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THE EFFECTS OF DRESSINGS CONTAINING GROWTH SUBSTANCES ON THE HEALING PROCESSES OF TREE WOUNDS

MICHEL DESSUREAULT

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by

MICHEL DESSUREAULT

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This thesis has been examined and approved.

Thesis director, Avery E. Rich, Prof. of Plant Pathology.

James E. Pollard, Asst. Prof. of Plant Sciences.

Douglas C. Routley, Prof. of Plant Sciences.

Alex L. Shigo, Adjunct Prof. of Plant Pathology.

Terry A. Tattar, Adjunct Asst. Prof. of Plant Pathology.

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ABSTRACT

THE EFFECTS OF DRESSINGS CONTAINING GROWTH SUBSTANCES ON THE HEALING PROCESSES OF TREE WOUNDS

by

MICHEL DESSUREAULT

Better dressings are needed to stimulate the tree's own responses to wounding and improve its chances for successful healing. The purpose of this study was to determine if exogenously applied growth substances could stimulate healing of wounds, thus preventing decay.

A commercial dressing and various growth substances in lanolin were applied to wounds on stems of red and sugar maples, white pines, and American beeches. The trees were dissected after one growing season. Wound closure, bark dieback around the wounds, and the amount of discolored wood were measured. Bacteria and fungi were isolated from the discolored tissues. Experiments were also conducted on apple seedlings.

The ethylene-releasing compound: 2-chloroethylphosphonic acid (CEPA), had no beneficial effects on healing. CEPA in lanolin promoted neither phenol production nor

phenylalanine ammonia-lyase (PAL) activity in apple seedlings unless it was applied daily without lanolin. The commercial dressing had the same effect as leaving the wounds without any dressing. Lanolin favored callus formation and prevented bark dieback, but did not prevent invasion of the discolored tissues by microorganisms. Callus formation was promoted on red maples and beeches by combining indole butyric acid (IBA) with gibberellic acid (GA) in a 5:2 ratio. On white pine, callus formation was promoted by adding benzyladenine (BA) to IBA and GA. Faster callus formation was generally not accompanied by increased resistance to invasion by microorganisms. Sugar maples did not respond to any of the growth substances. Indole acetic acid (IAA) prevented invasion of the discolored tissues by fungi, but inhibited callus formation.

Auxins and GA or BA applied together promoted callus formation but their practicability is restricted: their effectiveness is a function of many variables; they do not prevent invasion of the wood by microorganisms; and they are expensive. Auxins applied alone probably enhanced resistance to invasion by fungi by stimulating natural defense reactions. Although auxins used alone inhibit callus formation, they could be very useful in regulating the decay process in living trees.

INTRODUCTION

Wood decay in living trees is a major problem. It is the single largest source of losses through reduced growth, volume, and quality. Decay shortens the life of a tree and decreases its resistance to other diseases. A decayed tree is a weakened structure. It represents a potential hazard in populated areas as it may break under high winds or winter ice storms.

Decay results generally from wounds. Tree wounds concerned men as far back as 2000 B.C. About 100 years ago, Hartig was probably the first one to link decay to fungi and wounds. Since then, the concept of decay was expanded. Wounds still remain the most important factor in initiating the decay process.

Wound treatment is practiced to alleviate the adverse effects of wounds. The basic components of wound treatment consist in cleaning and shaping the wound, applying a wound dressing, and keeping the tree vigorous. Wound dressings are used for two reasons: to prevent decay and to promote rapid closure of the wound.

Conventional wound dressings have no beneficial effects and thus are practically worthless. Despite their continuous use, it appears that their effectiveness was never investigated. During the past five years, studies have indicated that commonly used wound dressings neither promote

callus formation nor prevent invasion of the wood by micro-organisms.

More effective wound dressings are needed. Whether it is from fire, snow, wind, animals, or men's activities, most trees are wounded at one time or another during their life. Although wound treatment is not practiced on forest trees, it could become important in the future. In view of the increasing wood shortage, better methods for regulating decay are needed.

Wound treatment has long been practiced on shade trees and probably this practice will increase. Because green spaces around cities are growing smaller, and awareness and concern for the environment are increasing, shade trees are becoming more valuable sentimentally as well as monetarily. At the same time, shade trees are subjected to increasing pressures from people.

Since trees have defense and repair mechanisms the ideal wound dressing should stimulate these mechanisms to prevent invasion of the wood by mieroorganisms, thus reducing the chances for the occurrence of decay. In addition, it should promote callus formation to close the wound quickly and further protect the injured tissues. It should also be inexpensive, easy to apply, long lasting, and able to withstand a broad range of weather conditions without drying or cracking.

Past studies on wound dressings were limited to an external evaluation of the dressings. The amount of callus tissues formed was measured and that process was called wound healing. I submit that the term wound healing should encompass all processes including callus formation, defense reactions, and possibly other processes initiated at the time of wounding.

The objectives of this investigation were 1) to determine if exogenously applied ethylene could stimulate defense mechanisms, 2) to determine if combinations of growth substances or other compounds could stimulate callus formation and other wound responses better than auxins alone. More precisely, this study was undertaken to determine the effects of growth substances on callus formation, bark dieback around the wounds, discoloration of the wood, and invasion of the discolored tissues by microorganisms.

REVIEW OF LITERATURE

Wound Dressings: Past and Present Situation

Wound dressings have a long history. Bartlett (6) has extensively reviewed the historical background of tree wound treatment. Today, many different types of dressings are available. Asphalt-based paints, emulsions, and aerosol sprays are among the most popular and most commonly used (41, 49).

