other compounds with fruit loosening activity that are less dependent on temperature than is ethephon. The activity of such a compound would be much more strictly dose dependent and thus more predictable under varying field temperatures.

The most deleterious effect of ethephon-induced gummosis on sour cherry trees is probably an interference with xylem function. Gum extruded from the site of synthesis into the vessel lumen through the pits could both affect sap viscosity and occlude vessels. When a sufficient proportion of the vessels in a shoot is affected, xylem hydraulic conductance and tissue water potential would be

expected to decline. This could lead to shoot die-back observed with severe ethephon-induced gummosis. This hypothesis led to the studies in Sections I and II of this thesis, in which I investigated the effects of ethephon-induced gummosis on xylem function and shoot water status.

I was also interested in finding a means of avoiding the severe gummosis associated with ethephon use under conditions of high temperatures. The effect of temperature on ethylene action and on the subsequent steps leading to the expression of an ethylene-mediated response is not known. The effect of temperature and ethylene concentration on sour cherry fruit abscission (Stage III of fruit development) was studied in Section III of this thesis. Finally, in Section IV, several potential stimulators of sour cherry fruit abscission were compared in the hopes of finding one with more stable activity under varying field conditions.

Section I

ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (*PRUNUS CERASUS*). I. EFFECT ON XYLEM FUNCTION AND SHOOT WATER STATUS.

ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (*PRUNUS CERASUS*). I. EFFECT ON XYLEM FUNCTION AND SHOOT WATER STATUS.

Abstract

Ethephon, (2-chloroethyl)phosphonic acid, is commonly applied to cherries (*Prunus* sp.) as a harvest aid, but can also induce severe gummosis. Ethephon was sprayed at concentrations up to 69.2 mM on *Prunus cerasus* L. cv Montmorency trees and the effects of gummosis on one-year-old shoots were studied. The number of functional vessels and the hydraulic conductance were decreased as the extractable gum content increased. Some gum was flushed from the xylem of excised segments of control shoots, but the amount of flushed gum greatly increased as gummosis developed. Flushed and extracted gum had the same monomer composition as that reported in the literature for gum not induced by ethephon. Internode and leaf water potentials decreased as gummosis increased, resulting in shoot dessication and die-back in severe cases. Recovery of xylem function may occur in less severe cases. There was an increasing discrepancy between measured and predicted hydraulic conductance as measured hydraulic conductance was decreased, suggesting that gum in the vessels has effects on the flow of xylem sap in addition to vessel occlusion.

Plant gums are large polysaccharides, sometimes grouped with pectins because of their water solubility (Aspinall 1969, Esau 1965, Smith and Montgomery 1959). Gum production is a common ethylene-mediated response of many plants to aging, stress, wounding, and attacks by insects and pathogens. Wounds are sealed and xylem vessels are occluded by the gum, preventing the loss of water and the invasion of pathogens into the plant (Agrios 1969, Butler 1911, Esau 1965, Higgins 1919, Martin and Nelson 1969, Nelson 1978, Shigo and Hillis 1973, Smith and Montgomery 1959, Talboys 1968). Gummosis is a descriptive term for the exudation of gum from plants, and is especially common to species of Citrus and Prunus (Butler 1911, Esau 1965, Rawlins and Takahashi 1952, Schneider 1973). Lacunas, formed by cell lysis in the immature secondary xylem and periderm of the stem, are sites of active gum synthesis and accumulation (Bradley et al. 1969, Bukovac 1979, Stosser 1978a, b, c, Wilde and Edgerton 1975). Gum synthesis is probably similar to that described for noncellulosic cell wall polysaccharides and polysaccharide root cap slimes (Albersheim 1976, Aspinall 1970, Darvill et al. (in press), Delmer 1977, Paull and Jones 1975, Robinson 1977). Aromatic compounds with the toxicity of phenolics are often entrapped in the gum matrix and aid in defense of the plant (Talboys 1968). The presence of polyphenols results in a yellow or brown colored gum. Little is known concerning what other functions plant gums may have. Arabinoxylan gums inhibit the growth of ice and

thus contribute to the cold hardiness of winter cereals (Olien 1965, 1967, 1978, Olien et al. 1968). The exudation of root cap slimes may aid in the advance of the root through the soil matrix (Esau 1965).

Sour cherry (Prunus cerasus L.) gum is a highly branched acidic arabinogalactan (Smith and Montgomery 1959), similar in structure to the hemicellulosic arabinogalactan of larch (Aspinall 1969, Timmell 1965) and the pectic arabinogalactan of suspension cultured sycamore cells (Keegstra et al. 1973).

The ethylene-generating compound ethephon, (2-chloroethyl) phosphonic acid, is commonly used for the promotion of cherry fruit abscission as an aid to mechanical harvest (Anderson 1969, Looney and McMechan 1970, Bukovac 1979, Bukovac et al. 1969, Bukovac et al. 1971). However, the rate of ethylene release from ethephon is highly dependent on temperature, with an apparent energy of activation of approximately 30 kcal $mole^{-1}$ (Biddle et al. 1976, Gregory and Higgins 1974, Olien and Bukovac 1978). Severe ethephon-induced gummosis and shoot die-back have occurred when high field temperatures followed ethephon application (Bukovac and Kesner 1973, Wilde and Edgerton 1975). Responses are more severe on trees already under stress (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Wilde and Edgerton 1975). Ethephon is sprayed over the entire tree and can therefore induce gummosis throughout the tree. The intensity of the gummosis response, wherever it occurs, is dependent on the amount of ethylene released as well as the sensitivity of the tree to

ethylene (Hall et al. 1977). Severe gummosis is associated with the death, and therefore the loss, of future fruit bearing wood. An interference with the water relations of the tree is probable. Stosser (1978a) found histologic evidence of vessel occlusion by gum. The present study was designed to determine the effect of ethephon-induced gummosis on xylem function and water relations of one-year-old shoots of sour cherry.

Materials and Methods

Plant Material and Ethephon Treatments. Selected branches of vigorous, established Prunus cerasus L. cv Montmorency trees, growing at the Michigan State University Horticultural Research Center near East Lansing, were sprayed with 0 to 10,000 μg ml^{-1} (69.2 mM) ethephon to induce gummosis. The surfactant X-77 (alkyl aryl polyethoxy ethanol and free fatty acids, Chevron Chemical Corp., San Francisco, CA) was included at 0.1% (v/v). Treatments were applied on eight dates between May 16, 1979 and August 31, 1979.

One-year-old shoots of uniform diameter and internode length were collected at several times after treatment and transported to the laboratory, taking care not to let the shoots dry out. The shoots were then held with the cut ends in water on the laboratory bench. Measurements were made on the day of collection in the sequence presented here. Shoots were collected based on the following criteria:

Control shoots were untreated with no visible injuries. Among the ethephon-treated shoots, both live gummed shoots, which had visible gum blisters or gum extrusions, and dead gummed shoots, which were defoliated, darker in color, and severely desiccated, were collected. Shoots collected in Exp. I were grouped into: a) Controls (untreated), b) Gum-E (sprayed prior to June 25, 1979 at 500 $\mu g\ ml^{-1}$ ethephon), c) Gum-L (sprayed on August 9 or August 22, 1979 at 5000 $\mu g\ ml^{-1}$ ethephon), and d) Dead (from both spray periods). Ethephon treatments in Exp. II were made on August 9 and August 22, 1979. Shoots collected in Exp. II were: a) Controls (untreated), b) Gum-F (the majority from branches sprayed with 2000 $\mu g\ ml^{-1}$ ethephon), and c) Gum-S (the majority from branches sprayed with 5000 $\mu g\ ml^{-1}$ ethephon).

Water Potential. Water potentials (ψ) were determined after the shoots were brought to the laboratory with a Wescor Model HR-33 Dew Point Microvoltmeter (Wescor, Inc., Logan, Utah) and two Wescor C-52 sample chamber psychrometers. Water potential of leaves was determined from leaf discs (6.35 mm diam.) excised with a paper punch and from 8 mm lengths of internode split longitudinally just before placing both halves in the sample chamber. The data presented were determined after 15 min equilibration. In most cases this was not sufficient time to allow for complete equilibration necessary for the best estimate of

tissue water potential, but was sufficient to show differences between treatments. Values are given in mega-Pascals.

Hydraulic Conductance. Hydraulic conductance (HC) of shoot segments was determined on the same shoot and at the same time as water potentials. Laminar flow of fluid through capillaries is described by the Hagen-Poiseuille equation:

$$F = \frac{P \pi \Sigma r^4}{8 \eta L}$$

where F is the volume flow rate, P is the pressure difference applied across the length L, r is the capillary (vessel) radius, and η is the viscosity coefficient of the fluid. The assumption of laminar flow was verified by calculation of the Reynolds number (Table I). The measured HC for each shoot segment was determined from the slope of applied pressure gradient vs the resulting flow rates.

$$\text{Measured HC} = \frac{F}{P/L} .$$

Pressure gradients were restricted to the range from 0 to 0.5 MPa m^{-1} for this purpose and all plots were linear within this range.

The apparatus used to measure HC is shown in Figure 1. Dionized, distilled water was forced through shoot segments at various pressures and the resulting volume rates of flow were determined. Shoot segments were excised under water, fitted through the rubber bung, and positioned in the

Table I. Reynolds Number at Maximum Flow Rates for Sour Cherry Internode Segments

Flow is laminar if Reynolds numbers are less than 2100. Viscosity was assumed to be that of water at 25 C in these calculations.

Gummosis Response Group	Maximum Reynolds Number
Control	2.89
Gum-E	3.36
Gum-L	1.01
Dead	8.04

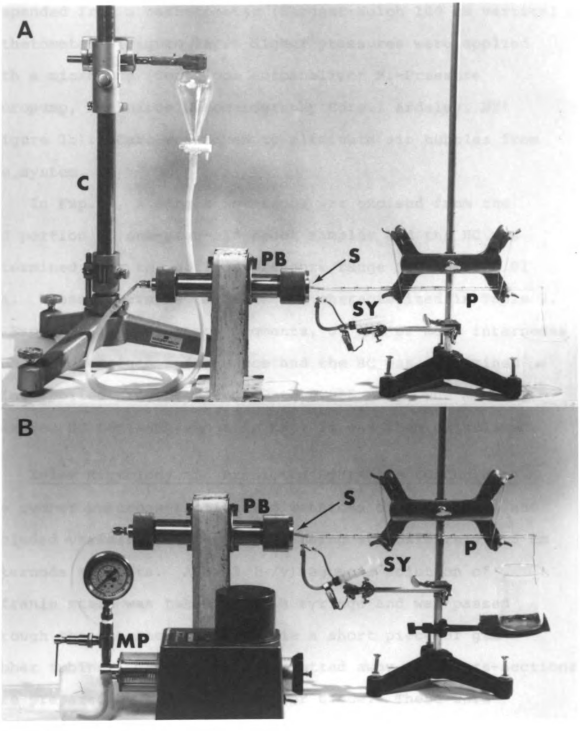
pressure chamber.[1] The pressure chamber was filled with water and pressures between 0 and 0.01 MPa were applied by the

[1]The hydraulic pressure chamber used in these studies was designed for studies of forced guttation by Dr. C. R. Olien, USDA-ARS, Crops and Soil Science Dept., Michigan State University, East Lansing, Michigan.

Figure 1. Apparatus used to determine pressure-flow relationships and hydraulic conductance. Shoot segments (S) were held in a rubber bung in a pressure chamber (PC). Volume rate of flow was measured by timing the advance of the meniscus in a 0.1 ml pipet (P). The meniscus was drawn back to the 0.00 ml mark at the start of each run with a syringe (SY).

a. Pressure supplied by head height of solution suspended from a cathetometer (C).

b. Pressure supplied by a micropump (MP).


A
C
PB
S
SY
P
B
PB
S
SY
P
MP

head pressure of a water reservoir (separatory funnel) suspended from a cathetometer (Sargent-Welch 100 cm vertical cathetometer) (Figure 1a). Higher pressures were applied with a micropump (Technicon AutoAnalizer Hi-Pressure Micropump, Technicon Chromatography Corp., Ardsley, NY) (Figure 1b). Care was taken to eliminate air bubbles from the system.

In Exp. I, a single internode was excised from the mid portion of one-year-old shoot samples and the HC was determined over the applied pressure range from 0 to 0.01 MPa. These internode segments are characterized in Table 2. In Exp. II, several shoot segments, of one or more internodes each, were excised in sequence and the HC was determined using applied pressures from 0 to 0.005 MPa. The mean measured HC for each shoot in Exp. II was then calculated.

Xylem Histology and Predicted Hydraulic Conductance. The number and cross-sectional dimensions of functional and occluded vessels were determined using approximately 1.5 cm internode segments. A 0.2% (w/v) aqueous solution of safranin stain was taken up in a syringe and was passed through the internode segment via a short piece of gum-rubber tubing. The excess was blotted away and cross-sections were prepared by hand with a razor blade. These were mounted in mineral oil to reduce diffusion of the dye and were photographed under a compound or dissecting microscope. The color photographic slides obtained were later projected on a screen and the number and radii (r) of both functional

Table II. Characteristics of Internode Segments From One-Year-Old Sour Cherry Shoots.

These data indicate a mean vessel diameter of 20.58 μm.

Parameter	Mean ± SD	Number of Observations
Internode diameter	$3.044 \times 10^{-3} \pm 0.334 \times 10^{-3}$ m	40
Internode length	$1.980 \times 10^{-2} \pm 0.250 \times 10^{-2}$ m	40
Number of vessels	1054.3 ± 204.7	7
vessel Σr	$10.848 \times 10^{-3} \pm 1.843 \times 10^{-3}$ m	7
vessel Σr^2	$126.2 \times 10^{-9} \pm 24.5 \times 10^{-9}$ m^2	7
vessel Σr^4	$20.19 \times 10^{-18} \pm 4.2 \times 10^{-18}$ m^4	7

and occluded vessels were recorded. Vessels surrounded by the red stain were considered functional and those without stain were counted as occluded. All vessels in five randomly selected radial sectors ("pie-slices") of 10° were measured for each cross section. The Σr, Σr^2, and Σr^4 was calculated for functional and occluded vessels in these five areas and the means were multiplied by 36 to give estimated values for the total cross section. Total numbers of functional and occluded vessels were arrived at in the same way. These determinations allowed calculation of a predicted HC from the Hagen-Poiseuille equation. When this is done using only functional vessels, the predicted HC can be compared with the measured HC.

$$\text{Predicted HC} = \frac{\pi(\text{functional } \Sigma r^4)}{8\eta} .$$

For a constant viscosity, this value is directly proportional to the Σr^4 term.

Gum Extraction. Stem tissue remaining after samples were removed for determination of water potential, HC, and histologic measurements, was frozen for later extraction of gum. The shoot samples were bulked by treatment group as designated in Exp. I. Gum was extracted from the wood based on methods of gum extraction from larch (Adams 1965) and cereals (Meredith et al. 1953, Olien 1965, Preece and MacKenzie 1952, Smith and Montgomery 1959). The stem tissue was cut into small pieces with a razor blade, further chopped in a Servall Omni-Mixer, and lyophilized.

The tissue was then refluxed in 85% ethanol to destroy hydrolytic enzymes, air dried, and ground to 20 mesh in a Wiley mill. One to 2 g of the milled wood was extracted three times with water at room temperature on a rotary shaker for 5 hr., yielding a total extract of 30 ml. The extract volume was reduced over a steam table and ethanol was added to make 40 ml of 85% (w/w) ethanol to precipitate the gum.

In Exp. II, gum was flushed from the xylem by pumping deionized distilled water through the shoot segments at pressures up to 0.07 MPa after first determining the measured HC. Even higher pressures were used for shoots with very low values of HC. The flushate was collected, frozen, and stored at -20 C. Subsequently, the bulked flushates were reduced in volume and the gum was precipitated with ethanol as before. Shoot segments, after being flushed, were stored frozen and the bulked samples were subsequently extracted for gum as in Exp. I. Dry weights of the gum precipitates were recorded. The gum obtained from milled wood will be referred to as "extracted gum" and gum flushed from intact shoot segments will be referred to as "flushed gum."

Some of the precipitates were hydrolyzed and the component neutral sugar monomers were derivatized to alditol acetates for gas chromatographic assay by the

method of Albersheim et al. (1967)[2]. Uronic acids are not assayed in this procedure. Optimum hydrolysis time for cherry gum with 2 N TFA was determined to be 90 min under standard autoclave conditions. Inositol was added as an internal standard prior to hydrolysis. After derivativization, the sugars were assayed on a Varian Aerograph, series 2100 gas chromatograph using a 1.8 m x 2mm i.d. glass column packed with 3% SP-2340 on Supelcoport (100 to 200 mesh). Column temperature was programmed from 150 to 210 C at 6 C/min. Helium was used as a carrier gas at 31 ml min^{-1} and detection was by flame ionization. Peak areas were determined with a Spectra Physics System 1 Computing Integrater. In addition to the internal standard, an external standard containing a reagent grade, equimolar mix of the sugars of interest was included. Good separation of the sugars was obtained from standards as well as gum samples. Extracted gum precipitates were compared with the monomer composition of extruded gum balls collected from the field and with published values for Prunus cerasus gum.

Results and Discussion

Field Observations. The appearance of trees after treatment with ethephon is illustrated in Figure 2 and 3.

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[2]GLC assay of neutral sugar composition for the gum polysaccharides isolated in this study was performed in the laboratory of Dr. D. P. Delmer, DOE, Michigan State University, East Lansing, Michigan.

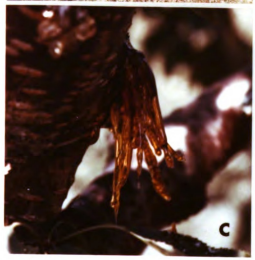
Figure 2. Gummosis of sour cherry.

a. Severe gummosis and die-back on ethephon-treated portion of tree.

b. Gum extrusion and die-back on current-seasons shoot.

c. Naturally occurring (not ethephon-induced) gummosis on a branch in response to a pruning wound.


A


C


B

Figure 3: Extrusion of gum from sour cherry.

a. Gum extruded from the cut end of an ethephon-treated one-year-old shoot.

b. Clear gum exuded from an ethephon-treated shoot.

c. Pigmented gum exuded from the cut end of an ethephon-treated shoot.


A
B
C

Control shoots remained normal, while ethephon caused extrusion of gum and die-back. Degree of gummosis was related to ethephon concentration and date of treatment. Higher concentrations of ethephon were required to induce the same degree of gummosis as the season progressed and growth rate decreased. Lacunas were observed in cross sections of gumming shoots (Figure 4). Gum extrusion was often associated with nodes of current season shoots, but any portion of one-or-more year-old shoots seemed capable of gum extrusion. Gum also oozed from the cut ends of affected shoots when samples were collected. The color of the extruded gum ranged from clear to yellow to dark brown, probably varying with the amount of phenolic material extruded along with the cherry gum. Exudation of gum occurred from severely affected trees for as long as two months after ethephon application.

