Ethylene Production by Loblolly Pine Seedlings During Cold Storage and Water Stress

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(ABSTRACT)

The effect of date and method of lifting on ethylene production by 1-0 loblolly pine (Pinus taeda L.) seedlings during cold storage, the dose-response relationship between ethylene and loblolly pine seedlings during cold storage, and the effect of water stress on ethylene and aminocyclopropane-1-carboxylic acid (ACC) production in two half-sib loblolly families were investigated.

Seedlings stored in Kraft-Polyethylene (K-P) bags showed a general trend of increasing ethylene concentrations from November through February, with an abrupt drop in March. Production rates may be related to the level of dormancy of the seedlings, with the peak in production corresponding to fulfillment of the chilling requirement.

Ethylene concentrations within the K-P bags generally declined over the twelve weeks in cold storage. Roots produced significantly higher levels of ethylene while stored in the K-P bags; however, when incubated under light, the needles produced higher concentrations.

Roots of machine-lifted seedlings produced significantly higher levels of ethylene than roots of hand-lifted seedlings. However, rates tended to moderate during storage and differences in production between HL and ML whole seedlings were not significant, which suggests that mechanical lifting is not a source of increased ethylene production.

A dose-response study indicated that ethylene fumigation during cold storage tended to slightly enhance growth of outplanted seedlings.

The effects of water stress on a Virginia Coastal Plain (CP) and an East Texas Drought Hardy (DH) loblolly family were also investigated. Ethylene production during severe stress (-2.8 MPa) appeared to be related to drought hardiness, with the CP seedlings producing much higher levels. Roots of both families produced greater levels of ethylene than the needles and may be due to an enhanced ability to convert 1-Aminocyclopropane-1-carboxylic acid (ACC) to ethylene.

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INTRODUCTION AND JUSTIFICATION

Over the past twenty years artificial regeneration of forest land has become increasingly common. In 1979, 96 percent of the forest land in the South was artificially regenerated by planting of nursery-grown bare-root seedlings (USDA Forest Service 1980). The number of acres of forest land harvested in the South has increased to over 1.5 million per year (Williston 1980), which is reflected in increased demand for nursery grown seedlings. Between 900 million and 1.2 billion pine seedlings are produced and planted each year and this number is expected to double by 1985 (Johnson et al. 1982).

Along with the increased production and planting of seedlings though, has been a decline in seedling survival. There are several causes for this reduction, one of which may be changes in nursery practices brought about to meet the increasing demand for seedlings (Johnson et al. 1982). The primary changes have been reduction or elimination of grading and longer rotations before allowing fields to lie fallow (Johnson et al. 1982). Another factor may be improper handling and storage of seedlings, which can be a

very critical aspect of the survival and hardiness of the seedlings.

Most seedlings pass through a period of cold storage between the time they are lifted and planted. Those that are not cold stored go to the field immediately after lifting, without refrigeration, where they may be stored at ambient temperatures for several days before planting. Within the cold storage environment the seedlings are subjected to the following conditions: 1) cool temperatures (3 to 5°C), 2) no light, and 3) a closed atmosphere. Noxious gases may accumulate as a result of the closed atmosphere, potentially causing a decline in seedling vigor.

Among the gases found to acculmulate is ethylene, the only identified plant hormone known to occur in the gas phase. It has been called the "aging hormone" (Spencer 1982) because its primary physiological effect is growth inhibition and inhibition of stem elongation (Abeles 1973, Moore 1979, Morgan 1982). However, ethylene is involved in other physiological responses throughout the entire life cycle of the plant and is not just involved in inhibition. Among these responses are: seed and bud dormancy, (breaking and perhaps initiation), root initiation, tropistic responses, latex secretion, flower initiation in pineapple, and sex expression in certain species (Abeles 1973, Morgan 1982, Spencer 1982.)

Ethylene production has also been found to be associated with and stimulated by mechanical wounding, disease and other stresses such as drought, excess water, wind and perhaps lifting and handling of seedlings (Cooper 1970, Abeles 1973, Kimmerer and Kozlowski 1982). Kimmerer and Kozlowski (1982) suggest that ethylene may be a useful indicator of the onset of stress and/or the degree of stress which a plant is experiencing. However, they point out that ethylene is also produced by non-stressed plants and that the level may be affected by the plant's stage of development and by environmental conditions.

Concentrations of ethylene in pine seedling cold storage facilities have reached physiologically significant levels (Johnson 1982) according to the general dose-response curve for ethylene developed by Abeles (1973). Abeles found that usually there is no effect at concentrations below 0.01 ppm, half maximal effects at 0.1 ppm and saturation at 1 to 10 ppm. Recent studies have shown that ethylene may affect field survival, seedling vigor and growth (Barnett 1980, Hinesley and Saltveit 1980), and that in general, levels of stress ethylene have been correlated with decreased growth (Abeles 1973). However, specific knowledge of pine seedling responses to ethylene during cold storage is limited.

Reforestation success could potentially be enhanced with better understanding of physical and physiological changes which occur during handling and storage of seedlings. The primary purpose of this study was to determine the role of ethylene in the handling and storage process, its effect on seedling vigor and physiology, and to determine the potential benefits of controlling ethylene levels in cold storage facilities.

Objectives

The primary objectives of this study were to:

1. determine the effect of the time loblolly pine (Pinus taeda L.) seedlings are lifted from the nursery bed on the rate of ethylene production by the seedlings during cold storage.
2. determine the site of ethylene production in seedlings during cold storage.
3. determine the effect of hand lifting versus machine lifting on ethylene production during cold storage.
4. develop the dose-response relationship between ethylene concentration during cold storage and growth and survival of loblolly pine seedlings.
5. determine the effect of water stress on ethylene production by loblolly pine seedlings.

LITERATURE REVIEW

Introduction

It has long been known that ethylene has an effect on plant growth. The first recorded effect of ethylene occurred in Germany, in 1864, when it was observed that leaking illuminating gas from gas mains defoliated shade trees. It wasn't until forty years later, in 1901, that Neljubov showed that ethylene was the active compound in the illuminating gas which caused horizontal growth and inhibition of cell elongation. In 1910 Knight et al. performed experiments similar to those of Neljubov and also found that ethylene was the active compound in illuminating gas. They also observed that ethylene caused horizontal growth and prevented plumular hook opening and leaf expansion. Many others have since observed that ethylene retards growth and is involved in many other physiological processes (Abeles 1973).

Due to the effect of ethylene on many physiological processes during the life of the plant, it has been categorized as a plant growth regulator (hormone). Although up until twenty years ago there were those who did not

follow this line of thinking since ethylene does not meet the definition of a plant hormone in the strictest sense of the word. A (plant) hormone is defined as an endogenous chemical, active in low concentrations, produced in one site of the organism and transported to another to bring about its physiological effect (Abeles 1973). However, according to Waring and Phillips (1973) ethylene does not move in physiologically significant amounts between different parts of the plant. Jackson and Campbell (1975), on the other hand, observed that ethylene applied to the roots of tomato plants grown in water culture moved to the shoots where it promoted various ethylene related responses. It appears that changes in ethylene levels in one area of the plant can cause a change in another (Bradford and Yang 1980). Some of the skepticism towards accepting ethylene as a plant hormone was also due to the fact that no other known hormone is a gas or such a simple compound. Gradually though, as more effects of ethylene were documented, ethylene came to be accepted as a plant hormone (Spencer 1982).

As with other hormones, the level of ethylene produced by the plant tissues is quite low, usually less than 10 ng per gram (Ward et al. 1978). This, along with the fact that ethylene is a gas, made it very difficult to quantitatively measure its rate of production. Prior the development of

the gas chromatograph, bioassays, such as the triple pea response, were the standard techniques used to detect the presence of ethylene (Abeles 1973). The advent of the flame ionization gas chromatograph made ethylene detection and measurement much easier and much more precise. Concentrations of ethylene as low as one part per billion (ppb) can accurately be detected.

Physical Properties of Ethylene

Ethylene, or ethene, is an unsaturated hydrocarbon consisting of two carbons, four hydrogens and a double bond ($CH = CH$, mol. wt. 28.05). It is colorless, flammable, has a sweet ether-like odor and is lighter than air. It is about five times as soluble in water as oxygen, with an absorption coefficient of 0.266 at 0°C, and 0.108 at 30°C (Abeles 1973, Windholz 1976).

Physiological Effects

Physiological responses to ethylene can be seen in all aspects of metabolism throughout the life cycle of most plants (Lieberman and Kunishi 1970). It is often considered a growth inhibitor because of its well known abilities to: 1) hasten ripening, 2) cause premature senescence of leaves and flowers, 3) stimulate abscission, 4) inhibit elongation

of stems and roots, and 5) induce epinasty (Abeles 1973). These, however, are only a few of the wide variety of effects that ethylene can have on the physiology of plants. Other observed effects of ethylene include:

1. effects on dormancy of seeds and buds
2. effects on geotropism
3. root initiation
4. hook opening
5. latex secretion
6. stimulation of flowering
7. sex expression
8. tissue proliferation

(Abeles 1973, Moore 1979, Morgan 1982).

Ripening

The involvement of ethylene in the ripening process of fruit is probably the most thoroughly studied aspect of ethylene physiology. Ethylene in the form of smoke was used for a long time to promote ripening of some fruits, while removal of ethylene was carried out to delay ripening (Abeles 1973).

Until the 1960's many researchers debated whether ethylene was a by-product of ripening and not a cause of it. However, since then, many workers, including Burg, Pratt,

and Goeshl have shown that ethylene does stimulate ripening. According to Abeles (1973), ethylene can promote ripening in any fruit as long as the tissue is in a receptive state.

It appears that there are two major biochemical processes involved in ripening on which ethylene has an effect: 1) changes in membrane permeability, which leads to altered compartmentation and release of enzymes and 2) increases in enzyme synthesis (Moore 1979). Ethylene may also be involved in changes in respiration rates in climacteric fruit (Abeles 1973, Moore 1979). Abeles (1973) points out though, that there is no reason to believe that ethylene is the sole controller of ripening.

Senescence

Ethylene can accelerate leaf and flower senescence (Abeles 1973, Gepstein and Thimann 1981). Senescence involves the breakdown of chlorophyll and chloroplasts, and the loss of RNA, protein and other constituents of the leaf (Abeles 1973). Ethylene may inhibit RNA and protein synthesis (Wareing and Phillips 1973) although it appears that it has little effect on RNA degredation (Abeles et al. 1967).

In general, it appears that the involvement of ethylene in the degradation of tissues is indirect. It seems to

regulate some master reaction such as control of auxin levels which maintain juvenility in the plant (Abeles 1973).

Abscission

Ethylene can stimulate fruit, flower and leaf abscission (Abeles 1973, Moore 1979) and is involved in the dehiscence of pecan and walnut fruits (Abeles 1973).

Ethylene, however, is not considered to be the major hormone involved in abscission (Moore 1979), because the ability of ethylene to hasten cell separation ultimately depends on the level of auxin in the tissue (Abeles 1973). It appears that the levels of auxin and perhaps other "senescence factors" must drop before ethylene can stimulate cellulase synthesis which is a major stage of abscission (Abeles 1973, Moore 1979).

Ethylene may play a passive role in abscission, in that tissues gradually become more sensitive to ethylene as they age, or it may play an active role through increased production rates (Abeles 1973). Liebermann and Kunishi (1970) believe that abscission is hastened by the anti-growth hormone effects of ethylene which bring into play the metabolic pathways associated with senescence.

Cell Elongation

Ethylene can induce inhibition of stem and root elongation in many species. Tissue response is rapid and apparently reversible (Abeles 1973); however, Abeles points out that Andrae et al. found that inhibition of growth was irreversible.

According to Burg et al. (1971), inhibition does not take place due to a decrease in water uptake, but to a re-orientation of cell microfibrils from a longitudinal to a radial direction. Steen and Chadwick (1981) also concluded that ethylene causes a re-orientation of cell wall microfibrils.

Effects of re-orientation can be observed in the increased radial expansion or swelling that usually accompanies decreased elongation of shoots or roots (Abeles 1973, Moore 1979). What growth occurs is in a radial direction as opposed to a longitudinal one (Abeles 1973).

Inhibition of leaf expansion is another example of ethylene's effects on elongation. Abeles (1973) presents data observed by Middleton, et al. in 1954, which shows that ethylene concentrations of 0.05 ppm caused abnormal growth in marigold and that 0.1 ppm caused leaf abnormalities in tomato.

Ethylene usually inhibits root growth; however, in one case Kays, Nicklow and Simon (1974) found that very low concentrations of ethylene can stimulate root elongation.

Epinasty

The effect of ethylene on stimulating epinastic responses in plants depends on the species and variety, and on the age of the leaf petioles involved. In general, younger leaves are much more responsive (Abeles 1973).

Epinasty is brought about by an increase in cell size (swelling) in the upper part of the petiole (Abeles 1973). Ethylene reverses the gravity-directed movement of auxin, causing an accumulation of ethylene in the upper side of the petiole (Kang and Burg 1973).

Dormancy

Ethylene has been observed to increase germination rates of seeds of several species (Abeles 1973, Vancura and Stotsky 1976). Ketring and Morgan (1970) felt that the rate of ethylene evolution was the factor controlling the natural dormancy of peanut seed, and that the ability of various chemicals, such as gibberellins and cytokinins, to promote germination was due to their ability to increase ethylene production.

It has been proposed that ethylene plays an ecological role in the seed germination process in that sensitivity to ethylene may allow germination of seeds only when seeds are suitably buried in the ground (Esashi and Leopold 1969). Ethylene has even been considered as an allelopathic chemical due to its effect on buried seed and on other soil-borne organisms (Putnam 1983).

The exact role of ethylene in the initiation and breaking of dormancy is unknown; however, it is generally agreed that growth promoters and inhibitors are involved in the process (Perry 1971, Wareing and Saunders 1971). It has been suggested that hormones are involved in gene repression and release (Perry 1971, Zimmerman and Brown 1980). Few studies have been done to determine the role of ethylene in dormancy. Perry (1971) reported that ethylene is one of several growth stimulators which decrease in concentration in the fall and increase with chilling treatments and reinitiation of growth. In a study of ethylene production by apple trees, Blanpied (1972) found that the highest production rates occurred in the dormant buds. Ethylene has been found to stimulate bud break in birch, beech and some oaks; however, high levels of ethylene can prevent further elongation of buds (Abeles 1973). Burg and Burg (1968) found that low concentrations of ethylene were very

effective in retarding bud development and that the effect was irreversible if treatment was continued for three or more days.

Other hormones, such as abscisic acid (AbA), gibberellins and cytokinins have also been implicated in dormancy. Eagles and Wareing (1964) suggest that the regulation of dormancy is brought about by changes in the balance of growth inhibitors and promoters. They suggest that a build-up of growth promoting hormones may offset the effects of growth inhibitors.

Root Initiation

Initiation of roots from leaves, stems and pre-existing roots in various species including monocots, dicots, herbaceous and woody, can be brought about by ethylene (Abeles 1973). According to Abeles, relatively high concentrations of 10 ppm ethylene are needed for root induction. However, Jackson and Campbell (1975) observed that 100 ppb ethylene applied to the roots of tomatoes stimulated growth of adventitious roots near the base of the stem. Abeles (1973) suggests that the most effective treatment appears to be exposing the plant to ethylene for several days then restoring a fresh air atmosphere.

Ethylene has also been observed to induce "root hair-like" outgrowths on cells which have experienced radial expansion (Abeles 1973, Moore 1979).

Stress Ethylene

Plant tissues normally produce low levels of hormonal ethylene, but when subjected to stress, production in the injured cells can increase tremendously (Tingey 1980). There is a substantial amount of evidence to support that ethylene production increases rapidly following stress caused by chemicals, insect damange, temperature extremes, drought, gamma irradiation, disease and mechanical wounding (Abeles 1973). Yang and Pratt (1978) state that any condition deviating from the normal environment of the intact plant can lead to increased stress ethylene production.

Abeles (1973) stated that ethylene production by plants can be controlled by a variety of mechanical stimuli such as separation of organs, incision, bruising and pressure. Several researchers have observed that increases in ethylene production in sliced leaf and stem tissues were not as great as in sliced fruit and other tissue (Abeles 1973). Physical stress can also cause increased ethylene production in trees, as observed by Brown and Leopold (1973). They found

that the application of bending stress to branches can increase levels of ethylene within the branches, and that this may result in increased diameter growth. Kays, Nicklow and Simon (1974) found that ethylene production by broad bean (Vicia faba L. var. Windsor) roots would increase as much as six times when physically obstructed. Neel and Harris (1972) reported that simply handling or shaking sweetgum (Liquidambar styraciflua L.) and corn (Zea mays L.) led to decreased growth and suggested that since mechanically disturbed plants produce higher levels of ethylene, and since ethylene is known to reduce growth, it followed that ethylene could be responsible for the decreased growth in their experiment.

Water stress can also have a profound effect on ethylene production. The amount of ethylene produced by wheat (Triticum aistivum L. cv. Eclipse) is generally quite low, but levels increased when excised leaves were subjected to water stress (Wright 1977, Hoffman et al. 1983). El-Beltagy and Hall (1973) also observed increased levels of internal ethylene in water-stressed Vicia faba L.. Wright (1977) and Apelbaum and Yang (1981) observed that the amount of ethylene was positively correlated with the amount of stress until leaf water potential reached approximately -12 bars (approximately 9-10% water loss) after which ethylene

production declined with increases in stress. Hoffman et al. (1983), on the other hand, reported that as water loss continued from 0 to 13.5% that ethylene levels continued to raise. Hoffman et al. (1983) reported that exposing excised wheat leaves to a relatively rapid water deficit resulted in a rapid increase in ethylene production followed by a decrease in production rates. This is in agreement with Apelbaum and Yang's (1981) report that following water stress ethylene production in excised wheat leaves increased 30-fold and then declined.

In general, the high rates of ethylene production brought about by stress begin within 35 minutes to one hour after the trauma occurs, and can persist for several hours (Ward et al. 1978, Abeles 1982). Rates of production usually return to normal within 24 hours of removing the stress (Tingey 1980, Abeles 1982). Hoffman et al. (1983) reported that rehydrating following water stress resulted in a large drop in ethylene production.

Wright (1981) suggested that leaf aging, which could involve the depletion of an ethylene substrate and changes in the level of endogenous cytokinin, is probably involved in determining the amount of ethylene produced by water stressed leaves.

In a study involving prolonged water stress (nine days) in whole plants of Vicia faba L. El-Beltagy and Hall (1974) observed three phases in levels of internal ethylene. The first stage involved an increase in ethylene production correlated with an increase in water saturation deficit (WSD). Closure of stomates was not considered to be correlated with this rise in ethylene production. The second phase involved a decrease in ethylene production followed in the third phase by another increase in production correlated with rapid senescence. They postulated that increased accumulation of ethylene within the plant tissue, as well as increased ethylene synthesis were responsible for the higher levels of ethylene.

It has been suggested that the role of stress ethylene is to help plants cope with stress (Yang and Pratt 1978). Its function may be to cause abscission of damaged plant organs, regulate seedling growth through the soil, increase disease resistance and influence ripening (Abeles and Abeles 1972). Wright (1977) suggested that the overall imbalance of growth hormones, including ethylene, which occurs during water stress results in inhibited growth, partial or complete stomatal closure, epinasty, increased permeability of roots and increased senescence and abscission of lower leaves and together increase the plant's chance of survival. Cooper

(1970) states that although there is a tendency to think of stress ethylene as the cause rather than the product of tissue damage, one should not overloook the possibility that stress ethylene may be a healing substance. Ethylene can produce intumescences in apple twigs and thereby may fulfill Habertandt's 1913 definition of a wound hormone: "A product of damaged cells which diffuses to neighboring cells and induces cell division leading to healing of the wound" (Cooper 1970, Yang and Pratt 1978). Yang and Pratt point out that ethylene may function indirectly in wound healing and disease resistance by influencing respiration and metabolic rates.

Biosynthesis

Several substances have been considered as possible precursors for ethylene. Of these, the amino acid methionine is considered to be the most probable one (Abeles 1973, Wareing and Phillips 1973, Spencer 1982).

Adams and Yang (1981) have suggested the following biosynthetic pathway for the conversion of methionine to ethylene: Methione → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene. They also suggest that the conversion of SAM to ACC is the rate controlling step and is controlled by the enzyme ACC

synthase (see Appendix A). Bradford and Yang (1980) determined that ACC may serve as a "signal" and be transported through the xylem if ethylene production is blocked at the site of synthesis, and be subsequently converted to ethylene.

Many substances have been observed to enhance and/or inhibit ethylene synthesis along this pathway. Auxin can induce plants to increase levels of ethylene production (Spencer 1982) and in vegetative plants the highest rates of ethylene production generally occur in actively growing regions of stems and leaves where auxin levels are high (Cooper 1970, Wareing and Phillips 1973). Burg and Burg (1968) found that pea stems would not convert methionine to ethylene unless first treated with indole acetic acid (IAA). In 1971 Kang et al. reported that continued presence of auxin is required for high production rates of ethylene. Yu et al. (1979) found that auxin stimulated ethylene production by stimulating production of ACC synthase.

Bufler and Bangerth (1983) found that ACC did not accumulate in apples stored in low oxygen and postulated that if oxygen is needed for ethylene action, and if ethylene is required for activation of ACC synthase, that perhaps low production of ethylene in low oxygen is a result of lowered stimulation of its own biosynthesis.

Another gas which is known to have an effect on ethylene synthesis and/or action is carbon dioxide. Abeles (1973) reported that carbon dioxide could inhibit ethylene production but that it could also enhance production or have no effect at all. Burg and Burg (1967) reported that it is a competitive inhibitor of ethylene production in etiolated pea (Pisum sativum L.). Beyer (1979) also observed classical inhibition kinetics for carbon dioxide and ethylene in etiolated pea seedling tissue. Kao and Yang (1982) and Horton et al. (1982), on the other hand, reported that a source of carbon dioxide is necessary for continued ethylene production. Horton et al. observed that photosynthesizing plant tissue in sealed flasks under a source of light produced less ethylene than tissue in the dark or tissue supplied with carbon dioxide. They felt that the amount of measured ethylene can be controlled by the internal pool of carbon dioxide. Kao and Yang (1982) reported that production of ethylene by excised segments of rice leaves increased with increasing levels of carbon dioxide until concentrations reached 2-3%, after which ethylene production leveled off. Their data indicated that carbon dioxide promotes conversion of ACC to ethylene through activation of the enzyme involved and not through synthesis of the enzyme.

Light has also been reported to have various effects on ethylene production. Kimmerer and Kozlowski (1982) reported that ethylene production by red pine (Pinus resinosa Ait.) and paper birch (Betula papyrifera Marsh.) was faster in the light. However, Wright (1981) reported that ethylene production by water-stressed wheat leaves was inhibited by light and suggested that one of the precursors requires an obligatory dark stage. Kao and Yang (1982) and Horton et al. (1982) also reported that light inhibited ethylene production but suggested that the effect was mediated through carbon dioxide. In separate experiments they found that lower rates of ethylene production by green tissue in the light, compared to tissue in the dark, was due to the reduction of the carbon dioxide pool by the photosynthesizing tissue. It was also reported that light itself actually could stimulate the conversion of ACC to ethylene when carbon dioxide levels were not limiting. Other inhibitors of ethylene synthesis include aminoethoxyvinylglycine (AVG), cycloheximide, Co^{+2}, uncouplers, free radical scavengers, inorganic phosphate (Chalutz et al. 1980), and temperatures greater than 35°C (Abeles 1973, Yang 1982) (see Appendix A). Abeles (1973) reported that storing plants under refrigeration can also reduce ethylene production.

Stress ethylene is believed to be synthesized in the same manner as hormonal ethylene (Tingey 1980), and the site of action of stress agents appears, like auxin, to be at the step between SAM and ACC (Adams and Yang 1982, Abeles 1982). Abeles and Abeles (1972) showed that the role of stress agents was to cause cells to synthesize increased amounts of ACC synthase. In a 1982 study Konze and Kwiatkowski confirmed that stress ethylene is indeed dependent on ACC formed after subjection to stress and not on stored ACC. According to McKeon et al. (1982) increased ethylene production by excised wheat leaves subjected to water stress was due to increased activity of what they called the "ethylene-forming enzyme" (EFE). Unlike ACC synthase, which catalyses the conversion of SAM to ACC, the EFE is reported to catalyze the conversion of ACC to ethylene.

Rates of production of ethylene in plant tissues vary from organ to organ and with stage of development and environmental factors, including stress (Wareing and Phillips 1973). El-Beltagy and Hall (1974) observed that the levels of internal ethylene in Vicia faba L. varied with time of day. Wareing and Phillips found that production rates were generally highest in the most actively growing areas, particularly meristematic tissues which are high in auxin. Johnson (1982) suggested that rates of ethylene

production in loblolly pine seedlings may be related to their stage of dormancy.