Until now, the concept of wound dressing for shade trees has been practically limited to the idea of protection against microorganisms. Protection has been attempted by applying to wounds materials supposedly acting as physical or chemical barriers against microorganisms. The tree's own responses to wounding were totally discounted. The most recent research in this area is still exclusively oriented toward products providing an effective physical (5,50) or chemical (30) protection against microorganisms as was sought in the past.

Based on recent findings, Shigo and Wilson (72) have seriously questioned the usefulness of common wound dressings. Neely (48) showed that neither asphalt nor shellac promotes callus formation. Shigo and Wilson (73) reported that neither of these dressings prevented discoloration or had any effect on invasion of the discolored tissues by pioneer microorganisms.

Wound Dressings: Future Outlook

Insisting on the fact that trees have natural defense and repair mechanisms that are often successful, Shigo and Wilson (72) have stressed the need for research aimed at finding ways to help the tree help itself. One way they pointed out would be to use materials that could stimulate existing mechanisms in the tree. Future research should also be concerned less by the external aspects of wounds and more by what happens inside the tree. It is inside the tree that the final outcome: either successful healing or decay, is determined.

Decay Process

Decay is only the end product of a long process which may stop at any stage (71). Succession of microorganisms plays an important role in that process. Of all the microorganisms present at the surface of the wound, only a few bacteria and fungi can penetrate the discolored wood. After these pioneers have changed the substrate, probably detoxifying chemical inhibitors present in these tissues (63,75), and only afterward, can wood-rotting fungi establish themselves in the discolored tissues.

Wounds do not always lead to decay. Coupled with the inability of decay fungi to successfully establish themselves on freshly wounded tissues, wound responses finally make the difference between successful healing or decay. A vigorous tree reacts rapidly and effectively to prevent microorganisms

from penetrating uninjured tissues, and the wound closes quickly. Even if a tree is vigorous, factors such as the severity of the wound or the time of the year the wound occurs reduce its chances for successful healing. Defense mechanisms are overcome, callus formation is delayed, and decay results. The decay process in living trees has been reviewed by Shigo and Hillis (70).

Wound Responses

As wounding occurs, cells are broken and their content is exposed to the air. Phenols are oxidized causing the brown discoloration characteristic of injured plant tissues (57). The oxidized phenols prevent or retard the growth of most microorganisms invading the wounded tissues (25,38).

When microorganisms begin to penetrate into the injured tissues, the tree responds to prevent further invasion. Phenol content and enzyme activity increase in tissues beneath and around the wound (64). This is the critical period while the enzyme systems of the tree succeed or fail in overcoming the enzyme systems of invading microorganisms.

The "protection zone" is then laid down and the affected tissues are compartmentalized. Vessels above and below the wound are occluded with plugs (54). Modified xylary tissues containing thick-walled parenchyma cells also occluded with pigmented materials develop toward the outside of the tree (54,66). These wound-associated tissues are

referred to collectively as "protection zone" (70) or "reaction zone" (36,61,62). They form a barrier between the affected tissues and the tissues formed after wounding.

The affected tissues are compartmentalized vertically by the plugged vessels and radially by the "protection zone". Compartmentalization is practically always effective horizontally, between the affected tissues and the tissues formed after wounding. It is the weakest along the vertical axis (68,69).

Finally, repair mechanisms are initiated to close the wound and seal it off permanently from the outside environment. Callus tissues develop almost exclusively along the vertical edges of the wound (48) and grow inward toward the center of the wound.

Previous Attempts to Stimulate Callus Formation

Forest and Shade Trees

Audus (3) has reviewed the early works relating to the application of growth substances on tree wounds. All previous attempts to stimulate callus formation on stems of trees have been unsuccessful.

Tilford (79) reported that a lanolin dressing containing 0.2% indole butyric acid (IBA) was slightly injurious on red maple, white oak, American elm, and hickory. Davis (20) tested various auxins, 0.01%-20% in a talc carrier, on sugar maple. The auxins included indole acetic acid (IAA),

2-chlorophenoxy proprionic acid, and 2,4-dichlorophenoxy acetic acid (2,4-D). Davis (21) also tested IBA, naphthalene acetic acid (NAA), and 2-chlorophenoxy acetic acid (2-CPA) at 0.1%, 1%, and 5% in lanolin. Callus formation was not promoted by any auxin. IBA and NAA inhibited callus formation at all concentrations and caused some bark dieback at the highest concentrations. Davis (20,21) reported that cysteine and glutathione stimulated callus formation. He suggested that compounds with sulfhydryl groups could have a stimulatory effect on callus formation. McQuilkin (43) tested IAA, IBA, NAA, and various other compounds at 0.1%-4%. He used many different carriers and treated wounds on stems of red maple, black gum, beech, white and scarlet oak, pitch and Virginia pine. Auxins failed to promote callus formation. McQuilkin (43) recognized the beneficial effect of lanolin on callus formation.

Fruit Trees

Many workers (17,35,67) have reported enhanced callus formation after applying auxins to pruning wounds on various fruit trees. Most recently, Lavee and Haskal (39) showed that IBA or IAA, when applied to pruning wounds, was particularly effective in promoting callus formation on many species of fruit trees. IBA was equally effective at 0.25% to 2%. Zinc oxide, 2%, alone or in combination with auxins promoted callus formation. They reported that gibberellic acid (GA) did not promote callus formation. They also reported that 200

ug/g kinetin in combination with either IAA or IBA inhibited callus formation or had no effect. Samish, Tamir, and Spiegel (58) reported that cysteine stimulated callus formation when it was applied to pruning wounds on apple trees.