Gum Extraction. Small quantities of gum were present even in control shoots (Table III). The amount of extracted gum per g milled wood increased with increasingly severe gummosis. Dead shoots yielded less gum than live gumming shoots, but more than control shoots. Either dead shoots contained less gum than did live, gumming shoots, or the gum in the desiccated, dead shoots was more difficult to extract. Only total extractable gum was measured in Exp. I. In Exp. II, there was an increase in both the flushed and extracted gum fractions with induced-gummosis. Of the gum present, the percentage of flushed gum increased as total

Figure 4. Shoot cross-sections illustrating functional and nonfunctional vessels. A 0.2% safranin solution was forced through the shoot. Functional vessels are surrounded by the red stain, nonfunctional vessels are not.

a. Control shoot.

b, c, d. Gummed but living shoots, Note lacunas.

e, f. Dead shoots.

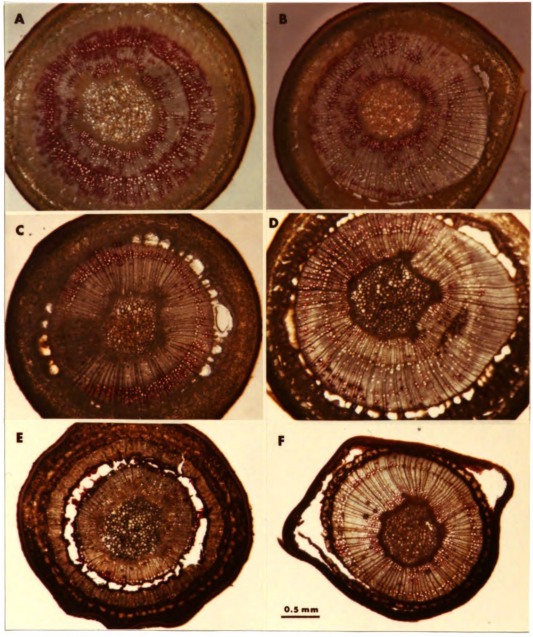

A
B
C
D
E
F
0.5 mm

Table III. Effect of Ethephon-Induced Gummosis on Gum Content of One-Year-Old Sour Cherry Shoots.

Experiment	Gummosis Response Group	Gum Yield per Wood Dry Weight (g/g)	
		Extracted Gum	Flushed Gum
I	Control	0.0110	---
	Gum-E	0.0474	---
	Gum-L	0.0677	---
	Dead	0.0332	---
II	Control	0.0081	0.00026
	Gum-F	0.0100	0.00381
	Gum-S	0.0166	0.00393

gum content increased (Table IV). Thus, more of the total extractable gum was found in the vessels of gummed than control shoots.

The neutral sugar monomer composition of extracted and flushed gum (Table V) agreed well with that of cherry gum balls collected from the field and with published values (Table VI). However, extracted and flushed gum from control shoots contained over 50% glucose. When the sugar ratios for control shoots were recalculated without glucose (Table V), they correspond with values obtained from gummed shoots, indicating that the gum from control shoots was extracted together with an unidentified glucose-containing polymer.

Xylem Histology. The average vessel diameter of one-year-old shoots was 20.6 μm. The percentage and Σr^4 for functional vessels were lower in gumming than control shoots (Table VII, Figure 4). There were few functional vessels in dead shoots. However, the flow of safranin solution was not restricted to the xylem of the excised segments from dead shoots as it was for control and live gummed shoots (Figure 4). This is probably related to extensive damage of the surrounding tissue due to cell lysis and tearing stresses of shoot desiccation followed by rehydration during measurement.

Hydraulic Conductance. Measured HC decreased with increasing severity of gummosis (Table VIII). The value of

Table IV. Effect of Ethephon-Induced Gummosis on Gum Flushed From One-Year-Old Sour Cherry Shoots as Percentage of Total Gum Obtained from These Shoots.

Gummosis Response Group	Flushed gum (%)
Control	3.08
Gum-F	27.6
Gum-S	19.1

Table V. Monomer Composition of Extracted and Flushed Gum.

Ratios for Control (+ Glc) were calculated including glucose, ratios for Control (- Glc) were calculated excluding glucose.

Gum Fraction	Gummosis Response Group	Mole % Neutral Sugal Sugar Monomers				
		L Ara	D Gal	D Man	D Xyl	D Glc
Extracted (Exp. I)	Control (+ Glc)	21.8	14.6	2.5	5.0	56.1
	(- Glc)	49.7	33.3	5.7	11.4	---
	Gum-E	52.3	30.7	5.6	7.0	4.4
	Gum-L	48.3	36.0	6.3	6.9	2.5
	Dead	52.6	31.7	5.8	7.5	2.5
Flushed (Exp. II)	Control (+ Glc)	27.3	15.4	Trace	6.3	51.1
	(- Glc)	55.7	31.4	Trace	12.9	---
	Gum-F	55.1	30.2	5.3	6.6	2.8
	Gum-S	54.7	32.2	5.5	6.3	1.3

Table VI. Composition of Sour Cherry Gum

Published values for gum not induced by ethephon from Smith and Montgomery (1959). Molar percentage of neutral sugars were compared with clear, extruded gum balls collected from branches of ethephon-treated sour cherry trees. Glucuronic acid content was not assayed.

	Mole % of Neutral Sugars	
Sugar	Published	Gum Ball
L Ara	68	59
D Gal	22	28
D Man	10	6
D Xyl	0	7
D Glc	0	1
D GlcA	10	--

Table VII. Effect on Ethephon-Induced Gummosis on Number of Functional Vessels of One-Year-Old Shoots.

Mean separation by Duncan's multiple range test. Means within columns followed by the same letter are not significantly different at P = 0.05.

Gummosis Response Group	Functional Vessels (%)	Functional Σr^4 $(m^4) \times 10^{18}$
Control	87.5 a	18.62 a
Gum-E	62.8 b	12.16 a
Gum-L	60.2 b	10.56 a, b
Dead	31.8 b	2.27 b

Table VIII. Effect of Ethephon-Induced Gummosis on Measured and Predicted Hydraulic Conductance (HC) of One-Year-Old Sour Cherry Shoot Segments

Mean separation within measured and predicted hydraulic conductance by Duncan's multiple range test. Means with the same letters are not significantly different at P = 0.05.

Significance of difference between measured and predicted hydraulic conductance at P = 0.05 (*) and P = 0.01 (**) by t-test.

Experiment	Gummosis Response Group	Hydraulic Conductance ($m^4 s^{-1} MPa^{-1}$) x 10^9			Ratio of Measured to Predicted HC
		Measured	Predicted		
I	Control	5.64 a	8.8 a	**	0.690
	Gum-E	2.42 b	5.34 a	*	0.453
	Gum-L	0.71 b,c	4.64 a,b	**	0.153
	Dead	0.86 c	1.00 b	ns	0.866
II	Control	4.95 a			
	Gum-F	1.15 b			
	Gum-S	0.62 b			

HC for dead shoots was probably overestimated since flow was not restricted to the xylem.

Since sequential samples from the same shoot were taken in Exp. II, trends in measured HC along the shoot could be examined (Figure 5). A generally increasing trend in HC occurred from the apical to the basal portion of one-year-old wood. Also, the mean measured HC and the scatter around the mean were less in gummed as compared to control shoots.

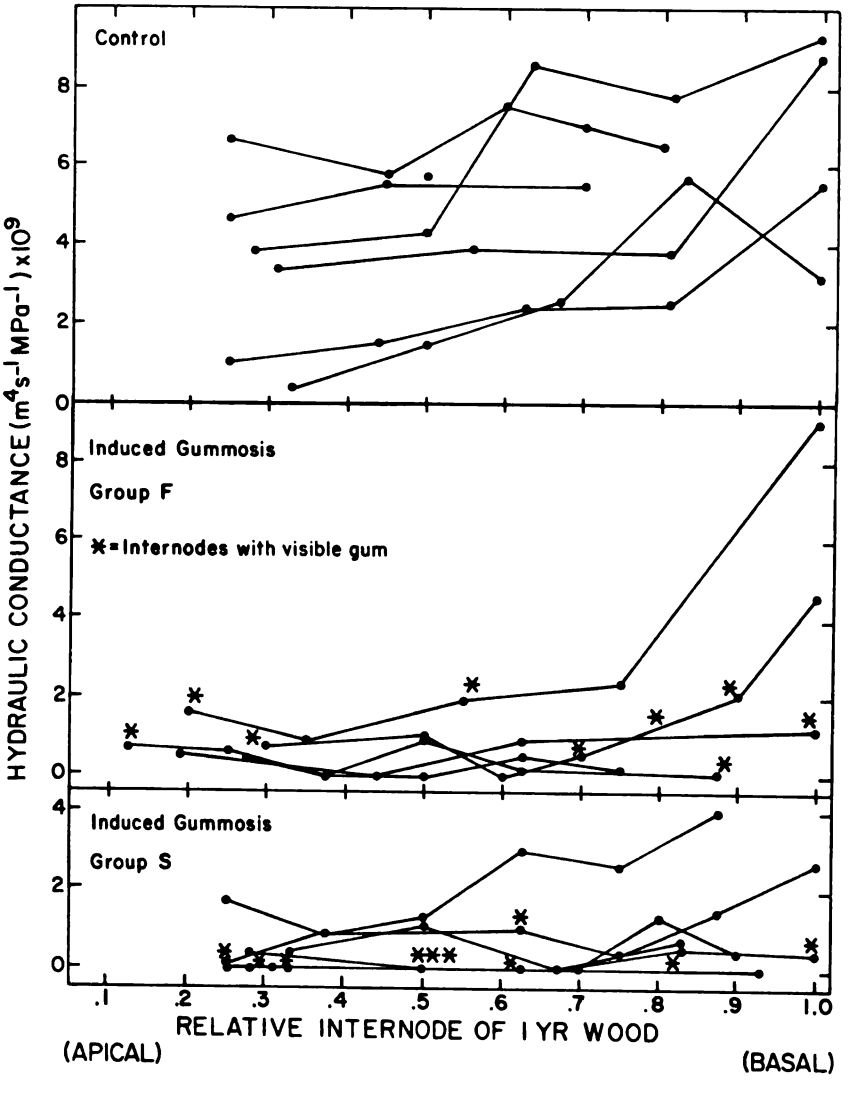
Determination of functional vessel Σr^4 (Exp. I) allowed calculation of predicted HC (Table VII). For this calculation it was assumed that the viscosity of water was unaltered as it passed through the internode segment, being 0.8937 cp at 25 C. Thus, predicted HC was directly proportional to the functional Σr^4. Predicted HC also decreased as the measured HC decreased and was significantly higher than measured HC in all cases, except for dead shoots where flow was not restricted to the xylem and, thus, the measured HC was probably overestimated. Except for dead shoots, the ratio of measured to predicted HC decreased with decreasing measured HC (Table VIII). There are several probable reasons for this. First, flow in the vessels may not be laminar. Reynolds numbers for the maximum flow rates observed were calculated assuming the viscosity of water and indicated that laminar flow occurred in all cases (Table I). All values were three orders of magnitude less than the upper limit (2100) for laminar flow.

Figure 5. Increase in hydraulic conductance from apical to basal end of gummed one-year-old sour cherry shoots. Asterisks indicate points of gum exudation.

a. Control shoots.

b. Gum-F shoots (Experiment II).

c. Gum-S shoots (Experiment II).

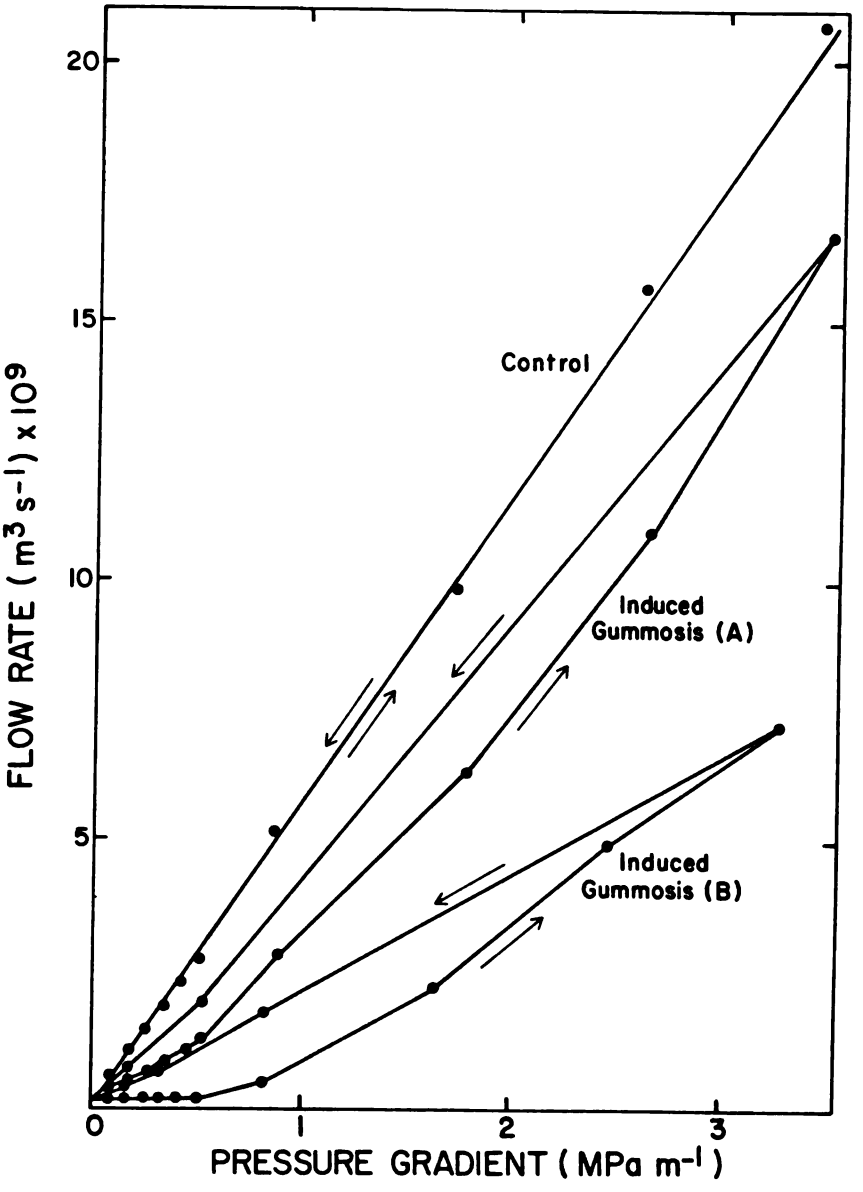

Control
Induced Gummosis
Group F
*=Internodes with visible gum
Induced Gummosis
Group S
HYDRAULIC CONDUCTANCE ($m^4s^{-1}MPa^{-1}$) x 10^9
RELATIVE INTERNODE OF 1 YR WOOD
(APICAL)
(BASAL)

Greater solution viscosity would only decrease the calculated Reynolds values. Thus, turbulent flow is unlikely.

Second, our measurements of functional vessel Σr^4 may be overestimated. The dye solution used to distinguish functional from nonfunctional (occluded) vessels required very little pressure to pass through the internode explants, so it is unlikely that plugged vessels were cleared by this procedure. Pressure gradients up to 14 MPa m^{-1} were insufficent to unplug the xylem of highly gummed shoots.

Third, and most likely, the viscosity of the solution in the vessels may have been greater than that of water. This seems to be the most likely alternative. When internode segments from gummed shoots were subjected to pressure gradients over 3 MPa m^{-1}, after initially determining flow rates at pressure gradients between 0 and 0.5 MPa m^{-1}, an increase in flow rate was observed when the pressure gradient was returned to the low range (Figure 6). This increase was not observed for control shoots, suggesting that material was being flushed from the xylem of gummed shoots. This suggestion is supported by the finding that a higher percentage of extractable gum was flushed from the xylem of shoots with decreased measured HC in Exp. II (Table IV). Thus, the increasing discrepancy between measured and predicted HC probably occurs because the viscosity of the fluid in functional (nonoccluded) vessels is increasingly greater than that of water, due to the increasing gum content of those vessels.

Figure 6. Pressure-flow curves for control and gummed one-year-old sour cherry shoot segments. The control internode segment was untreated. Curves A and B were obtained from the Gum-E and Gum-L ethephon-induced gummosis treatments of Exp. 1, respectively. Arrows indicate the order of data obtained for individual single internode shoot segments as the pressure gradient was first increased and then returned to a low range. The data do not indicate hysteresis because flow rates are irreversibly increased by the high pressure gradients. It is likely that flow rates are increased in gummed shoots because gum is flushed from the xylem with higher pressures.


20
15
10
5
0
1
2
3
FLOW RATE ($m^3 s^{-1}$) x 10^9
PRESSURE GRADIENT (MPa m^{-1})
Control
Induced Gummosis (A)
Induced Gummosis (B)

Water Potential. Water potential of both internode and leaf tissue was lower in gummed than in control shoots (Table IX). Shoots killed by gummosis were defoliated and severely desiccated. The internode water potential of dead shoots was below the detection capability of our instrument, the limit being about - 6 MPa.

Correlations. All parameters measured were correlated with the mean measured HC (Table X). Correlation coefficients obtained using mean values were high, but were generally not significant due to the small number of degrees of freedom. The highest correlation coefficients for means were obtained with extracted and flushed gum. Correlation coefficients for individual shoots were lower, but all were significant (Table X). Because the shoots were bulked prior to gum extraction, these data could not be correlated with HC on an individual shoot basis. Since the meaning of measured HC for dead shoots is questionable, this group was not included.

Time-Course of Hydraulic Conductance. The possibility of recovery from the observed decrease in HC is suggested by the composite time-course of measured HC, based on time after ethephon application (Figure 7). Measured HC did not change with time in control shoots. Measured HC declined rapidly in gummed shoots to a low plateau level. Shoots that were killed remained at this low level (Figure 7, line B). Two dead shoots showed higher HC values near 90 days, but

Table IX. Effect of Ethephon-Induced Gummosis on Water Potential of One-Year-Old Sour Cherry Shoots.

Water potential was determined with a dew point thermocouple hygrometer. Dead shoots had no leaves and the water potential of internode tissue from dead shoots was below the detection capability of our instrument.

Mean separation by Duncan's multiple range test. Means with the same letter are not significantly different at P = 0.05.