The exact site of ethylene biosynthesis is not known (Abeles 1973); however, Apelbaum et al. (1981) feel that the conversion of ACC to ethylene may be associated with the cellular membrane.

Mode of Action

The specific mode(s) of action of ethylene has not been positively established; however, several theories have been developed. There is evidence that ethylene works through interactions with other hormones (Lieberman and Kunishi 1970, Mullins 1970), through changes in respiration rates and metabolic activities (Yang and Pratt 1978) and/or through re-orientation of cell membrane microtubules (Steen and Chadwick 1981).

Interaction with Other Hormones

A significant amount of interaction takes place between auxin and ethylene. As mentioned earlier, auxin can stimulate the synthesis of ethylene through inducing ACC synthase production (Adams and Yang 1981), and may be required for continued high rates of ethylene production (Yu et al. 1979). In fact, many plant responses previously

attributed to auxin may be due to auxin-induced ethylene. However, ethylene does not play an intermediary role in all auxin-induced responses (Abeles 1973, Muir and Richter 1970), and in some cases (ripening and abscission) auxin can block the effect of ethylene.

Auxin and ethylene often have opposing effects as seen in ripening and abscission where auxin has a delaying influence and ethylene a promotive effect (Abeles 1973). Abeles feels that this suggests that one mechanism of ethylene action may be control of auxin levels in plant tissues. Ethylene may control auxin through lowering the tissue's ability to synthesize auxin (Valdovinos et al. 1970), or through inhibiting auxin transport (Morgan et al. 1970, Valdovinos et al. 1970). Lieberman and Kunishi (1970) came to the conclusion that ethylene acts through modulation of growth-promoting hormones (auxin, as well as cytokinins and gibberellins), thereby preventing over growth or over development of tissues and organs. They describe a feedback relationship where levels of auxin and cytokinins above the threshold point for balanced growth are brought under control by the increased levels of ethylene which they induce.

Enzyme Synthesis

Another aspect of ethylene action may be regulation of RNA and protein synthesis (Abeles 1973). Wareing and Phillips (1973) state that exposure of tissues to ethylene may result in both quantitative and qualitative changes in enzymes. Cycloheximide, an inhibitor of protein synthesis, has been shown to block the activity of ethylene (Abeles 1973). However, some of the responses which ethylene induce occur much too rapidly to be controlled by nucleic acid and protein synthesis (Hill 1973, Wareing and Phillips 1973). Wareing and Phillips suggest that the action of ethylene probably includes short-term effects on cell membranes and longer-term effects on nucleic acid and protein synthesis.

Respiration and Metabolism

Another possible aspect of ethylene action may be an influence on respiration rates and metabolic activities (Yang and Pratt 1978). According to Yang and Pratt ethylene, particularly stress ethylene, can induce increased respiration rates in fruits, flowers, storage organs and vegetative tissues; however, Goldney and Van Stevenvick (1970) found that respiration rates in leaves fell during increased ethylene production.

In the case of wound ethylene, ethylene's fuction may be to stimulate adjoining tissues to begin metabolic changes to increase resistance, and perhaps begin healing processes (Stahman et al. 1966, Cooper 1970, Yang and Pratt 1978).

Beyer (1979) suggested that the metabolism of ethylene itself is an important aspect in the mechanism of ethylene action. Working with purified carbon-labeled ethylene he determined that the metabolic system involved consists of one pathway in which ethylene is incorporated into the tissue and another in which ethylene is oxidized to carbon dioxide. Based on studies with deuterated ethylene Abeles et al. (1972) argued that the metabolism of ethylene is not an important aspect of its mode of action, but that ethylene, like many other hormones, acts through causing conformational changes in cells.

Re-orientation of Microtubules

In some cases ethylene may act through re-orientation of cell membrane microtubules, and other changes in cell wall structure (Osborne et al. 1970, Steen and Chadwick 1981). The effect of ethylene on radial expansion and inhibition of elongation may be due to the deposition of new microtubules on the cell wall in a more longitudinal direction than normal, restricting cell elongation but allowing radial expansion (Moore 1979, Steen and Chadwick 1981).

Osborne et al. (1970) reported that changes in cell wall plasticity due to increased cell wall depositions of certain proteins and increased cross-linking could also account for the changed orientation of cell growth in response to ethylene.

Ethylene in Pine Seedlings

Only a few studies dealing with the effect and/or production of ethylene on species of pine have been carried out. The following three studies looked at ethylene production by conifer seedlings during cold storage.

Hinesley and Saltveit (1980) did a study with bare-root three-year-old fraser fir (Abies fraseri (Pursh) Poir.) seedlings, in which they fumigated half of the population with a continuous exposure to 17.5 ppm of ethylene for eight weeks of cold storage at 38 °C. Terminal shoot elongation during the first season of the fumigated seedlings, as compared to the control seedlings (cold storage), was reduced by 22 percent. This was a significant reduction at $p=0.05$. Percentage of abnormal or aborted buds was higher in seedlings exposed to ethylene but the difference was not significant at $p=0.05$.

Barnett (1980) did a study with 1-0 loblolly pine seedlings, held in cold storage (34 to 36 °F) for 21 and 42

days, with and without an ethylene absorbent. The absorbent used was Purafil ES which consists of potassium permanganate absorbed on an aluminum medium. It oxidizes ethylene to water and carbon dioxide, and according to Abeles (1973) will also absorb O_3, SO_2, NO, H_2S and NH_3 from air.

Evaluation of root generation potential (number of new roots per seedling) was carried out on a sample of seedlings from each treatment which were potted in sand and grown in a growth chamber (75 °F, 18 hr photoperiods of 1500 footcandles). Barnett found that adding Purafil sachets to bags containing loblolly pine seedlings did not affect root generation potential after three weeks of storage; however, after six weeks the seedlings with Purafil produced significantly more new roots.

Survival and height growth of a sample of seedlings from each treatment, outplanted in silt loam soil, were measured during the dormant season one year after outplanting. Barnett found that whereas length of storage time did not affect survival, Purafil did improve survival by an average of six percentage points.

Johnson (1982) observed that ethylene can accumulate to physiologically significant levels in cold storage facilities storing loblolly pine seedlings and that type of seedling packaging and type of forklift used by facilities

can have an effect on ethylene levels. Ethylene levels in the atmosphere at the Virginia Division of Forestry, New Kent Forestry Center's facility, which uses open-ended seedling bales and gasoline-powered forklifts, varied significantly ($p=0.001$) over the 14-week storage period. A maximum concentration of 2369 ppb ethylene was reached in late December, and was followed by a large drop in concentration to 174 ppb. The low corresponded with a cessation of seedling lifting due to cold weather and frozen soil during the last week of January. Upon resumption of lifting ethylene levels increased slightly.

Levels of ethylene in the atmosphere of Union Camp's cold storage facility at their hardwood nursery in Capron, Virginia, where seedlings are packaged in Kraft-Polyethylene (K-P) bags and only hand-powered forklifts are used, did not vary significantly ($p=0.05$). Ethylene concentrations remained fairly constant at, or slightly above, the control concentration of 200 ppb.

Johnson felt that the low levels of ethylene in the Union Camp facility may possibly be due to ethylene being retained within the K-P bags. He felt that the variation in ethylene levels in the VDF facility could be due to differences in the amount of forklift activity (ethylene is present in exhaust of gasoline-powered engines) and/or the open ended

bales. However, he found that a lot of forklift activity and high ethylene concentration did not correspond. He felt that a possible explanation for this may be that the majority of ethylene is from the seedlings and that ethylene production may be a function of seedling dormancy and therefore vary with time of lifting.

Control of Ethylene Levels During Storage

Ethylene can be removed from the atmosphere of storage facilities with the use of brominated activated charcoal (BAC). There are several problems associated with BAC, such as the release of corrosive substances (HBr and Br_2) and it is hygroscopic and therefore gets wet in humid conditions (Abeles 1973). A more effective ethylene absorbent is permanganate-alumina (Purafil). Permanganate scrubbers are non-corrosive and are effective against other air pollutants as well (Abeles 1973).

The concept of a controlled atmosphere (CA) to limit ethylene action was developed in 1933 by Kidd and West (Moore 1973). They determined that since oxygen is necessary for ethylene action and carbon dioxide inhibits ethylene action they would store fruit in an atmosphere rich in carbon dioxide (5 to 10%), low in oxygen (1 to 3%) and with as little ethylene as possible (ethylene scrubbers)

(Moore 1973). Most of the ethylene around the fruit is removed; however, it is difficult to remove the ethylene within the fruit that is involved in the ripening process. Hypobaric ventilation can be used along with low oxygen, high carbon dioxide and ethylene scrubbers to make the system even more efficient at delaying ripening and lengthening storage life.

MATERIALS AND METHODS

Study 1

In order to address objectives one and two twelve hundred 1-0 loblolly pine seedlings were hand-lifted monthly beginning in November 1982 and continuing through March 1983 from the Virginia Division of Forestry's New Kent Nursery near New Providence, Virginia. Two hundred intact seedlings were sealed into a Kraft-Polyethylene (K-P) bag, while 400 seedlings were dissected at the root collar, and the roots and shoots sealed into separate K-P bags. A duplicate sample was established with the remaining 600 seedlings.

The K-P bags were stored in a cold storage room at 5°C. Two gas samples from within each K-P bag were taken weekly during the first month of cold storage, monthly thereafter for two additional months. Samples were taken in 13 ml VACUTAINER brand tubes containing a 10 ml vacuum (22.4 in. Hg). The stoppers supplied with the tubes were found to leak ethylene and ethane at high rates and were replaced with alternate sleeve stoppers. The tubes were flushed with nitrogen and a new vacuum was pulled. All samples were analyzed on a Bendix 2500 gas chromatograph (see Appendix B).

After sampling was completed xylem water potential of a sample of seedlings from each of the bags containing whole seedlings and shoots was determined using Scholander's Pressure Chamber technique (Scholander, et al. 1965). Contents of each K-P bag were then oven-dried for weight determination. Ethylene concentrations were expressed on a dry-weight basis.

Wound ethylene, produced by cutting the seedlings in half, was accounted for using the following equation, which is in terms of dry-weights: wound ethylene = (root concentration + shoot concentration) - intact concentration. The concentration of wound ethylene was then partitioned, according to the proportion of ethylene produced by the roots and shoots, and subtracted from the concentrations of ethylene produced by the roots and shoots respectively. "Hormonal ethylene" was equated to the original shoot or root concentrations minus the respective wound ethylene. This "hormonal ethylene" could also include wound ethylene from other sources besides cutting the seedlings in half. None the less, this value proved useful in comparing ethylene production between roots, shoots and whole seedlings and in providing information on the origin of hormonal ethylene in pine seedlings.

Statistical Analysis

Analysis of variance techniques were used to determine if there were significant differences between ethylene production rates of the three plant parts over time in cold storage and over lifting dates (see Appendix C). Peak ethylene, date of peak ethylene production, total ethylene produced (sum of values recorded over cold storage), and date and amount of the smallest level of ethylene produced were the response variables used in the analysis of variance. Effect of lifting date alone, without the influence of cold storage, was determined through analysis of ethylene production after the first week in cold storage. These variables were determined from response surfaces of ethylene production versus time in cold storage developed for each bag of each lifting date. As a result, the analysis of variance was based on two replications (one value for each of the two bags). Duncan's Multiple Range test was used to separate the means.

Study 2

This study also addressed the effect of lifting date and site of production of ethylene during cold storage. Seedlings collected with those in studies one and three were kept in cold storage and sampled weekly for the first month

of storage. Three needle fascicles and three lateral roots were removed from each of five seedlings and placed in one of two 40 ml vials. In addition, 1/2 ml of water was added to the vials to prevent desiccation. The vials were incubated under light (30 umol/m^2/sec) at 25°C. Gas samples (0.5 ml.) were taken after three and six hours, and analyzed by gas chromatography. The samples were replicated twice. Ethylene production was expressed on a dry-weight basis.

Xylem water potential of the seedlings was measured at the time of sample preparation in order to determine whether water stress was a factor in the observed levels of ethylene production.

Statistical Analysis

Rates of ethylene production over time were expressed as average production rates per hour after three and six of incubation by dividing the three-hour values by three and the six-hour values by six.

Analysis of variance techniques were used to determine if lifting date, part and week had significant effects on synthesis rates and if there were significant differences between rates at three and at six hours.

Study 3

For the comparison of hand versus machine lifting (objective three), 2400 seedlings were taken from the grading table in January and March 1983 and treated as in study one.

Statistical Analysis

Analysis of variance procedures were used to determine if lifting method had a significant effect on ethylene production by the different plant parts over time in cold storage (see Appendix C). Response variables were determined from response surfaces, as described in Study 1. Duncan's Multiple Range test was used to separate the means.

Study 4

The dose-response relationship of ethylene and loblolly pine seedlings during cold storage was investigated through the introduction of known concentrations of ethylene into K-P bags containing 400 1-0, March machine-lifted, loblolly pine seedlings. To insure a more ethylene tight system, the bags were wrapped in polyvinyl sheeting and sealed within two polyethylene bags. The following treatments were administered to two sets of seedlings:

1. control, no treatment

2. ethylene absorbent (Purafil) added to bag
3. 500 ppb ethylene injected into K-P bag
4. 1000 ppb ethylene injected into K-P bag
5. 2000 ppb ethylene injected in K-P bag
6. 4000 ppb ethylene injected into K-P bag

Concentrations of ethylene within the fumigated bags were measured periodically (approximately every two weeks) and adjusted as needed. After a storage period of six weeks, a subsample of 192 seedlings per treatment were outplanted in a randomized block design at the Reynolds Homestead Research Center near Critz, Virginia.

Rate of bud break as well as first year survival and height growth was determined by classifying the level of bud elongation on a scale of 0 to 3, where 0 equaled no swelling and 3 indicated a totally elongated bud. Measurements were taken on three occassions through late spring (May 13, May 27, and June 11,1983). Height, to the nearest 0.5 cm, and survival were measured on December 17,1983.

In addition to the field outplanting, a subsample of 16 seedlings per treatment were potted in nursery soil and grown in a greenhouse. Seedlings were fertilized approximately every two weeks with 20-20-20 Peters General Purpose fertilizer (Fogesville, Pa.) and approximately once a month with Peters Soluble Trace Element Mix. Time to bud

break,height, root collar diameter, as well as root and shoot dry weights were measured. Time to bud break was determined in the manner discussed above, with the exception that measurements were taken every two to three days. Heights and diameters were measured just prior to harvest.

A subsample of 30 seedlings per treatment were also studied for root growth capacity (RGC). A variation of the technique introduced by Stone (1955) was used to measure the RGC and is described below. Seedling root systems were pruned to 12 cm below the root collar and the seedlings from each treatment were planted in two replicate 46 x 10 x 41 cm^3 (length x width x height) trays containing Pro-Mix BX Premier growth medium. Seedlings were grown in a greenhouse under ambient air temperatures, soil temperature of 27° C and a 16-hour photoperiod. After 24 days the seedlings were carefully removed from the trays and washed to expose the new white roots. Total number of new roots in two categories (between 0.5 and 1.5 cm and greater than 1.5 cm) and cumulative length of the new roots were measured, then root and shoot dry weights were determined.

Statistical analysis

Regression analysis of the field and greenhouse data, based on both the average ethylene concentrations within the K-P bags two weeks after each fumigation and on the actual ethylene treatment levels were performed to determine if there were significant relationships between the plant responses and the two ethylene levels, and if so, which relationship was stronger.

Analysis of variance techniques were also used. Actual levels of ethylene within the bags were difficult to control due to differential rates of leaking; therefore, for the analysis of variance it was assumed that the ethylene fumigations were doses to which the seedlings were exposed on several occasions, and not continuous levels of exposure.

To determine if there were significant differences in the root growth potentials of seedlings exposed to different levels of ethylene, the variables number of new roots less than 1.5 cm, number of new roots greater than 1.5 cm, total length of new root tissue and average length of new roots were analyzed with analysis of variance and regression techniques. Duncan's Multiple Range test was used to separate the means.

Study 5

Seedlings from two half-sib loblolly pine families were used to investigate the effect of water stress on production of ethylene by loblolly pine seedlings. Seed from an east Texas drought-hardy family (female parent clone CR1-8) and from a Virginia Coastal Plain family (female parent clone R-523) were sown in nursery soil amended with peat moss and grown in Ray Leach tubes in a greenhouse. Seedlings were fertilized approximately every two weeks with 20-20-20 Peters General Purpose ferlizer (Fogelsville, Pa.) and approximately once a month with Peters Soluble Trace Element Mix. Five months after germination, seedlings were transplanted to larger pots (11 cm. diameter) containing nursery soil. The seedlings were then moved to a growth chamber room with a 16-hour photoperiod, day temperature of 25° C, night temperature of 22° C, and relative humidity of 50 percent. Light was supplied from high output fluorescent lamps from which the photosynthetic photon flux density (ppfd) equaled 100 umol/m^2/sec. Treatment was initiated one month after moving the seedlings to the growth chamber.

A uniform population of 90 seedlings per family was selected for treatment. Seventy-five seedlings from each family were subjected to water stress while the remainder were a watered control. Water was withheld from the

seedlings for fifteen days, after which they were rewatered. Five seedlings from each family were destructively sampled every three days (for eighteen days), with control seedlings also sampled on days zero, nine and eighteen. Data collected from the sample seedlings were: xylem water potential, stomatal conductance, ethylene production and concentration of 1-Aminocyclopropane-1-carboxylic acid (ACC) in the root and shoot tissue.

Xylem water potential was measured on one needle fascicle per sample seedling with Scholander's Pressure Chamber technique (Scholander et al. 1965).

Stomatal conductance was measured with a steady state porometer (Interface Instruments, Corvalis, Oregon), on one sample of needles per seedling. Cuvette humidity was maintained at the ambient growth chamber humidity of approximately 50 percent.

Seedlings were carefully removed from their pots and washed. Ethylene production was then measured according to the incubation method described in study two. Temperatures remained at 25°C, while the light intensity was 18 umol/ m^2/ sec. All gas samples were analyzed on a Varian Model 3700 gas chromatograph (see Appendix A).

1-Aminocyclopropane-1-carboxylic acid Extraction

The remaining roots and shoots of the five seedlings were then frozen in liquid nitrogen and homogenized with a mortar and pestle. The ground tissue was freeze-dried then finely ground with a Wiley mill. Two 0.5 gram samples of both the roots and shoots were placed in test tubes for extraction using a variation of the technique described by McKeon et al. (1982). Eight ml of 80% ethanol (10:1 v/v, solution:tissue) was added to each of the test tubes which were then capped with a marble and placed in a 75°C water bath. After one hour 4 ml of liquid was pipetted off each test tube and dried under a stream of nitrogen in a 60°C bath. The tissue samples were re-extracted with another eight ml of ethanol under the same conditions, for one hour. An additional four ml of liquid were pipetted off and added to the now dry sample, and dried in the same manner. When the samples had dried to a crust-like material, 2 ml of chloroform and 2 ml of water were added to each sample and vortexed until the crust had dissolved. The mixture was then centrifuged for 15-20 minuted at 10,000 g., after which a 0.5 ml aliquot of the acqueous supernatant was placed in a test tube and diluted with 4.5 ml of water.

The amount of ACC in the samples was measured through conversion to ethylene using a variation of the technique

developed by Lizada and Yang (1979). An internal standard was used to determine the efficiency of conversion.

One-half ml of the diluted solution was placed in each of two test tubes, one of which served as the standard. Three tenths of a ml of water and 0.1 ml of $HgCl_2$ were added to the sample test tube, while 0.1 ml of water and 0.2 ml of 5 micromolar ACC and an equal amount of $HgCl_2$ were added to the standard. The tubes were then capped and a mixture of saturated NaOH (2:1,v/v) plus 5% NaOCl was added, with a syringe, to each. The test tubes were shaken for a few moments, then were placed in a 30°C water bath. Six hours later, 0.5 ml gas samples were taken and analyzed by gas chromatography.

Ethylene concentrations were converted to values of ACC (nmol/ g dry wt) through the use of formulas presented in Appendix D.

Statistical analysis

Analysis of variance techniques were used to determine if treatment, family, part and day had significant effects on ethylene and ACC production rates. Rates of production were also looked at in terms of stomatal conductance and xylem water potential.

Rates of ethylene production were expressed as an average production rate per hour by dividing the value of ethylene after three hours by three. Rates of production measured for control seedlings were averaged to give a mean value for day zero.

Regression techniques were used, when appropriate to show the change in ethylene and ACC production with changes in both stomatal conductance and xylem water potential.

RESULTS

In studies one and three, original ethylene measurements from within the bags were analyzed. In addition, the original values were divided into "wound" and "hormonal" ethylene as described in the Materials and Methods. Wound ethylene will refer to the ethylene produced as a result of cutting the seedlings in half. Hormonal ethylene will refer to the remaining ethylene production, and may include ethylene stimulated from wounding aside from the cutting. When used alone, ethylene will refer to the original measurements.

Study 1

The maximum ethylene concentration measured for each group of seedlings was significantly affected by the month that the seedlings were lifted from the nursery. Seedlings lifted in February produced the highest concentrations of ethylene (0.434 ppb/g dry wt), followed by January seedlings (0.187 ppb/g dry wt) (Table 1). A significant lifting date-part interaction was primarily due to the February-lifted seedlings. February was the only month in which a

significant difference was observed between the peak ethylene levels in bags of roots, shoots and whole seedlings (Table 1). Bags of roots contained the highest peak concentrations of ethylene, followed by bags of whole seedlings, then shoots. All months tended to follow this same general trend (Table 1). Replications were not significant for any of the lifting dates.

The peak ethylene concentration produced by February seedlings occurred after just one week in cold storage, while the peaks of the other lift dates occurred, on average, near the third week in cold storage (data not shown). The different seedling parts did not show a significant difference in the date of their peak productions.

Cumulative ethylene measured for each group of seedlings over the twelve weeks in storage was also significantly affected by the lift date. The bags of February seedlings generally produced the greatest amount of ethylene (0.712 ppb/g dry wt) while December and March seedlings produced the least (0.516 and 0.366 ppb/g dry wt respectively). There was, however, a significant lift date-part interaction which was especially evident where the January concentrations of whole seedlings were greater than those of February (Table 2). The interaction also resulted from the

Table 1. Maximum concentration of ethylene (hormonal and wounnd) measured in K-P bags of hand-lifted roots, shoots and whole seedlings by lift dates. Values are expressed on a dry weight basis.

ldate	roots	shoots	whole	x
	ppb/g			
Nov	0.12 FE[1]	0.09 F	0.12 DE	0.11
Dec	0.15 DE	0.11 FE	0.13 DEF	0.13
Jan	0.19 CDE	0.14 DEF	0.22 CD	0.19
Feb	0.60 A	0.28 C	0.43 B	0.43
Mar	0.13 DEF	0.09 F	0.09 F	0.10
x	0.24	0.19	0.14	

1- Values within the table with the same letter are not significantly different (Alpha=0.05).

fact that roots, shoots and whole seedlings within January and February lift dates produced significantly different amounts of ethylene; while amounts of ethylene produced by shoots and whole seedlings lifted in December and March did not differ significantly. The cumulative amounts of ethylene produced by the roots were higher than totals for shoots and whole seedlings for every lift date except January (Table 2).

Lifting date and plant part also had significant effects on the date and concentration of the minimum quantity measured for each group of seedlings over time in cold storage. The minimum quantity produced by shoots and whole seedlings were significantly lower than those produced by roots, and they tended to occur after a longer period of cold storage (Table 3). The lowest concentration was recorded for March seedlings near the end of the twelve week storage period. Minimum quantities for December, January and February seedlings were not significantly different.

Concentrations of ethylene within the bags after one week of cold storage were used to compare the effect of lifting date, without the influence of prolonged cold storage. Concentrations of ethylene within bags of seedlings lifted in December, January and March ranged from a mean of 0.097 ppb/g dry wt for December seedlings to 0.079 ppb/g dry wt

Table 2. Cumulative ethylene measured in K-P bags of seedlings from different lift dates over 12 weeks of cold storage. Values are expressed on a dry weight basis.

ldate	roots	shoots	whole	x
	ppb/g			
Nov	--	--	--	--
Dec	0.66 D [1]	0.44 G	0.45 G	0.52
Jan	0.79 C	0.51 F	0.84 B	0.71
Feb	1.20 A	0.58 E	0.79 C	0.66
Mar	0.51 F	0.30 H	0.29 I	0.36
x	0.79	0.46	0.59	

1 - Values within the table with the same letter are not significantly different (Alpha=0.05).