Roles of Growth Substances and Other Compounds in Cambial Activity

Many workers (7,8,34) have reached the conclusion that auxins play an important role in the regeneration and repair of the tissues altered by wounding. More recent research into the regulatory mechanisms of cambial activity and xylem differentiation in higher plants indicates that not only auxins, but gibberellins (4,46,55,82) and possibly cytokinins (19,45,55,56,80) play a role in these growth processes.

Auxins appear to be primarily responsible for xylogenesis while the effect of gibberellins is limited to the production of cambial derivatives (46,74). Not only the presence of both auxins and gibberellins seems necessary, but the ratio between them is important for normal growth and development (81,84). New tissues initiated in dormant shoots resembled most normal growth when IAA and GA were used in a 5:1 ratio (22).

Although sucrose may not directly regulate xylem formation, it could be a limiting factor due to its importance as substrate (80). The addition of sucrose enhanced the effect of auxins on cambial activity in pine (85) and

willow (55) tissues. It has been reported (37) that ascorbic acid, 100 ug/ml, may act as a growth substance in promoting growth.

Roles of Ethylene in Defense Reactions

Although ethylene may not be the key trigger to disease resistance in plants (59), it is often associated with increased resistance (64,76). Ethylene is considered as a plant hormone although its role and mode of action are not fully understood (27,40). Ethylene is produced by healthy plant tissues (9) but is more characteristic of stressed, wounded, or infected plants where its production increases drastically (51).

The increase in ethylene production coupled with higher phenol content and enhanced activity of enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase, and polyphenoloxidase (PPO) is now considered a characteristic plant response to injuries and diseases (27).

All the evidence indicates that ethylene exerts a regulatory role on the synthesis, either directly (23) or indirectly (33), and possibly on the activity (13) of PAL. This is the key enzyme for the production of phenols in most plants.

Exogenously applied ethylene stimulated PAL activity in swede and parsnip root tissues (52), in pea seedlings (31, 32), and in carrot roots (13). In all cases, inhibitors of protein synthesis prevented the effect of ethylene, and the

increase in enzyme activity was attributed to de novo enzyme synthesis. Similar results were obtained in excised tissues (23).

Ethylene may also have a regulatory role on other enzymes involved in resistance mechanisms. Enhanced peroxidase (26,65,76) and PPO (76) activity due to ethylene has been reported. Ethylene also causes an increase of the phenol content (14,53). Phenols play an important role in resistance by inhibiting the growth of microorganisms (16, 29,61,62,63,75,77).

In summary, all attempts to stimulate callus formation on forest and shade trees have failed, but only auxins were tested. Gibberellins, cytokinins, and possibly other compounds could enhance the action of auxins on cambial activity thus promoting callus formation. All previous investigations have been limited to the effects of auxins on callus formation. The effects of growth substances on other wound responses and on the decay process are not known. Ethylene apparently was never applied to wounds. It could stimulate defense mechanisms and increase resistance to invasion by microorganisms.

MATERIALS AND METHODS

Wound Dressing Trials in the Greenhouse

Wound Dressing Preparation

The compounds tested were incorporated in lanolin. Each compound was first dissolved in the appropriate mixture of alcohol and water. Just enough solvent to obtain complete dissolution was used. CEPA was diluted in propylene glycol. The solutions were stirred in anhydrous lanolin (U.S.P. grade) liquefied by preheating to approximately 45 C. The solvent was then evaporated under vacuum until the lanolin had returned to its original consistency. The dressings were used within a few days and stirred immediately before they were applied to the wounds. The dressings tested either in the greenhouse or in the field were prepared the same way.

Plant Material and Growing Conditions

Actively growing McIntosh apple seedlings were used for the trials in the greenhouse. The seedlings were grown in six-inch pots at approximately 24 C day (14 hr), and 15 C night (10 hr). The seedlings were two to three-months old, approximately 0.6-1.0 m in height, and 5-10 mm in diam at the largest point on the stem. Seedlings of approximately the same height and stem diameter were chosen for each trial.

Wounding Procedure

The seedlings were wounded on the lower portion of the stem by removing a segment of bark and xylem tissues with a tool designed for that purpose. The tool consisted of two razor blades fixed to a wooden block approximately 1.3 X 1.3 X 5 cm. The distance separating the blades was 1.5 cm. It corresponded to the length of the wound on the longitudinal axis of the stem. Unless otherwise stated, all wounds were 1.5 cm long. The cutting edges protruded approximately 1.5 mm from the surface of the block. That distance corresponded to the depth of the wound on the radial axis of the stem.

Rating System

The bases for evaluating the effects of the dressings in the greenhouse trials were 1) the closing period or the period of time elapsed until callus tissues completely closed the wounds, and 2) the external appearance of the callus tissues formed compared to callus tissues formed over nontreated wounds. The wounds were examined at least every two or three days until completion of the trial period.

Wound Dressing Trials on the Field

Trial of 2-Chloroethylphosphonic Acid (CEPA) on Red Maple

Wounds on red maples (Acer rubrum L.) growing at the Massabesic Experimental Forest in Alfred, Maine, were treated with dressings containing various concentrations of CEPA.

The trees, 10-15 cm in diam at 1.4 m aboveground, were wounded on 13 July 1972. The wounds were elliptical with pointed tips. Large, 5 X 10 cm, and small, 2.5 X 5 cm, wounds were made. The longer axis of the wounds was parallel to the longitudinal axis of the stem. The wounds were made by placing a metal template on the stem and cutting the bark around it with a knife. The bark was cut to the cambium and removed. The exposed wood was scraped. Approximately 7 cc and 2 cc of the lanolin dressings were applied to the large and small wounds, respectively.