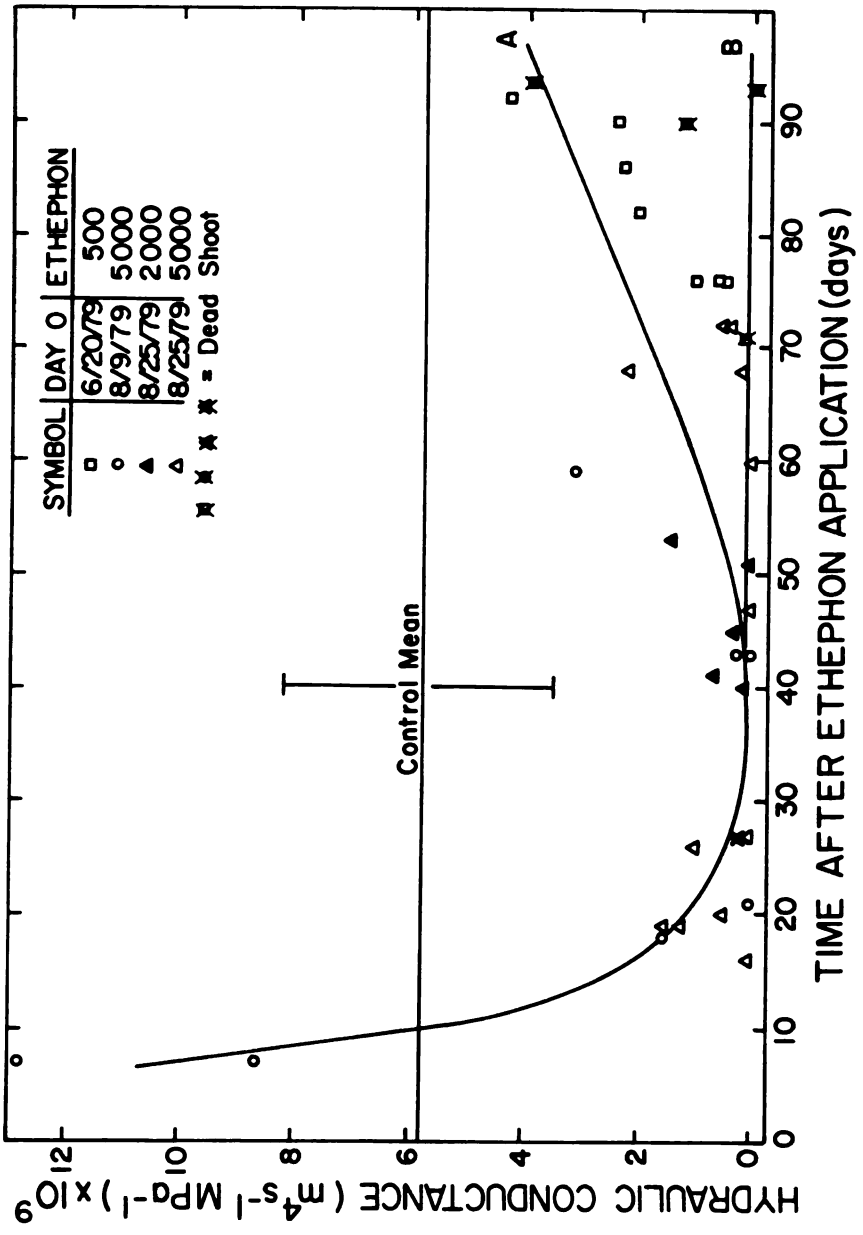
Experiment	Gummosis Response Group	Water potential (MPa) Internode		Leaf	
I	Control	- 0.74	a	- 1.83	a
	Gum-E	- 1.70	ab	- 3.84	b
	Gum-L	- 2.87	b	- 3.49	b
	Dead	Dry		---	
II	Control	- 0.99	a	- 2.31	a
	Gum-F	- 2.61	a	- 3.81	a
	Gum-S	- 1.63	a	- 3.03	a

Table X. Correlation of Effects of Ethephon-Induced Gummosis With Measured Hydraulic Conductance.

Dead shoots were not included in these calculations. The significance of the correlation coefficient (r) is indicated at P = 0.05 (*) and P = 0.01 (**).

Experiment	Parameter	Shoot Means (r)	Individual Shoots (r)
I	% Functional vessels	0.966 ns	0.679 **
	Σ(Functional vessel radius)^4	0.909 ns	0.660 **
	ψ Internode	0.985 ns	0.444 *
	ψ Leaf	0.871 ns	0.777 **
	Extracted gum	- 0.996 *	
II	ψ Internode	0.727 ns	
	ψ Leaf	0.791 ns	
	Extracted gum	- 0.935 ns	
	Flushed gum	0.977 *	

Figure 7. Time-course of measured hydraulic conductance with time after ethephon application. Curve A indicated recovery and curve B indicated no recovery in hydraulic conductance.

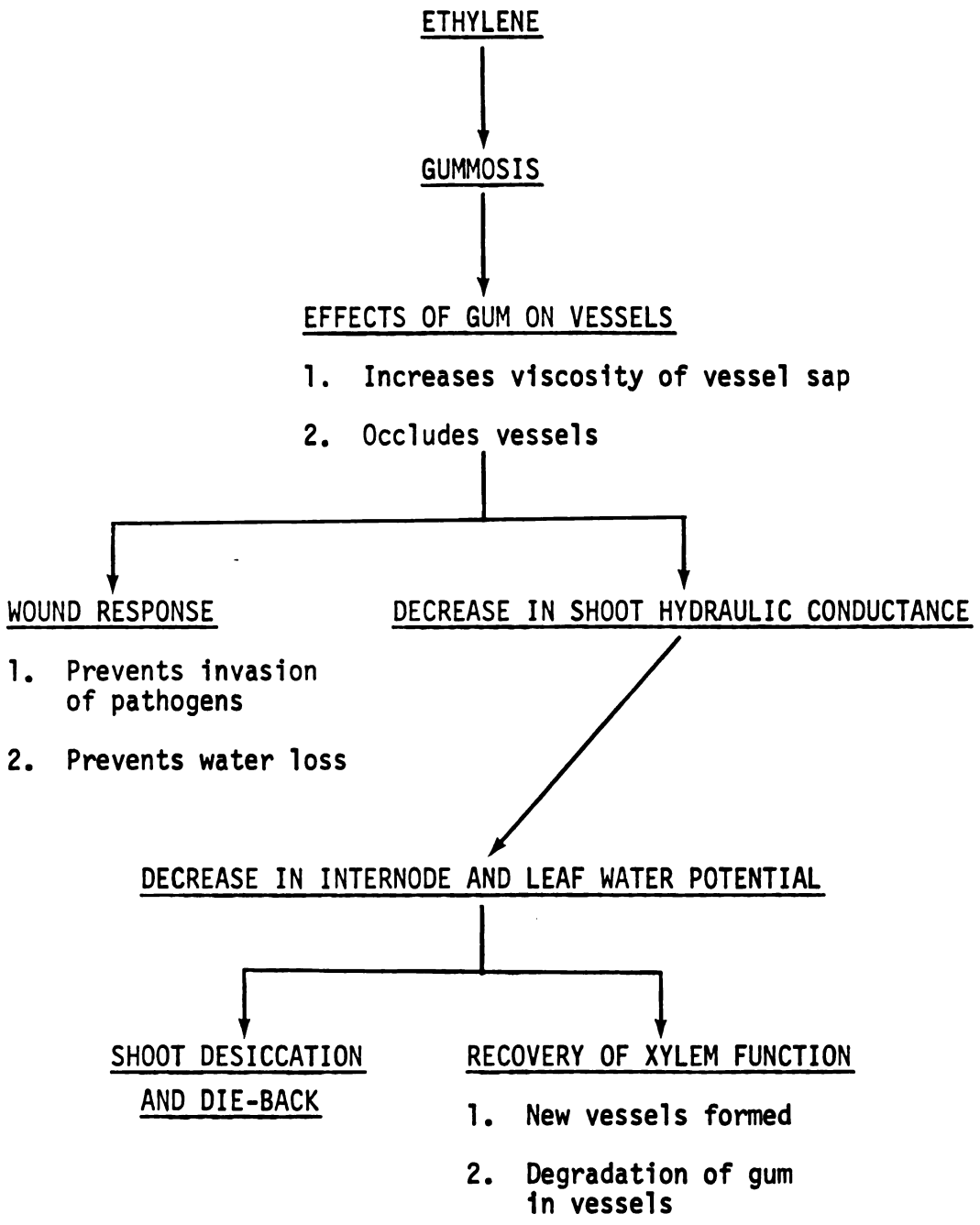

SYMBOL | DAY 0 | ETHEPHON
6/20/79 500
8/9/79 5000
8/25/79 2000
8/25/79 5000
= Dead Shoot
Control Mean
A
B
HYDRAULIC CONDUCTANCE ($m^4 s^{-1} MPa^{-1}$) x 10^9
TIME AFTER ETHEPHON APPLICATION (days)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90

flow was not restricted to the xylem in these shoots. Shoots that were not killed showed a generally increasing trend in measured HC with time after treatment (Figure 7, line A), regardless of the time of ethephon application. This recovery could be due to decreased gum content of existing vessels, perhaps by enzymatic degradation of the gum in those vessels, or by the formation of new functional vessels when the cambium is active. Terminal growth ceases in mature sour cherry trees during early July. This event has been correlated with the cessation of cambial activity (Esau 1965). Thus, while the formation of new xylem could explain the increasing trend in measured HC for shoots sprayed in mid-June, it is an unlikely explanation for shoots treated in late summer. Enzymes which degrade gum polysaccharides are present in cereals (Bass et al. 1957, Preece and MacKenzie 1952) and similar enzymes may be responsible for the recovery of measured HC observed in sour cherry in this study.

Conclusions

The results obtained in this study can be summarized by the following scheme (Figure 8). Gummosis is induced by ethylene. As a result, gum moves into the vessels and causes an increase in vessel sap viscosity. There was an increase in the amount of gum flushed from the xylem of intact shoot segments as total gum content increased. Gum extracted from ground stem samples and flushed from excised

Figure 8. The effects of ethylene-induced gummosis on sour cherry trees.


ETHYLENE
GUMMOSIS
EFFECTS OF GUM ON VESSELS
1. Increases viscosity of vessel sap
2. Occludes vessels
WOUND RESPONSE
1. Prevents invasion of pathogens
2. Prevents water loss
DECREASE IN SHOOT HYDRAULIC CONDUCTANCE
DECREASE IN INTERNODE AND LEAF WATER POTENTIAL
SHOOT DESICCATION AND DIE-BACK
RECOVERY OF XYLEM FUNCTION
1. New vessels formed
2. Degradation of gum in vessels

shoot segments had the same composition as gum not induced by ethephon. The effects of sour cherry gum on the viscosity and flow characteristics of aqueous solutions were subsequently studied (Olien 1980, Section II). As gum content of the vessel increases, the gum can occlude the vessel and prevent further flow. This is to the plants advantage at wounds, preventing the invasion of pathogens and the loss of water from the plant. Balanced against this is the reduction of hydraulic conductance. Ethephon is applied to the entire tree as a harvest aid and can therefore induce gummosis throughout the tree, as opposed to the localized effect of a wound. This has a major effect on tree hydraulic conductance, with no advantage to the plant as a wound response. Thus, a process evolved as a protective mechanism, now becomes a serious detriment to the tree. The decrease in hydraulic conductance results in decreased internode and leaf water potential. When severe, shoot desiccation and die-back occur. In less severe cases, recovery of xylem function may be possible. When the cambium is active, new functional vessels can be formed. The plant may also be able to regain function of existing vessels, perhaps by enzymatic degradation of the gum within affected vessels.

The die-back caused by severe ethephon-induced gummosis results in the loss of fruit bearing wood. Secondary and long term effects of gummosis are unknown. The dead wood and weakened condition of a tree suffering from severe

gummosis may make the tree more susceptible to attack by pathogens and insects and to winter injury. The effects of less severe gummosis, when induced over much of the tree and perhaps over several seasons, might be more subtle but no less important in determining tree productivity and longevity. The generalized decrease in the water status of the tree can decrease factors contributing to yield (Hall et al. 1977, Hsiao 1973, Kaufmann 1972). Thus a process which under normal conditions allows the tree to survive can result in injury and even death of the tree.

The increasing discrepancy between predicted and measured hydraulic conductance, as gum content increased, indicated that an increase in the number of vessels occluded by gum was not sufficient to account for the observed decrease in measured hydraulic conductance. The parallel increase in the amount of gum flushed from the xylem of excised shoot segments suggested that gum is present in functional vessels, thus, also affecting flow by altering vessel sap viscosity. This was further investigated (Olien 1980, Section II).

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Section II

ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (*PRUNUS CERASUS*) II. FLOW CHARACTERISTICS OF GUM SOLUTIONS

ETHEPHON-INDUCED GUMMOSIS IN SOUR CHERRY (*PRUNUS CERASUS*). II. FLOW CHARACTERISTICS OF GUM SOLUTIONS.

Abstract

Viscosity of dilute aqueous sour cherry gum solutions is greatly increased by small increases in gum concentration. Hydraulic conductance of gum solutions through a glass capillary was correspondingly decreased. Flow of gum solutions was Newtonian for pressure gradients up to 1.8 MPa m^{-1}, but became plastic at greater pressure gradients, with a resulting decline in apparent viscosity. The magnitude of this effect diminished as gum concentration increased. Flow of water, a solution of the component sugar monomers of sour cherry gum, and sucrose solutions remained Newtonian over the entire pressure gradient range examined (0 to 4.3 MPa m^{-1}). Assuming Newtonian behavior, the concentration of gum in functional (non-occluded) vessels was predicted which would account for the discrepancy between measured and predicted hydraulic conductance of internode segments excised from control and ethephon-treated one-year-old sour cherry shoots. We conclude that gum restricts flow in the xylem both by increasing vessel sap viscosity and by occlusion of vessels.

I established (Olien 1980) that ethephon-induced gummosis in one-year-old shoots of sour cherry (Prunus cerasus L. cv Montmorency) resulted in increased extractable gum and in occlusion of xylem vessels. Also, more gum was flushed from the xylem of internode segments excised from gummed shoots than from untreated shoots. However, the ratio of measured HC to predicted HC decreased with increasing severity of gummosis, suggesting that the effects of gum on HC are not restricted to vessel occlusion.

Low concentration of aqueous sour cherry gum solutions are very viscuous. Thus the presence of gum in small amounts could be expected to markedly alter sap viscosity in vessels and could therefore account for the increasing discrepancy between observed and predicted HC as degree of gummosis increased. A simple determination of viscosity over a concentration range for bulk gum solutions would not be sufficient to investigate this question, however. Solutions of polymers generally are non-Newtonian in their flow characteristics due to their large size and tendency to interact with each other (Siegel 1962). Thus, it is important to determine the changes in apparent viscosity that may occur over a range of applied stresses.

Flow is Newtonian when the rate of shear is proportional to the applied stress (McBain 1950). The proportionality constant is the viscosity coefficient. Non-Newtonian flow can be of several types. "Plastic flow" implies that there

is a threshold value of stress (yield value) before flow can occur. In "Bingham plastic flow" the rate of shear is linearly related to stress above the yield value and rarely occurs in the strictest sense (Dinsdale and Moore 1962). Commonly the rate of shear of polymer solutions increases faster than the increase in applied stress, creating an increasingly positive deviation from Newtonian behavior (McBain 1950). This behavior is referred to as "pseudo-plastic" or "thixotrophic" flow (McBain 1950, Dinsdale and Moore 1962) and is often due to the breaking of weak intermolecular forces between colloid particles as the applied stress is increased (McBain 1950, Jirgensons 1958). A clear yield value may not be observed, but a theoretical yield value may be obtained by extrapolation of the upper part of the curve to the stress axis. "Thixotrophy" is the isothermal, reversible interconversion of colloid solutions between the sol and gel states (Dinsdale and moore 1962, Houwink 1958). Gel structures formed at low stresses tend toward sols as the applied stress is increased. Negative deviations from linearity are also possible and are referred to as "dilatant flow" or "shear thickening" (Dinsdale and Moore 1962, Scott Blair 1969). In true dilatancy the space between colloid particles must expand for flow to occur (Scott Blair 1969). Flow can be Newtonian at low stresses, but as the driving stress increase, the particles pack. Flow reaches a maximum and increasing stress will not increase the flow rate. The purpose of

this study was to determine the flow characteristics of sour cherry gum solutions and relate these to the observations made for flow of water through sour cherry shoots where gumming was induced to various extents.

Materials and Methods

Test Solutions. Clear, non-pigmented gum balls, extruded from the branches of sour cherry trees (Prunus cerasus L. cv Montmorency) were collected following treatment with high concentrations of ethephon (up to 10,000 μg ml^{-1}, or 69.2 mM) and stored frozen. Gum solutions of 1.90 and 3.81% (w/v) were prepared by dissolving gum in warm, deionized, distilled water while mixing with a magnetic stirrer. The solutions were then spun in a clinical centrifuge to remove debris and stored at 4 C. Gum concentration was determined from the residue dry weight of a 2 ml aliquot of solution. Gum solutions of 1.91% and 1.09% were obtained by dilution of the 3.8% solution. Solution density was determined at 25 C in a 25 ml glass pycnometer. Viscosity was determined at 25 C with an Ostwald viscometer in a temperature controlled water bath. Deionized distilled water, solutions of 44 and 66% (w/v) sucrose, and a solution of the component neutral sugar monomers of sour cherry gum at the equivalent concentrations to a gum of a solution of 1.90% were also prepared for comparison with the gum solutions. The concentrations, densities, and viscosities were determined as described above (Table I).

Table I. Concentration, Density, and Viscosity of Sugar and Sour Cherry Gum Solutions.

Density and viscosity were determined at 25 C. Values for water are from the Handbook of Chemistry and Physics.

Solution	Solution Concentration (%, w/v)	Density (g cm^{-3})	Viscosity (cp)
Water	---	0.997	0.894
Sugar Monomers	1.90	1.000	0.922
Sucrose	44	1.160	4.55
	66	1.238	16.35
Gum	1.09	0.996	7.17
	1.90	1.000	13.23
	1.91	1.000	13.35
	3.81	1.007	35.10

Flow Experiments. Pressure-flow characteristics of these solutions were determined in a glass capillary (4.0 cm x 0.1 mm i.d.) using the appartus described previously (Olien 1980, Section I). Pressure gradients from 0 to 0.15 MPa m^{-1} were applied by adjusting the head height of a solution reservoir. Higher pressure gradients were attained with a micropump (Technicon AutoAnalizer Hi-Pressure Micropump, Technicon Chromatography Corp., Ardsley, NY). The test solution was placed ahead of a steel piston equipped with an O-ring in a specially designed hydraulic pressure chamber.[1] The piston and walls of the chamber were lubricated and the frictional resistance of the piston was measured. Water was pumped through the micropump, driving the piston forward to provide the required pressure. Flow rates through the capillary were measured by timing the rate of meniscus advance in a 0.1 ml pipet, calibrated in 0.01 ml divisions. Measured and predicted hydraulic conductance (HC) and the Reynolds numbers were determined for the test solutions in the capillary as previously described (Olien 1980, Section I).