Table 3. Minimum quantities of ethylene measured for hand-lifted seedlings and week of storage when minimums occurred. Values are expressed on a dry weight basis.

ldate	roots		shoots		whole	
	week	ppb/g	week	ppb/g	week	ppb/g
Nov	10 A[1]	0.69 a[2]	8 B	0.39 a	10 AB	0.35 ab
Dec	12 A	0.77 a	12 A	0.40 a	12 B	0.46 ab
Jan	3 A	0.69 a	8 B	0.34 a	8 B	0.56 a
Feb	4 A	0.78 a	3.5C	0.49 a	12 B	0.54 a
Mar	8 A	0.44 b	12 A	0.24 a	12 B	0.18 b

1- Values in a column with the same upper case letter are not significantly different (Alpha=0.05).

2- Values in a column with the same lower case letter are not significantly different (Alpha=0.05).

for January and March seedlings, but did not differ significantly. Ethylene concentrations within bags of February seedlings were significantly higher than the concentrations other months. Values for February seedlings after one week ranged from 0.275 ppb/g dry wt to 0.596 ppb/g dry wt for roots and shoots, respectively (Table 4).

Wound and Hormonal Ethylene

Levels of wound ethylene produced by the roots after cutting the seedlings in half tended to be higher than levels produced by the shoots (Figure 1). Levels produced by both tended to decrease over time in cold storage (Figure 2). Lifting date also had a significant effect on wound ethylene, with January and February seedlings (roots and shoots) producing the highest levels of wound ethylene while March seedlings produced the lowest (Tables 5 and 6). In addition, there were also significant week-lifting date interactions for root and shoot wound ethylene production which appeared to result from the widely fluctuating values observed for bags of January roots and shoots, and from the very high values recorded for both roots and shoots of February-lifted seedlings. In general, the levels of wound ethylene produced by the seedlings lifted in the earlier months tended to fluctuate during cold storage while

Table 4. Concentration of ethylene in K-P bags of hand-lifted seedlings after one week of cold storage (on a dry weight basis).

ldate	roots	shoots	whole	x
	ppb/g			
Nov	--	--	--	--
Dec	0.12 D[1]	0.07 D	0.10 D	0.10
Jan	0.08 D	0.08 D	0.07 D	0.08
Feb	0.60 A	0.28 C	0.43 B	0.43
Mar	0.08 D	0.08 D	0.08 D	0.08
x	0.22	0.13	0.17	

1- Values within the table with the same letter are not significantly different (Alpha=0.05).

concentrations of seedlings lifted in the later months tended to decline over cold storage. Bags (replicates) and interactions of bags with lifting date and weeks in cold storage also had significant effects on the levels of wound ethylene produced by the roots. These effects were not considered to be biologically significant and were not analyzed; however, the appropriate degrees of freedom were removed from the error term. Bags and interactions did not have significant effects on shoot wound ethylene, therefore the appropriate degrees of freedom were added to the error term.

Analysis of variance of the hormonal ethylene data for the roots indicated that all two-way interactions between lifting date, weeks in cold storage and bags, as well as the three-way interaction had significant effects on root hormonal ethylene. Only the week-lifting date interaction was analyzed; however, the degrees of freedom from the other interactions were removed from the error term. The week-lifting date interaction may be explained by the lack of significant changes over weeks in cold storage for November and December seedlings while the seedlings of later months showed significant fluctuations. It was also influenced by the disproportionately high values observed for February roots after the first week in cold storage (Table 7). Over

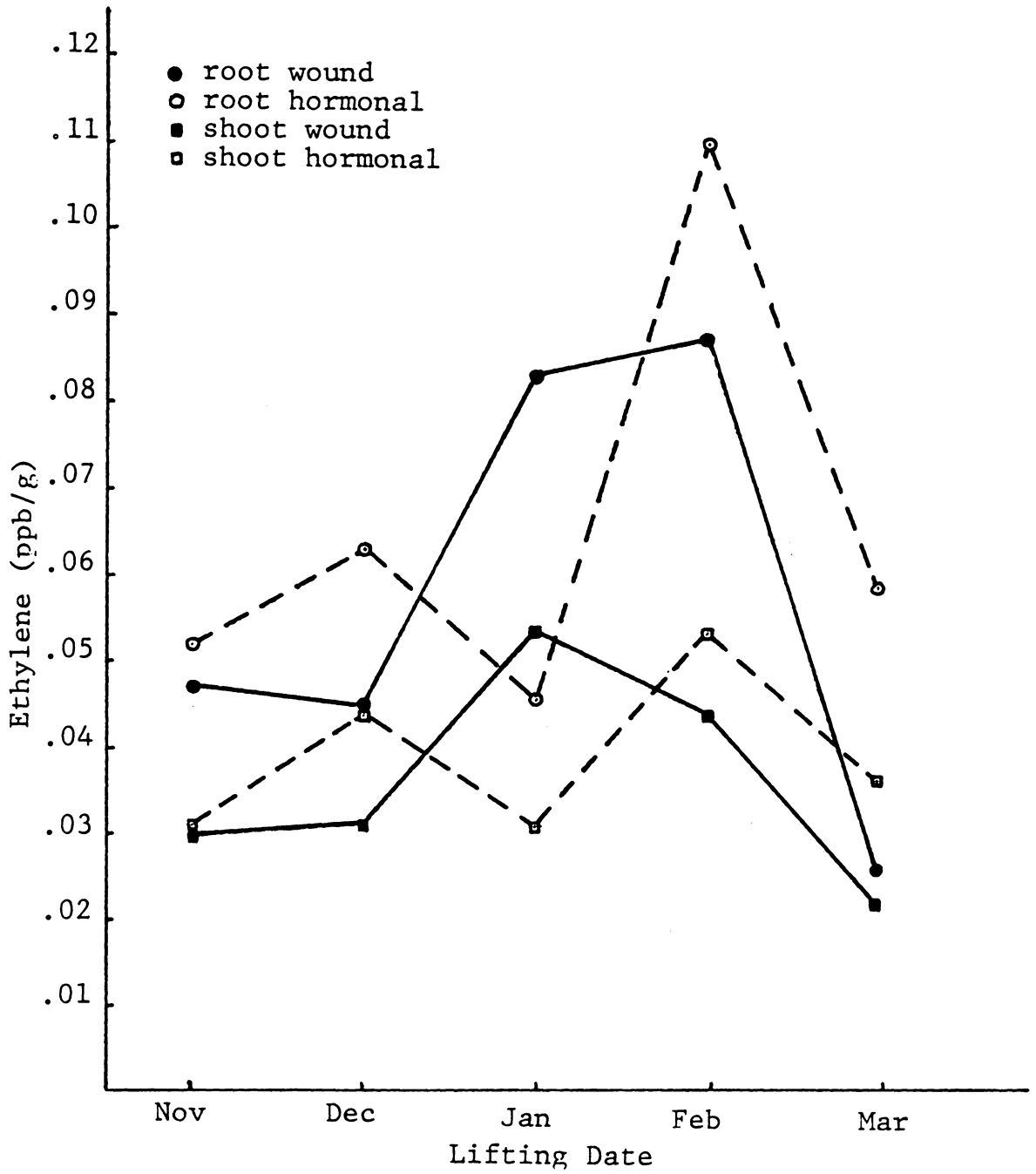

Figure 1. Wound and hormonal ethylene produced by roots and shoots of seedlings lifted on different dates.

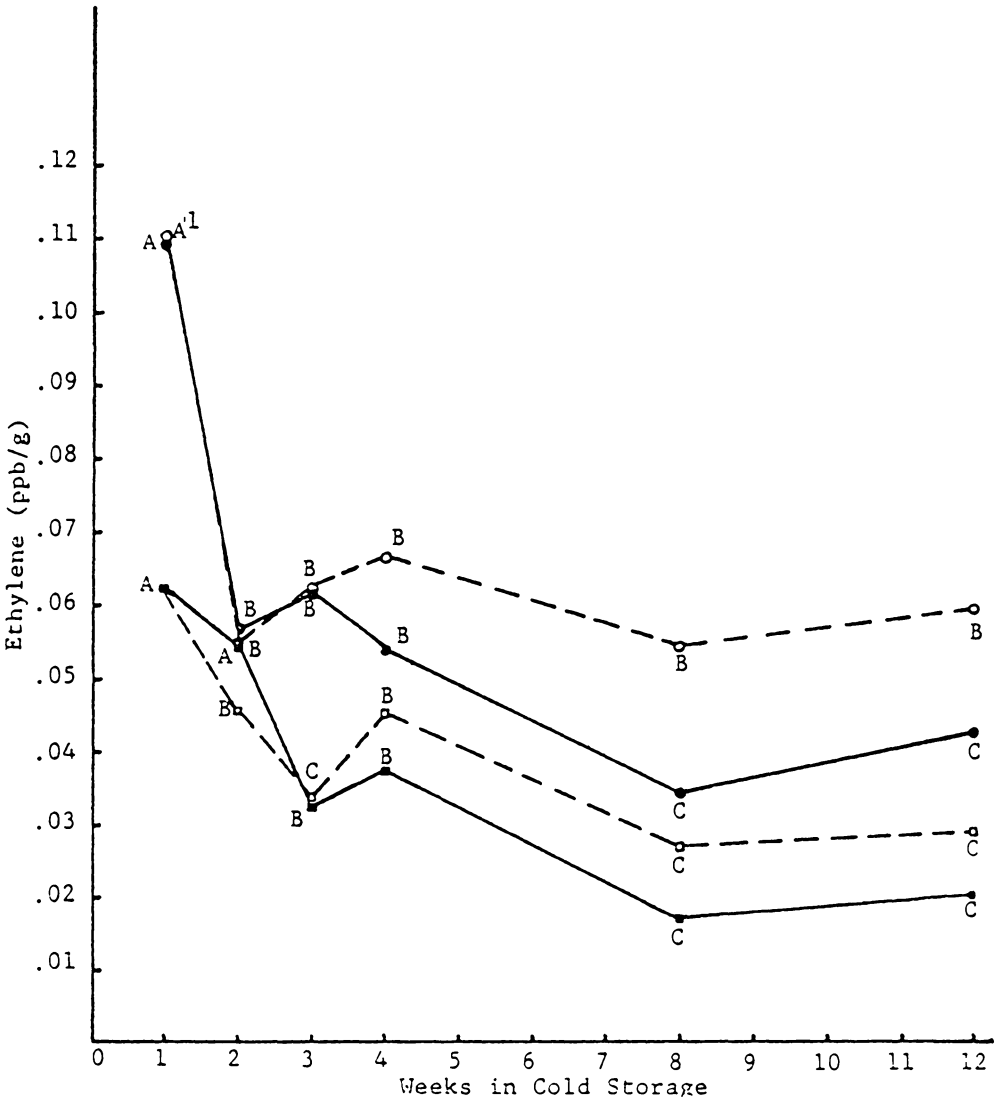

Figure 2. Concentrations of wound (—) and hormonal (- -) ethylene (dry weight basis) produced by roots (o) and shoots (□) over weeks in cold storage.

1- Concentrations within a treatment with the same letter are not significantly different.

Table 5. Root wound ethylene of hand-lifted seedlings from different lift dates over weels in cold storage (dry weight basis).

Weeks	Nov	Dec	Jan	Feb	Mar	$\bar{x}$
	ppb/g					
1	--	0.07 A b	0.04 C c	0.29 A a	0.04 A c	0.11
2	--	0.04 BC b	0.09 B b	0.06 B b	0.04 A b	0.06
3	0.07 A[1] b[2]	0.03 BC c	0.14 A a	0.05 BC b	0.02 BC c	0.06
4	0.06 A b	0.06 A b	0.09 AB a	0.04 BC bc	0.03 B c	0.05
8	0.03 B ab	0.05 AB a	0.04 C ab	0.04 BC a	0.02 C b	0.03
12	0.03 B bc	0.03 C bc	0.11 AB a	0.04 C bc	0.01 C c	0.04
$\bar{x}$	0.05	0.04	0.08	0.09	0.03	

1- Values in a column with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values in a row with the same lower case letter are not significantly different (Alpha = 0.05).

Table 6. Shoot wound ethylene of hand-lifted seedlings from different lift dates over weeks in cold storage (dry weight basis).

Weeks	Nov	Dec	Jan	Feb	Mar	$\bar{x}$
			ppb/g			
1	--	0.04 AB b	0.03 BC b	0.14 A a	0.04 A b	0.06
2	--	0.03 ABC b	0.11 A a	0.03 B b	0.04 A b	0.05
3	0.05 A[1] a[2]	0.03 ABC bc	0.05 B a	0.02 B bc	0.01 B c	0.03
4	0.04 A ab	0.05 A ab	0.06 B a	0.03 B bc	0.02 B c	0.04
8	0.01 B a	0.02 BC a	0.02 C a	0.02 B a	0.02 B a	0.02
12	0.01 B b	0.02 C b	0.05 BC a	0.02 B b	0.01 B b	0.02
$\bar{x}$	0.03	0.03	0.05	0.04	0.02	

1- Values in a column with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values in a row with the same lower case letter are not significantly different (Alpha = 0.05).

lifting dates, February roots had a significantly higher mean level of hormonal ethylene (0.111 ppb/g) than any of the other months which showed no significant differences.

The interactions of lifing date, weeks in cold storage and bags also had significant effects on hormonal ethylene produced by the shoots. The week-lifting date interaction appeared to result from the very high value for the first week for February seedlings and the general crisscrossing of all values during cold storage (Table 8). Without the high concentrations of February shoots in the first week, only a slight decrease in hormonal ethylene values would be seen between the beginning and end of cold storage. Lifting date alone also had a significant effect (Figure 1). February and December shoots generally produced higher mean concentrations followed by March, November and January shoots (Table 8).

A plot of the mean values of wound and hormonal ethylene produced by the roots and shoots over lifting dates indicated very similar trends between the roots and the shoots (Figure 1). Similar trends were also seen between hormonal and wound ethylene production by roots and shoots over weeks in cold storage (Figure 2).

Xylem water potential of the whole seedlings at the end of the storage period ranged from mean values of -0.45 MPa

Table 7. Root hormonal ethylene levels of hand-lifted seedlings from different lift dates over weeks in cold storage (dry weight basis).

Weeks	Nov	Dec	Jan	Feb	Mar	$\bar{x}$
			ppb/g			
1	--	0.05 A b	0.04 B b	0.30 A a	0.04 B b	0.11
2	--	0.06 A a	0.02 B b	0.09 B a	0.05 B a	0.05
3	0.04 A[1] bc[2]	0.06 A bc	0.02 B c	0.08 B ab	0.11 A a	0.06
4	0.04 A bc	0.08 A ab	0.10 A a	0.03 B c	0.09 A ab	0.07
8	0.06 A abc	0.08 A ab	0.04 B bc	0.07 B abc	0.03 B c	0.05
12	0.06 A bc	0.05 A c	0.07 AB abc	0.08 B abc	0.04 B c	0.06
$\bar{x}$	0.05	0.06	0.04	0.08	0.06	

1- Values in a column with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values in a row with the same lower case letter are not significantly different (Alpha = 0.05).

Table 8. Shoot hormonal ethylene levels of hand-lifted seedlings from different lift dates over weeks in cold storage (dry weight basis).

Weeks	Nov	Dec	Jan	Feb	Mar	$\bar{x}$
	ppb/g					
1	--	0.03 C b	0.04 AB b	0.13 A a	0.04 A b	0.06
2	--[1] [2]	0.06 A a	0.03 AB b	0.05 B ab	0.04 A ab	0.05
3	0.04 A b	0.05 AB ab	0.01 B c	0.04 BC b	0.04 A ab	0.03
4	0.04 A BC	0.06 A A	0.06 A AB	0.02 C C	0.05 A ABC	0.04
8	0.03 A a	0.04 BC a	0.02 AB a	0.04 BC a	0.02 B a	0.03
12	0.03 A a	0.02 C a	0.03 AB a	0.04 BC a	0.02 B a	0.03
$\bar{x}$	0.03	0.04	0.03	0.05	0.04	

1- Values in a column with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values in a row with the same lower case letter are not significantly different (Alpha = 0.05).

to -0.83 MPa. The shoots tended to be slightly drier, with mean values ranging from -0.58 MPa to -1.18 MPa. March seedlings (hand-lifted and machine-lifted) were generally drier than seedlings lifted in earlier months.

Study 2

After three hours of incubation, needles from seedlings hand-lifted (HL) in December, February and March, on average, were producing significantly higher levels of ethylene (5.97 ul/g dry wt/hr) than hand-lifted roots (3.59 ul/g dry wt/hr). Appendix F shows production rates after six hours of incubation. Roots and needles from seedlings lifted by machine (ML) in January and March did not produce significantly different amounts of ethylene during incubation.

Marginal effects of weeks in cold storage on ethylene production by needles of HL seedlings were not significant. However, there was a significant week-month interaction which masked cold storage effects (Table 9). For instance, ethylene production by needles from December-lifted seedlings did not change significantly over the four-week storage period; while production by needles from February-lifted seedlings fluctuated and production by needles of March-lifted seedlings increased significantly over the cold

storage period (Table 9). Roots of February and March-lifted seedlings generally produced ethylene at lower rates than the needles, except during the first week of cold storage (Table 9). Needles and roots of December-lifted seedlings tended to produce ethylene at similar rates. Production rates of the roots fluctuated in December and tended to decline over cold storage in February and March.

Significant differences between synthesis rates of January and March ML seedlings over cold storage only occurred after the first and fourth weeks of storage when March rates were significantly higher than January rates.

On average, needles tended to produce ethylene at higher rates than roots, and February and March needles generally produced ethylene at the highest rates. On average, there were no significant changes in production rates over cold storage; however, individual months did show changes in production rates over storage (Table 9).

A comparison of synthesis rates of March ML and HL seedlings after three hours indicated that ML seedlings tended to synthesize ethylene at higher rates (4.032 nl/g dry wt/hr) than HL seedlings (2.421 nl/g dry wt/hr).

Table 9. Average hourly ethylene production rates of roots and needles from handlifted seedlings lifted on different dates, over weeks in storage (3 hours of incubation; dry weight basis).

WEEKS	DEC		JAN		MAR			
	SHOOTS	ROOTS	SHOOTS	ROOTS	SHOOTS	ROOTS	SHOOTS	ROOTS
	UL/G DRY WT/HR							
1	4.84	3.76	6.31	8.42	2.42	3.19	4.52 BC[2]	5.12 BC
2	1.64	1.45	8.79	5.33	5.68	3.22	5.37 B	3.34 CD
3	3.41	3.04	12.60	3.66	7.18	3.32	7.75 A	3.34 CD
4	2.88	3.58	3.44	2.08	12.48	2.01	6.26 AB	2.55 D
$\bar{X}$	3.20C[1]	2.96C	7.78A	4.88B	6.94A	2.93C		

1- Week means with the same letter are not significantly different (Alpha = 0.05).
2- Month means with the same letter are not significantly different (Alpha = 0.05).

Study 3

Results from the third study indicated that machine-lifted seedlings tended to produce more ethylene than hand-lifted (HL) seedlings. The maximum concentrations of ethylene measured for ML seedlings ranged from 0.178 ppb/g to 0.448 ppb/g for roots and shoots respectively (Table 10). Only the concentrations produced by the ML roots were significantly higher than any of the HL values. Cumulative ethylene produced by the samples of ML roots were also significantly higher than the totals determined for HL seedlings (Table 11). The levels of ethylene measured for the roots of ML seedlings during the first week of storage were also higher (Table 12). Hormonal and wound ethylene levels also tended to be higher in the ML seedlings (Tables 13 and 14).

Within ML seedlings, the roots produced significantly higher maxima and totals over storage than the whole seedlings or shoots. In fact, the peak ethylene values recorded for the shoots and whole ML seedlings did not differ significantly from the values measured in the HL shoots and whole seedlings (Table 10). Maximum levels of ethylene measured for the different plant parts of January ML seedlings tended to occur after two to three weeks of cold storage; however, maximum levels for March ML seedlings

Table 10. Maximum ethylene concentrations in bags of January or March hand-lifted and machine-lifted seedlings (dry weight basis).

lift method	roots	shoots	whole	x
	ppb/g			
hand-lift	0.16 B[1]	0.12 B	0.16 B	0.14
machine-lift	0.45 A	0.18 B	0.21 B	0.28
x	0.30	0.15	0.18	

1- Values with the same letter are not significantly different (Alpha=0.05).

Table 11. Cumulative ethylene measured for hand-lifted and machine-lifted seedlings over weeks in cold storage (dry weight basis).

lift method	roots	shoots	whole	x
	ppb/g			
hand-lift	0.65 B[1]	0.41 B	0.56 B	0.54
machine-lift	1.11 A	0.51 B	0.60 B	0.74
x	0.88	0.46	0.58	

1- Values within the table with the same letter are not significantly different (Alpha=0.05).

Table 12. Ethylene concentrations in bags of January and March hand-lifted and machine-lifted seedlings after one week in cold storage (dry weight basis).

	lift method		
ldate	hand-lift	machine-lift	x
	ppb/g		
Jan	0.08 C[1]	0.11 B	0.10
Mar	0.08 C	0.29 A	0.18
x	0.08	0.20	

1- Values within the table with the same letter are not significantly different (Alpha=0.05).

Table 13. Wound ethylene of roots and shoots of January March seedlings (dry weight basis).

lift date	hand-lift roots	hand-lift shoots	machine-lift roots	machine-lift shoots
	ppb/g			
Jan	0.08 A[1]	0.05 a[2]	0.07 B	0.03 b
Mar	0.04 C	0.02 b	0.07 B	0.03 b

1- Values with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values with the same lower case letter are not significantly different (Alpha = 0.05).

Table 14. Hormonal ethylene of roots and shoots of January and March hand-lifted and machine-lifted seedlings (dry weight basis).

lift date	hand-lift roots	hand-lift shoots	machine-lift roots	machine-lift shoots
	ppb/g			
Jan	0.05 B[1]	0.03 b[2]	0.02 A	0.06 a
Mar	0.06 B	0.04 b	0.07 B	0.03 b

1- Values with the same upper case letter are not significantly different (Alpha = 0.05).
2- Values with the same lower case letter are not significantly different (Alpha = 0.05).

occurred after the first week in cold storage (data not shown). The seedlings lifted by hand and by machine in January tended to produce higher maximum concentrations of ethylene than March HL and ML seedlings.

Seedlings lifted by machine in March produced significantly higher levels of ethylene during the first week of storage than January ML seedlings (Table 12) First week values for HL seedlings over these two months did not change and there were no significant differences in concentrations produced by the different seedling parts.

During cold storage the minimum values were recorded for shoots and whole seedlings of HL seedlings. Lows recorded for ML and HL seedlings were significantly lower in March than in January. The lows for both HL and ML seedlings tended to be recorded after the second or third month in cold storage (data not shown).

Study 4

Concentrations of ethylene within the fumigated bags were measured periodically (approximately every two weeks) and adjusted as needed. The K-P bags were not totally ethylene-tight and lost ethylene at different rates. Concentrations in many of the bags dropped to below 500 ppb two weeks after fumigation. Therefore, the given treatment levels were not

continuously maintained and exposures may have begun to overlap. However, the seedlings were exposed to the given treatment concentrations on several occassions during the six week storage period. What varied then, was the length of time that the seedlings were exposed to a given treatment.

Mean concentrations of ethylene in the bags after fumigation (based on values two weeks after the first two fumigations and five days after the last) are presented in Table 15.

Regression analysis of the data based on both the mean concentrations and on the given treatment levels were performed. In general, stronger relationships were indicated by the regression analysis based on the given treatment values. However, the coefficients of determination were quite low in all cases, as were the slope parameters. Therefore, analysis of variance techniques using the given ethylene treatment concentrations as dose treatments were also used to analyze the data.

Bud Activity

Three weeks after outplanting (May 13), there was very little difference among levels of bud activity in seedlings exposed to different concentrations of ethylene. Levels of

Table 15. Ethylene concentrations within K-P bags the day before outplanting, five days after refumigation.

Rep	Treatment					
	Control	Purafil	500ppb	1000ppb	2000ppb	4000ppb
	ppb					
Bag 1	596.8	265.6	586.8	458.6	1107.0	578.3
Bag 2	512.9	161.8	613.1	416.3	535.6	1122.4

bud activity ranged from a mean low of 0.65 for seedlings stored with Purafil to a mean high of 0.89 for seedlings exposed to 4000 ppb ethylene (Table 16). The following significant linear models were fit with the analysis using the mean concentrations and using the given ethylene treatment levels respectively:

1. Bud Activity = 0.55 + 0.0003ppm(Mean Concentraiton)
2. Bud Activity = 0.57 + 0.0368ppm(Treatment)

The coefficients of determination were quite low at 0.02 and 0.08 respectively (see Appendix E for the scattergrams); however, the parameters were significant. There were no significant differences between any of the different treatments, except for the 4000 ppb (Bag 2) treatment which had significantly more bud activity (Table 16). In general, the control and Purafil treatments tended to have the least bud activity.