Each tree received groups of two wounds at two levels on the stem. The lowest wounds were made at 0.6 m and 0.9 m and the highest ones at 1.2 m and 1.5 m aboveground. At each level, a large wound faced either East or West and a small wound faced in the opposite direction at 30 cm below or above it. Wounds on the same tree received the same dressing and each dressing was applied on two trees. The trees were harvested from 7 to 21 August 1973.

Trial of Growth Substances on Red Maple

Four red maples (Acer rubrum L.), 11.5-18.2 cm in diam at 1.4 m aboveground, growing at the Massabesic Experimental Forest, were wounded at the time of leafing out, on 1 May 1973. Each tree received 16 wounds. The wounds, 1.5 cm in diam and about 1 cm deep, were made with a drill. Wounds were made at 20 cm intervals beginning at 0.3 m up to 1.7 m aboveground. At each height, two wounds were opposite

each other. Each alternate group of wounds was made in a direction perpendicular to the preceding group so that wounds facing the same direction were at 40 cm intervals. The wounds were assigned dressings at random and treated immediately after wounding by filling the cavity with a dressing, about 2 cc. A different dressing was applied to each wound on a tree and each dressing was applied on four trees. The trees were harvested three months later.

Trial of Growth Substances on White Pine, American Beech, Sugar Maple, and Red Maple

Three white pines (Pinus strobus L.), 4.5-5.8 cm, three American beeches (Fagus grandifolia Ehrh.), 5.5-6.9 cm, eight sugar maples (Acer saccharum Marsh.), 5.7-10.0 cm, and one red maple (Acer rubrum L.), 10.0 cm in diam at 1.4 m aboveground, were selected in the College Woods at the Experimental Forest of the University of New Hampshire, in Durham.

The trees were wounded by removing a disc of bark, 1.5 cm in diam, with a plug cutter. The exposed wood was scraped and a chisel, 5 mm wide, was driven into the wood to a depth of approximately 2 mm. Each tree received 16 wounds disposed as described above for the red maples. In this trial, only eight dressings were tested. The dressings were assigned at random to eight groups of two wounds. At each height, the two wounds facing opposite directions received the same dressing. Within each group, one wound at random was treated immediately after wounding, and the other, two weeks later

by filling the cavity with the dressing, about 1 cc.

The beeches, white pines, two sugar maples, and one red maple were wounded at the time of leafing out, on May 8. Three sugar maples were wounded about one month before, on March 30, and three more, one month after the time of leafing out, on 8 June 1973. The trees were harvested seven months after wounding.

Wound Dressings Evaluation

When the trial period was over, the trees were cut in sections and brought to the laboratory. The condition of the dressings and of the wounds was noted. The wounds were cleaned, and the size of exposed wood was measured horizontally at its largest point. The sections were then split longitudinally through the wound with a disinfested axe to expose the discolored wood.

Isolations of the microorganisms were carried out in a clean room by excising chips, approximately 3 X 10 mm, with a sterile gouge. The chips were placed in an upright position in petri dishes containing a growing medium. The medium consisted of 10 g malt extract, 2 g yeast extract, and 20 g agar/liter. The plates were incubated in the dark at 25 C, and examined at three to four-day intervals for at least 20 days. The presence of either bacteria or fungi was recorded.

For the CEPA trial, six chips were taken throughout the discolored tissues behind each wound. For the trial of

growth substances on red maple, one chip was taken behind the wounds, one above, and one below the wounds: at mid-distance between the wounds and the tips of the columns of discolored tissues. For the trial on white pine, American beech, red maple, and sugar maple, two more chips were taken at the extremities of the columns.

The length of the columns was measured. The thickness of the columns behind the wounds was measured only for the CEPA trial. The amount of dead bark above and below the wounds was measured along the longitudinal axis of the stem. When vigor was considered, the average growth in diam for the last five years was measured at 1.4 m aboveground. Readings were taken at four positions equally spaced around the stems.

Laboratory Experiments

Total Phenol Determination

Sample preparation. A 2.5-cm long portion of stem containing the wound was cut. The bark and the wood were separated, oven-dried overnight at 105 C, and ground separately in a Wiley mill to pass a 20-mesh screen.

Extraction. Approximately 0.3-0.5 g of wood or 0.1 g of bark was weighed and placed in a 10-ml beaker. Cold methanol, 10 ml, was added and the solution was stirred for 5 hr. Some methanol was added periodically to replace that lost by evaporation. The solvent was collected and combined with two additional methanol washings. The combined extract was

centrifuged at 750 X g for 20 min in a clinical centrifuge and the volume was made up to 10 ml. Portions of the extracts were used for the spectrophotometric assay that was run the same day.

Spectrophotometric assay. An aliquot, 0.5 ml or less, was placed in a test tube, diluted to 3.5 ml with distilled-deionized water, and mixed. The Folin-Ciocalteu reagent, 2 N, was diluted with distilled-deionized water in a 1:1 ratio. The diluted reagent, 0.5 ml, was added to the diluted extract and mixed. After 3 min, 1 ml of Na_2CO_3 solution, 14% w/v in water, was added and mixed. The absorbance was read 1 hr later against a reagent blank at 725 nm. The results were compared each time to a gallic acid standard.