Hydraulic conductances obtained with the glass capillary were compared with those obtained by forcing deionized, distilled water through excised segments of one-year-old

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[1]The hydraulic pressure chamber used in these studies was designed for studies of forced guttation by Dr. C. R. Olien, USDA-ARS, Crops and Soil Science Dept., Michigan State University, East Lansing, Michigan.

shoots (Olien 1980, Section I). These shoots had been induced to gum by the application of ethephon,(2-chloroethyl) phosphonic acid. Control shoots were untreated while shoots in the gummosis response groups Gum-E and Gum-L had been sprayed with 2000 or 5000 $\mu g\ ml^{-1}$ ethephon. The majority of the Gum-E shoots had been sprayed with 2000 $\mu g\ ml^{-1}$ and the majority of the Gum-L shoots had been sprayed with 5000 $\mu g\ ml^{-1}$.

Results and Discussion

Viscosity and Gum Concentration. The viscosity of dilute sour cherry gum solutions was greatly increased by small increases in gum concentration (Figure 1). The viscosity of a 1.9% gum solution was 13 cp, 15 times more viscous than water at 25 C (Table I). By comparison, a 66% solution of sucrose was required to obtain a viscosity of 16 cp.

Hydraulic Conductance. The pressure-flow data are presented in Figures 2 and 3. Data for water in Figure 3 with (line A) and without (line W) the piston in the pressure chamber indicated that the piston resistance resulted only in a shift in the abscissa intercept value (yield value) from the origin and did not alter the slope of the line. Thus, the piston moves without measurable resistance at pressures above the yield value and the only correction necessary of the test solution data obtained

Figure 1. Viscosity of sour cherry gum solutions as a function of gum concentration. Viscosity determined in an Ostwald viscometer at 25 C.

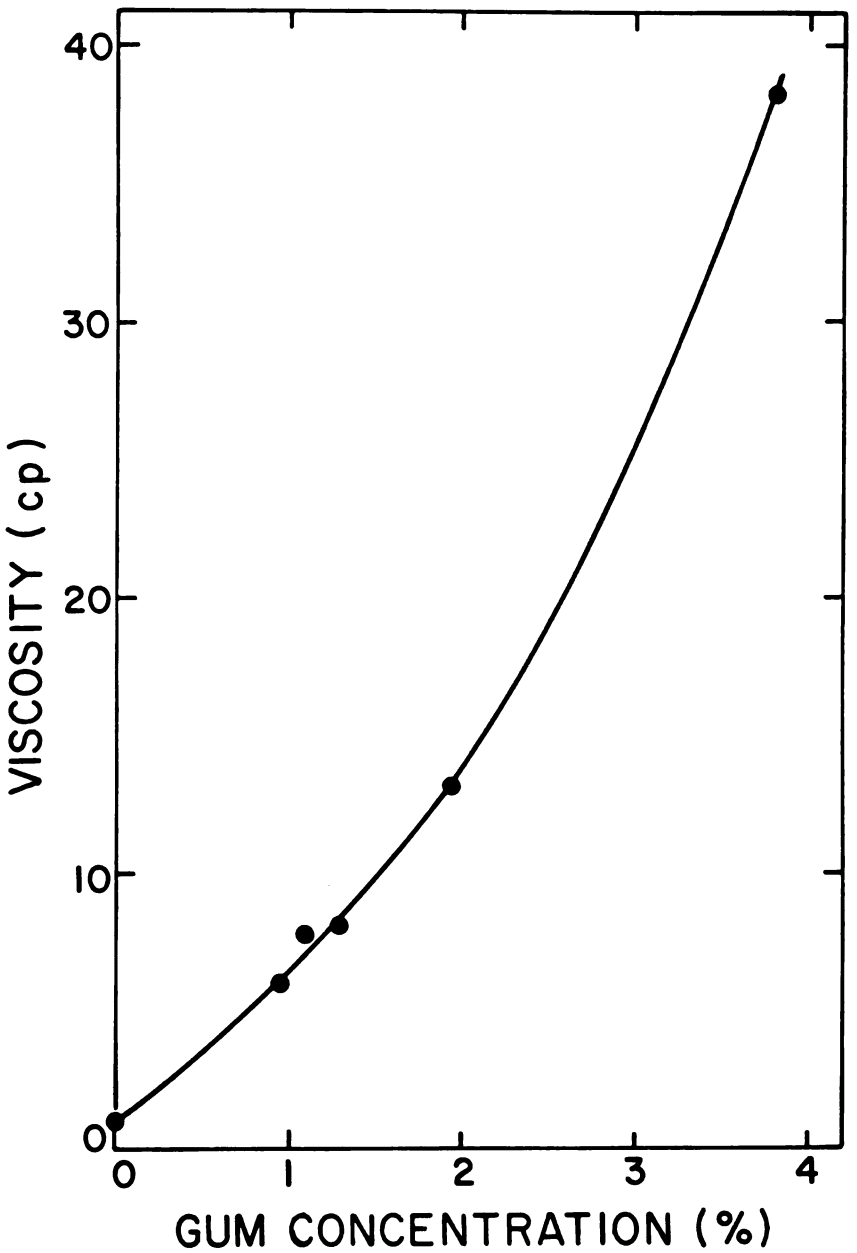

40
30
20
10
0
VISCOSITY (cp)
0
1
2
3
4
GUM CONCENTRATION (%)

Figure 2. Effect of low pressure gradients on flow rate of water, gum component sugar monomers, sucrose, and sour cherry gum solutions through a glass capillary. Monomers in solution at equivalent concentrations to gum at 1.90%. Capillary dimensions were 4.0 cm x 0.1 mm i.d. Pressure applied by height of solution head.

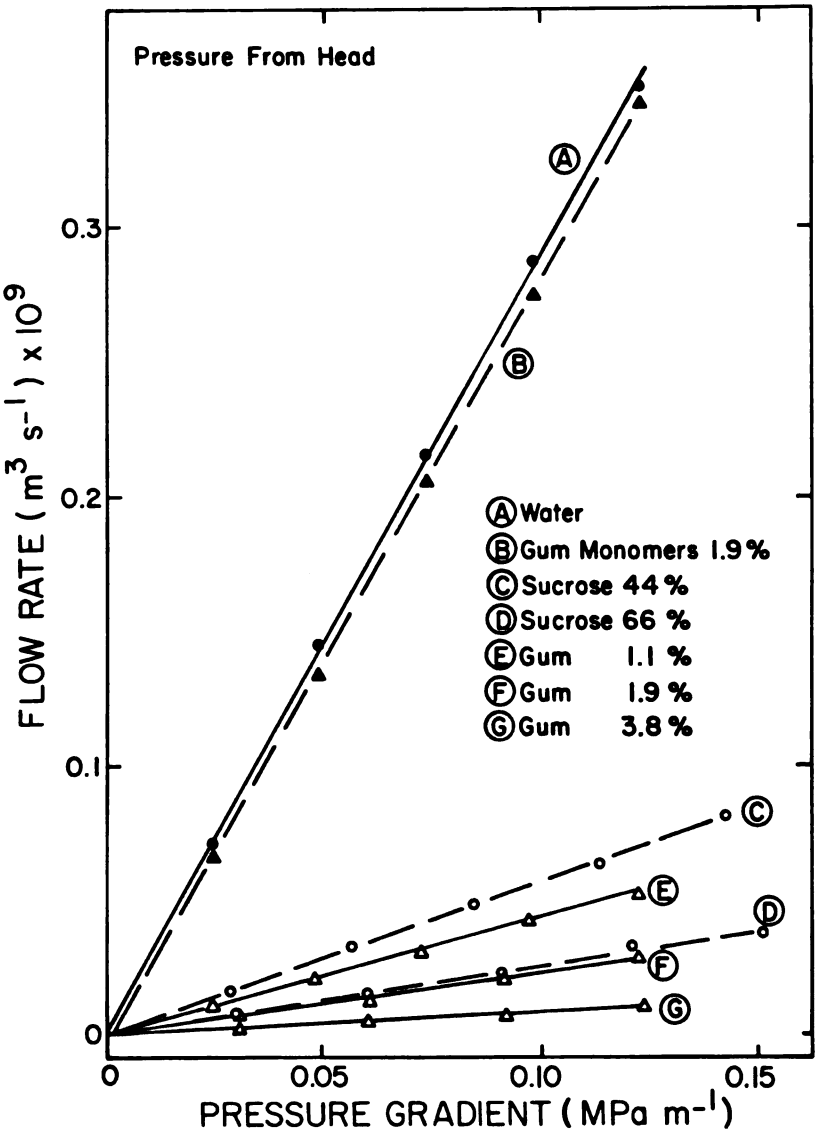
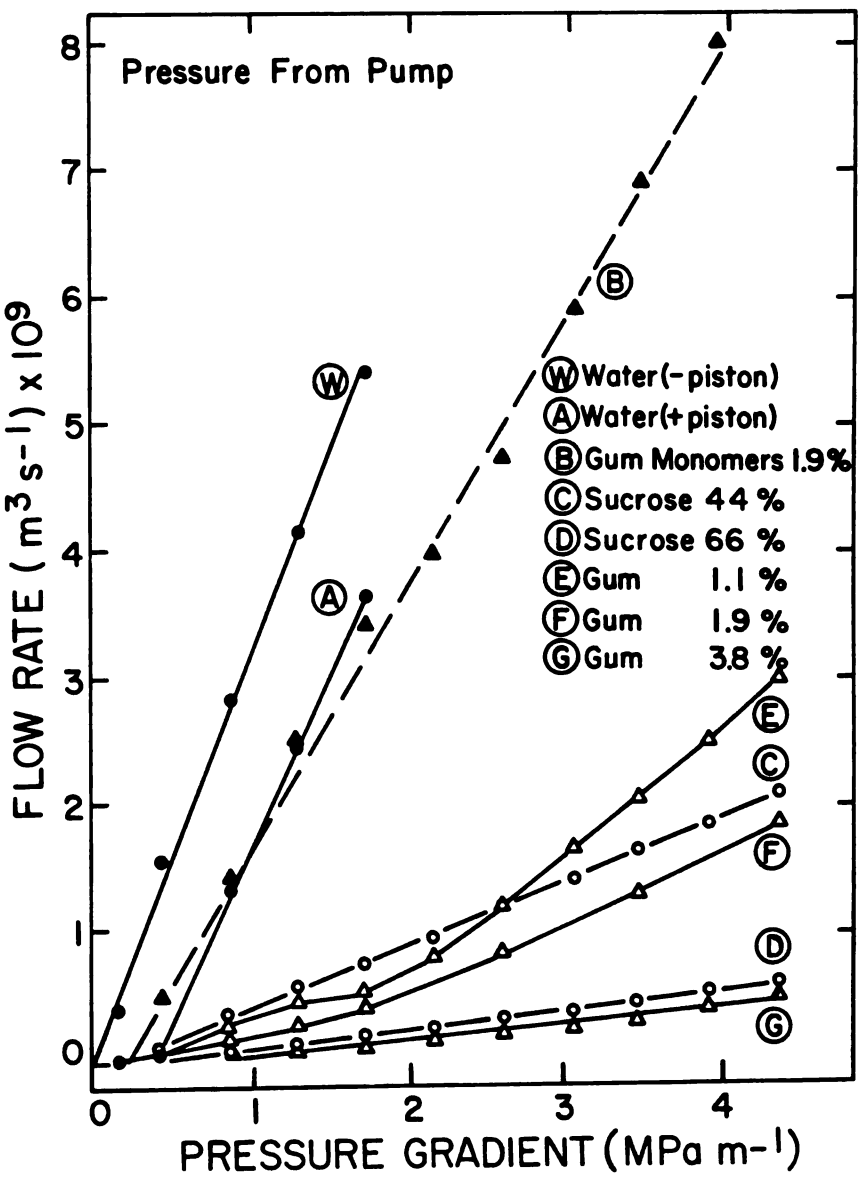

Pressure From Head
FLOW RATE ($m^3 s^{-1}$) x 10^9
0.3
0.2
0.1
0
0
0.05
0.10
0.15
PRESSURE GRADIENT (MPa m^{-1})
Ⓐ Water
Ⓑ Gum Monomers 1.9 %
Ⓒ Sucrose 44 %
Ⓓ Sucrose 66 %
Ⓔ Gum 1.1 %
Ⓕ Gum 1.9 %
Ⓖ Gum 3.8 %

Figure 3. Effect of pressure gradient on flow rate of water, gum component sugar monomers, sucrose, and sour cherry gum solutions through a glass capillary. Pressure applied by a micropump. Capillary dimensions were 4.0 cm x 0.1 mm i.d. Line W obtained without piston in pressure chamber for water. Lines A through G obtained with the piston in the chamber.


Pressure From Pump
FLOW RATE ($m^3 s^{-1}$) x 10^9
PRESSURE GRADIENT (MPa m^{-1})
0
1
2
3
4
5
6
7
8
Ⓦ Water(- piston)
Ⓐ Water(+ piston)
Ⓑ Gum Monomers 1.9%
Ⓒ Sucrose 44 %
Ⓓ Sucrose 66 %
Ⓔ Gum 1.1 %
Ⓕ Gum 1.9 %
Ⓖ Gum 3.8 %

using the piston is the subtraction of this artifact resistance value. For line A the yield value was 0.402 MPa m^{-1} and the mean value for all solutions was 0.328$\pm$ 0.144 MPa m^{-1}. The assumption of laminar flow through the capillary was verified by calculation of the Reynolds number for the maximum flow rates (Table II).

The flow rates of all solutions were directly proportional to the applied pressure gradient between 0 and 0.15 MPa m^{-1} (Figure 2). Thus, all solutions exhibited Newtonian flow in this pressure gradient range. In addition, there was no apparent yield value to overcome before flow occurred for any of the test solutions. Data for the gum monomer solution were essentially identical to those for water, while the flow rates for the sucrose and gum solutions were markedly lower, as expected from the greater viscosities of these solutions.

Flow characteristics of water, gum monomer, and sucrose solutions remained Newtonian over the entire pressure gradient range measured, from 0 to 4.0 MPa m^{-1} (corrected for piston resistance). Mean measured HC for Newtonian flow, obtained by both pressure systems, is presented in Table II.

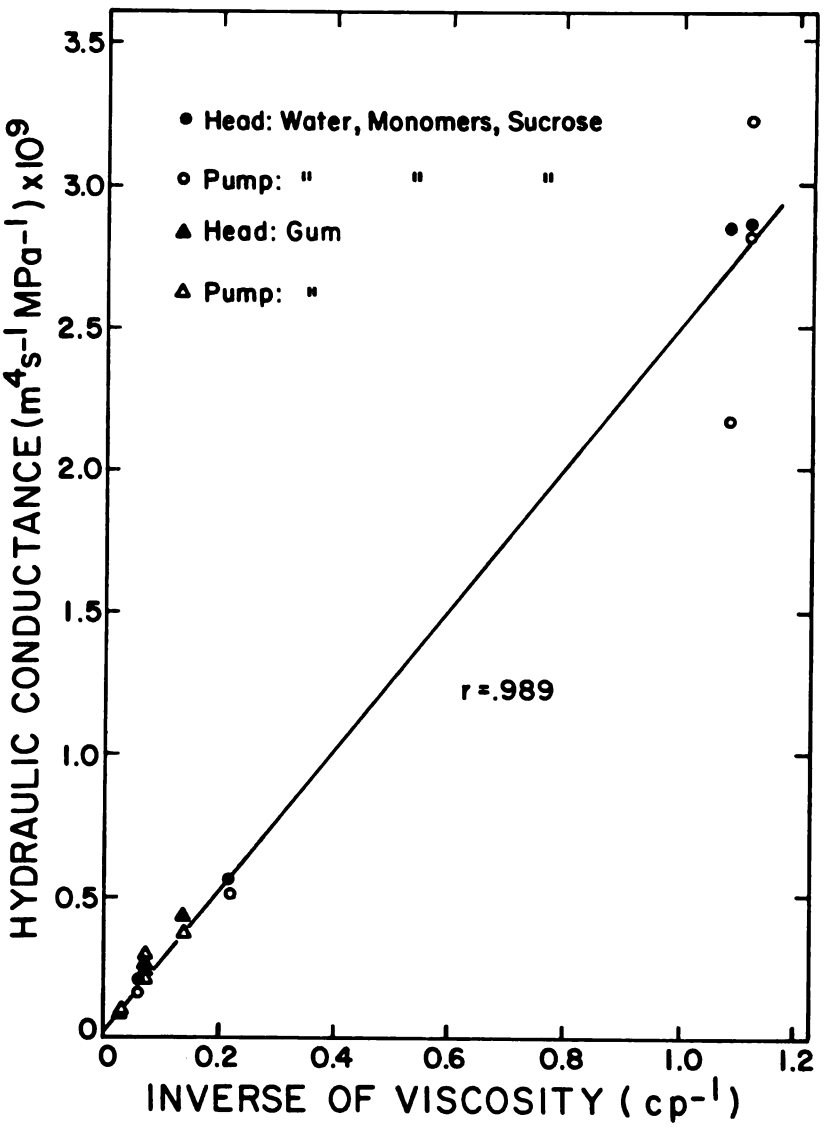
Hydraulic Conductance and Viscosity. HC for all solutions correlated well with the inverse of viscosity over the region of Newtonian flow (Figure 4), as expected from the Hagen-Poiseuille equation. The correlation coefficient ($r = 0.989$) was significant at $P = 0.01$.

Table II. Effect of Sugar and Gum Solution Concentration on Hydraulic Conductance and Reynolds Number at Maximum Flow Rates.

Hydraulic conductance was measured as the solutions were forced through a glass capillary (4.0 cm x 0.1 mm i.d.) over a range of pressure gradients from 0 to 4.3 MPa m^{-1}. Calculated values in this table for gum solutions are restricted to data conforming to Newtonian flow (0 to 1.8 MPa m^{-1}). Flow is laminar for Reynolds numbers less than 2100.

Solution	Solution Concentration (%, w/v)	Reynolds Number	Mean Measured HC ($m^4 s^{-1} MPa^{-1}$) x 10^9
Water	---	767	2.930
Sugar Monomers	1.90	1062	2.464
Sucrose	44	682	0.536
	66	2	0.204
Gum	1.09	53	0.937
	1.90	14	0.260
	1.91	18	0.212
	3.81	2	0.086

Figure 4. Relationship of hydraulic conductance in a glass capillary and viscosity as obtained for water, sucrose, and gum component sugar monomer solutions (circles) and sour cherry gum solutions (triangles). Open symbols represent data from pressure gradients obtained with a micropump, closed symbols represent data from pressure gradients obtained by solution head height. Capillary dimensions were 4.0 cm x 0.1 mm i.d.