By May 27 there were still no significant differences among treatments within Bag 1. Bag 2 seedlings exposed to 4000 ppb had significnatly more bud activity (2.12) than any of the other seedlings except those exposed to 2000 ppb (Table 16). Control seedlings continued to show the least amount of bud activity, while Purafil seedlings had reached levels of activity similar to the other treatments. Regression analysis indicated that the relationship between

Table 16. Field bud activity measured periodically after outplanting. Bud activity is based on a scale of 0 to 3.

Bag	Cont.	Pur.	500	1000	2000	4000
MAY 13						
1	0.70 B[1]	0.71 B	0.78 B	0.72 B	0.58 B	0.62 B
2	0.61 B	0.58 B	0.57 B	0.62 B	0.77 B	1.17 A
MAY 27						
1	1.46 B	1.68 B	1.63 B	1.70 B	1.49 B	1.72 B
2	1.54 B	1.57 B	1.54 B	1.45 B	1.84 AB	2.12 AB
JUNE 11						
1	2.71 BC	2.82 BA	2.74 ABC	2.86 AB	2.68 BC	2.69 BC
2	2.56 C	2.67 BC	2.97 AB	2.73 ABC	2.87 AB	2.96 A

1- Values within a section with the same letter are not significantly different (Alpha=0.05).

the stage of bud break on May 27 and mean ethylene concentrations in the bags two weeks after fumigation was not significant. However, a significant linear model (Bud Activity=1.43 + 0.06(TRT)) was fit to the data using the given treatment concentrations. (See Appendix E for the scattergram).

By the final measurement date (June 11), most seedlings had a bud activity of near three and hence, there were no significant differences between any of the treatments except in Bag 2 where bud activity of the control seedlings was significantly lower (2.56) than activity in seedlings exposed to 4000 ppb, 1000 ppb and 500 ppb (Table 16). The seedlings stored with Purafil also maintained lower bud activity than seedlings stored under a concentration of 4000 ppb ethylene. As with levels of bud activity on May 27, levels on June 11 were not positively related to the mean levels of ethylene in the bags, but a significant linear model (Bud Activity=2.66 + 0.03(TRT)) was fit to the data using the given treatment concentrations.

Results from the parallel greenhouse study also indicated that the seedlings stored under 4000 ppb broke bud at a significantly faster rate (Figure 3). There were no significant differences among bud activities of any of the other treatments; however, control seedlings did tend to have the least amount of bud activity.

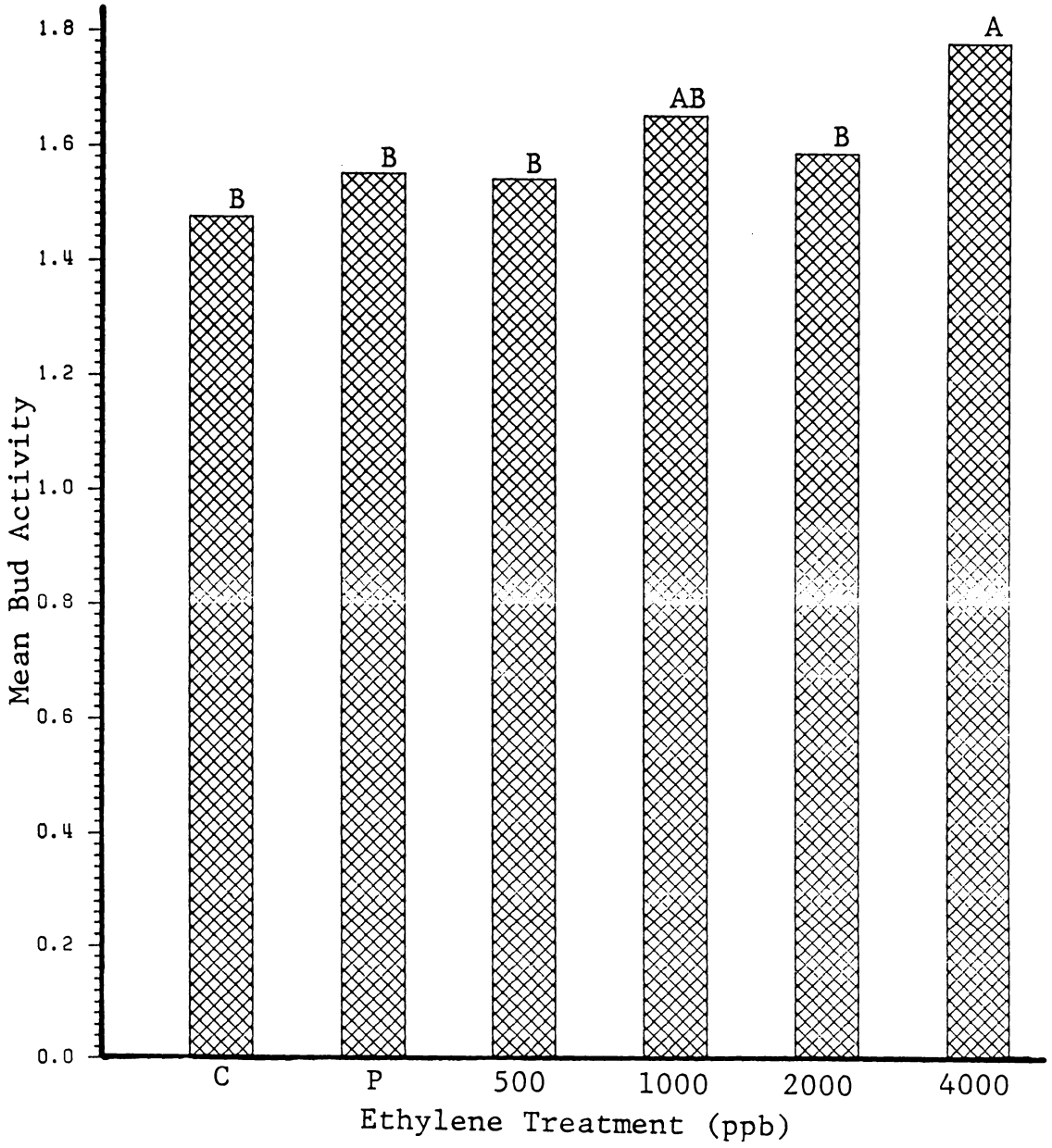

Figure 3. Mean bud activity of seedlings exposed to ethylene treatments during cold storage and grown in a greenhouse. Bars with the same letters are not significantly different (Alpha = 0.05).

Survival

Field survival for all treatments was very high, ranging from 94 percent to 99 percent. However, regression analysis indicated that there was not a significant relationship between mean ethylene concentrations after fumigation and survival or between ethylene treatment levels and survival. Analysis of variance also indicated that treatments, blocks and reps did not have significant effects on survival; however a mean separation with Duncan's Multiple Range test indicated that survival of seedlings exposed to 4000 ppb ethylene was significantly higher at 99 percent than survival after treatments of 500 ppb, 1000 ppb, Control and 2000 ppb at 95.8, 95.8, 95.8 and 94.3 percent respectively (Figure 4).

Seedling Height

In order to obtain a better representation the effect of treatments, blocks and reps on the height growth of seedlings exposed to different amounts of ethylene, the means of the 24 seedlings in each plot were used in the regressions and analysis of variance. The use of means removed the effect of the inherent variation among seedling growth. The analysis indicated that the relationships between mean ethylene concentrations and mean height and

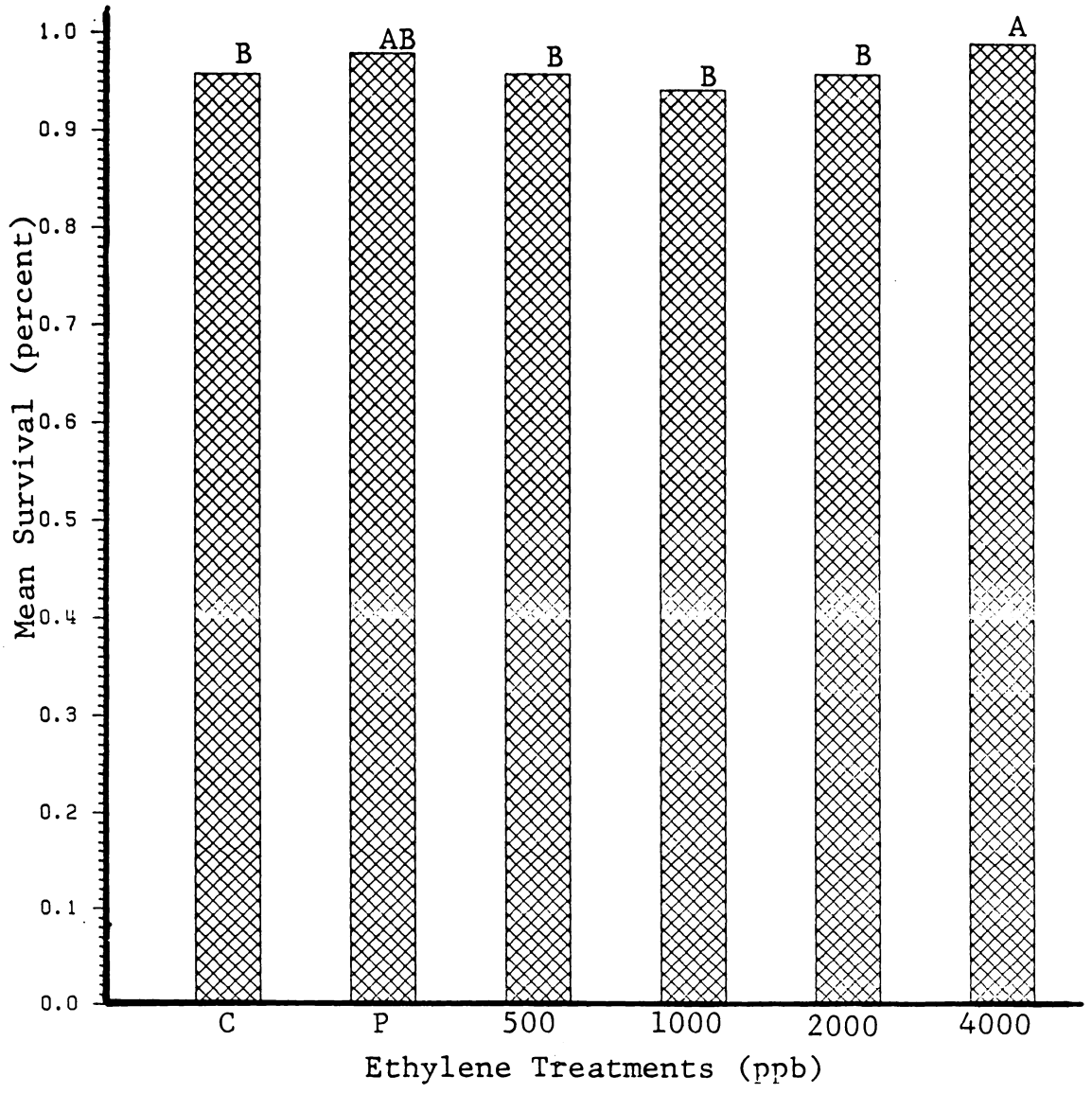

Figure 4. Survival after one year in the field, by ethylene treatment. Bars with the same letter are not significantly different (Alpha = 0.05).

between ethylene treatments and mean height were significant. The following linear models were fit for the mean concentration analysis and for the actual treatment analysis respectively:

1. Ht = 28.23 + 0.004ppm(Mean Concentration)
2. Ht = 30.98 + 1.210ppm(Treatment)

The coefficients of determination were quite low at 0.09 and 0.39 respectively (see Appendix E for the scattergrams); however, the parameters were significant. Analysis of variance indicted that blocks and reps were not significant but that treatment effects were significant. Seedlings exposed to 4000 ppb averaged 36.1 cm in height after one year in the field and were significantly taller than any of the other seedlings which ranged from 28.1 to 31.1 cm in height. No significant differences were apparent between these other treatments; however, control and Purafil seedlings tended to have less height growth than the others.

The data from the parallel greenhouse study was inconclusive due to a problem with severe needle chlorosis. There was no apparent correlation between the ethylene treatments and the chlorosis, which may have been "summer chlorosis". Summer chlorosis occurs sporadically in nursery-grown loblolly pine seedlings and may be due to a combination of high temperature and pockets of unincorporated organic matter (Mengel and Kirby 1978).

A sample of the outplanted seedlings were lifted at the end of the first growing season in order to gather height, root collar diameter and dry weight data. The seedlings treated with 4000 ppb ethylene were found to have significantly greater height (38.4 cm) than any of the other treatments except 2000 ppb. The control and Purafil seedlings, which averaged 32.3 cm and 31. 3 cm respectively, were significantly shorter than the seedlings exposed to 4000 and 2000 ppb (Figure 5).

Root collar diameters of the subsample of seedlings ranged from a low of 6.4 mm for seedlings exposed to 500 ppb ethylene to a high of 7.4 mm for seedlings stored under 4000 ppb (Figure 6). Regression analysis indicated that there was a significant relationship between mean ethylene concentrations and root collar diameters and between ethylene treatment levels and root collar diameters. Linear models fit for each relationship were quite similar:

1. Diameter = 6.17 + 0.001(Mean Concentration)
2. Diameter = 6.14 + 0.177(Treatment)

Although the coefficients of determination for each equation were quite low at 0.03 (see Appendix E for the scattergrams), the parameters in each equation were highly significant.

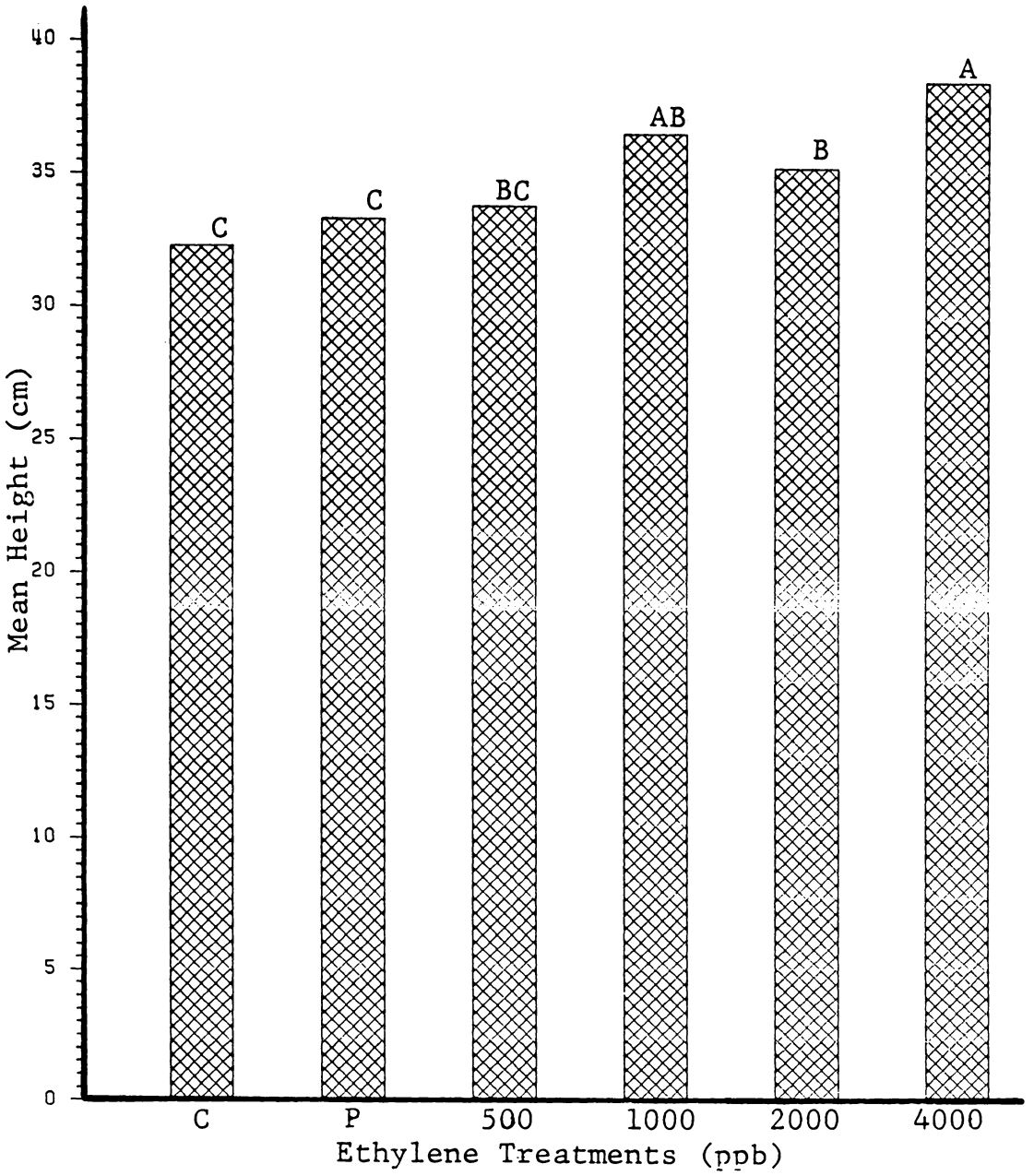

Figure 5. Mean seedling height after one year in the field, by treatment. Bars with the same letter are not significantly different (Alpha = 0.05).

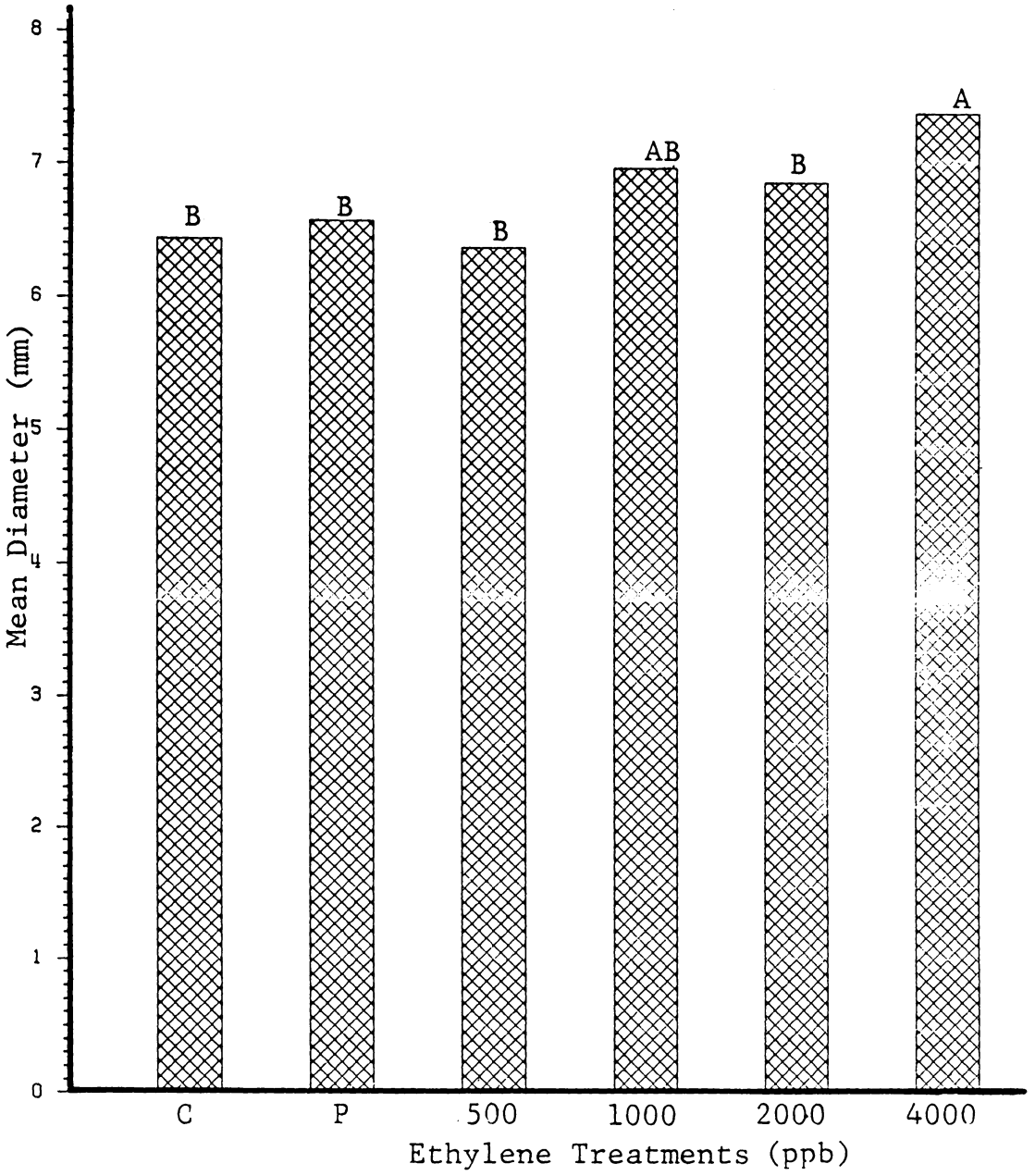

Figure 6. Mean root collar diameter of seedlings grown in the field for one year, by ethylene treatment. Bars with the same letter are not significantly different.

Root dry weights of the seedlings ranged from a mean of 2.75 grams for control seedlings to a mean of 4.42 grams for seedlings treated with 4000 ppb. The following significant linear models were fit to the root dry weight data using mean ethylene concentrations and the ethylene treatment levels:

1. Root dry wt = 2.42 + 0.002(Mean Concentration)
2. Root dry wt = 2.38 + 0.291(Treatment)

Although the coefficients of determination were quite low at 0.04 and 0.05 respectively (see Appendix E for the scattergrams), the parameters were highly significant. Analysis of variance indicated that the mean dry weight of the seedlings exposed to 4000 ppb was significantly higher than the dry weights of any of the other treatments (Table 17).

The mean shoot dry weight of the seedlings exposed to 4000 ppb were also significantly higher than the shoot dry weights of any of the other treatments. Shoot weights ranged from a mean of 8.30 grams for controls to a mean of 14.18 grams for seedlings exposed to 4000 ppb (Table 17). The following significant linear models were fit to the shoot dry weight data:

1. Shoot dry wt = 7.26 + 0.005(Mean Concentration)
2. Shoot dry wt = 6.83 + 1.00(Treatment)

Table 17. Seedling root and shoot dry weights after one year in the field, by ethylene treatments.

treatment	roots	shoots
	g	
Control	2.75 B[1]	8.30 B
Purafil	3.21 B	9.22 B
500 ppb	2.87 B	9.04 B
1000 ppb	3.57 B	10.68 B
2000 ppb	3.58 B	10.56 B
4000 ppb	4.42 A	14.18 A

1- Values in a column with the same letter are not significantly different (Alpha=0.05).

Although the coefficients of determination were quite low at 0.05 and 0.07 respectively (see Appendix E for the scattergrams), the parameters were highly significant.

As stated earlier, the data from the harvest of the parallel greenhouse study was inconclusive.

Root Growth Capacity

The root growth capacity of a subsample of the seedlings stored under different concentrations of ethylene were analyzed using the following response variables: total number of new roots, number of new roots less than 0.5 cm, cumulative length of new roots and average length of new roots.

Seedlings exposed to 500 ppb had the greatest number of new roots and the greatest number of small roots. Total number of new roots per seedling ranged from a low of 16.4 for 1000 ppb to 31.7 for the 500 ppb treatment (Table 18). The average number of small roots on seedlings exposed to 500 ppb was 18.8, which was significantly higher than the average number of small roots on seedlings of other treatments (Table 18). Seedlings exposed to 1000 ppb had the fewest number of small roots; however, this value was not significantly lower than any of the other treatments except 500 ppb. Regression analysis indicated that there

was not a significant relationship between mean ethylene concentrations and number of roots or between given ethylene concentrations and number of roots.

Cumulative lengths ranged from 31.6 cm to 63.7 cm for 2000 ppb and 4000 ppb respectively; however, significant relationships between mean ethylene concentrations and total length and between given treatment levels and total length were not indicated by regression analysis. Total lengths of new roots on seedlings exposed to 1000 ppb and 2000 ppb were significantly lower than on seedlings exposed to 500 ppb and 4000 ppb (Table 18).

Average length of new roots ranged from 1.65 cm/new root for seedlings stored with Purafil to 2.4 cm/new root for seedlings exposed to 4000 ppb (Table 18). Average lengths of new roots on controls and on seedlings treated with 1000 ppb were not significantly different than lengths of roots on seedlings exposed to 4000 ppb, while 500 ppb and 2000 ppb treatments were not significantly different than the Purafil treatment (Table 18). The seedlings with the lower average new root length (500 ppb, 2000 ppb and Purafil) had a higher ratio of small roots to large roots.