Phenylalanine Ammonia-Lyase (PAL) Activity

Enzyme extraction. The wounds were scraped and wiped with methanol to remove the dressings. A 40-cm long portion of stem containing the 30-cm long wound was cut and the bark was removed. The stem portion was weighed and cut in small pieces. The tissues, approximately 1 g, and polyvinyl-pyrrolidone (PVP), 1.5 g/gram of tissues, were placed in 10 ml of 0.1 M Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer at pH 8.8, containing 5 mM 2-mercaptoethanol. The tissues were homogenized in an ice bath with a Sorvall homogenizer for 1.5 min. The extract was passed through cheesecloth. The filtrate was used to rinse out the tissues left in the homogenizer, and passed through cheesecloth a second

time. The filtrate was centrifuged at 27,000 X g for 20 min. The supernatant was used for the enzyme assay.

Enzyme assay. The reaction mixture contained 1.5 ml of enzyme extract, 1 ml of 0.5 M Tris buffer at pH 8.8, and 0.2 ml of 0.1 M L-phenylalanine in 0.1 M Tris buffer at pH 8.8. The reaction was carried out in three different test tubes placed in a water bath at 30 C. The reaction was stopped after 20, 40, and 60 min by adding 0.3 ml of 5 N HCl. The increase in absorbance was measured at 290 nm against a blank containing buffer instead of substrate. The amount of protein in the extract was determined by Lowry's technique (42).

Chromatography of Extractable Phenols

Extraction. The tissues were ground with a Wiley mill to pass a 20-mesh screen. Each sample, exactly 2.0 g, was extracted in 250 ml cold water for 12 hr with a magnetic stirrer. The water extracts were filtered through Whatman no. 40 cellulose paper and extracted with 100 ml ethyl acetate by stirring for 1 hr. The water layer was removed in a separatory funnel, acidified with 1 N HCl to pH 2, and placed in a boiling water bath for 1 hr. The acidified water extract was extracted with ethyl acetate and separated as before. Ethyl acetate extracts were concentrated to 5 ml at about 50 C in a rotary evaporator.

Chromatography. Portions, 50 and 100 uliters, of the non-hydrolysed and hydrolysed extracts were chromatographed on cellulose thin-layer chromatography plates with butanol:

acetic acid:water 6:1:2 in the first direction and 7% acetic acid in water (v/v):0.03% sodium acetate in water (w/v) in the second direction. After drying at room temperature, the plates were examined under ultraviolet light after exposure to NH_3.

RESULTS

Ethylene

Greenhouse Trial

Callus formation. CEPA in lanolin did not affect callus formation, or inhibited it, on apple seedlings (Table 1). Wounds dressed with lanolin closed faster than nontreated wounds. CEPA, 1,000 ug/g, caused some swelling above the wounds. CEPA, 10,000 ug/g caused even more swelling, and the stems became flattened at the wounding site.

Laboratory Experiments

Total phenol content. CEPA in lanolin did not affect the phenol content of the wood, or the bark, in the vicinity of wounds on apple seedlings (Table 2). Phenol content of the wood did not change upon wounding as it did in bark where it doubled.

Phenylalanine ammonia-lyase (PAL) activity. CEPA in lanolin, 0-500 ug/g, did not significantly affect PAL activity in woody tissues beneath the wounds. PAL activity was not significantly different at 2, 5, or 10 days after wounding (Fig. 1). CEPA in propylene glycol, 10 ug/ml, slightly increased PAL activity when it was applied daily. Below that concentration, PAL activity decreased; and it was clearly inhibited above 1,000 ug/ml CEPA (Fig. 2).

Table 1. Effect of lanolin dressings containing 2-chloroethylphosphonic acid (CEPA) on callus formation over wounds on the stems of apple seedlings.

Dressings[a]	Rate (ug/g)	Closing period[b] (days)
None	———	84
Lanolin	———	40
CEPA	10	164+
CEPA	100	113
CEPA	1,000	70[c]
CEPA	10,000	164+[d]

[a]CEPA was incorporated in lanolin.

[b]Period of time elapsed until callus tissues completely closed the wounds. Means of 2 replications.

[c]Swelling of the stems was noted above the wounds.

[d]Much swelling was noted above the wounds and the stems were flattened at the level of the wounds.

Table 2. Effect of lanolin dressings containing 2-chloroethylphosphonic acid (CEPA) on the phenol content of tissues proximal to wounds on the stems of apple seedlings.

Tissues	Days after wounding	Total phenol content (mg/g dry wt)[a]						
		No wound	No dressing	CEPA (ug/g)				
				0	10	100	1,000	10,000
Wood[b]	—	6.5	—	—	—	—	—	—
	2	—	7.8	5.3	6.9	8.7	6.9	5.4
	10	—	7.2	4.5	5.3	4.6	6.3	5.9
Bark[c]	—	35.3	—	—	—	—	—	—
	1	—	72.5	79.6	75.7	61.9	69.1	76.6
	3	—	57.6	83.3	63.6	64.5	69.1	72.0

[a]Means of 2 replications.

[b]LSD (P=0.05) = 3.2 mg/g.

[c]LSD (P=0.05) = 18.1 mg/g.