HYDRAULIC CONDUCTANCE ($m^4s^{-1}MPa^{-1}$) x 10^9
INVERSE OF VISCOSITY (cp^{-1})
● Head: Water, Monomers, Sucrose
○ Pump: " " "
▲ Head: Gum
△ Pump: "
r=.989
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0.2
0.4
0.6
0.8
1.2

Agreement of Measured and Predicted Hydraulic Conductance. The HC predicted by the Hagen-Poiseuille equation can be calculated from the capillary radius raised to the fourth power and the solution viscosity. The mean of the ratio of measured to predicted HC over the pressure gradient range giving Newtonian flow was close to unity, 1.155 $\pm$ 0.207. Thus, there is good agreement between observed and predicted HC for the flow of the test solutions through the glass capillary, so long as flow is Newtonian.

Plastic Flow of Gum Solutions. Flow of gum solutions, especially when dilute, became plastic at pressure gradients above 1.8 MPa m^{-1} (Figure 3). This was not an artifact of the experimental system since flow remained Newtonian for the other solutions over the entire pressure gradient range examined. Above 1.8 MPa m^{-1}, the measured HC for gum solutions was 2.71 $\pm$ 0.33 times greater than the predicted HC. The changes in measured HC with increasing applied pressure for the four gum concentrations is illustrated in Figure 5. The data obtained above 1.8 MPa m^{-1} gave a good linear fit (r from 0.995 to 0.999). The assumption of two linear portions of the curve allowed calculation of the pressure gradient where the deviation from Newtonian flow occurred for each gum solution. The transition point was similar for all gum concentrations (Table III), with a mean (corrected for piston resistance) of 1.78 $\pm$ 0.30 MPa m^{-1}. Pressure gradients in the xylem of trees at maximum rates of transpiration have been estimated to be between 0.02 and

Figure 5. Transition from Newtonian to plastic flow of sour cherry gum solutions in a glass capillary and diminishing effect with increasing gum concentration. Pressure gradients applied with micropump. Capillary dimensions were 4.0 cm x 0.1 mm i.d.

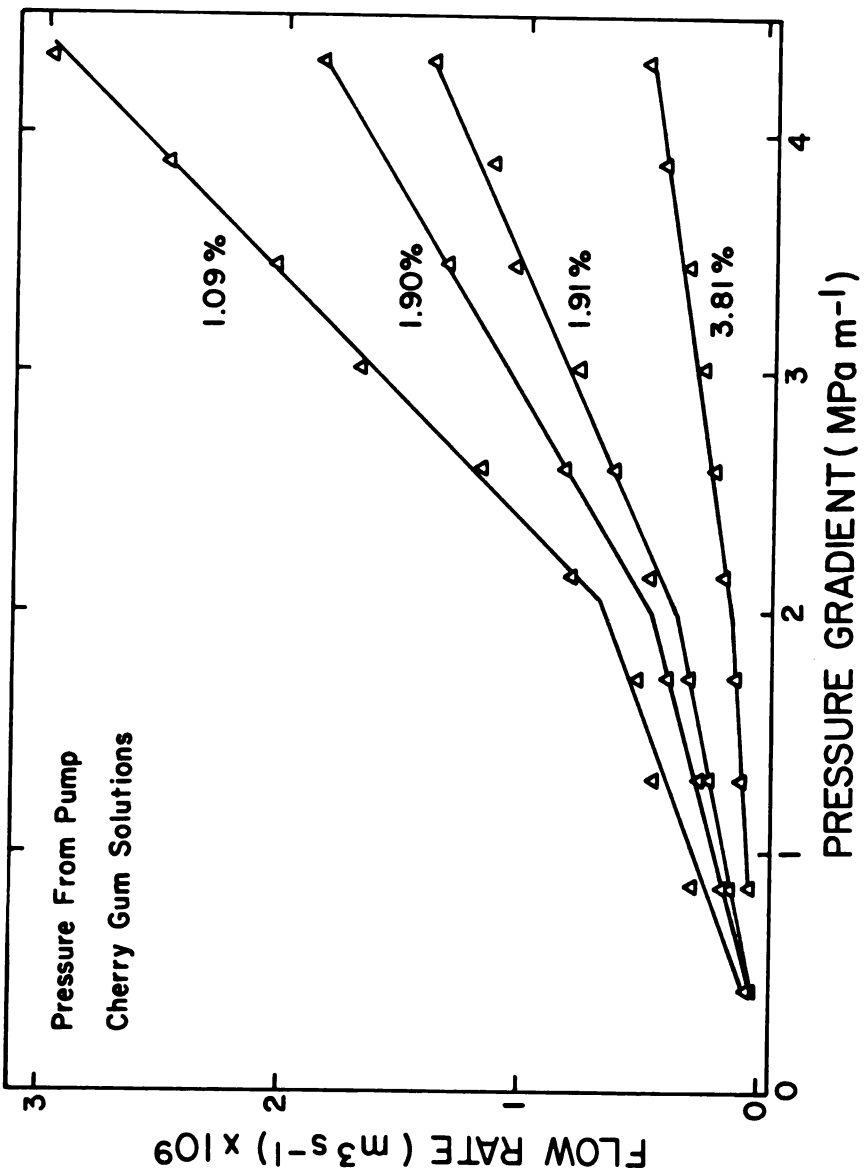

Pressure From Pump
Cherry Gum Solutions
1.09%
1.90%
1.91%
3.81%
FLOW RATE (m3s-1) x 109
PRESSURE GRADIENT (MPa m-1)
0
1
2
3
4

Table III. Pressure Gradient of Transition from Newtonian to Plastic Flow for Sour Cherry Gum Solutions.

Values are corrected for resistance of the pressure chamber piston driving the solutions.

Gum Concentration (%, w/v)	Transition Pressure Gradient (MPa m^{-1})
1.09	1.876
1.90	1.448
1.91	1.645
3.81	2.134
Mean	1.776 $\pm$ 0.296

0.05 MPa M^{-1} (Meidner and Sheriff 1976, Nobel 1974, Zimmermann and Brown 1971). These values are, however, averaged over the height of the tree. While flow of gum solutions are Newtonian in this pressure gradient range, pressure gradients sufficient to cause plastic flow might easily occur over short lengths of high resistance to flow in the xylem, as would be caused by the presence of gum or tyloses in the vessels (Zimmermann and Brown 1971). When corrections are made for differences in Σr^4, a 10-fold increase in the pressure gradient would be required for plastic flow to occur in control shoots. Plastic flow of gum solutions may therefore occur in intact shoots at such constrictions.

The occurence of plastic flow at high gradients would to some extent compensate for the presence of gum in the vessel because the apparent viscosity decreases as the pressure gradient increases. This effect, however, diminishes with increasing gum concentration. Not only did the magnitude of the change decrease, but there was also a decreasing trend in the ratio of the pressure-flow slopes above and below 1.8 MPa m^{-1} with increasing gum concentration (Table IV). This ratio represented a 2.0 to 2.5-fold decrease in the apparent viscosity. The correlation coefficient between this ratio and gum concentration was -0.778, and between the ratio and the inverse of viscosity was 0.952. The apparent decrease in viscosity could result from the breaking of weak intermolecular bonds between

Table IV. Ratio of Plastic to Newtonian Pressure-Flow Slope for Sour Cherry Gum Solutions.

Correlation coefficient (r) significant at P = 0.05 (*) or not significant (ns).

Gum Concentration (%, w/v)	Viscosity (cp)	Ratio
1.09	7.17	2.549
1.90	13.23	2.158
1.91	13.35	2.071
3.81	35.10	2.000

r (ratio x gum concentration) = 0.778 ns

r (ratio x viscosity^{-1}) = 0.952 *

gum polymers, as in thixotrophy, and also from a more streamlined orientation of the gum molecules in the vessel (Jirgensons 1958, McBain 1950).

Prediction of Gum Content in Functional Vessels of Sour Cherry Shoots. There was an increasing discrepancy between measured and predicted HC as the gum content of one-year-old sour cherry shoots increased following ethephon-treatment (Olien 1980, Section I). This was thought to result from the solution viscosity in functional vessels being greater than the assumed viscosity of water due to the presence of gum. Flow of solution through excised shoot segments was Newtonian over the pressure gradients applied (Olien 1980, Section I). The viscosity in non-occluded vessels necessary to account for the discrepancy of measured and predicted HC was calculated. The correlation coefficient between HC measured as solutions were forced through the glass capillary and the inverse of solution viscosity was significant at P = 0.01 and the regression equation was expressed by equation I: (I)

$$HC = (3.68 \times 10^{-11} m^4 s^{-1} MPa^{-1}) + (2.45 \times 10^{-9} cP\ m^4 s^{-1} MPa^{-1})_\eta{}^{-1}$$

This is in agreement with the relationship predicted by the Hagen-Poiseuille equation:

$$HC = 0 + (2.45 \times 10^{-9}\ cP\ m^4 MPa^{-1})_\eta{}^{-1} \quad \text{(II)}$$

If the HC values obtained with the glass capillary are divided by the capillary r^4, they can be compared with the HC values from shoots when divided by their respective Σr^4 values for functional vessels, where r is capillary or

vessel radius. Making this correction in equation (I) and rearranging, solution viscosity in functional vessels of the one-year-old internode shoot segments was predicted:

$$\eta = \frac{(3.921 \times 10^{8} \text{ cp } s^{-1}MPa^{-1}}{(HC)/(r^{4}) - (5.887 \times 10^{6} \ s^{-1}MPa^{-1})} \qquad \text{(III)}$$

This prediction assumes that the discrepancy between measured and predicted HC is entirely due to the viscosity term. The viscosity values thus calculated (Table V) are notably higher than that for water at 25 C (Table I). The viscosity predicted for control shoots was 0.43 cp greater than that for water, while for the most gummed but living shoots the difference was 5.52 cp.

Under conditions of Newtonian flow, the gum concentrations required for these viscosities were estimated from the data in Figure 1 and are presented in Table V. The measured HC values were obtained by forcing dionized distilled water through the internode segments and the vessel sap would, therefore, be diluted in the process of measurement. Thus the predictions made here are probably underestimates of sap viscosity in the functional vessels of intact shoots. If the pressure gradient exceeded 1.8 MPa m^{-1}, the apparent viscosity for these predicted gum concentrations would be decreased because of plastic flow.

Table V. Predicted Viscosity and Gum Concentration in Functional Vessels of One-Year-Old Sour Cherry Shoots.

One-year-old sour cherry shoots had been induced to gum by application of 2000 or 5000 $\mu g\ ml^{-1}$ ethephon, (2-chloroethyl) phosphonic acid. Control shoots were untreated. The majority of the shoots in gummosis response group Gum-E were sprayed with 2000 $\mu g\ ml^{-1}$ and the majority of the Gum-L shoots had been sprayed with 5000 $\mu g\ ml^{-1}$ ethephon.

Gummosis Response Group	Viscosity (cp)	Gum Concentration (%, w/v)
Control	1.32	0.05
Gum-E	2.03	0.20
Gum-L	6.41	0.95

Conclusions

Small increases in the concentration of sour cherry gum greatly increased solution viscosity, and thus decreased HC. Newtonian flow of sour cherry gum solutions through a glass capillary occurred with pressure gradients from 0 to 1.8 MPa m^{-1}. Plastic flow occurred at pressure gradients over 1.8 MPa m^{-1}, but the deviation from Newtonian to plastic flow decreased with increasing gum concentration. The solution viscosity in functional (non-occluded) vessels was calculated which would account for the discrepancy between measured and predicted HC of the internode segments from one-year-old sour cherry shoots (Olien 1980, Section I). The gum concentrations required for these predicted viscosities under conditions of Newtonian flow were then estimated. The apparent viscosities for these predicted gum concentrations would be decreased at pressure gradients greater than 1.8 MPa m^{-1} because of plastic flow.

There is probably a distribution of gum concentrations within and among vessels. When ethylene induces an increase in gum synthesis, more gum enters the vessels, increasing sap viscosity and, at high enough concentrations, occluding vessels. The apparent viscosity of a gum solution may decrease if plastic flow occurs. We have shown that the effect of gum on vessel sap viscosity is a complex function of gum concentration and the pressure gradient driving the solution.

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Section III

SOUR CHERRY (*PRUNUS CERASUS*) FRUIT ABSCISSION: INTERACTION BETWEEN TEMPERATURE AND ETHYLENE.

SOUR CHERRY (PRUNUS CERASUS) FRUIT ABSCISSION: INTERACTION BETWEEN TEMPERATURE AND ETHYLENE.

Abstract

Sour cherry fruit explants (Prunus cerasus L. cv Montmorency) were collected during Stage III of fruit development and incubated with or without exogenous ethylene at temperatures between 15 and 35° C. Exogenous ethylene was applied either by preincubation in a 1.0 mM ethephon bath or by enriching a flow-through gas system with 7 ppm ethylene. Fruit-pedicel abscission layer development was subsequently indexed by measuring the decrease in fruit removal force from the initial value. The effect of temperature on the promotion of fruit abscission was relatively low for all treatments, with a mean Q_{10} (20 to 30° C) of 1.20. There was no detectable interaction of ethylene concentration with temperature. A given concentration of exogenous ethylene, regardless of source, promoted abscission to the same extent over the temperature range assayed. Thus, ethylene action is independent of temperature between 15 and 35° C. The effect of temperature is restricted to the steps subsequent to ethylene action which lead to development of the fruit abscission layer.

Footnote:

Appreciation is expressed to Dr. D. R. Dilley for allowing us use of the gas flow-through system and controlled temperature rooms.

Introduction

The role of ethylene in sour cherry fruit abscission is well established (Wittenbach and Bukovac 1972, 1974). The ethylene-generating compound ethephon, (2-chloroethyl) phosphonic acid, is commonly used to promote cherry fruit abscission for mechanical harvest. With ethephon, fruit removal is improved and damage to the fruit and tree are minimized (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Bukovac et al. 1971, Looney and McMechan 1970). The response induced by ethephon is determined not only by the amount of ethephon applied, but also by the temperatures during and after application (Bukovac and Kesner 1973, Micke et al. 1973, Wilde and Edgerton 1975). A major impact of temperature is its marked effect on the rate of ethylene release from ethephon (Olien and Bukovac 1978). The apparent energy of activation for this reaction is about 30 kcal mole $^{-1}$ (Biddle et al. 1976, Gregory and Higgins 1974, Olien and Bukovac 1978).

The development of any hormone-mediated process first requires the accumulation of the hormone, secondly the action of the hormone, and finally the subsequent steps leading to the expression of the response. The net effect of temperature on the process will be determined by the temperature effect on each of these phases. Determining the effect of temperature on the ethylene-mediated promotion of cherry fruit abscission would provide a basis for a better understanding and more efficient use of ethylene or ethylene-generating compounds. The development of the fruit-pedicel

abscission layer is an easily indexed response and may be representative of other ethylene-mediated responses in cherry, such as gummosis. Some data are available on the effect of temperature on abscission layer development in cherry in the absence of exogenous ethylene (Wittenbach and Bukovac 1973) and on endogenous ethylene production (Olien and Bukovac 1978), but not on the combined effect of temperature and ethylene on promotion of cherry fruit abscission. This study was designed to determine whether or not there was an interaction between temperature and ethylene concentration in this process.

Materials and Methods

Assay of abscission layer development. Sour cherry (Prunus cerasus L. cv Montmorency) fruit in Stage III of development were collected with pedicels attached from vigorous, mature trees at East Lansing, Michigan. The initial mean force required to remove the fruit from its pedicel (FRF) was determined using a Hunter Force Gauge (Ametek, Inc. Hatfield, PA) for samples of 100 fruit each at the time of explant collection. The development of the fruit-pedicel abscission layer was indexed by the decrease in the FRF (Stosser et al. 1969, Cain 1967) after incubation of the explants under conditions described below.

Ethylene and temperature treatments. Fruit explants were incubated with exogenous ethylene either by pretreatment with ethephon or by enriching a flow-through gas system with ethylene. Control explants received no exogenous ethylene.

When ethephon was used as a source of ethylene, the explants were preincubated for 24 hr with the pedicels in deionized distilled water or 1.0 mM ethephon at room temperature (about 21° C). The explants were then removed from the preincubation baths and placed with pedicels in 25 ml scintillation vials containing deionized, distilled water. Fifty explants were incubated in this manner (2 fruit per vial) in the dark at 15, 25, or 35° C. FRF was determined after 4 days.

For ethylene gas experiments, five 25 ml scintillation vials containing deionized, distilled water were placed in a 1.75 liter jar with a screw cap lid. The fruit explants were positioned on a screen, with the pedicels of 2 or 4 explants submerged in each vial. Air or air enriched with ethylene to about 7 ppm was passed through the jars at about 50 ml min^{-1} by the method described by Saltveit (1978). Gas flow through each jar was independently regulated and the flow rates were established and monitored with capillary flow meters as described by Claypool and Keefer (1942). Gas samples were collected daily and assayed for ethylene by gas chromatography. Five jars with and five jars without ethylene enrichment were incubated at 15, 22.5, or 30° C, for a total of 30 jars and 150 vials. Each jar was considered to be an experimental unit, making five replications. After the prescribed period of incubation, the FRF was determined for 10 fruit explants from each jar and the mean FRF was calculated. In Ethylene Gas Experiment I, four explants were used per

vial. The FRF for 2 fruit explants from each vial was measured after 4 days and the remaining fruit were assayed after 7 days. In Ethylene Gas Experiment II, 2 explants were placed in each vial and FRF was assayed after 7 days.

Explants were collected at successively later times in Stage III of fruit development. The experiments were initiated in the following order: (a) Ethephon Experiment (7/3/78, early-mid Stage III), (b) Ethylene Gas Experiment I (7/5/78, early-mid Stage III), (c) Ethylene Gas Experiment II (7/13/78, mid-late Stage III). The effect of temperature was expressed as the Q_{10}, which was calculated for 20 and 30° C from Arrhenius plots of the data. Differences in FRF from initial values were used as rates.

Results and Discussion

Initial FRF. Initial FRF decreased with succeeding dates of explant collection as the fruit continued to mature in the field (Table I).

Table 1. Fruit removal force (FRF) at initiation of experiments.

Experiment	Date	Initial FRF (g)
Ethephon	7/3/78	979 ± 208
Ethylene Expt. I	7/5/78	762 ± 183
Ethylene Expt. II	7/13/78	488 ± 143

This decrease was reflected in smaller differences in FRF from initial values at the completion of succeeding experiments (Table 2, 3). There was a large inherent degree of error associated with the FRF, the mean coefficient of variation for initial values being 27.3%.