Table 18. Root growth capacity of seedlings exposed to ethylene treatments.

Treatment	No. roots 0.5< x <1.5 cm.	Total No. of roots	Total length (cm)	Avg length (cm)
Control	9.2 B[1]	18.8 BC	48.9 ABC	2.4
Purafil	12.8 B	21.6 BC	43.0 BC	1.6
500 ppb	18.8 A	31.7 A	59.9 AB	1.9
1000 ppb	8.9 B	16.4 C	36.3 C	2.2
2000 ppb	12.3 B	18.4 BC	31.6 C	1.7
4000 ppb	11.8 B	25.0 AB	63.7 A	2.4

1- Values in a column with the same letter are not significantly different (Alpha=0.05).

Study 5

Xylem water potential declined steadily during the fifteen days of water stress, from -0.99 MPa to -2.87 MPa for the Coastal Plain (CP) seedlings and from -0.94 MPA to -2.82 MPa for the Drought Hardy (DH) seedlings (Table 19). Xylem water potential of seedlings from both families returned to near control levels after rewatering.

Stomatal conductance rates also declined steadily over the water stress treatment, and improved slightly upon rewatering (Table 20). Values of stomatal conductance for seedlings from the two families were not significantly different. Figure 7 shows the relationship between xylem water potential and stomatal conductance for each family over the stress period.

Ethylene Production

After three hours of incubation, the average hourly rate of ethylene production by the needles was significantly affected by family, days of drying, and a family-day interaction (see Appendix F for the six-hour production rates). In general needles from seedlings in the CP family tended to produce ethylene at higher rates (0.28 ul/g dry wt/hr) than needles from DH seedlings (0.20 ul/g dry wt/hr), however; the difference was significant only on day zero and

Table 19. Xylem water potential of CP and DH seedlings during the water stress study.

	Days of Drying						
Family	0	3	6	8	12	15	18[1]
	MPa						
CP	-0.99	-1.14	-1.26	-2.47	-2.87	-2.87	-0.88
DH	-0.93	-1.20	-1.31	-1.63	-2.52	-2.82	-0.85

1- Rewatered.

Table 20. Levels of stomatal conductance for CP and DH seedlings during the water stress study.

	Days of drying						
Family	0	3	6	9	12	15	18[1]
	cm/sec						
CP	0.099	0.095	0.039	0.014	0.014	0.011	0.040
DH	0.092	0.092	0.028	0.012	0.012	0.013	0.020

1- Rewatered.

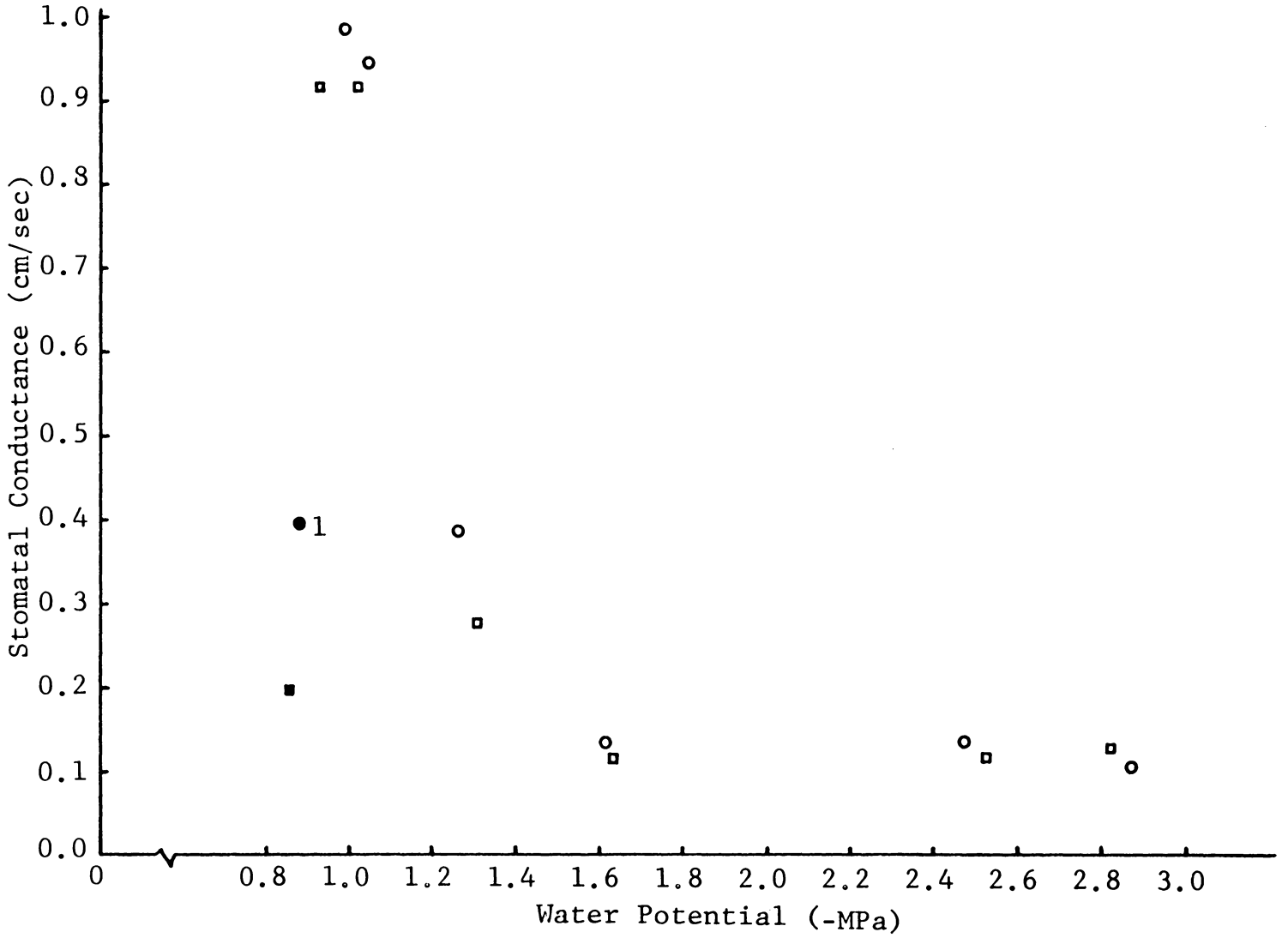

Figure 7. Stomatal conductance as affected by xylem water potential in two families of loblolly pine: Coastal Plain of Virginia (o); Drought Hardy of East Texas (▫). 1- Rewatered.

on the last day of drying (Table 21). The general trend with increasing stress for the CP needles was an increase in production until the sixth stress day followed by a decrease at day nine and twelve, then a significant rise in production on day fifteen. The rates declined steeply upon rewatering. Needle rates of DH seedlings also increased slightly until day nine, but dropped through day fifteen, and increased significantly upon rewatering (Table 21).

Production rates generally remained high until water potential dropped to below -1.3 MPa at which time the rates fell. The rates rose drastically in CP needles when water potential fell to -2.5 MPa (Figure 8). Needle ethylene production in DH seedlings was also significantly affected by water potential. In general, ethylene production rates decreased as water potential decreased (Figure 8).

The rate of ethylene production by the roots of water stressed seedlings tended to be much higher than the rates of the needles. Like the shoots, the production rates of the roots after three hours were significantly affected by days of drying, and a family-day interaction; however, family alone did not have a significant effect (see Appendix F for six hour rates). Production rates of the roots were not significantly different between the two families except on the third day of drying and after rewatering. Rates of

Table 21. Needle ethylene production rates of water stressed seedlings by family. Rates are based on an incubation time of three hours.

Family	Days of Drying							
	0	3	6	9	12	15	18[1]	$\bar{x}$
	UL/G DRY WT/HR							
CP	0.28 BC[2]	0.32 B	0.28 BC	0.14 EF	0.19 ED	0.47 A	0.29 BC	0.28
DH	0.22 CDE	0.24 BCD	0.23 CD	0.19 EFD	0.15 EF	0.11 F	0.25 BCD	0.20
$\bar{x}$	0.25	0.28	0.26	0.17	0.17	0.29	0.27	

1- Rewatered.
2- Values within the table with the same letter are not significantly differnt (Alpha =0.05).

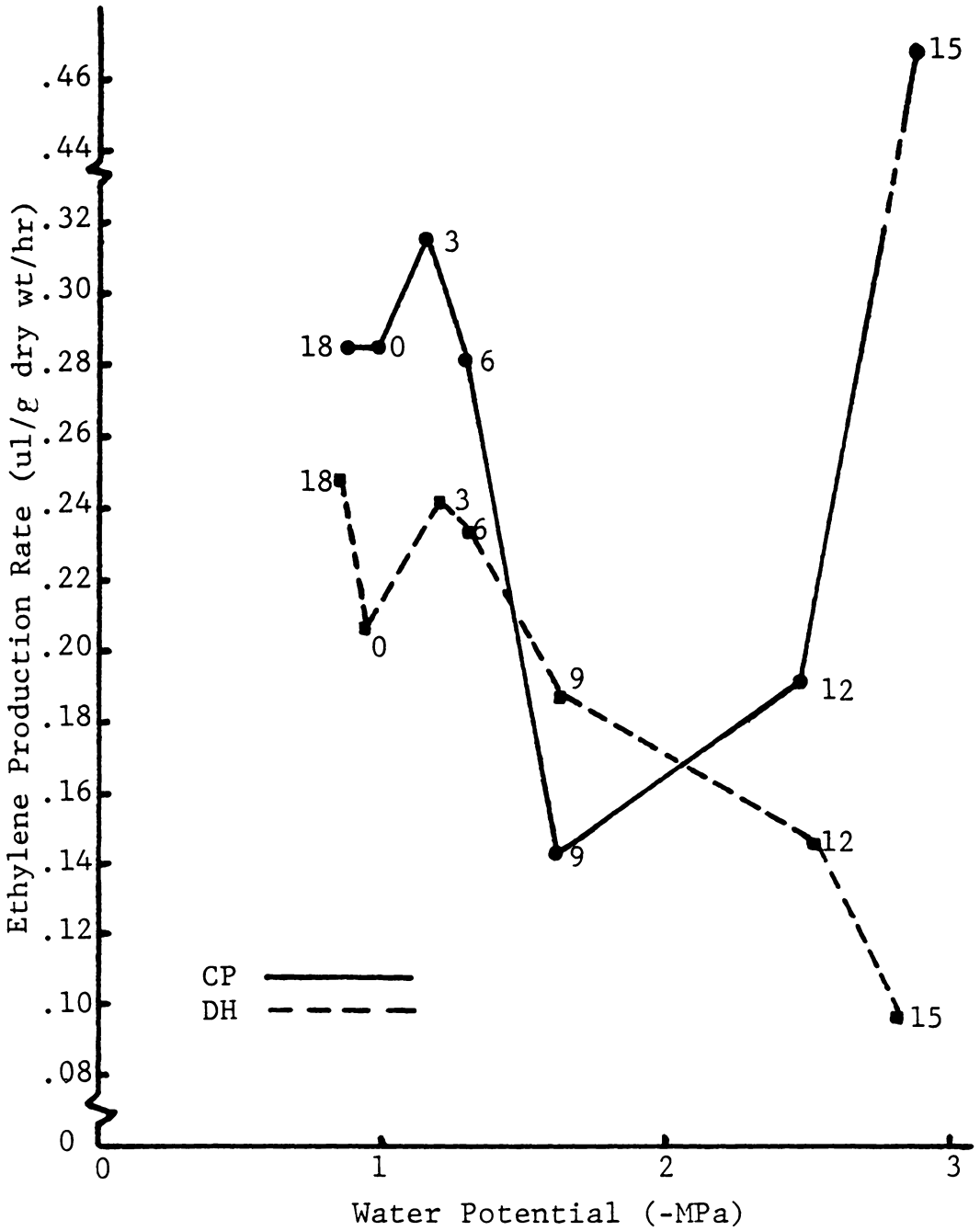

Figure 8. Relationship between ethylene production rates of needles of Coastal Plain (CP) and Drought Hardy (DH) seedlings and xylem water potential. Numbers indicate days of drying.

the CP roots did not decline between days zero and three, while rates of DH roots declined from 1.34 ul/g dry wt/hr to 0.68 ul/g dry wt/hr (Table 22; Figure 9). After rewatering, production rates of the roots of the DH seedlings were significantly higher (2.54 ul/g dry wt/hr) than rates of the CP seedlings (1.83 ul/g dry wt/hr). In general, rates of production for roots of both families tended to decline with water stress, and increased significantly upon rewatering.

1-Aminocyclopropane-1-carboxylic Acid Levels

Levels of ACC within the seedling tissue were not significantly different between the two families, although levels were significantly higher in CP seedlings on the last day of drying (Table 23) The mean levels of ACC in the root tissue (122.0 nmol/g dry wt) were significantly higher than in the shoots (92.8 nmol/g dry wt). Levels of ACC also changed significantly with days of drying.

ACC in the needles ranged from a high of 158.7 nmol/g dry wt on the last day of drying to a low of 52.8 nmol/g dry wt on the sixth day of drying. However, the low was not significantly different for values measured on the ninth and twelfth days of drying or after rewatering (Table 23). Values tended to decline through the sixth day of drying and level out before increasing significantly on the fifteenth day of drying. Values declined upon rewatering (Table 23).

Table 22. Root ethylene production rate of Coastal Plain (CP) and Drought Hardy (DH) seedlings. Values are based on three hours of incubation.

Family	Days of Drying							
	0	3	6	9	12	15	18[1]	$\bar{x}$
	UL/G DRY WT/HR							
CP	1.07 CD[2]	1.11 CD	0.98 ED	0.58 GF	0.63 GF	0.46 G	1.83 B	0.95
DH	1.33 C	0.68 GEF	0.80 DEF	0.74 GEF	0.61 GF	0.50 GF	2.54 A	1.03
$\bar{x}$	1.21	0.89	0.89	0.66	0.62	0.48	2.19	

1- Rewatered.
2- Values within the table with the same letter are not significantly different (Alpha = 0.05).

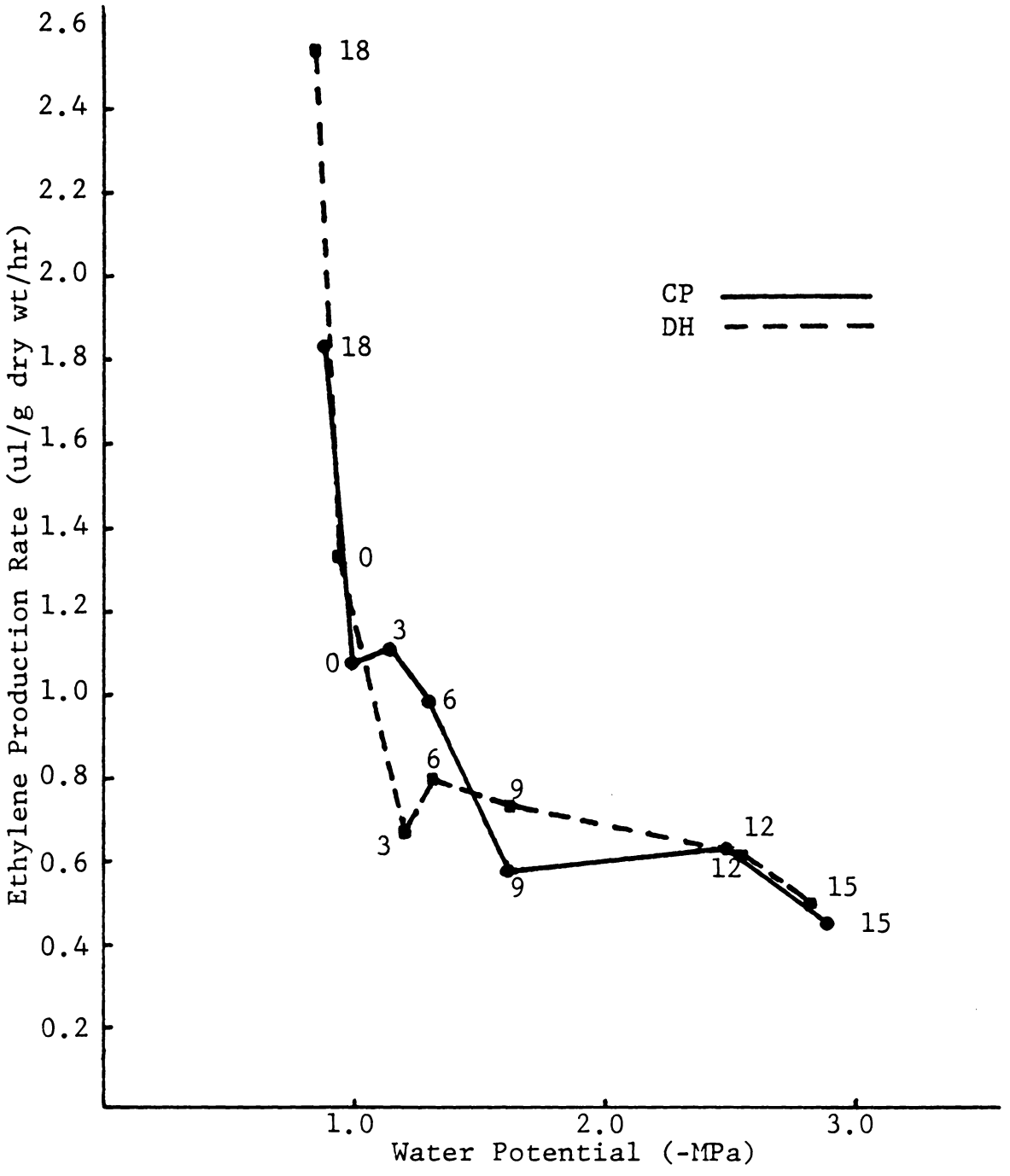

Figure 9. Relationship between ethylene production rates of roots of Coastal Plain (CP) and Drought Hardy (DH) seedlings and xylem water potential. Numbers indicate days of drying.

Table 23. Levels of ACC within the needles of Coastal Plain (CP) and Drought Hardy (DH) seedlings during water stress.

Family	Days of Drying							
	0	3	6	9	12	15	18[1]	$\bar{x}$
	nmole/g							
CP	111.0	80.6	60.6	77.3	67.9	190.9	70.7	94.2 A[2]
DH	127.9	105.7	45.1	60.4	97.6	126.4	77.5	91.5 A
$\bar{x}$	119.5 B[3]	93.2 BC	52.8 C	68.9 C	82.8 BC	158.7 A	74.1 C	

1- Rewatered.
2- Values in a column with the same letter are not significantly different (Alpha = 0.05).
3- Values in a row with the same letter are not significantly different (Alpha = 0.05).

Figures 10 and 11 show the changes in ACC levels in the needles with declining water potential in relationship to changes in ethylene production with declining water potential.

Levels of ACC did not appear to be correlated with changes in stomatal conductance. Increases in ACC corresponded with both increases and decreases in stomatal conductance (Figure 12).

The highest mean levels of ACC in the roots (181.2 nmol/g dry wt) also occurred on the last day of drying, and the low (88.7 nmol/g dry wt) occurred on the sixth day of drying (Table 24). Levels of ACC in the roots fluctuated but did not change significantly until the last day of drying when concentrations increased substantially. ACC levels in roots of CP seedlings tended to be higher and fluctuated more widely than levels in roots of DH seedlings. Figures 13 and 14 show changes in levels of ACC in root tissue of seedlings of each family with declining water potential and changes in ethylene production with declining water potential.

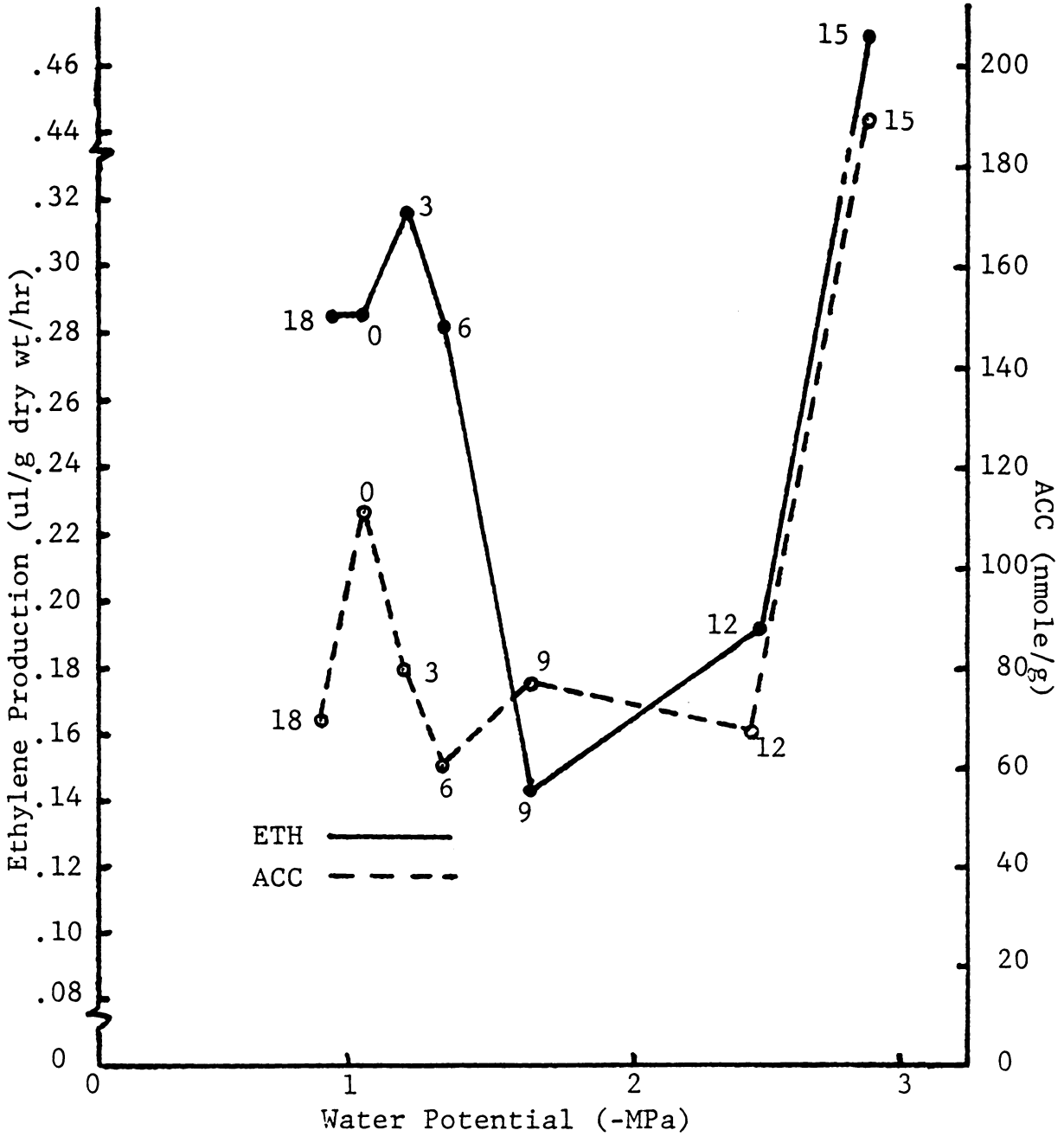

Figure 10. ACC levels in needle tissue of Coastal Plain seedlings with declining water potential in relation to ethylene production. Numbers indicate days of drying.