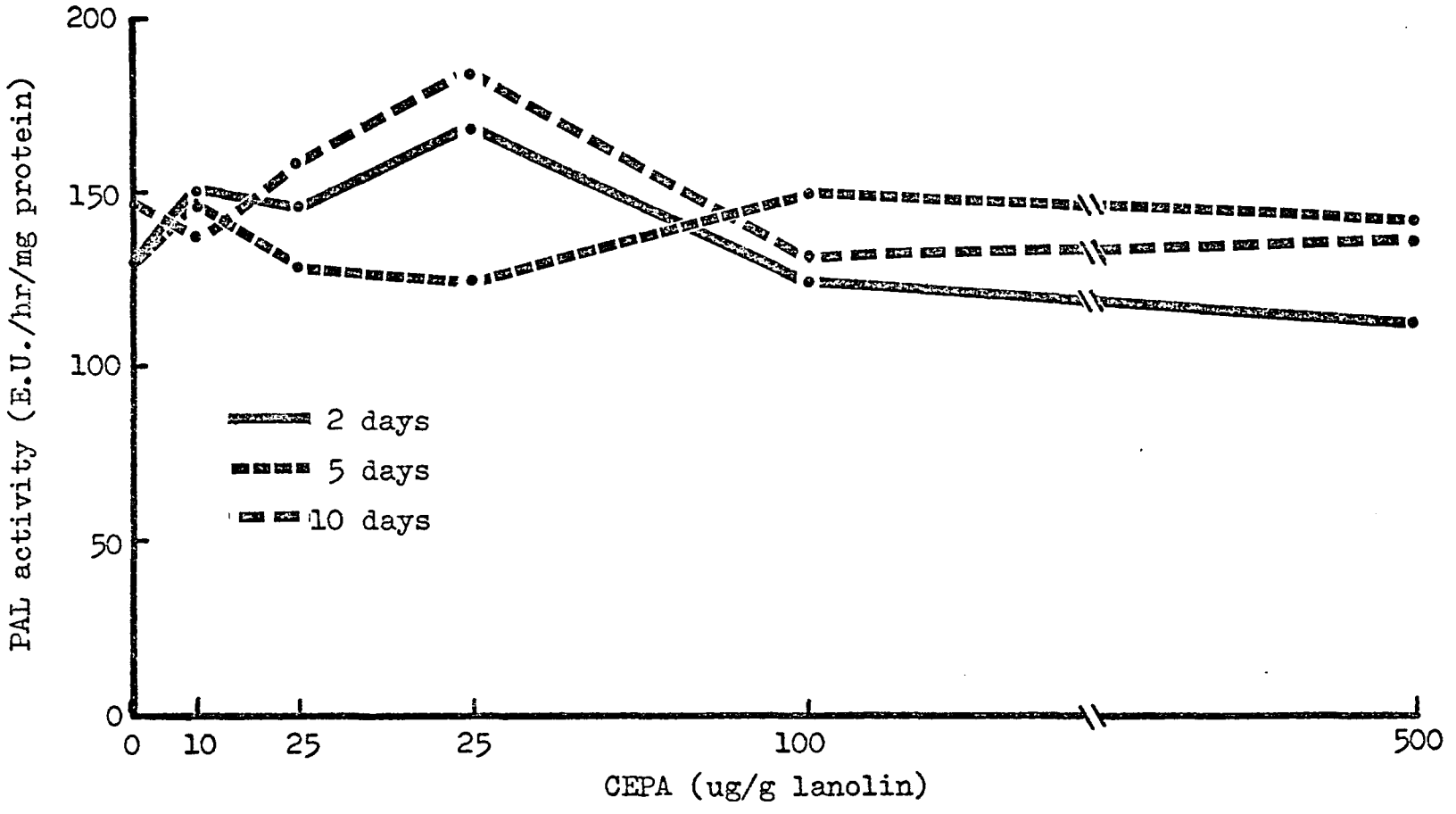


Fig. 1. Phenylalanine ammonia-lyase (PAL) activity in woody tissues proximal to wounds on stems of apple seedlings 2, 5, and 10 days after applying 2-chloroethylphosphonic acid (CEPA) in lanolin. Each point represents the mean of 3 replications. LSD (P=0.05) = 55 E.U./hr/mg protein.