Table 2. Ethephon Experiment: Effect of temperature and preincubation in 1 mM ethephon on abscission of sour cherry fruit explants.

	Δ FRF after 4 days (g)	
Temperature (°C)	Control	Ethephon
15	521	552
25	630	666
35	669	719

Table 3. Ethylene Gas Experiments: Effect of temperature and air enriched with 7 ppm ethylene on abscission of sour cherry fruit explants.

Experiment	Time (days)	Temperature (°C)	Δ FRF from initial value (g)	
			Control	Ethylene
I	4	15	275	405
		22.5	334	523
		30	465	583
	7	15	352	484
		22.5	400	531
		30	462	582
II	7	15	168	228
		22.5	141	277
		30	203	309

Effect of temperature. Temperature had a small effect on the rate of abscission layer development (Fig. 1). The Q_{10} for explants with and without exogenous ethylene were similar, with an overall mean of 1.20 $\pm$ 0.10 (Table 4). There was no detectable difference in Q_{10} between explants treated with ethephon or with ethylene gas. Wittenbach and Bukovac (1973) incubated similar sour cherry fruit explants at 15, 25, and 35° C and measured the reduction in FRF after 3 days. No exogenous ethylene was added. We calculated

Fig. 1. Effect of temperature on abscission layer development in sour cherry fruit explants. Ethephon Expt.: lines with solid circles labeled + and - ethephon. Ethylene Gas Expt. I: lines with open circles labeled + and - C_2H_4. Solid lines represent data from explants incubated at designated temperatures for 4 days. Dashed lines indicate 7 day incubations. Abscission layer development was indexed by decrease in fruit removal force (FRF) from the initial values. Data for Ethylene Gas Expt. II not shown.

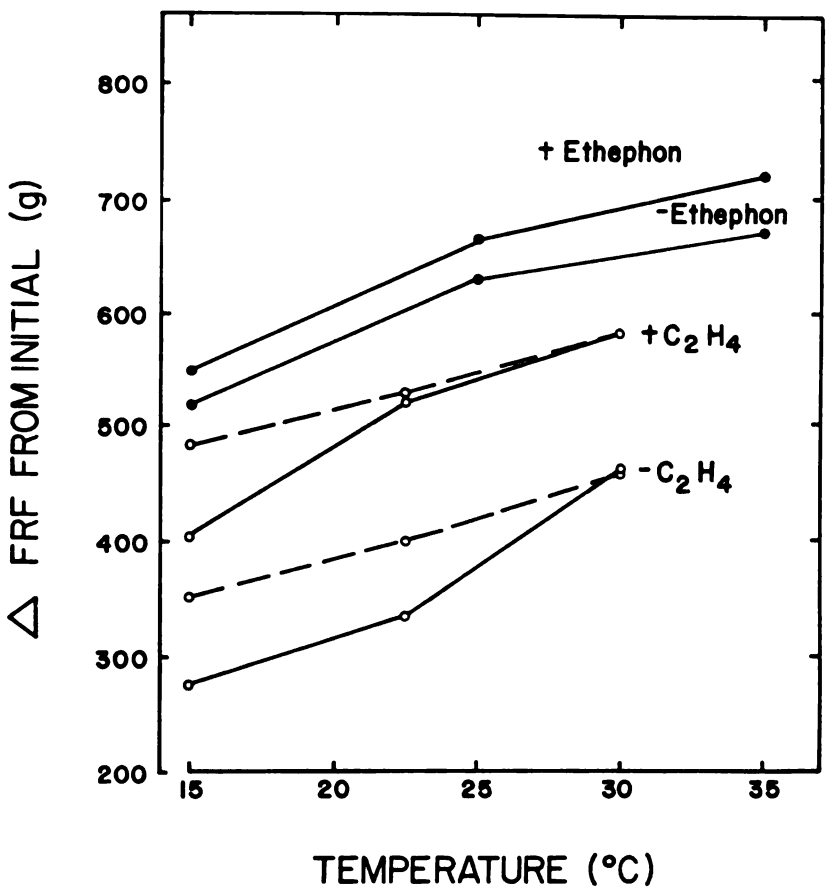

800
700
600
500
400
300
200
15
20
25
30
35
+ Ethephon
-Ethephon
+C2 H4
-C2 H4
Δ FRF FROM INITIAL (g)
TEMPERATURE (°C)

Table 4. Q_{10} for abscission of sour cherry fruit explants and effect of ethephon (1 mM) or ethylene (7 ppm) treatment.

Experiment	Time (days)	Calculated Q_{10} (20 to 30°C)	
		Control	+Ethylene
Ethephon Expt.	4	1.13	1.14
Ethylene Gas Expt. I	4	1.41	1.27
	7	1.20	1.13
Ethylene Gas Expt. II	7	1.13	1.22

the Q_{10} (between 20 and 30°C) for these data, in the manner previously described, and obtained a Q_{10} of 1.33. This was within the range of Q_{10} values obtained in this study. Thus, temperature does affect abscission layer formation in sour cherry fruit, but the effect is relatively small.

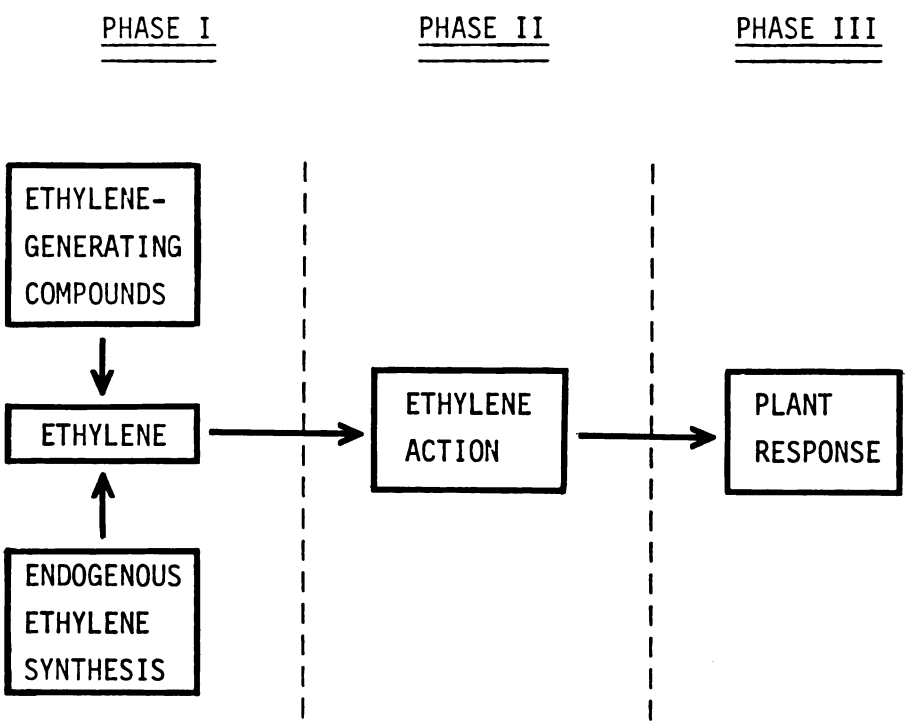
Ethylene-temperature interaction. One of the most striking features of the data in Fig. 1 is that there is no significant interaction of temperature and ethylene concn on the decrease in FRF (data from Gas Experiment II not shown). The effect of ethylene on fruit abscission is apparently independent of temperature over the temperature range examined. This is also reflected in the fact that there was little difference in the Q_{10} values of treatments with and without

exogenous ethylene (Table 4). A given concentration of exogenous ethylene promoted abscission to the same extent at all temperatures assayed. Thus, ethylene action is not temperature dependent between 15 and 35° C. If ethylene action were affected by temperature, the slope of temperature vs Δ FRF plots would be expected to increase with increasing ethylene concentration. The effect of temperature on the rate of sour cherry fruit abscission layer development is apparently restricted to the events subsequent to ethylene action.

Conclusions

A three phase model is proposed to relate the temperature dependencies of the processes involved in sour cherry fruit abscission (Fig. 2). Ethylene is generated in Phase I. The rate of endogenous ethylene evolution is low from sour cherry fruit in Stage III of development (Blanpied 1972, Wittenbach and Bukovac 1974) and from leaves (Olien and Bukovac 1978). Also, the rate of endogenous ethylene evolution from several plant species that have been examined has a low dependence on temperature, with a mean Q_{10} (20 to 30° C) of 2.1 (Olien and Bukovac 1978). Ethylene can also be generated from exogenous sources, such as ethylene-generating compounds. Ethylene action occurs during Phase II of the model. It is dependent only on ethylene concn and is not influenced by temperature. The subsequent development of the abscission layer (Phase III) has a relatively

Fig. 2. Proposed model of an ethylene-mediated response. Phase I: ethylene generation from endogenous or exogenous sources (rate of ethylene release is dependent on temperature). Phase II: ethylene action (not affected by temperature). Phase III: subsequent steps leading to the expression of the ethylene-mediated response (temperature sensitive).


PHASE I
PHASE II
PHASE III
ETHYLENE-GENERATING COMPOUNDS
ETHYLENE
ENDOGENOUS ETHYLENE SYNTHESIS
ETHYLENE ACTION
PLANT RESPONSE

low temperature dependence (Q_{10}, 20 to 30° C, of 1.20), which is independent of ethylene concn. Examination of several ethylene-mediated responses in a number of species would be necessary to determine whether this model might apply generally to plants.

From this model it can be seen that the amount of ethylene released from an ethylene-generating compound and the temperature dependence of that rate of release are the overriding factors affecting the stimulation of sour cherry fruit abscission. An ethylene-generating compound with all of the characteristics of ethephon, but with a lower dependence on temperature would give more consistent results under varying field temperatures. Promotion of fruit abscission would then be more directly related to the concentration of the applied compound.

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Section IV

RELATIVE BIOLOGICAL ACTIVITY AND TEMPERATURE DEPENDENCE OF SEVERAL COMPOUNDS WITH POTENTIAL FOR PROMOTING ABSCISSION OF SOUR CHERRY (*PRUNUS CERASUS*) FRUIT

RELATIVE BIOLOGICAL ACTIVITY AND TEMPERATURE DEPENDENCE OF SEVERAL COMPOUNDS WITH POTENTIAL FOR PROMOTING ABSCISSION OF SOUR CHERRY (PRUNUS CERASUS) FRUIT

Abstract

Ethylene generation from ethephon, (2-chloroethyl) phosphonic acid, is highly dependent on temperature and thus, gives inconsistent promotion of cherry fruit abscission under field conditions. A promoter of fruit abscission which was less dependent on temperature would be preferable. Several compounds were compared for relative rates of ethylene evolution, effect of temperature on ethylene evolution, and ability to promote fruit abscission when applied to sour cherry foliage (Prunus cerasus L. cv Montmorency) at equimolar concentrations. These compounds were: (a)ethephon, (b) GAF 7767141 (structure not released), (c) CGA 13586 (2-chloroethyl-tris-(2-methoxyethoxy)-silane), (d) CGA 15281 (2-chloroethyl-methylbis-(phenylmethoxy)-silane), (e) ER 3952 (1,1,5,5-tetra-methyl-3-dimethylaminodithiobiuret), and (f) BOH (beta-hydroxy-ethylhydrazine). CGA 15281 was a good promoter of sour cherry fruit abscission with minimal dependence on temperature. ER 3952 did not release ethylene, but was active as a fruit-loosener.

Footnote:

Ethephon was donated by Amchem Products, Inc. Ambler, PA, the CGA compounds by the Ciba-Geigy Corp., Greensboro, NC, GAF 7767141 by the GAF Corp., NY, NY and ER3952 by ESSO Res and Eng. Co., Linden, NJ. BOH was produced by the Olin Mathieson Chem. Corp., NY, NY.

Introduction

Ethephon (2-chloroethylphosphonic acid) is now commonly used to promote cherry fruit abscission for mechanical harvest. There are problems, however with inconsistent activity and under some conditions with phytotoxicity (Anderson 1969, Bukovac 1979, Bukovac et al. 1969, Bukovac et al. 1971, Looney and McMechan 1970, Wilde and Edgerton 1975, Olien 1980, Sections I and II). This is due to the marked effect of temperature on the rate of ethylene generation from ethephon, with an apparent energy of activation of about 30 kcal mole $^{-1}$ (Biddle et al. 1976, Gregory and Higgins 1974, Olien and Bukovac 1978). Tree response to ethephon is thus dependent on temperature in the field for several days after ethephon application. During cherry harvest high temperatures may become limiting while low temperature may restrict performance for color development in apples (Anonymous, 1980).

We have established that ethylene action is not affected by temperature between 15 and 35° C and that the subsequent steps leading to the development of the sour cherry fruit-pedicel abscission layer have a relatively low dependence on temperature, with a Q_{10} for 20 to 30° C of 2.10 (Olien 1980, Section III). The rate and temperature dependence of endogenous ethylene evolution from sour cherry fruit, in Stage III of development, and leaves are low (Blanpied 1972, Olien and Bukovac 1978, Wittenbach and Bukovac 1974). Thus, the major effect of temperature determining the response to a given dose of ethephon is on the rate of ethephon degradation.

A fruit-loosening compound with the characteristics of ethephon, but with a lower dependence of activity on temperature would give more consistent results under varying field temperatures. Promotion of fruit abscission would then be more strictly a function of the concentration of the compound applied. Our criteria for a good fruit-loosening compound was that it: (a) be an active ethylene generator over several days, (b) have a low dependence of activity on temperature, and (c) give satisfactory promotion of fruit abscission. Six potential compounds were applied to sour cherry trees at equimolar concentrations and evaluated by the above criteria.

Materials and Methods

Compounds and plant material. Six compounds were evaluated at equimolar conc on mature sour cherry trees. These compounds were ethephon, GAF 7767141 (structure not released), CGA 13586 (2-chloroethyl-tris-(2-methoxyethoxy)-silane), CGA 15281 (2-chloroethyl-methylbis-(phenylmethoxy)-silane), ER 3952 (1,1,5,5-tetramethyl-3-dimethylaminodithio-biuret), and BOH (beta-hydroxyethylhydrazine). Concn for evaluation were based on the recommended use of ethephon for promotion of fruit abscission.

Ethylene assays. Ethylene generation was measured from leaves or fruit by determining the amount of ethylene accumulated after a 1 hr incubation in sealed 260 ml jars or 75 ml test tubes. Five leaves or 10 fruit were incubated in jars. Two leaves were incubated in each tube with the

pedicels positioned in 1 ml of water. Tubes, but not jars, were incubated in controlled temperature water baths between 15 and 40° C. Jars were incubated at ambient laboratory temperature. Ethylene evolved after the incubation period was assayed by gas chromatography as previously described (Olien and Bukovac 1978). The effect of temperature on the rate of ethylene generation was determined using 3 temperatures and was expressed as apparent energy of activation (E_a) determined from the slope of Arrhenius plots.

Promotion of fruit abscission assay. Abscission layer development was indexed by the reduction in the force required to remove the fruit from its pedicel (FRF) (Cain 1967, Stosser et al. 1969). Mean FRF was determined for samples of 50 or 100 fruit with a Hunter Force Gauge (Ametek, Inc. Hatfield, PA) 7 to 8 days after application of the designated compounds and the data were expressed as percentage difference from control.

Ethylene generation time-course. In 1977, ethephon, CGA 13586, and CGA 15281 were sprayed to run-off on entire trees at 2.08 mM (300 ppm ethephon), with X-77 (alkyl aryl polyethoxy ethanol and free fatty acids, Chevron Chem. Corp. San Francisco, CA) added as surfactant at 0.1% (v/v). Control treatments consisted of both nontreated and 0.1% X-77 treated trees. Five trees were selected for each treatment and assigned to a randomized block design. Because of a limited quantity of CGA 13586, only 2 replications were used for this treatment. Ethylene generation from leaves was

determined in 260 ml jars 1,3,5,9,14,18, and 37 days after treatment and from fruit 9 days after treatment. Ambient laboratory incubation temperature ranged from 28 to 33° C.

All 6 compounds were compared in 1978 on each of 6 trees. A total of 1.7 pmole was applied without added surfactant to the upper surface of individual leaves in 10 droplets of 5 ml each. This assured application of a uniform dose to each leaf and was calculated to be equivalent to a dilute spray of 1.7 mM (250 ppm ethephon). Comparable non-treated leaves were used as controls. After 24 hr, residues were washed from the leaves with a water spray. Samples of 2 leaves each were collected at 1,2,4,8,16 and 32 days after treatment. Ethylene was measured after incubation in 75 ml test tubes at 25° C as previously described.

Effect of temperature on ethylene generation. E_a values for ethylene generation was determined in 1976 and 1978. Ethephon and CGA 15281 were compared in 1976. Branches on 5 sour cherry trees were sprayed with 1.73 mM ethephon or 3.12 mM CGA 15281. Control branches received no treatment. Leaves were collected and ethylene generation was determined in 75 ml test tubes at 1,6,17,24 and 140 hr after treatment. Incubations were at 21, 30 and 40° C, with controls assayed at 30° C.

In 1978, the E_a was determined after 2,8, and 16 days for all 6 compounds as part of the ethylene generation time course experiment. Ethylene evolution was assayed as previously described at 15, 25, and 35° C.

Reduction of fruit removal force. In 1977, the mean FRF was determined for samples of 100 fruit each at 8 days after treatment as part of the ethylene generation-time course study. In 1978, comparison of FRF was made in a separate experiment and was replicated over 5 trees. Marked branches were sprayed with 1.7 mM of ethephon, CGA 15281, GAF 7767141, or ER 3952. Sprays included 0.1% X-77. Controls were not treated. FRF was evaluated for samples of 50 fruit each 7 days after treatment.

Results

Ethylene generation time course. In 1977, ethephon and CGA 15281 generated ethylene most rapidly, with mean initial rates of 0.77 and 1.69 nmole $g^{-1}hr^{-1}$, respectively (Fig. 1). The initial rate for CGA 13586 was lower (0.44 nmole $g^{-1}hr^{-1}$) but was greater than for the controls. There was no difference between the untreated and surfactant treated controls (0.07 and 0.05 nmole $g^{-1}hr^{-1}$, respectively). Ethylene evolution rates for all treatments approached that of the controls after 14 days. An anomalous peak of ethylene generation occurred from the ethephon treatment on day 9, but was not evident for any of the other treatments. The relative rates of ethylene generation from fruit and leaves were similar 9 days after treatment, but greater differences were observed for fruit on both a fresh weight and a per fruit basis (Table 1).

In 1978, the mean initial rates of ethylene generation from ER 3952 and BOH treated leaves (0.18 and 0.13 nmole g^{-1}

Fig. 1. Time-course of ethylene generation from potential fruit-loosening compounds: 1977. The compounds were applied as dilute sprays at 2.08 mM and ethylene generation was monitored from detached sour cherry leaves.