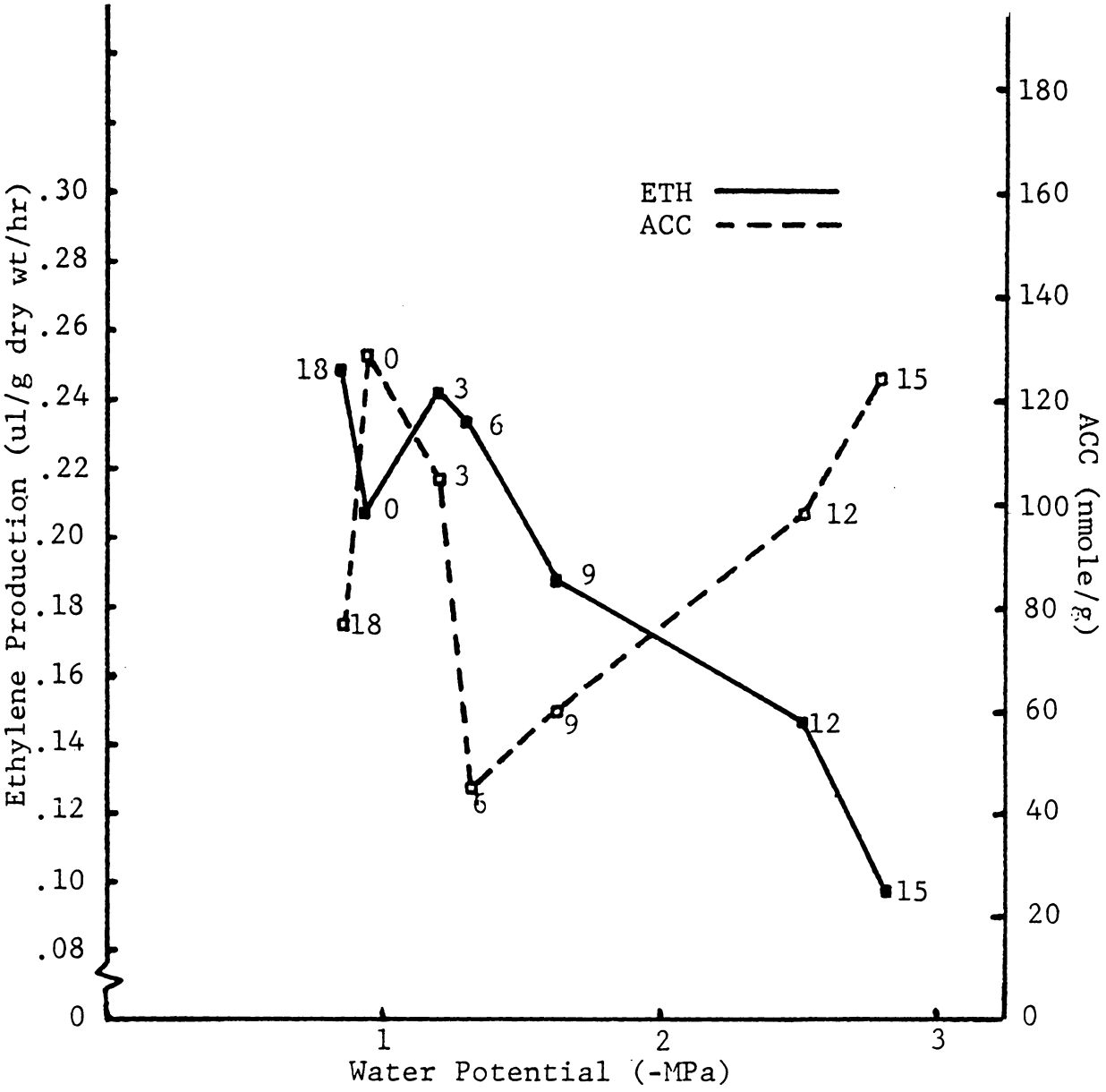

Figure 11. ACC levels in needle tissue of Drought Hardy seedlings with declining water potential in relation to ethylene production. Numbers indicate days of drying.

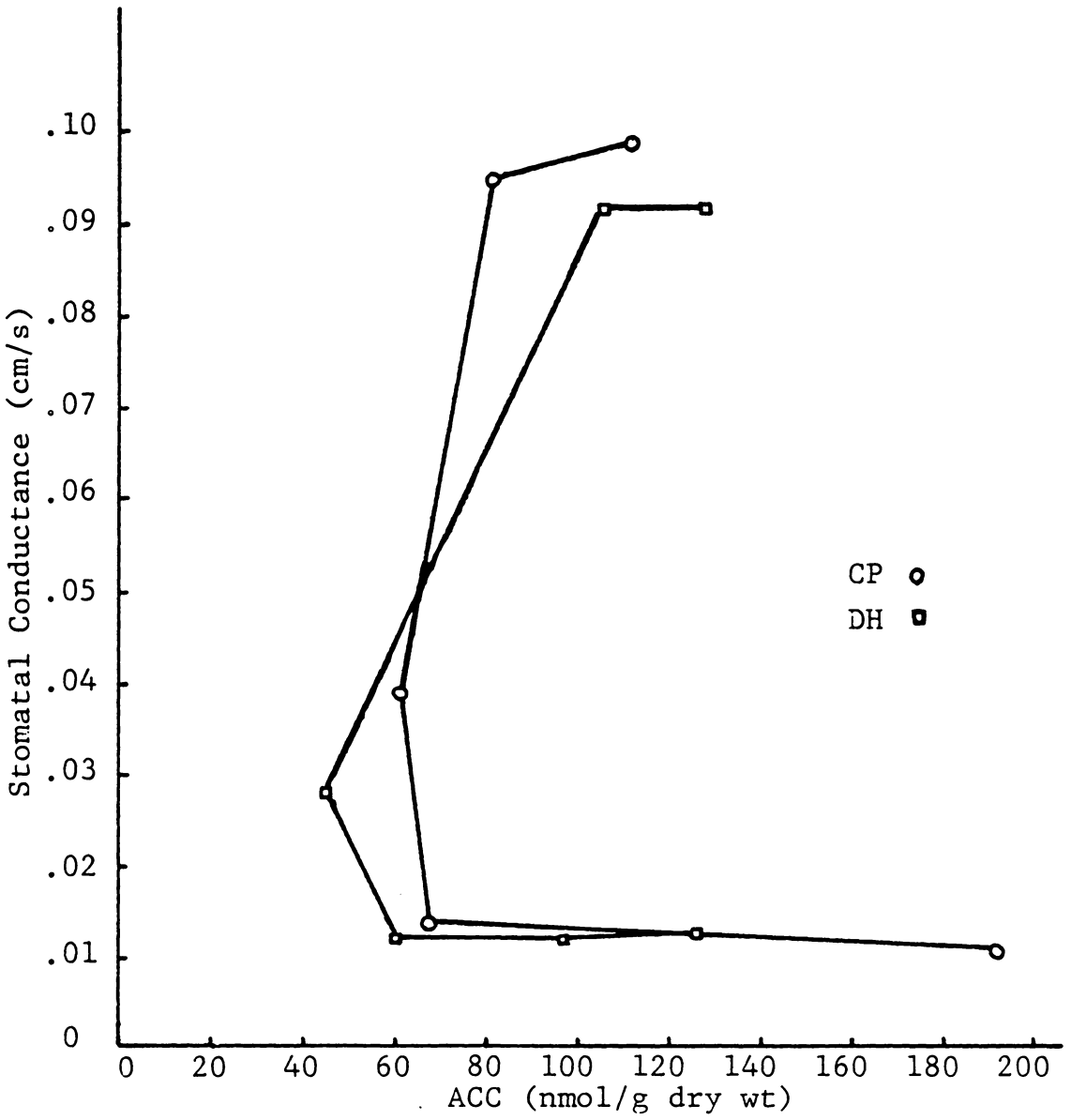

Figure 12. Levels of ACC in needles of Coastal Plain (CP) and Drought Hardy (DH) seedlings in relation to stomatal conductance.

Table 24. Levels of ACC within the roots of Coastal Plain (CP) and Drought Hardy (DH) seedlings during water stress.

Family	Days of Drying							
	0	3	6	9	12	15	18[1]	$\bar{x}$
	nmoles/g							
CP	107.8	162.4	95.8	156.9	91.3	197.3	124.8	133.7 A[2]
DH	150.6	92.5	81.6	105.2	86.4	165.0	93.0	110.6 A
$\bar{x}$	129.2 AB[3]	127.4 AB	88.7 B	131.0 AB	81.8 B	181.2 A	108.9 B	

1- Rewatered.
2- Values in a column with the same letter are not significantly different (Alpha = 0.05).
3- Values in a row with the same letter are not significantly different (Alpha = 0.05).

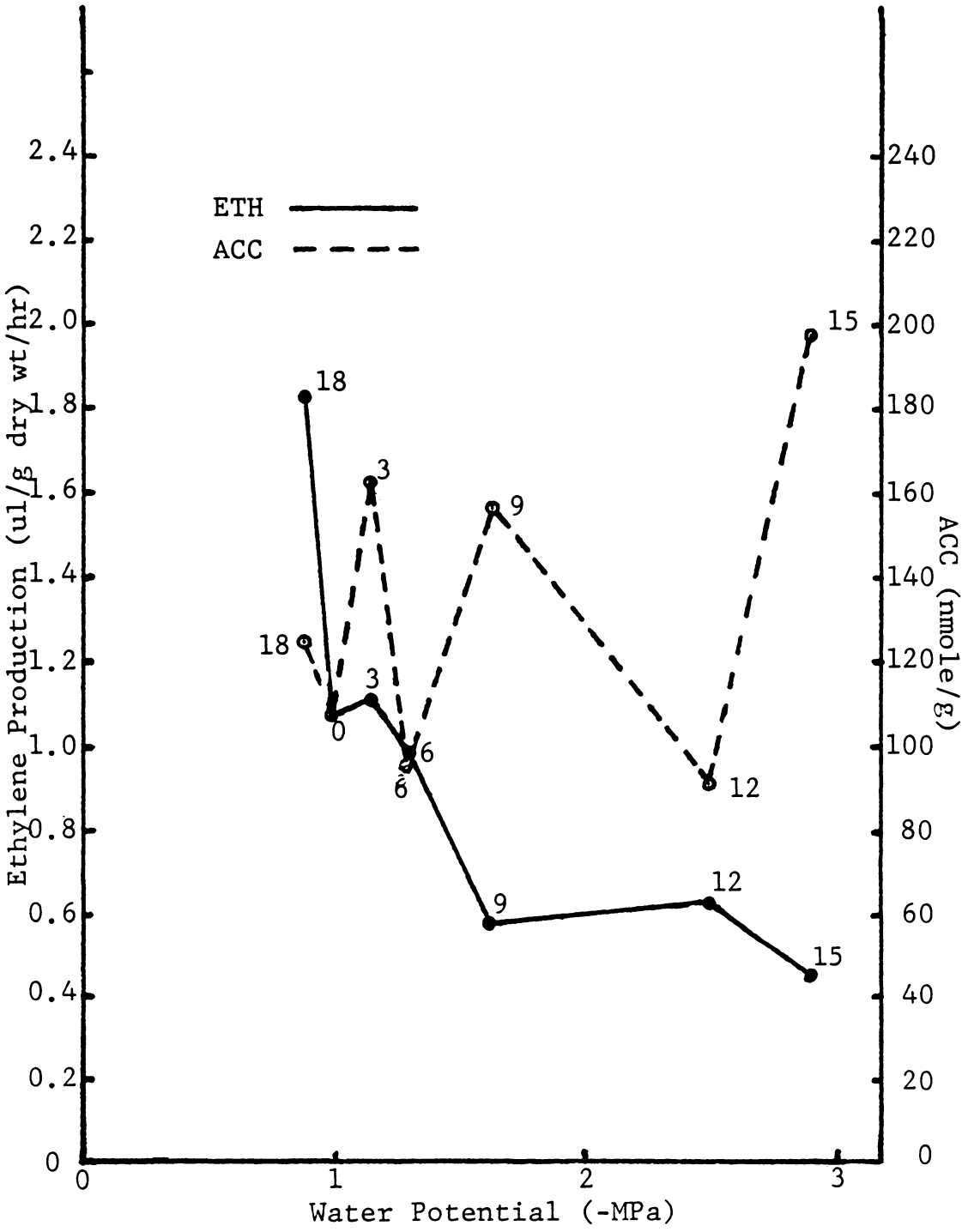

Figure 13. ACC levels in root tissue of Coastal Plain (CP) seedlings with declining water potential in relation to ethylene production. Numbers indicate days of drying.

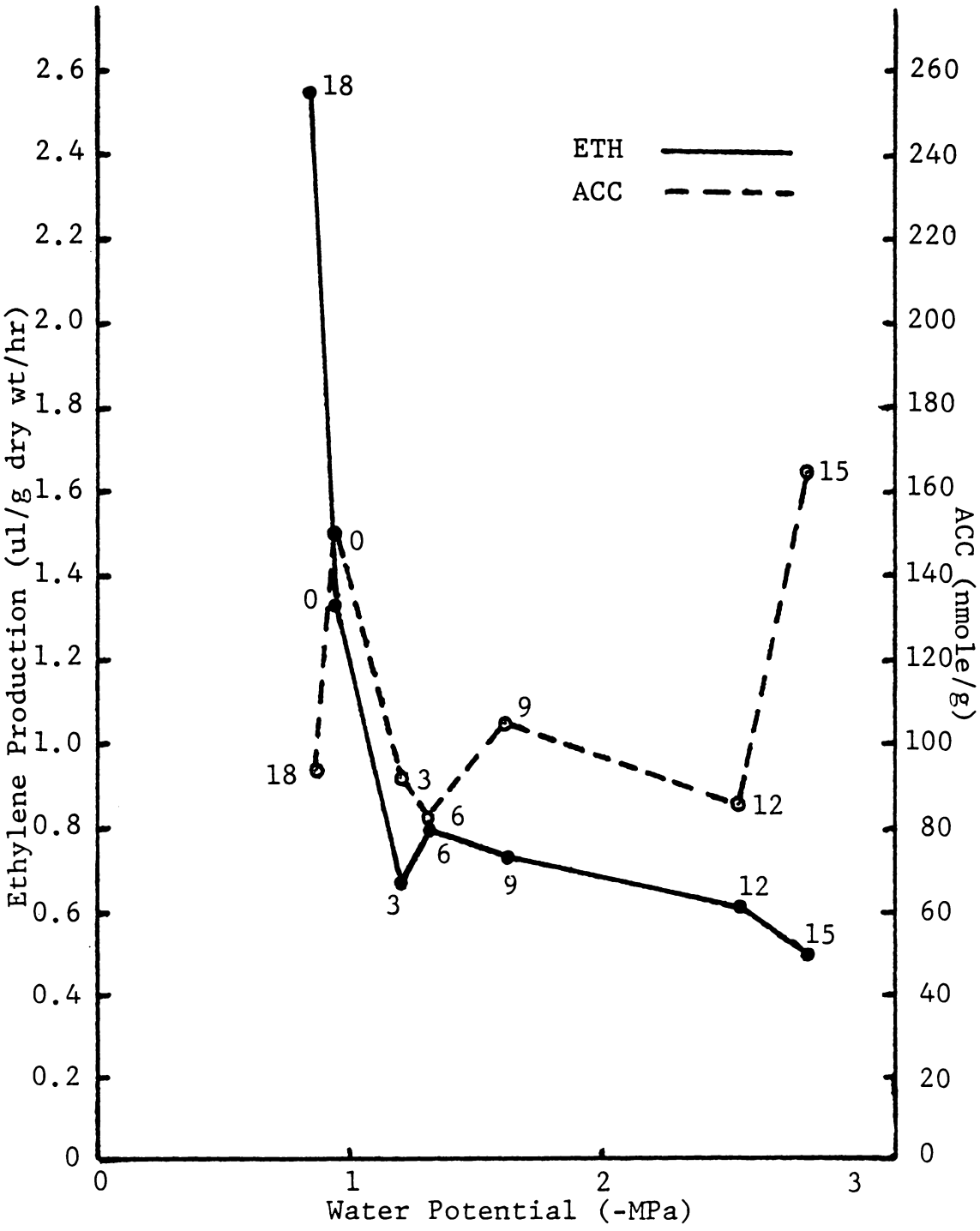

Figure 14. ACC levels in root tissue of Drought Hardy seedlings with declining water potential in relation to ethylene production. Numbers indicate days of drying.

DISCUSSION

Effects of Date and Method of Lifting

Ethylene was found to be produced by loblolly pine seedlings during cold storage, as suggested in earlier studies (Barnett 1978, Johnson 1982). Levels of ethylene produced were affected by the lift date, number of weeks in cold storage and lifting method.

Ethylene production was significantly influenced by lifting date with the general trend of increasing concentrations through February and an abrupt drop in March. Johnson (1982) reported a similar trend in the ethylene concentration of a loblolly pine cold storage facility, but the peak concentration was observed in late December. These findings suggest that ethylene may be related to seedling dormancy.

Dormancy, which has been defined as the temporary suspension of growth (Samish 1954), consists of several distinct physiological stages. Quiescence and rest are the two primary phases of dormancy (Samish 1954, Wareing and Saunders 1971). Quiescence is imposed by unfavorable external factors, such as unfavorable temperatures or water

supply, and may occur before and after rest. Rest is brought about by internal factors and a chilling treatment is required to break it. Temperatures near 5°C are generally the most effective for meeting the chilling requirements; however, this may vary with species and genotype (Perry 1971). Number of hours of exposure to the chilling temperatures also varies with species, genotype and with the previous seasons's weather (Perry, 1971).

Results from the present study suggest that levels of ethylene may be related to the stage of dormancy. The peak in ethylene production may be related to meeting the chilling requirement. Garber (1978) reported that the chilling requirement of an Arkansas progeny of loblolly pine seedlings was fulfilled by mid-December. As previously mentioned, the peak ethylene concentration in a VDF cold storage facility occurred in late December (Johnson 1982). The warm fall of 1982 may have delayed fulfillment of the chilling requirement, and the ethylene peak in the present study. The ethylene peaks in this study occurred during the first week of storage of the February-lifted seedlings and during the second and third week in storage of the January-lifted seedlings. It is possible that the temperatures within the cold storage room were more efficient at fulfilling the chilling requirement, and hence the earlier peak for January-lifted seedlings.

An alternative explanation for the extremely high peak after the first week in storage of February-lifted seedlings may be that the seedlings suffered cold damage prior the lifting date. Kimmerer and Kozlowski (1983) reported that chilling injury can induce production of wound ethylene. While temperatures on the day of lifting were near 50°F, Garber (1978) reported that mild weather following extremely cold weather can be harmful to loblolly seedlings. Therefore, the high value during the first week may have been a response to wounding. During cold storage, concentrations of ethylene within the bags of HL seeldings generally decreased. The peak ethylene concentrations for seedlings of all lift dates, except February, generally occurred near the third week of cold storage, while the minimum concentrations occurred after the second or third month of storage. The changes in levels of ethylene may be related to the differences in storage potential of loblolly seedlings that Garber and Mexal (1980) observed. They observed that early lifted seedlings (November and December) did not store well for extended periods (nine weeks). Height growth and survival of the seedlings declined as storage time increased from one to nine weeks. Seedlings lifted in January showed the lowest survival and height growth after five weeks of cold storage (Garber and Mexal,

1980). This may correspond with the peak in hormonal ethylene observed for January seedlings after the fourth week of storage. The relatively low survival (78% vs 87%) and height growth (13 cm vs 15 cm) for February seedlings after one week of storage in Garber and Mexal's study could also correspond with the high levels of ethylene recorded during the first week in bags of February seedlings.

Concentrations of wound ethylene produced by the seedlings after cutting them in half were also affected by cold storage. Levels generally declined over the weeks in cold storage (Figure 2). According to Tingey (1980) and Abeles (1982), rates of ethylene production return to normal within 24 hours after removing the stress. Therefore, it appears that the wound created by cutting the seedlings does not heal completely during storage, although levels of wound ethylene did decrease.

An interaction between lift dates and storage was also observed for wound ethylene production. Seedlings lifted in January and February produced significantly higher levels of wound ethylene and therefore, seemed more sensitive to wounding than seedlings lifted in the other months. Although wound ethylene decreased over cold storage for most seedlings, the highest levels of wound ethylene produced by January seedlings occurred after the second week of storage for the shoots and after the third week for the roots.

Seedlings lifted by machine generally produced higher concentrations of ethylene than HL seedlings, and roots of ML seedlings generally produced more than the shoots or whole Ml seedlings. In general, it appears that the ML seedlings were producing ethylene in the same manner as the Hl seedlings except that the roots of the ML seedlings produced higher levels of ethylene. This may have been a result of the wounding which seedlings may incur during the mechanized lifting process. The difference in ethylene production by the roots of HL and ML seedlings did not appear to be due to wound ethylene from cutting the seedlings in half. The highest levels of wound ethylene fluctuated from HL to ML, while the levels of "hormonal" ethylene were generally higher for ML seedlings. Whole ML seedlings tended to produce more ethylene than whole HL seedlings; however, for March seedlings the difference was only significant during the first week of storage. This suggests that wounding sustained during lifting caused the increased ethylene production during the first week of storage of the March-lifted seedlings. Furthermore, since ethylene levels soon moderated for both months, and since there were no significant differences in the mean levels of ethylene produced by ML and HL seedlings, there appears to be little problem of the accumulation of high ethylene levels for ML seedlings.

Site of Ethylene Production

Abeles (1973) reported that rates of ethylene production will vary from organ to organ and with time of development. In etiolated pea seedlings, highest rates were observed in the nodal and meristematic regions, while in apple stems highest rates were associated with dormant buds and senescing and abscising tissue.

Results from studies one and three indicate that the roots of 1-0 loblolly seedlings generally produce more "hormonal" and wound ethylene per gram dry weight than the shoots (Figures 1 and 2). Johnson (1982) also observed that concentrations of ethylene within K-P bags were slightly higher in the end of the bag containing the roots. However, after the first week of storage, only production by roots and shoots of February-lifted seedlings were significantly different. This suggests that the differences are not due to unequal amounts of wounding during lifting, but that the difference in production rates is physiological in nature. During cold storage, roots would have more meristematic tissue than the dormant shoots, which may partially explain the higher rates of production by the roots.

While studies one and three indicated that roots tended to produce more ethylene than the shoots; study two, where samples were incubated under light, indicated that the

needles generally produced more ethylene per gram dry weight than the roots. However, needle and root production rates were not significantly different for December-lifted seedlings and for the first week's sample of February and March seedlings (Table 9). Kimmerer and Kozlowski (1982) reported that ethylene production by leaves of paper birch (Betula papyrifera Marsh.) and shoots of red pine (Pinus resinosa Ait.) was much higher in the light. Wright (1981) and Kao and Yang (1982), on the other hand, reported that light inhibited ethylene production in photosynthesizing tissue. Kao and Yang determined that the inhibition was due to the depletion of carbon diaoxide by the photosynthesizing tissue. The light intensity in the incubation chamber in the present study was at or below the compensation point for loblolly pine, therefore, probably little or no change in carbon dioxide concentrations was occurring. The fact that December-lifted seedlings showed no differences in needle and root production rates may be a function of seasonal variations in photosynthesizing ability and pigment content. It has been reported (Martin et al. 1978) that frost hardening resulted in a decrease in the capacity of the electron transport system and the ability to photosynthesize declines. Levels of active pigments (esp. P700) also declined with frost hardening. The reduction of pigments

and photosynthetic capacity in the December-lifted seedlings was probably lower than in the seedlings of later lifting dates, and therefore these seedlings were able to photosynthesize at a higher rate, causing a slight decrease in carbon dioxide concentrations. This decrease in carbon dioxide may have resulted in the inhibition of the ethylene production in December. Some variation in ethylene levels may have been due to daily fluctuations in ethylene production. El-Beltagy and Hall (1974) reported a diurnal fluctuation in internal ethylene concentrations in broad bean leaves.

Dose-Response Study

Increasing levels of ethylene exposure during cold storage had positive effects on the rate of bud break, height and diameter growth, root growth potential and dry weights of outplanted seedlings. Seedlings exposed to 4000 ppb ethylene generally had significantly greater responses than the other seedlings. In most cases, there were no significant differences between the responses of the other treatments. A factor in the lack of clear-cut responses in the analysis of variance and lack of large coefficients of determination and slope parameters in the regression analysis was that the K-P bags were not totally ethylene-

tight and lost ethylene at different rates. Therefore it was difficult to determine and control the actual levels of ethylene in the bags and exposures may have overlapped. However, the seedlings were exposed to the given treatment concentrations on several occassions over the six week storage period. For discussion purposes, the treatments will be referred to as originally specified.

The survival of seedlings exposed to all treatments was very high with seedlings exposed to 4000 ppb showing significantly higher survival than all other treatments except the Purafil treatment. Barnett (1980) found that survival of seedlings stored for six weeks with Purafil averaged six percentage points higher than control seedlings. Although results from the present study also indicated that seedlings stored with Purafil had better survival than Control seedlings, the difference was not significant.

The rate of bud break appeared to be stimulated by exposure to ethylene. Rate of bud break of seedlings exposed to 4000 ppb was the highest while Purafil and control treatments tended to have the slowest rate of bud break. Reports concerning the effect of ethylene on bud break vary. According to Abeles (1973) the normal regulatory role of ethylene in bud development is unknown.

However, ethylene has been reported to stimulate bud development in several tree species, including oaks, beech and birch.

The rate of bud break may not have been a direct effect of ethylene on the buds, but may have been an indirect effect through root growth capacity. Seedlings exposed to 500 ppb and 4000 ppb ethylene had significantly more new roots than other seedlings, while seedlings exposed to 1000 ppb generally had the fewest new roots. It has been suggested that the rate of bud break is actually a reflection of the initiation of root growth in the spring and the associated translocation of water, nutrients and metabolites (Johnson and Stumpff, 1984). If this is true, then the seedlings exposed to 4000 ppb, which quickly initiated new roots, would have been more disposed to breaking bud. However, the seedlings exposed to 500 ppb which had a comparable number of new roots exhibited a significantly lower level of bud activity. This difference may be related to the average length of the new roots on the seedlings exposed to 500 ppb which were significantly lower than the average length of the 4000 ppb treatment. It is assumed that the longer roots were initiated earlier and thus had more time to grow (DeWald, unpublished thesis results). Once the seedling begins to break bud, root

elongation will be curtailed while root initiation may continue. This suggests that the roots of the 4000 ppb treatment were initiated much earlier, allowing the seedlings to become established, which in turn favored early bud break.