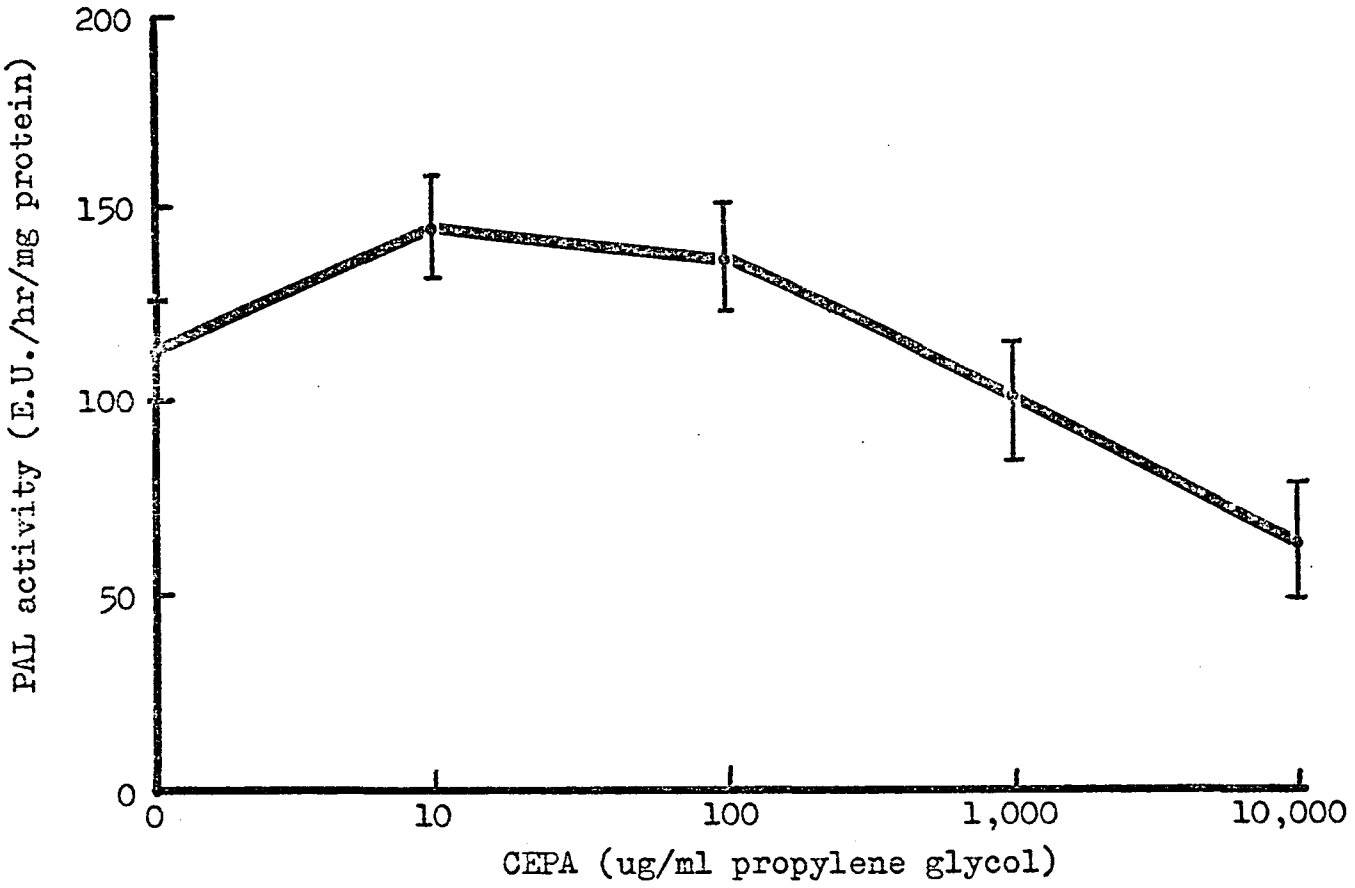


Fig. 2. Phenylalanine ammonia-lyase (PAL) activity in woody tissues proximal to wounds on stems of apple seedlings following daily applications of 2-chloroethylphosphonic acid (CEPA) in propylene glycol. Each point represents the mean of 3 replications at 2, 5, and 10 days after wounding. LSD (P=0.05) = 27 E.U./hr/mg protein.

Field Trial

Callus formation. Under field conditions, 100-10,000 ug/g CEPA in lanolin significantly inhibited callus formation on red maples (Table 3). Large wounds appeared to be more affected than small wounds. Although there was a large variation between trees, wounds dressed with lanolin alone consistently closed faster than nontreated wounds, or than wounds treated with CEPA. The size of the wound had no significant effect on callus formation although large wounds may have closed faster than small wounds. A significant interaction between trees and dressings indicated that some trees responded differently to some dressings.

Discoloration of the wood. CEPA in lanolin had little effect on the length or the thickness of the columns of discolored wood. Highest CEPA concentrations tended to increase the length and the thickness of the columns on large wounds (Table 4). Wounds dressed with lanolin alone had columns about three times shorter and thinner than nontreated wounds. Large wounds had longer columns than small wounds, but large wounds were two times longer than small wounds. Although cracking of the bark, above and below the wounds, occurred with most wounds, it was most severe with nontreated wounds and large wounds. The least cracking was associated with wounds dressed with lanolin alone.

Microorganisms. None of the dressings had any effect on the presence of microorganisms in discolored tissues associated with small wounds. On large wounds, some of the

Table 3. Effect of lanolin dressings containing 2-chloroethylphosphonic acid (CEPA) on callus formation 13 months after wounding red maples in July.

Dressings[a]	Rate (ug/g)	Wound closure (mm)[b]	
		Small wounds[c]	Large wounds[d]
None	———	6.8	7.5
Lanolin	———	8.3	9.5
CEPA	100	5.8	6.5
CEPA	1,000	5.8	4.8
CEPA	10,000	5.5	4.5

[a]CEPA was incorporated in lanolin.

[b]Size of the wounds at the time of wounding (2.5 or 5 cm) minus the size of exposed wood measured horizontally at the largest point 13 months later. Means of 4 wounds (2 wounds/tree).

[c]Elliptical wounds, 2.5 X 5 cm. LSD (P=0.05) = 2.1 mm.

[d]Elliptical wounds, 5 X 10 cm. LSD (P=0.05) = 2.6 mm.

Table 4. Effect of lanolin dressings containing 2-chloroethylphosphonic acid (CEPA) on discoloration of the wood 13 months after wounding red maples in July.

Dressings[a]	Rate (ug/g)	Column length (cm)[b]		Column thickness (mm)[c]	
		Small wounds[d]	Large wounds[e]	Small wounds[d]	Large wounds[e]
None	———	27.4	30.9	3.1	4.6
Lanolin	———	9.6	11.9	1.1	0.6
CEPA	100	10.4	13.4	1.8	0.9
CEPA	1,000	6.9	14.3	0.6	0.9
CEPA	10,000	8.8	15.5	1.3	2.3

[a]CEPA was incorporated in lanolin.

[b]Length of the columns of discolored wood measured along the longitudinal axis of the stem with the wound included. Means of 4 wounds (2 wounds/tree). LSD (P=0.05) = 4.7 and 6.4 cm for small and large wounds, respectively.

[c]Average thickness of the discolored wood beneath the wounds. Means of 4 wounds (2 wounds/tree). LSD (P=0.05) = 1.6 and 1.8 mm for small and large wounds, respectively.