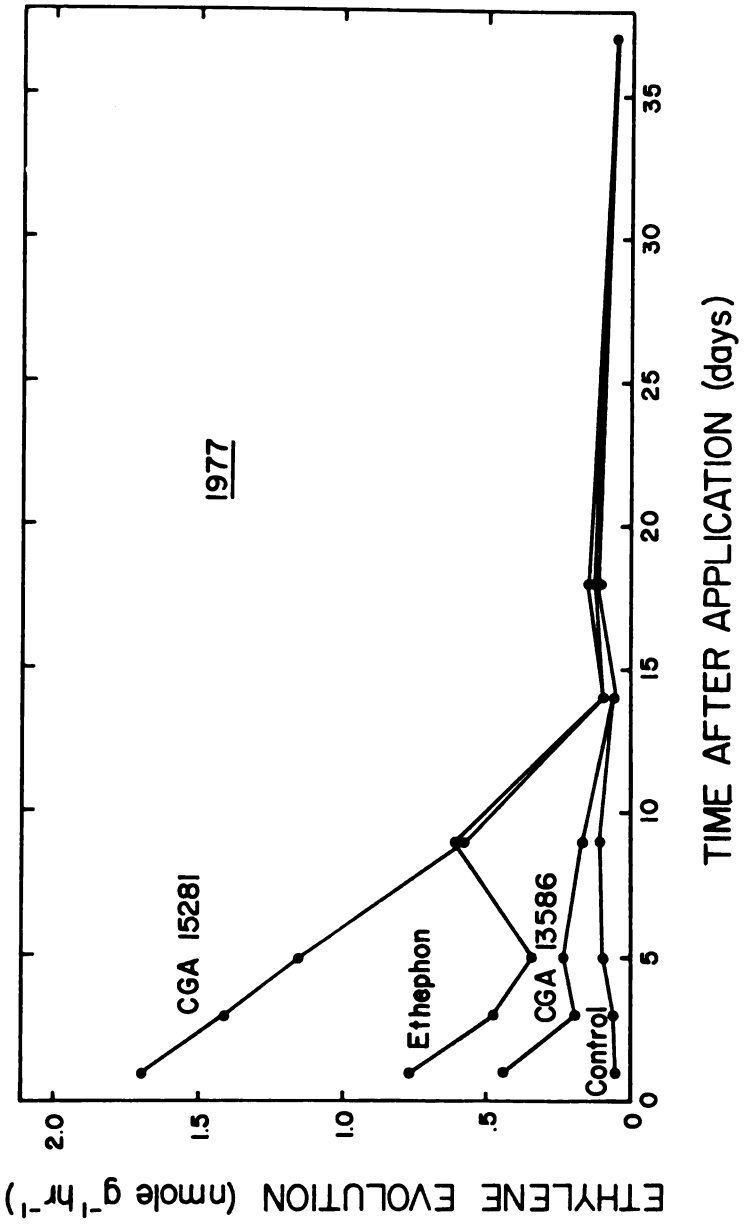

1977
CGA 15281
Ethephon
CGA 13586
Control
0
.5
1.0
1.5
2.0
0
5
10
15
20
25
30
35
TIME AFTER APPLICATION (days)
ETHYLENE EVOLUTION (nmole g⁻¹hr⁻¹)

Table 1. Comparison of ethylene generation from fruit and leaves following treatment with potential fruit loosening compounds.[z]

Treatment	Rate of ethylene generation: Per leaf fresh wt (nmole $g^{-1}hr^{-1}$)	Per fruit fresh wt (nmole $g^{-1}hr^{-1}$)	Per fruit (nmole hr^{-1})
Not sprayed	0.108	0.007	0.267
X-77	0.125	0.003	0.125
Ethephon	0.615	0.136	5.690
CGA 13586	0.166	0.019	0.835
CGA 15281	0.585	0.061	2.636

[z]Data collected in 1977, 9 days after treatments were applied.

hr^{-1}) by t-tests at P = 0.10 (Fig. 2). These rates remained fairly constant throughout the study. CGA 13586, CGA 15281, and GAF 7767141 were an intermediate group, with mean initial rates of 3.75, 3.58 and 3.51 nmole $g^{-1}hr^{-1}$ respectively. Ethephon had the greatest mean initial rate (8.69 nmole g^{-1} hr^{-1}). Near control rates of ethylene evolution were obtained for all compounds after 8 days.

Effect of temperature on ethylene generation rate. The effect of temperature on rates of ethylene generation at 2 days after treatment is summarized in Table 2. Ethephon and GAF 7767141 had higher E_a values, while CGA 13586 and CGA 15281 had lowest E_a values. ER 3952, BOH, and control treatments were intermediate in temperature sensitivity. As the ethylene generation rates in 1978 approached control levels,

Fig. 2. Time-course of ethylene generation from potential fruit-loosening compounds: 1978. 1.7 pmole of compound was applied to the upper surfact of sour cherry leaves in 10 droplets of 5 ul each.

ETHYLENE EVOLUTION (nmole g⁻¹ hr⁻¹)
TIME AFTER APPLICATION (days)
1978
Ethephon
CGA 13586
CGA 15281
GAF 7767141
ER 3952
βOHethylhydrazine
Control
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30

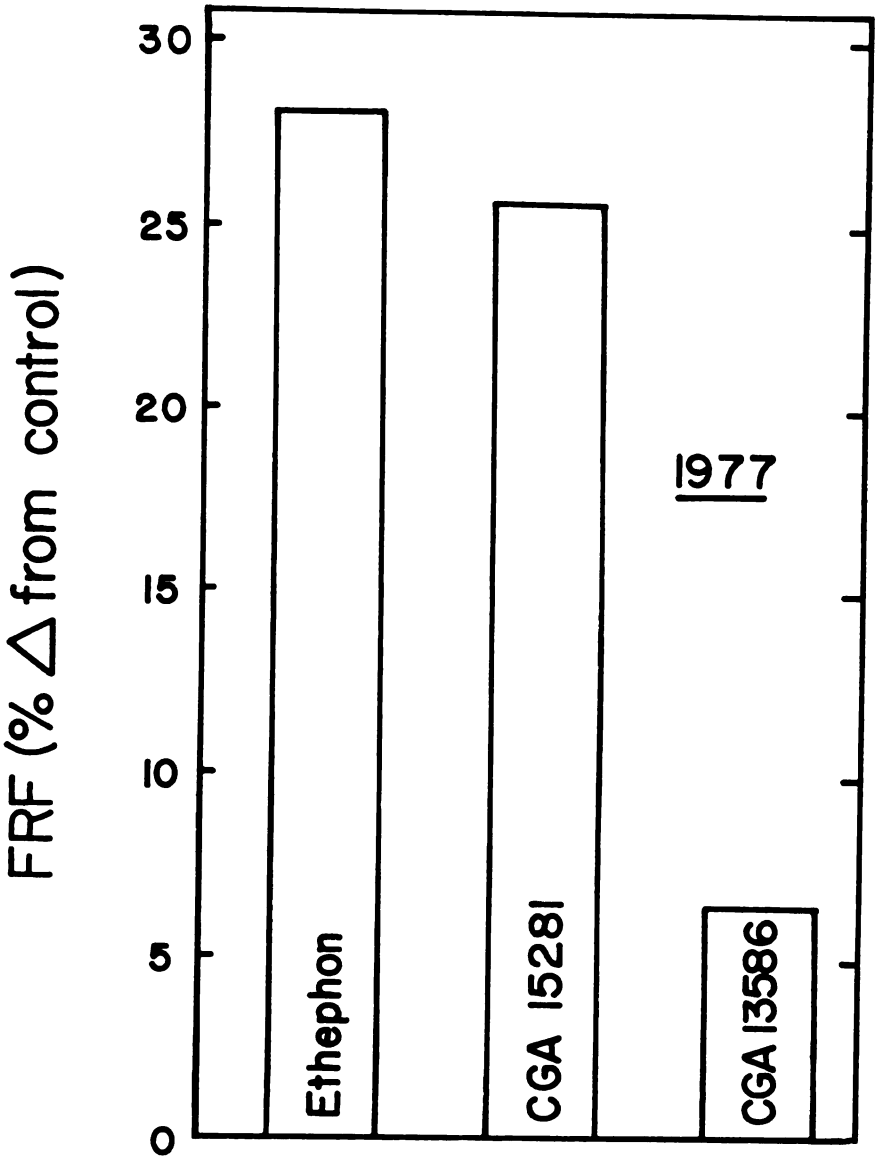
Table 2. Effect of temperature on generation of ethylene from potential fruit-loosening compounds.

Year	Assay temp (°C)	Apparent Energy of Activation (kcal $mole^{-1}$)			
		Rapid ethylene generators			
		Ethephon	CGA15281	CGA13586	GAF7767141
1976	21,30,40	25	11	---	---
1978	15,25,35	26	9	11	26
		Slow ethylene generators			
		Control	BOH	ER 3952	
1978	15, 25, 35	20	14	17	

the E_a values (measured on day 8 and 16) also approached the E_a of the control (data not shown).

Physiologic response. The reduction in FRF was generally consistent with ethylene generation. The correlation coefficient between FRF and initial ethylene evolution rate in 1977 was - 0.825 and in 1978 was - 0.817. ER 3952 gave good reduction in FRF in 1978 with no observed increase in ethylene generation. By deletion of the ER 3952 data, the correlation coefficient in 1978 was - 0.953. In 1977 (Fig. 3) ethephon and CGA 15281 induced a marked reduction in FRF (25 to 30%) relative to controls by 8 days after treatment. The response with CGA 13586 was considerably less (less than

Fig. 3. Effect of potential fruit-loosening compounds on reduction of FRF relative to controls: 1977. Difference in FRF from nontreated controls measured 8 days after spraying sour cherry trees with 2.08 mM of the designated compound.


FRF (% Δ from control)
30
25
20
15
10
5
0
Ethephon
CGA 15281
CGA 13586
1977

7%), which was consistent with the lower rate of ethylene generation. The FRF was reduced 15 to 20% by GAF 7767141, CGA 15281, and ER 3952 relative to control in 1978 (Fig. 4). Ethephon-treated leaves had the highest rates of ethylene generation in 1978 and ethephon also gave the greatest reduction in FRF (29%). No induced gummosis was observed for any of the treatments made.

Discussion

A second maximum of ethylene generation occurred from ethephon-treated leaves, but not from any of the other treatments, on day 9 of the 1977 time-course. It is known that the rate of ethylene generation from ethephon is highly dependent on temperature. Maximum ambient laboratory temperature (incubation temperature) also occurred on day 9. It seemed likely that the variation in assay temperature could have accounted for this peak in activity. Adjusting the data for ethephon to a constant assay temperature of 30° C, based on an energy of activation of 30 kcal $mole^{-1}$, effectively eliminated this anomalous peak (Fig. 5). Since none of the other compounds (Fig. 1) showed a similar peak, this suggested that ethephon has a much greater temperature sensitivity than the two CGA compounds. Differences in temperature sensitivity may also account for the inconsistency in the initial rates of ethylene evolution between the time-courses of 1977 and 1978. In 1977 the initial rate of ethylene evolution from ethephon-treated leaves was 2.43 fold greater than that from CGA 15281-treated leaves, while

Fig. 4. Effect of potential fruit-loosening compounds on reduction of FRF relative to controls: 1978. Difference in FRF from nontreated controls measured 7 days after spraying sour cherry trees with 1.7 mM of the designated compound.

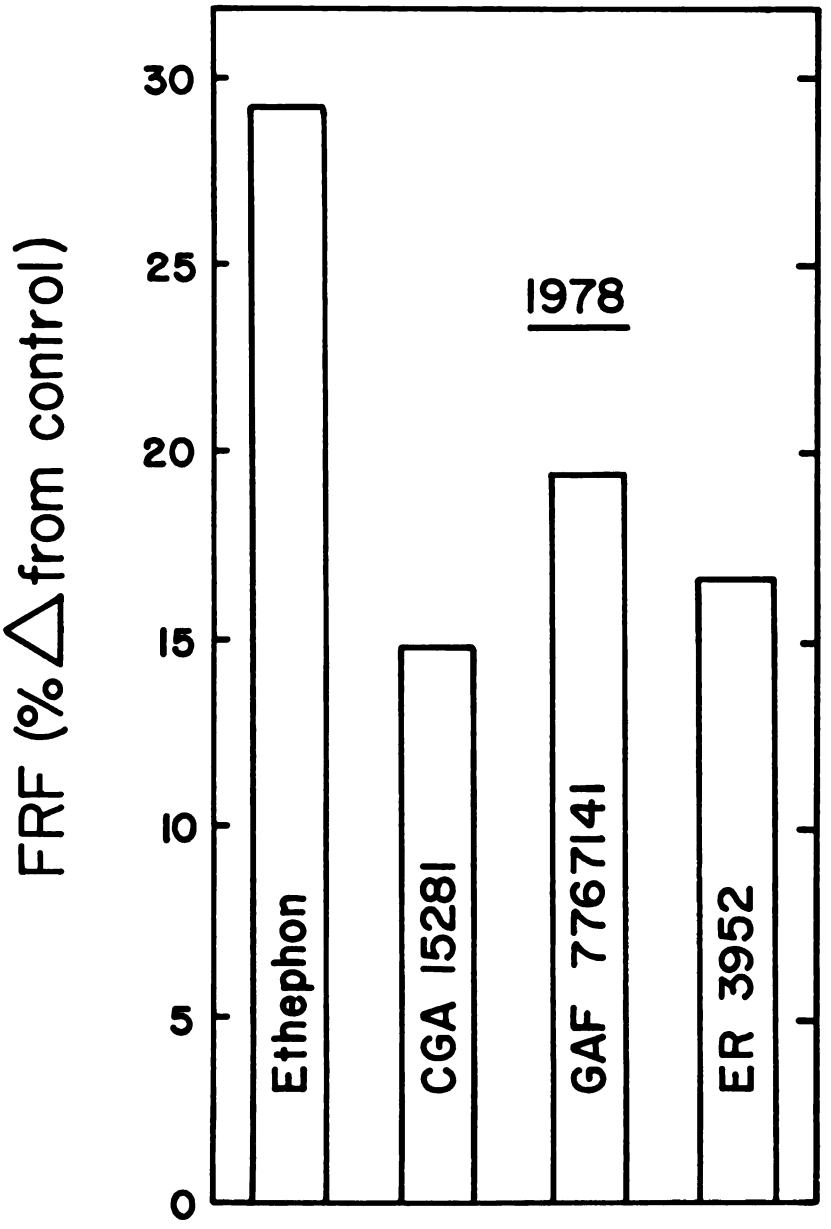
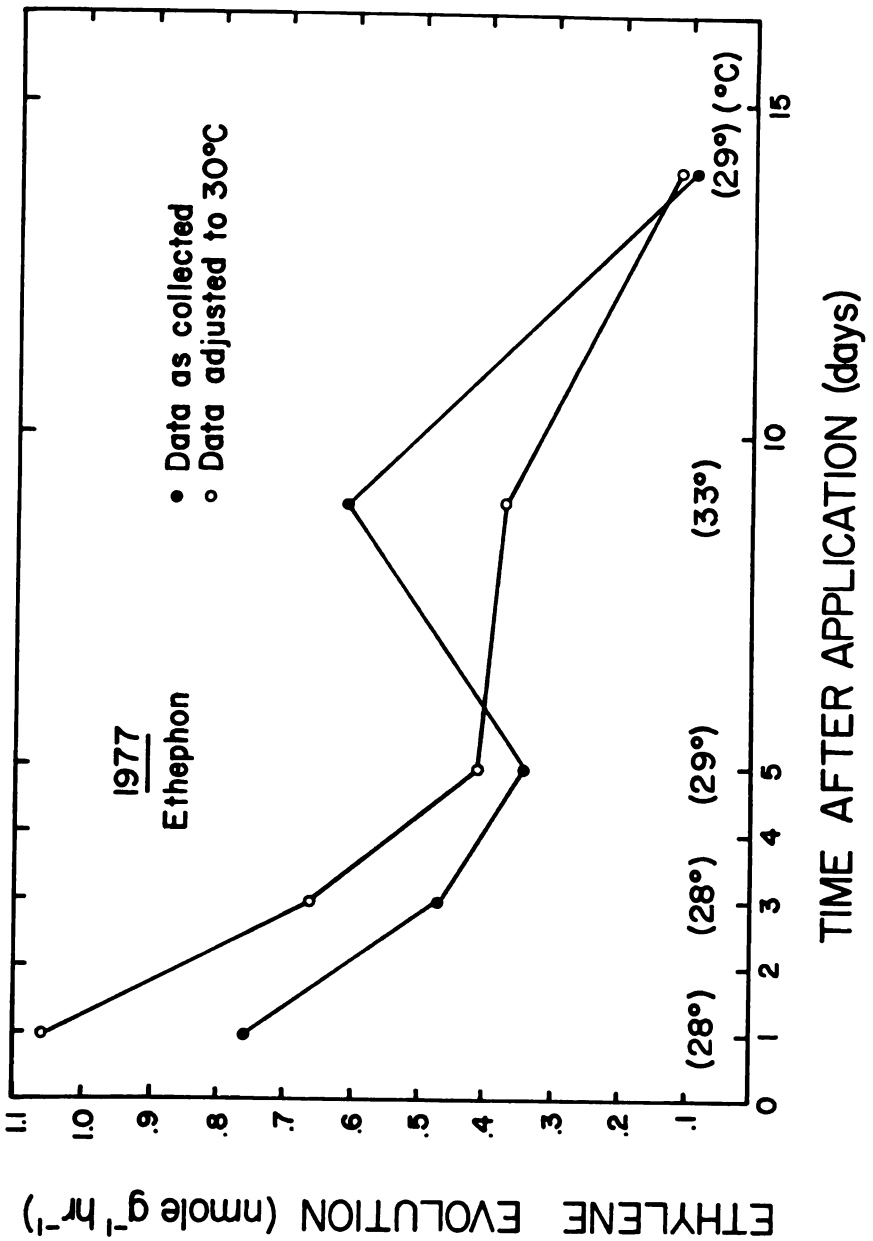

FRF (% Δ from control)
30
25
20
15
10
5
0
1978
Ethephon
CGA 15281
GAF 7767141
ER 3952

Fig. 5. Correction of 1977 ethephon ethylene-generation time-course to 30° C. Data (closed circles) collected at the temperatures designated in parentheses were adjusted (open circles) to 30° C based on an energy of activation value for ethephon of 30 kcal mole.