In addition to early bud break, the seedlings exposed to 4000 ppb were significantly larger in terms of average height, root collar diameter and dry weight by the end of the first growing season. There were no specific differences in the size of the seedlings of the other treatments, although controls, 500 ppb and seedlings stored with Purafil tended to be smaller overall. Ethylene has been associated with inhibition of cell elongation (Abeles 1973), but such inhibition has usually been reported for etiolated tissues, and the effect on light-grown tissue is not as strong. Reports also vary as to whether the effect on elongation is temporary or permanent (Abeles 1973).

The stimulated growth of the seedlings exposed to 4000 ppb may also be related to the accelerated bud activity seen in those seedlings. A portion of the enhanced growth would be due to the fact that the seedlings began growing earlier. Ethylene may also have had a direct effect on the ability of the buds to elongate. Apparently a relatively high concentration (4000 ppb) of ethylene is required to cause

this type of stimulation in growth, although Hinesley and Saltveit (1980) reported that exposure of three year old fraser fir seedlings to high concentrations (17.5 ppm) of ethylene resulted in abnormal bud break and a significant reduction in shoot elongation. In loblolly seedlings, Barnett (1980) found no difference in height growth between Controls and seedlings stored with Purafil.

Although firm conclusions about the dose-response relationship between ethylene and loblolly pine seedlings can not be made from this study, one important conclusion can be drawn. The study indicated that the levels of ethylene that accumulate within K-P bags were not detrimental to the seedlings. Although seedlings stored with Purafil had slightly higher survival than the controls, the growth of the seedlings was not enhanced by the Purafil treatment. While this may be the case for seedlings lifted in March, seedlings lifted during other months may respond differently to the ethylene treatments.

Water-Stress Study

The first significant change in ethylene production rates of needles of seedlings in the Coastal Plain (CP) family occurred after the ninth day of drying (-1.6 MPa), when production rates dropped by almost one third. Rates

gradually increased through day twelve before rising precipitously on the last day of drying (-2.87 MPa) (Figure 8). El-Beltagy and Hall (1974) observed a somewhat similar trend in internal levels of ethylene in intact, water-stressed broad bean. One significant difference with broad bean though, was that of a sharp increase in ethylene levels within 24 hours of the initial water stress. Falling concentrations were seen through the third day of stress, followed by gradually increasing concentratins to a high on the last day of drying. Other studies (Wright 1977, Kimmerer and Kozlowski 1982, and Hoffman et al. 1982) with excised wheat leaves exposed to a relatively rapid water stress showed steadily increasing ethylene levels with increasing water stress up to a 10 - 13.5 percent water loss (approximately -1.7 MPa to -2.0 MPa). Ethylene levels declined when water loss exceeded that point. It was suggested that the decrease may result from scarcity of substrate in the excised tissue. While levels of ethylene production by CP seedlings did increase with water stress, it was only after a water potential of -1.6 MPa was reached.

Levels of ethylene production by the CP needles were similar to the production rates of the DH needles; however, CP production was significantly higher on the last day of drying. In a study involving water stress of excised leaves

of a drought-resistant and a drought-sensitive cultivar of winter wheat (Triticum aestivum L. em Thell), Rose and Kirkham (1983) found that the leaves of the drought-resistant cultivar produced significantly more ethylene. They suggested that the drought-resistant cultivar had a greater ability to convert ACC to ethylene. This does not appear to be the case for loblolly seedlings since ACC levels in the DH needles increased after a water potential of -1.6 MPa was reached while ethylene production decreased. Since the drought tolerant seedlings produced less ethylene, this suggests that ethylene, in large quantities, does not help the seedlings cope with stress. However, it could also be possible that the sharp increase in ethylene does help the plants cope, but that since the DH seedlings are less sensitive to water stress, the ethylene increase may not occur until a lower water potential (less than -2.8 MPa) is reached. Another, perhaps related possibility, is that the increased ethylene production of ethylene by the CP seedlings on the last day of stress may be a function of cell injury (Kimmerer and Kozlowski 1982). It is possible that the DH seedlings do not sustain cellular injury due to water stress until a lower water potential is reached.

Upon rewatering, ethylene production by CP needles fell substantially. Hoffman et al. (1982) also reported that

rehydrating resulted in a large drop in ethylene production. However, DH production increased upon rewatering. In both cases though, production returned to near control rates. It appears that the activity of the "ethylene-forming enzyme" (EFE) (McKeon et al, 1982) in the two families is not affected until water potential is below approximately -2.0 MPa.

Levels of ACC in the needle tissue of the two families was not significanty different except on the last day of drying, when ACC levels in CP tissue was significantly higher. This suggests that the CP needles not only have enhanced ability to convert ACC to ethylene, but that the production of ACC is also enhanced. In both families ACC concentration began to increase when water potential reached approximately -1.4 MPa, which corresponded with stomatal closure (Figure 7). El-Beltagy and Hall (1974) reported that ethylene is not a cause of stomatal closure. Therefore, the increase in ACC may correspond to the increase in AbA which has been correlated with stomatal closure (Walton, 1980). McKeon et al. (1982),on the other hand, reported that AbA inhibited ACC production. However, their study involved exogenous applications of AbA to excised wheat leaves and the concentrations may have been in excess of that required for stomatal closure.

Ethylene production by the roots of both families was significantly higher than in the shoots. Although light intensity was low during incubation, it may have been sufficient to allow some photosynthesis in the needles. The concentration of carbon dioxide in the needle vials may have been reduced enough to inhibit ethylene production by the needles in relation to root production. In both families ethylene production by the roots decreased with increasing water stress. Perhaps, as shown in the dose-response study, lower concentrations of ethylene may stimulate root activity, increasing the plant's ability to acquire water. Levels of ACC in the roots were comparable to levels in the shoots, suggesting that the roots are more efficient in converting ACC to ethylene.

CONCLUSIONS

Levels of ethylene produced by 1-0 loblolly pine seedlings hand-lifted monthly between November and December generally increased through January and February and dropped abruptly in March. The highest level of ethylene recorded in the K-P bags occurred during the first week of cold storage of February seedlings. Levels of ethylene produced by January seedlings during the second and fourth weeks of cold storage were also high. Ethylene concentrations within the K-P bags generally declined over the twelve week storage period.

Concentrations of ethylene in K-P bags of roots (on a dry weight basis) were significantly higher than within bags of shoots or whole seedlings; however, when needle and root samples were incubated under low light, the needles of February and March seedlings produced higher levels of ethylene. This may be a reflection of the fact that the K-P bags were stored in a cold, dark environment where the shoots remained dormant and the roots were relatively active, while during incubation under light and warmer temperatures, the shoot metabolism may have been activated.

Wound ethylene production due to cutting the seedlings in half was greater for the roots. Concentrations of wound ethylene produced by roots and shoots were generally higher in January and February while concentrations of hormonal ethylene were highest in February alone, indicating that lift date has a significant effect on levels of ethylene production. It appears that fulfillment of the chilling requirement may be correlated with the peak in ethylene production.

During the first week of cold storage, roots of seedlings machine-lifted in January and March produced significantly higher concentrations of ethylene (per gram dry weight) than roots of hand-lifted seedlings. Lifting technique did not have an effect on the levels of ethylene production by the shoots and whole seedlings. This suggests that roots may sustain more injury during mechanical lifting; however, since whole seedlings were not significantly affected overall by the lifting technique, this does not appear to be a major source of increased ethylene production.

March-lifted seedlings were exposed to Purafil, 500 ppb, 1000 ppb, 2000 ppb, 4000 ppb ethylene and a Control treatment during six weeks of cold storage to determine the dose-response relationship of ethylene and loblolly pine seedlings. Concentrations of ethylene within the K-P

storage bags, which were additionally wrapped with polyvinyl sheeting and placed inside two polyethylene bags, were measured periodically and adjusted as needed. The bags were not completely ethylene-tight and lost ethylene at varying rates, which may have been a factor in the lack of clear-cut responses for all of the treatments except 4000 ppb.

The seedlings fumigated with 4000 ppb had significantly greater survival, root growth capacity, bud activity and height and diameter growth. Response to the other treatments varied; however, in general, ethylene had a slight positive effect on seedling growth. Unlike many studies which categorize ethylene as a growth inhibitor, this study indicates that ethylene treatments enhanced growth of loblolly pine seedlings. Further study, with rigidly controlled ethylene exposures is necessary to determine the actual dose-response relationship of ethylene and loblolly seedlings, and to determine if seedlings from different lift dates are equally sensitive to ethylene.

Half-sib loblolly seedlings from a Virginia Coastal Plain family and a East Texas Drought Hardy family were exposed to water stress by withholding water for fifteen days. Seedlings were rewatered on the sixteenth day.

Ethylene production rates of the needles of both families were not significantly different and generally declined with

increasing water stress; however, on the last day of drying (-2.8 MPa) production by the CP needles increased significantly while production by the DH needles continued to decline. Production rates of both families returned to near control levels upon rewatering.

Over the drying period the levels of ACC in the CP needle tissue generally followed the production of ethylene. Levels of ACC in the DH needle tissue, on the other hand, began increasing on the sixth day of drying while ethylene production was declining. Levels of ACC in needle tissue of both families began increasing when water potential reached -1.4 MPa which corresponded with stomatal closure.

Roots of seedlings from both families produced ethylene at significantly higher rates than the needles. Production rates of the roots declined over the drying period and increased significantly upon rewatering.

Levels of ACC in the root tissue were comparable to levels in the needle tissue, and generally followed the same trend, with increasing rates after the sixth day of drying. It appears that the roots of loblolly seedlings are more sensitive to water stress than needles and that the site of activation of the increased ethylene production is the conversion of ACC to ethylene.

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Appendix A

BIOSYNTHETIC PATHWAY

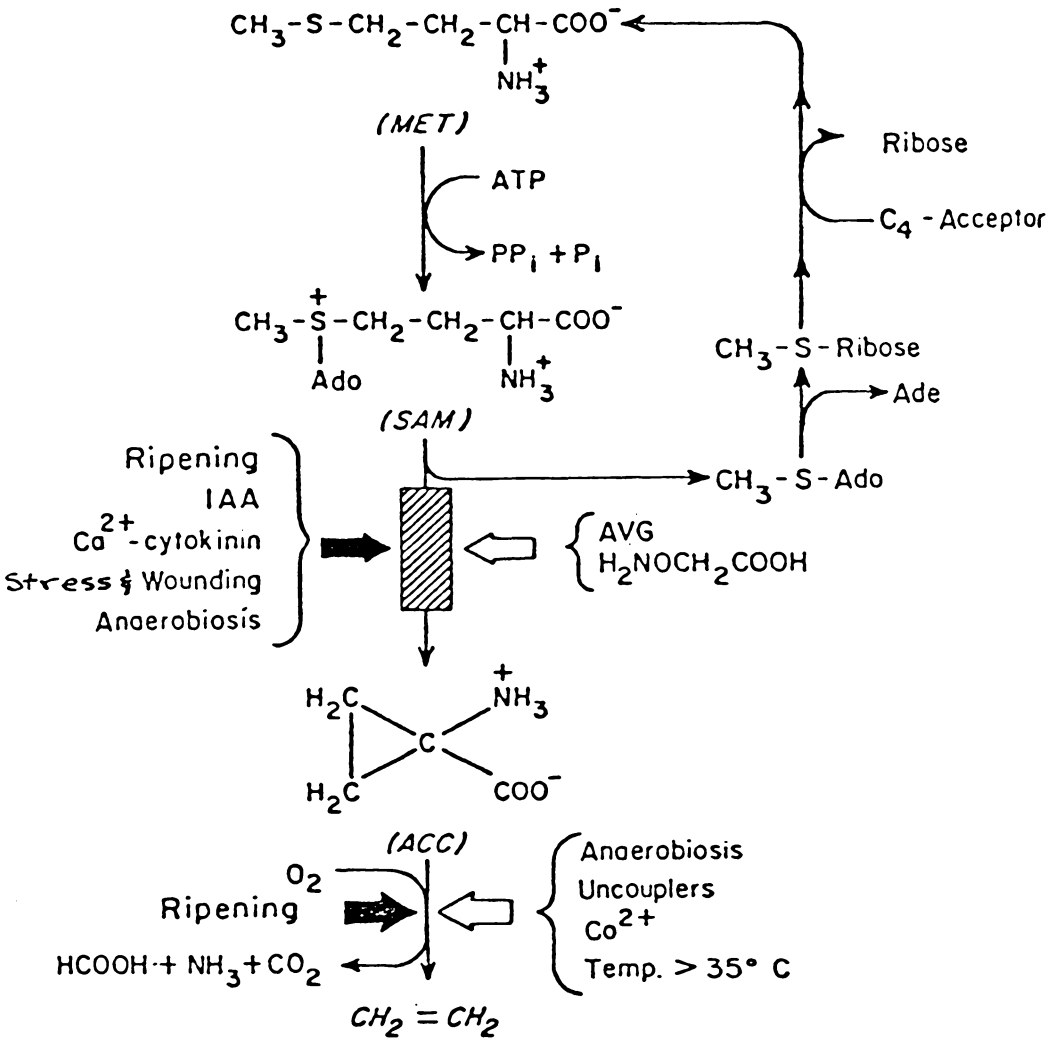

Fig. 1. Regulation of ethylene biosynthesis. ▨ : this reaction is normally supressed and is the rate-limiting step in the pathway; (−); induction of synthesis of the enzyme; (−): Inhibition of the reaction. Met, Ado, and Ade stand for methionine, adenosine, and adenine, respectively.

Yang 1981

Appendix B

GAS CHROMATOGRAPH

Bendix 2500

column: 6' x Poropak Q, 80/100 mesh

carrier gas: helium

ml/min: carrier:40, H:25, air:100

injection port: 110°C detector: 110°C

column conditions: 90°C programmed

chart speed: .5 ipm

detector: FID

sensitivity: recorder attenuation: 0.2

suppression r: 10X

input attenuation: 1X

Varian Model 3700

column: 6' x , Activated Alumina F-1, 80/100 mesh

carrier gas: helium

m/min carrier: 40

injection port: 120°C, detector: 130°C

column conditions: 95°C

chart speed: 1 cm/min

detector: FID

Appendix C

ANALYSIS OF VARIANCE TABLES

Appendix C1. Sources of variation and breakdown of degrees of freedom for Study 1.

Source of Variation	df
Lifting Date (n=5)	4
Plant Part (n=3)	2
Lifting Date * Plant Part	8
Error	15
Corrected Total	29

where Lifting Date= Date seedlings lifted from nursery
Plant Part= Whole, shoots or roots

Appendix C2. Sources of variation and breakdown of degrees of freedom for Study 3.

Sources of Variation	df
Lmet (n=2)	1
Ldate (n=2)	1
Lmet * Ldate	1
Part (n=3)	2
Lmet * Part	2
Ldate * Part	2
Part * Lmet * Ldate	2
Error	12
Corrected Total	23

where Lmet= Lifting Method (hand or machine)
Ldate= Lifting date (Jan. or Mar.)
Part= Whole, shoots or roots

Appendix C3. Sources of variation and breakdown of degrees of freedom for Study 4.

Sources of Variation	df
Blocks (n=4)	3
Treatments (n=6)	5
Blocks * Treatments	15
Error (reps and interactions)	24
Corrected Total	47

Appendix C4. Sources of variation and breakdown of degrees of freedom for Study 5.

Sources of Variation	df
Treatment (n=2)	1
Day (n=7)	6
Treatment * Day	6
Family (n=2)	1
Day * Family	6
Treatment * Day * Family	6
Part (n=2)	1
Treatment * Part	1
Day * Part	6
Treatment * Day * Part	6
Family * Part	1
Treatment * Family * Part	1
Day * Family * Part	6
Error	6
Corrected Total	55

Appendix D

ACC CONVERSION EQUATIONS

1) Conversion efficiency (based on internal standards)

$$\text{eff. factor} = \frac{(\text{pmoles sample} + \text{ACC}) - (\text{pmoles sample})}{1000 \text{ pmole ACC added}}$$

2) Convert ethylene pmoles to ACC pmoles

$$\text{ACC pmoles in incubation tube} = \frac{\text{pmoles sample}}{\text{efficiency factor}}$$

3) Convert to dry weight basis (based on 0.5g sample)

$$\text{ACC pmole/g dry wt} = (\text{ACC pmole})(0.320)$$

Appendix E

DOSE-RESPONSE STUDY SCATTERGRAMS

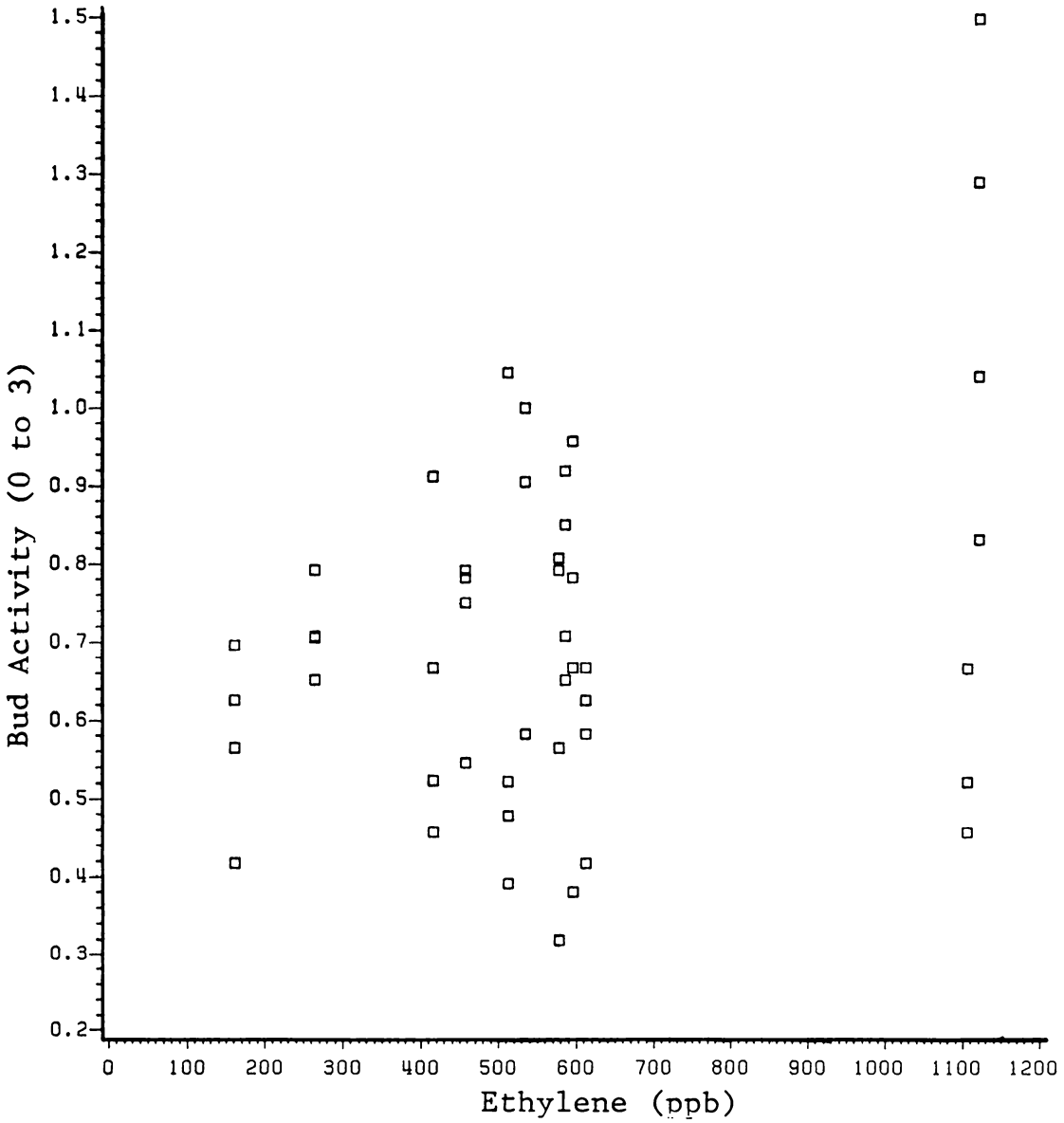

Figure E1. Mean bud activity as affected by mean ethylene concentrations in K-P bags two weeks after fumigations. (5/13/33)

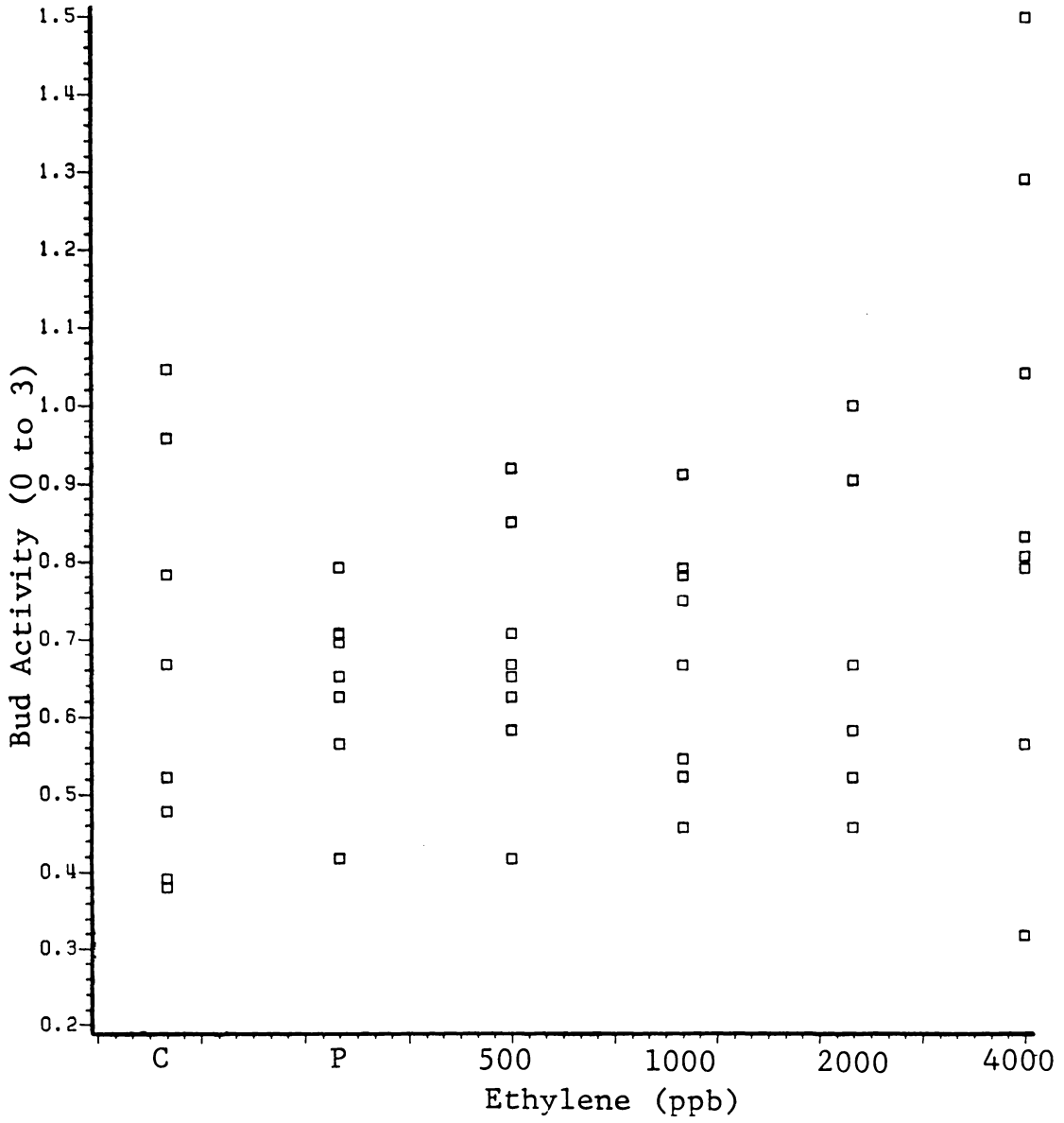

Figure E2. Mean bud activity as affected by given ethylene treatment levels. (5/13/83)

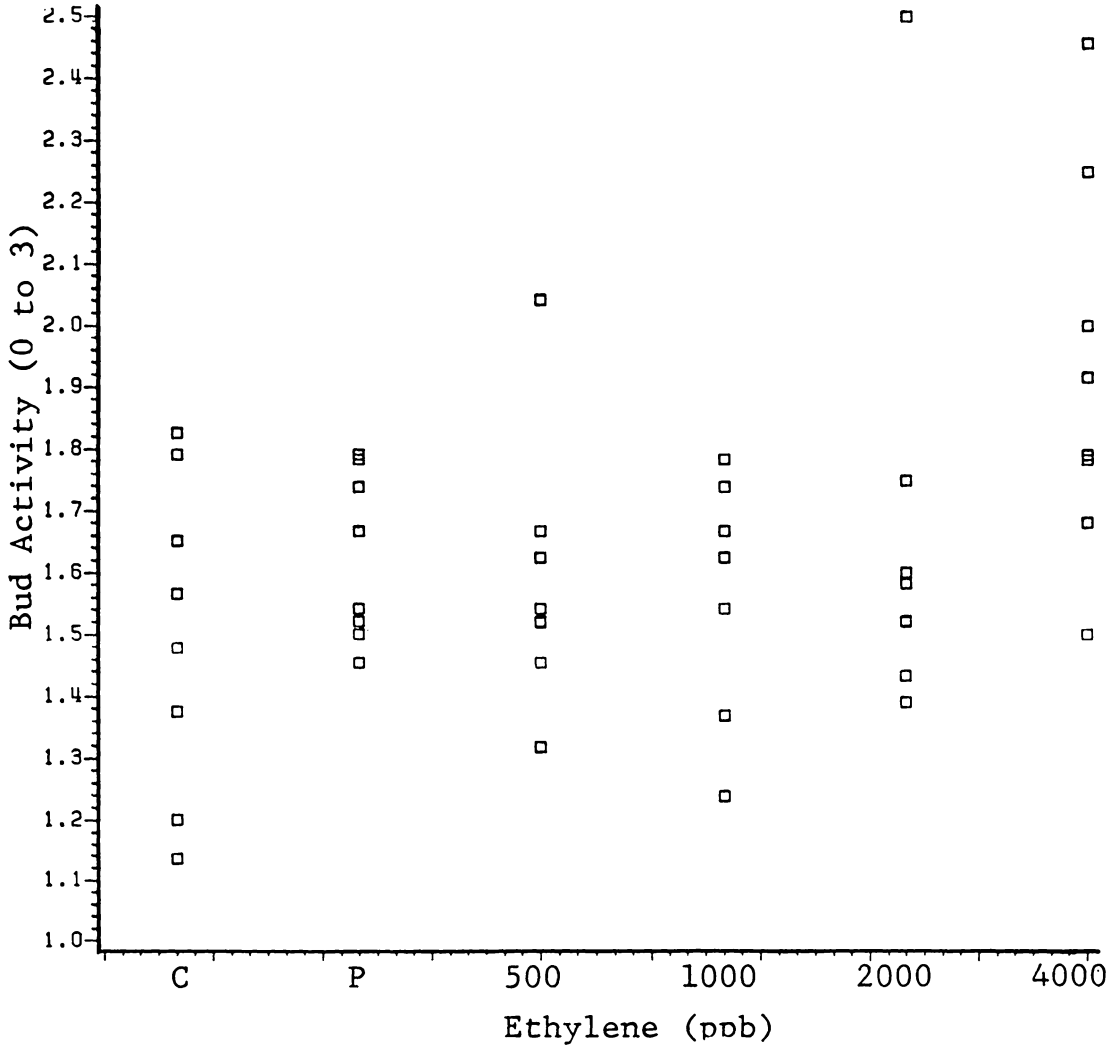

Figure E3. Mean bud activity as affected by given ethylene treatment levels (5/27/83).