1977
Ethephon
• Data as collected
○ Data adjusted to 30°C
(28°)
(28°)
(29°)
(33°)
(29°) (°C)
ETHYLENE EVOLUTION (nmole g⁻¹ hr⁻¹)
TIME AFTER APPLICATION (days)
0
1
2
3
4
5
10
15
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1

in 1978 the ethephon treatment was 2.22-fold less active than the CGA 15281 treatment. Differences in compound formulations may also account for some of the observed discrepancies.

A surprising result in 1978 was that the reduction in FRF with ER 3952 was equal to CGA 15281 and GAF 7767141, despite the fact that very little ethylene was generated from ER 3952 treatments relative to the other 2 compounds. The initial rate of ethylene evolution from ER 3952-treated leaves was not significantly different from controls ($P = 0.10$). No ethylene was accumulated over aqueous solutions of 4 mM ER 3952 unbuffered or buffered at ph 6 and incubated at 30° C for 2.5 hr (data not presented). The structure of ER 3952 does not suggest an obvious mechanism for ethylene evolution upon degradation of this compound. It would appear that ER 3952 is not an ethylene generating compound, but can induce ethylene-mediated responses. ER 3952 has been reported to promote pistillate flower formation in cucumber (Bukovac et al. 1972) and numerous other responses commonly induced with ethylene (Anonymous 1970).

Conclusions

The compounds tested can be categorized into 3 groups (Table 3). Ethephon and GAF 7767141 exhibited rapid initial rates of ethylene generation which are highly temperature dependent. CGA 13586 and CGA 15281 also had rapid rates of ethylene generation, but these rates were far less sensitive

Table 3. Summary of ethylene generation and biological activity of potential fruit-loosening compounds.

Compound	Ethylene generation	Temperature sensitivity	Reduction in FRF
Ethephon	rapid	high	good
GAF 7767141	rapid	high	good
CGA 15281	rapid	low	good
CGA 13586	rapid	low	limited
ER 3952	slow	intermediate	good
BOH	slow	intermediate	--[z]
Control	slow	intermediate	none

[z]Reduction in FRF for BOH was not assayed.

to changes in temperature. ER 3952 and BOH were not different from controls, having low rates of ethylene generation with an intermediate dependence on temperature. It has been suggested that BOH when applied to tissue degrades too rapidly to be effective in abscission of citrus fruits (Rasmussen and Cooper 1968). Reduction in FRF was proportional to ethylene generation for all compounds except ER 3952 which gave a significantly reduced FRF with no observed increase in ethylene generation. CGA 15281 most closely fit the criteria for a good fruit loosening compound as stated in the introduction. Compounds of this type could be expected to give more consistent results under varying tempera-

tures in the field than ethephon or GAF 7767141. ER 3952, while not an ethylene generator, may also have potential as a fruit-loosener. The mode of action of this compound is not known.

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DISCUSSION

DISCUSSION

This dissertation has established that xylem function and shoot water relations of sour cherry are affected by ethephon-induced gummosis. The number of conducting vessels is decreased and the xylem sap viscosity is probably increased as a result of the stimulation of gum synthesis by ethylene and the extrusion of gum into the vessel lumen. The composition of extracted gum and gum flushed from the xylem of shoot segments was the same as sour cherry gum extruded from the tree (gum balls) and as reported in the literature for gum not induced by ethephon. The amount of gum flushed from the xylem increased as the total gum content of the shoot increased. Increases in gum extracted from ground stem tissue and gum flushed from intact shoot segments correlated well with observed decreases in measured xylem hydraulic conductance.

There was an increasing discrepancy between observed and measured hydraulic conductance with increasing gum content. This suggested that gum may have other effects on xylem function than simply occluding vessels. It was found that sour cherry gum has a marked effect on solution viscosity. The normal range of pressure gradients occurring in the transpiration stream of trees is 0 to 0.05 MPa m^{-1} (Meidner and Sheriff 1976, Nobel 1974, Zimmermann and Brown, 1971). At pressure gradients within this range, aqueous gum solutions flowed as a Newtonian fluid. However, flow of gum solutions became plastic at pressure gradients over

1.8 MPa m^{-1}. Pressure gradients of this magnitude might easily occur across vessel lengths of high resistance, as would be caused by the presence of gum. The occurrence of plastic flow at high pressure gradients to some extent compensates for the presence of gum, because the apparent viscosity decreases as the pressure gradient increases. This effect decreases with increasing gum concentration, however. Assuming Newtonian behavior, the concentration of gum in non-occluded vessels was estimated which would account for the discrepancy between measured and predicted hydraulic conductance.

The water potential of internode and leaf tissue was decreased in gummed shoots, as might be predicted by the above results. Thus, it is not surprising that in severe cases, shoots desiccate and die back. In less severe cases, the time-course of hydraulic conductance indicated that the tree has recovery mechanisms to regain adequate xylem function and avoid shoot die-back. In periods when the cambium is active, new vessels may be produced which could increase the hydraulic conductance of the shoot, so long as these vessels remained free of gum. Additionally, the plant may be able to regain function of existing vessels, perhaps by enzymatic degradation of the gum in affected vessels. It would be interesting to determine if such enzyme activity occurs in the apoplast of intact tissues and if the amount of gum hydrolysis varied between different tissues of the shoot and with conditions such as decreased water potential.

The heat pulse method for measuring sap velocity, as described by Closs (1958) would allow a comparative, non-destructive study of gummed and control shoots. Preliminary work on sour cherry is described in Appendix C.

High field temperatures make the occurrence of ethephon-induced gummosis more likely. The main effect of temperature is on the rate of ethylene release from ethephon. In Section III of this thesis it was shown that there was no temperature effect on ethylene action and a very small effect of temperature on the subsequent steps leading to the expression of an ethylene-mediated response. In Section IV five other potential fruit-loosening compounds were compared with ethephon. One group of compounds, especially CGA 15281, had active rates of ethylene generation with minimal sensitivity to changes in temperature and also gave good reduction in fruit removal force. A compound of this type would be preferable to ethephon because the activity is more strictly dose dependent under varying field temperatures. In addition, ER 3952 had good fruit-loosening acitivity but there was no apparent ethylene evolution. The mechanism of action of this compound is unknown and should be studied further. Higher concentrations of this compound should be applied to determine whether it will induce gummosis.

While ethephon-induced gummosis can cause serious injury to the tree, more should be learned about the functions of gum polysaccharides in the normal physiology of

plants. For instance, is the ethephon-induced increase in wood and bud hardiness of cherry (Proebsting and Mills 1978) related to ethephon-induced increases in gum synthesis? Arabinoxylan gums are involved in the cold hardiness of winter cereals (Olien 1965, 1976, 1978, Olien et al. 1968).

More detailed studies of the biosynthesis of cherry gum could be carried out using in vitro callus cultures. It is now possible to obtain such cultures of Prunus species (Rosati et al. 1980, Tabachnik and Kester 1977). Typical gum polysaccharides have recently been obtained from embryo callus cultures of barley and rye by Dr. C. R. Olien and M.N. Smith (personal cummunication). The monomer composition of this gum was similar to gums extracted from the crowns and seeds. The arabinogalactan polysaccharide isolated from the medium of suspension cultured sycamore cells was similar to cherry gum (Albersheim 1976). In such an in vitro system, the induction of gum synthesis as well as the biosynthesis of gum could be studied apart from the complex events that occur in intact tissue, such as a cherry shoot.

A brief study was made of other gum producing plants (Appendices D and E). Some tulip varieties exude gum from the bulb scales after exposure to ethylene. This can occur in shipment of the bulbs as a result of Fusarium rot. Two susceptible varieties were obtained and induced to gum with ethephon (Appendix D), but the time period during which the bulbs were susceptible to ethephon-induced gummosis was short and no further work was done with tulip.

Gum production in barley and rye crowns was also studied. There is no cell lysis associated with gum synthesis in winter cereals, in contrast to Prunus species. The effect of ethylene on the production of these gums in cereals was not known. A small increase in arabinoxylan gum content could have a marked effect on the winter hardiness of cereals because of their role as inhibitors of freezing kinetics in critical meristematic tissues. The effect of ethephon sprays on crown gum content of both non-hardened and hardened barley and rye were assayed (Appendix E). Low ethephon concentrations (0 to 100 ppm) stimulated gum synthesis, as in Prunus and Tulipa. Higher concentrations (1000 to 10000 ppm) of ethephon decreased the gum content. Enzymes are known to occur in cereals which degrade these gums (Bass et al 1957, Preece and MacKenzie 1952). The activity of these enzymes may be increased by ethylene as the activity of cell wall degrading enzymes is increased by ethylene in Prunus.

Why do cereals not have a gummosis response similar to tulip and cherry? The answer may be that tulips are perennials in the sense that daughter bulbs are produced within the current mother bulb for the succeeding year. Cherries and tulips tend to preserve and heal their injured parts. Cereals are more apt to abandon non-essential injured tissue and regenerate new tissue, so long as the meristematic tissue is preserved. In both cases, protection of critical meristems is essential.

When high concentrations of ethylene are generated from ethephon, gummosis is induced in sour cherry to an excessive degree, in the absence of wounds, and over the major portion of the tree. Thus, a process which under normal conditions allows the tree to survive can result in the death of the tree. The wound protective function of the gum has been likened to the ability of blood to clot at wounds and form a protective scab (Smith and Montgomery 1959). Continuing the analogy, blood can also clot in the absence of wounds and result in a stroke.

The die-back caused by severe ethephon-induced gummosis results in the loss of present and future fruit bearing wood. Secondary and long term effects of gummosis are unknown. The dead wood and weakened condition of a tree suffering from severe gummosis may make the tree more susceptible to attack by insects, pathogens, and to winter injury. The effects of less severe gummosis, when induced over much of the tree and perhaps over several seasons, might be more subtle, but no less important in determining tree productivity and longevity.

APPENDICES

Appendix A

Derivation of the Hagen-Poiseuille Equation

A discussion of the Hagen-Poiseuille equation is given by several authors (Dinsdale and Moore 1962, Prandtl and Tietjens 1934, Scott Blair 1969). Flow through a capillary of length L and radius r is assumed to be laminar. It is also assumed that the rate of shear ($\frac{dv}{dr}$) is proportional to the stress applied (force/surface area). v is the mean velocity of fluid flow through the capillary. The proportionality constant of stress to rate of shear is the viscosity coefficient (η) of the fluid. When a pressure is applied to drive the fluid through the capillary:

$$\text{Force} = (\text{Pressure})\ (\text{Cross Sectional Area})$$
$$= P \pi r^2.$$

The surface area of the capillary = $2 \pi rL$,

so the applied stress = $\frac{Pr}{2L}$.

Thus: $\frac{dv}{dr} = \frac{1}{\eta} \frac{Pr}{2L}$

The volume rate of flow (F) = $v \pi r^2$, and by substitution:

$$dF = \frac{P \pi}{2L\eta} r^3 \, dr.$$

Integration gives the familiar Hagen-Poiseuille equation:

$$F = \frac{P\pi r^4}{8L\eta} .$$

This equation was arrived at independently by Hagen and Poiseuille at about the same time. A brief historical account is given by Prandtle and Tietjens (1934).

Appendix B

REYNOLDS NUMBER

$$R = \frac{2\ d\ f}{\eta\ \pi\ r}$$

R = Reynolds number

d = solution density

F = volume flow rate

η = viscosity

r = capillary radius

Laminar flow occurs at Reynolds numbers less than 2100. Above this value turbulent flow can be expected.

Appendix C

HEAT PULSE METHOD OF MEASURING SAP VELOCITY

The heat pulse method for measuring sap flow rate, as described by Closs (1958) would allow a comparative study of intact gummed and control shoots. This method was attempted using a flexible three ohm nichrome heating cable connected to a 1.5 volt dry cell. The heating cable was wrapped once around the shoot and two 3 mil copper-constantan thermocouples were fastened against the shoot, one at 1 cm above and one at 1 cm below the point where the heating cable was fixed. The difference in temperature between the two thermocouples was measured with a Wescor microvoltmeter (Wescor, Inc., Logan Utah) after giving a heat pulse of a few seconds. Recording the difference in temperature with time after the heat pulse is applied corrects for conduction of heat through the wood and xylem sap and indicates heat convection due to the flow of the sap. Attempts were made to calibrate this method by simultaneously measuring flow rate with a porometer as water was pulled through shoot segments with a vacuum aspirator. The large degree of variability obtained with the heat pulse method made it impractical to pursue at the time. However, the method might be refined so that comparative data could be obtained from intact trees. This would also allow repeated measurements of the same shoots. Differences in environmental conditions affecting transpiration rate in the field might be compensated for by

expressing the sap velocities of gumming shoots relative to those of nearby control shoots.

Appendix D

ETHEPHON-INDUCED GUMMOSIS IN TULIP BULBS.

Tulips are another group of monocots besides cereals that form gum. Gum synthesis and cell lysis are both induced by ethylene in tulips and result in the formation of gum blisters on the outer bulb scales (Rees 1973, Kamerbeek et al. 1971) similar to the gummosis response of cherry. The gums produced by members of the Liliaceae consist mainly of mannose and glucose (Smith and Montgomery 1959).

Limited work was done with Apledoorn and Gander bulbs, generously supplied by Dr. August DeHertogh. These varieties are especially apt to show ethylene-induced gummosis. This is a particular problem when bulbs become infected with Fusarium during shipment from Holland. The bulbs arrived from Holland in the fall of 1977 and preliminary experiments showed a good gummosis response after suspending the bulbs over ethephon baths or injecting ethephon solutions into the bulbs. Gum occurred in blisters on the outer scales. However, sensitivity of the response to ethylene decrease rapidly as floral initiation was completed. Respiration also decreases at this time. Due to the short period when gummosis is readily inducible, further experiments were not conducted with tulip bulbs.

Appendix E

EFFECT OF ETHEPHON SPRAYS ON GUMS OF CEREAL CROWNS.

Introduction

Arabinoxylan gums of winter cereals are important components of the cold hardiness of these plants (Olien 1965, 1967, 1978, Olien et al. 1968). Effective gums of this type act as inhibitors of freezing kinetics by forming a cohesive film over the growing ice lattice, preventing liquid water from reaching the growing crystal. Protection of the meristematic tissue in the cereal crown from freeze injury by this mechanism is essential to the survival of the plant.

Gum synthesis is stimulated by ethylene and ethephon sprays in Prunus species (Abeles 1973) and in tulips (Rees 1972, Kamerbeek et al. 1971). The purpose of the study was to find out if gum production in cereals is stimulated by application of ethephon and therefore similar in this respect to Prunus and tulip. It also seemed important to determine if there were qualitative differences in the gum composition of ethephon-sprayed and control plants, in both the non-hardened and hardened states.

Methods and Materials

Two experiments were conducted using Rosen rye and Hudson barley. In the first experiment, plants were grown in sand filled flats outdoors under non-hardening conditions from April 27 to June 13, 1978. In the second experiment, plants were grown in the same manner under hardening

conditions from September 3, to November 16, 1978. Plants were fertilized with Hoaglands solution. Each flat contained 144 plants and was considered as an experimental unit. The non-hardened experiment consisted of one replication and the hardened experiment consisted of two replications. Ethephon was sprayed on the foliage at 0, 10^2, 10^3, and 10^4 ppm once at the mid point of the growing period and once three to four days before the plants were harvested. Sprays included 0.01% X-77 (alkyl aryl polyethoxy ethanol and free fatty acids, Chevron Chem. Corp., San Francisco, CA) as surfactant. After harvesting, the crowns were immediately isolated and kept frozen until they could be lyophilized. The extraction of gum from the crowns followed the procedure described by Olien (1965). The crowns were milled to 40 mesh prior to refluxing in 85% ethanol. The gum was then extracted in water and fractionally precipitated from solution with ammonium sulfate. Highest yield of gum was at 30% salt. The precipitates were desalted with 72% ethanol and the dry weight of the precipitate recorded. Finally, the gum was hydrolyzed and the alditol acetate derivatives of the sugar monomers were assayed by GLC as described by Albersheim et al. (1967). Most of this work was performed in the laboratory of Dr. C.R. Olien. The GLC assay was done in Dr. D.P. Delmer's laboratory.

Results and Discussion

In general, ethephon caused a small increase in barley and rye gum production at low concentrations (up to 10^2 ppm) (Table E 1). Thus production of both cherry gum (an arabinogalactan) and barley and rye gums (arabinoxylans) are enhanced

by ethylene. Higher concentrations of ethephon reduced gum content of the crowns. Ethephon at 10^4 ug ml^{-1} decreased the amount of gum below control levels. Enzymes exist in cereals which degrade the gums (Bass et al. 1957, Preece and MacKenzie 1952). This may be a means of converting these polysaccharides to metabolic energy necessary during the recovery period (C.R. Olien, personal communication). Higher levels of ethylene may increase activity of these enzymes in the same manner that the activity of cell wall degrading enzymes are increased in the ethylene-induced of lacunas in Prunus.

There were no apparent differences in monomer composition between ethephon treatments. The monomer composition of the arabinoxylan gums measured in these experiments did differ from published values (Table E 2). Our assays generally indicated less xylose and more galactose on a mole per cent basis. The mole per cent of xylose was about the same for non-hardened and hardened barley and rye. In both barley and rye, gum from hardened plants contained less arabinose and galactose than gum from non-hardened plants. There was also more glucose from hardened plants than from non-hardened plants and than published values indicated. Thus, there seemed to be more differences in gum monomer composition between non-hardened and hardened plants than between ethephon treatments.

Conclusions

Ethephon sprays may increase the gum content of barley and rye up to 10^2 ppm, but decrease gum content at

higher concentrations. Ethephon sprays did not affect the monomer composition of the extracted gums.

Table E 1. Mean dry weight of desalted gum precipitate per dry weight of barley and rye crowns.

Ethephon Concentration (ppm)	Gum Yield per Crown Dry Weight ($mg\ g^{-1}$)			
	Hudson Barley		Rosen Rye	
	NH[z]	H[z]	NH	H
0	4.07	5.58	---	6.45
10^2	---	6.27	---	7.39
10^3	3.00	5.68	---	6.02
10^4	3.40	5.06	---	5.62

[z]NH = Non-hardened, H = hardened.

Table E 2. Sugar monomer composition of barley and rye gums.

	Mole Percent Values of Sugar Monomers					
	Published[y]		Experimental			
	Barley	Rye	Barley		Rye	
Sugar			NH[z]	H[z]	NH	H
L ara	23	29	26.9	15.3	37.3	16.3
D xyl	61	60	42.2	35.3	45.8	48.9
D man	--	--	3.5	0.7	0.3	0.8
D gal	--	--	11.1	5.2	9.7	5.8
D glu	12	5	16.2	43.4	6.9	28.4
ara/xyl	.38	.48	.63	.43	.81	.33

[y]from: Smith and Montgomery 1959.

[z]NH = Non-hardened, H = hardened.

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