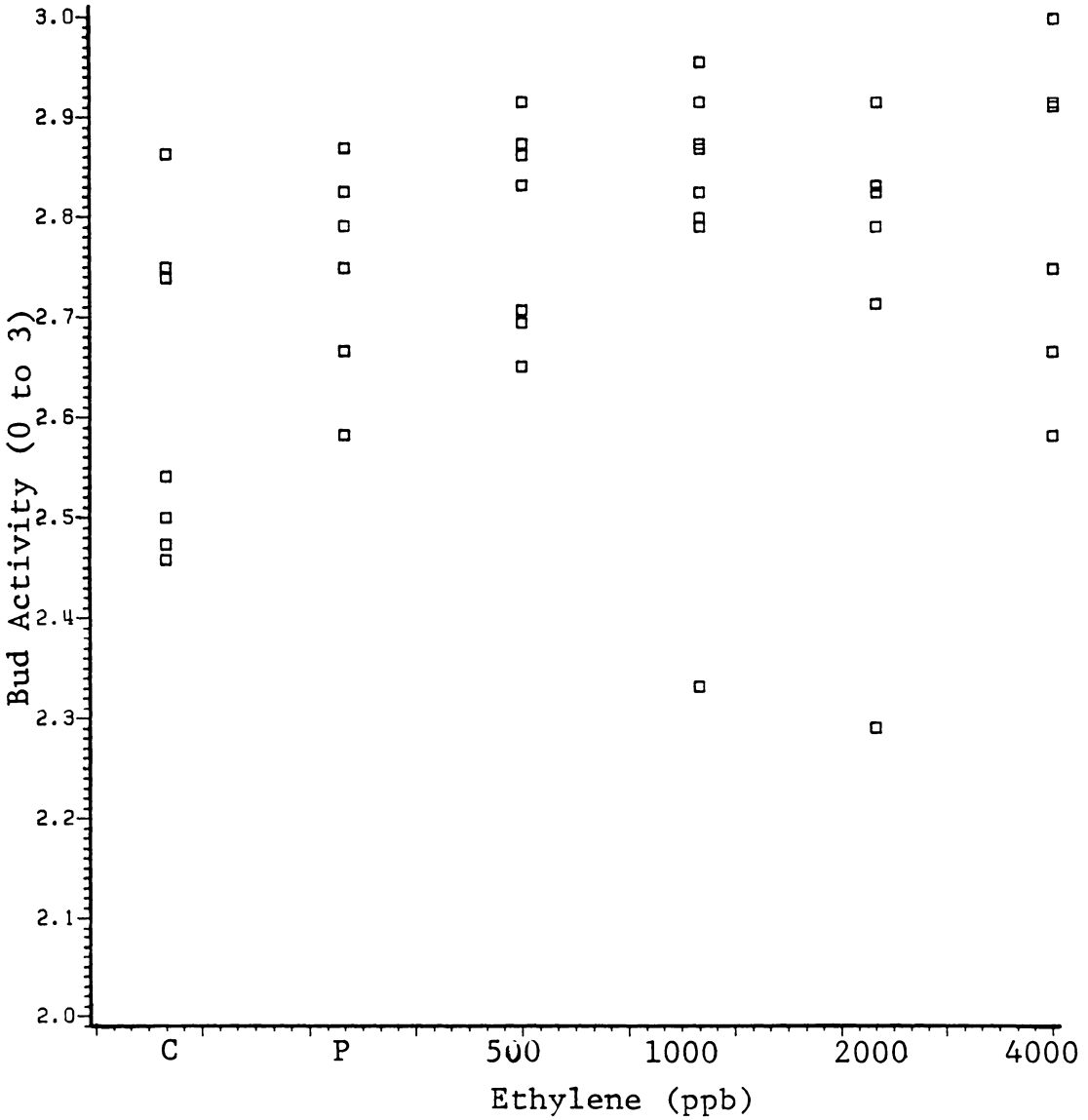

Figure E4. Mean bud activity as affected by given ethylene treatment levels (6/11/83).

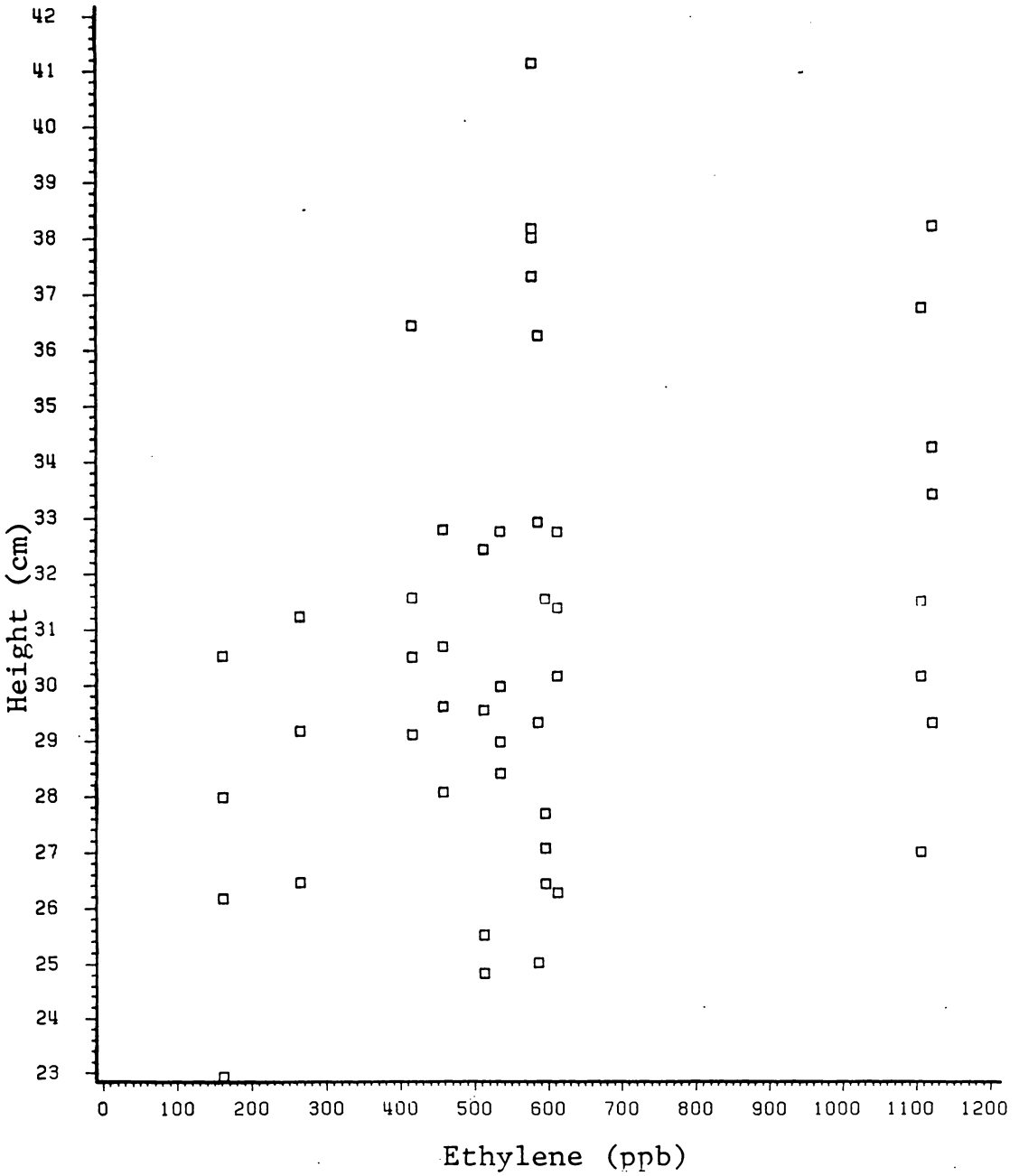

Figure E5. Mean seedling height after one year in the field as affected by mean ethylene concentrations in K-P bags during cold storage.

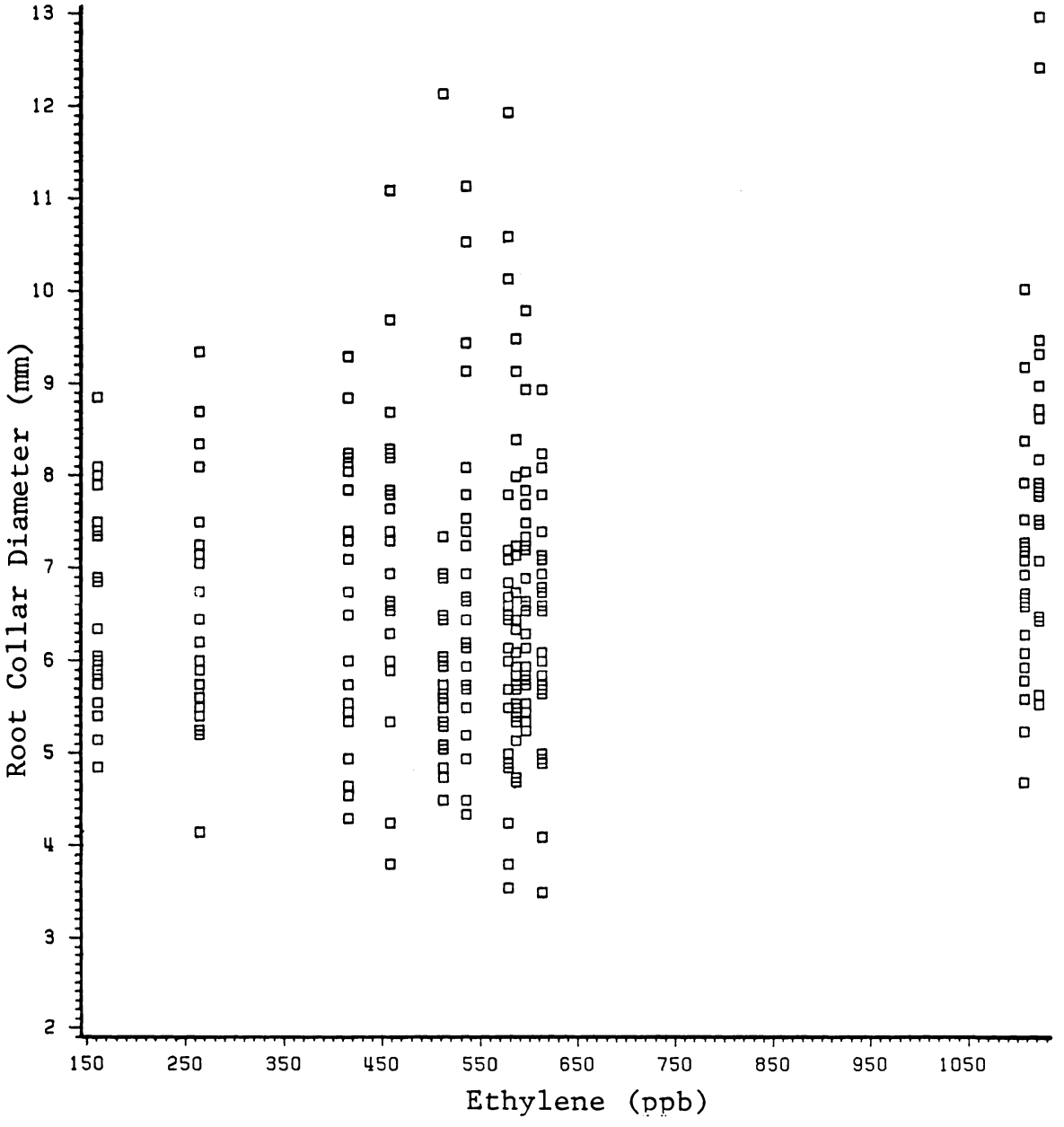

Figure E6. Root collar diameters of seedlings after one year in the field as affected by mean ethylene concentrations in K-P bags during cold storage.

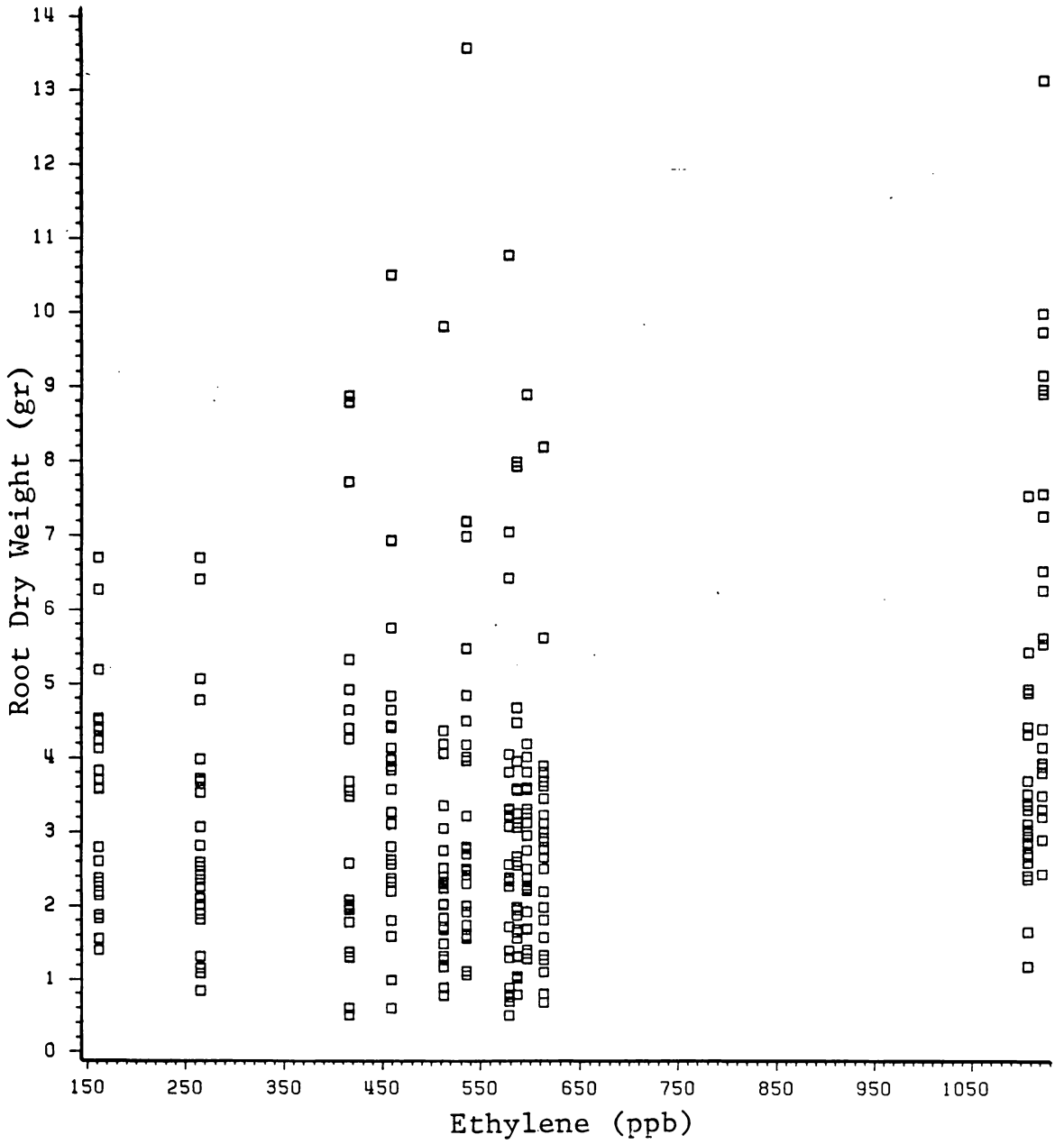

Figure E7. Root dry weights of seedlings after one year in the field as affected by mean ethylene concentrations in K-P bags during cold storage.

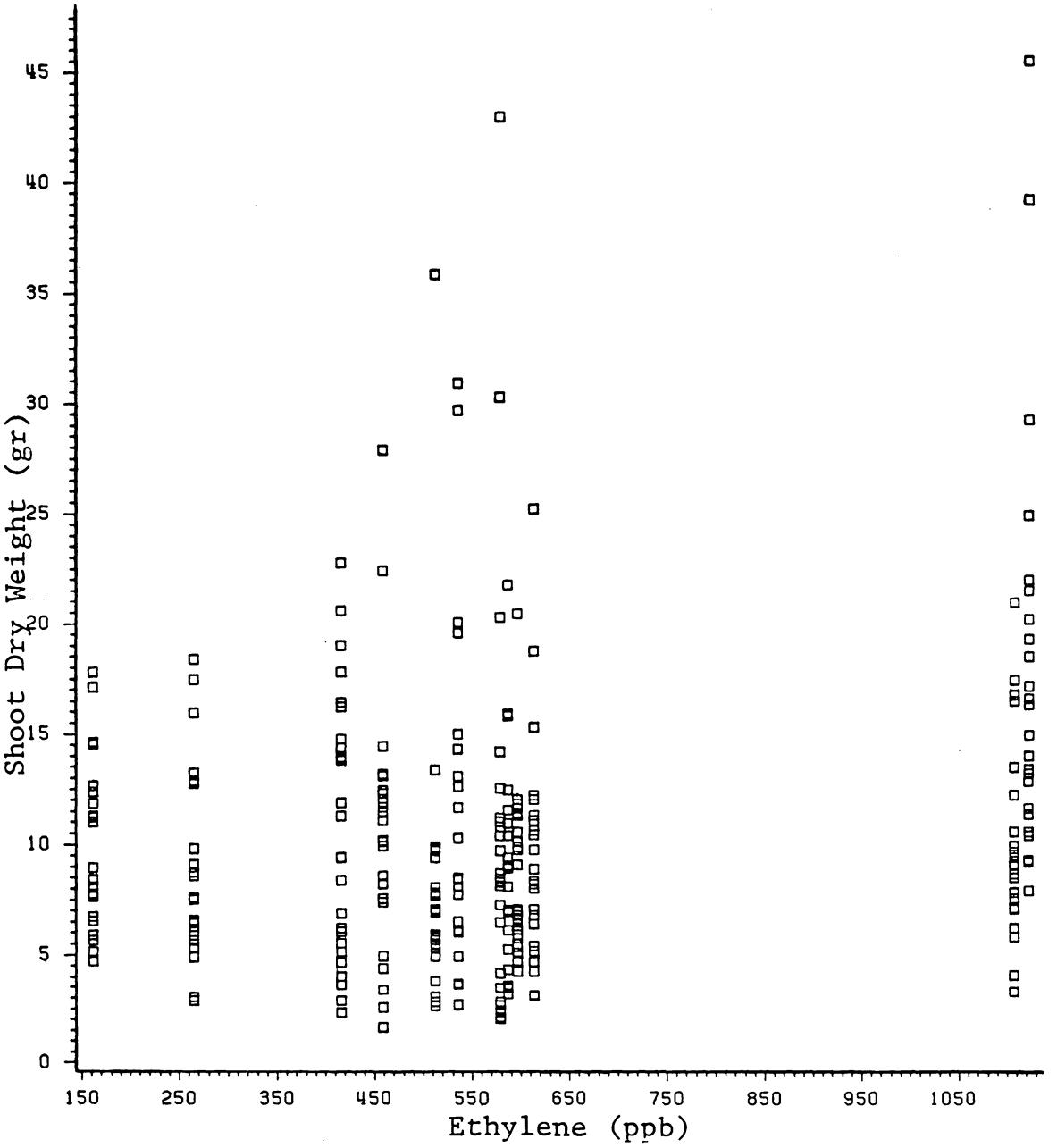

Figure E8. Shoot dry weights of seedlings after one year in the field as affected by mean ethylene concentrations in K-P bags during cold storage.

Appendix F

SIX HOUR PRODUCTION RATES

Appendix F1. Hourly needle and root ethylene production rates by seedlings after six hours of incubation (Study 2).

	Dec		Feb		Mar	
week	shoots	roots	shoots	roots	shoots	roots
	ul/g dry wt/hr					
1	4.34	2.05	7.54	7.57	3.75	2.05
2	2.85	2.46	9.59	3.72	11.28	2.23
3	6.26	1.97	11.84	2.97	15.84	2.19
4	2.87	1.99	3.67	1.50	10.82	2.04

Appendix F2. Needle ethylene production rates of water stressed CP and DH seedlings after six hours of incubation (Study 5).

Fam	0	3	6	9	12	15	18[1]
	ul/g dry wt/hr						
CP	0.20D[2]	0.22CD	0.33B	0.13F	0.14EF	0.41A	0.15EF
DH	0.16E	0.15EF	0.23C	0.14EF	0.13F	0.09G	0.16E

1- Rewatered.
2- Values within the table with the same letter are not significantly different.

Appendix F3. Root ethylene production rates of water stressed CP and DH seedlings after six hours of incubation (Study 5).

Fam	0	3	6	9	12	15	18[1]
	ul/g dry wt/hr						
CP	0.63EF[2]	0.72ED	1.10B	0.38G	0.35G	0.25G	1.09BC
DH	0.89CD	0.43GF	0.91BCD	0.63EF	0.38G	0.35G	1.64A

1- Rewatered.
2- Values within the table with the same letter are not significantuly different (Alpha = 0.05).

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