[d]Elliptical wounds, 2.5 X 5 cm.

[e]Elliptical wounds, 5 X 10 cm.

dressings, including the lanolin dressing, reduced the number of isolations yielding fungi. The dressings containing 100 ug/g and 1,000 ug/g CEPA also reduced the number of isolations yielding bacteria (Table 5).

Properties of the dressings. Lanolin was more difficult to put on wounds from which sap was exuding than on wounds that stayed dry, but nevertheless, it could be applied. The dressings stayed on the wounds during part of a growing season, through the winter, and during most of the next growing season without falling off. After 13 months, the lanolin had hardened but it did not crack. The dressings became thinner as part of the lanolin was absorbed by the wood. Compared to nontreated wounds, the wood under the lanolin dressings stayed very moist and firm.

Compounds Other than Growth Substances

Dressings containing compounds other than growth substances inhibited callus formation or had no effect (Table 6). Ascorbic acid may have promoted callus formation but the response was not as good as that of growth substances.

Auxins, Gibberellin, and Cytokinin

Greenhouse Trials

Auxin and gibberellin interaction. A combination of IAA and GA in a 5:2 ratio gave the best results. Callus formation was consistently promoted, and the tissues that formed over the wounds most closely resembled those formed over

Table 5. Effect of lanolin dressings containing 2-chloroethylphosphonic acid (CEPA) on the presence of microorganisms in discolored tissues 13 months after wounding red maples in July.

Dressings[a]	Rate (ug/g)	% of isolations yielding[b]			
		Fungi		Bacteria	
		Small wounds[c]	Large wounds[d]	Small wounds[c]	Large wounds[d]
None	———	20	54	40	42
Lanolin	———	29	12	33	33
CEPA	100	21	12	38	8
CEPA	1,000	17	4	33	12
CEPA	10,000	46	50	29	29

[a]CEPA was incorporated in lanolin.

[b]% based on 24 attempts (6 attempts/column of discolored tissues).

[c]Elliptical wounds, 2.5 X 5 cm.

[d]Elliptical wounds, 5 X 10 cm.

Table 6. Effect of lanolin dressings containing compounds other than growth substances on callus formation over wounds on the stems of apple seedlings.

Dressings[a]	Rate (ug/g)	Closing period[b] (days)
None	———	72
Lanolin	———	61**
L-cysteine-HCl	100	116+
"	1,000	58**
"	10,000	51*
Cystine	100	92**
"	1,000	55**
"	10,000	116+
Methionine	100	60**
"	1,000	63**
"	10,000	80**
Glutathione	100	52**
"	1,000	31**
"	10,000	91**
Ascorbic acid	10	40
"	100	104**
"	1,000	41
Sucrose	5%	56*
"	10%	58***

[a]All compounds were incorporated in lanolin.

[b]Period of time elapsed until callus tissues completely closed the wounds. Means of 4 wounds (2 wounds/seedling). The number of asterisks indicates the number of wounds not closed after 116 days.

nontreated wounds. They were flat and covered with bark almost as they appeared (Table 7). As the ratio of IAA to GA became smaller, the response was not consistent. A 1:2 ratio produced disorganized callus tissues without bark. As the ratio increased, the tissues covering the wounds also became disorganized or had no bark.

IAA alone, 500 or 1,000 ug/g, induced rapid closure of the wounds, but the tissues that covered the wounds had the appearance of a random outgrowth rather than well organized callus tissues. GA alone, 200 ug/g, also induced rapid closure of the wounds, but only portions of the wounds were closed and the tissues closing the wounds resembled those formed under the influence of IAA. The addition of GA to IAA resulted in faster production of well organized callus tissues. In combination with GA, IAA was effective at a lower concentration than it was without GA.

Trial of various growth substances on apple seedlings. Almost all dressings containing growth substances promoted callus formation when they were applied to wounds on apple seedlings (Table 8). Most dressings stimulated the production of somewhat disorganized tissues. The dressings that promoted well organized callus tissues contained IAA or IBA, in combination with GA.

The response to 100 ug/g BA was inconsistent while it was consistent when 1,000 ug/g BA was used. NAA and indole proprionic acid (IPA) definitively inhibited callus formation while the response to IBA was inconsistent. When GA was

Table 7. Effect of lanolin dressings containing various combinations of indole acetic acid (IAA) and gibberellic acid (GA) on callus formation over wounds on the stemsof apple seedlings.

Dressings[a]		Closing period[b]	Bark formation[c]	External characteristics
IAA (ug/g)	GA (ug/g)	(days)	(days)	of the callus
0	0	60+	—	no callus
100	0	60+	—	1/2 complete
200	0	60+	—	3/4 complete
500	0	38	72	overgrown
1,000	0	31	62	overgrown
0	100	60+	—	nearly complete
100	100	20*	52	complete
200	100	20*	52	complete
500	100	24	62	slightly overgrown
1,000	100	27	no bark	complete
0	200	23*	39	slightly overgrown
100	200	24	no bark	overgrown
200	200	60+	—	no callus
500	200	22	30	complete
1,000	200	20	61	overgrown

[a]IAA and GA were incorporated in lanolin.

[b]Period of time elapsed until callus tissues completely closed the wounds. Means of 2 wounds (1 wound/seedling). The number of asterisks indicates the number of wounds not closed after 60 days.

[c]Time elapsed until bark appeared over the callus tissues.

Table 8. Effect of lanolin dressings containing growth substances on callus formation over wounds on the stems of apple seedlings[a].

Dressings[b]	Rate (ug/g)	Closing period[c] (days)
None	———	72
Lanolin	———	61**
BA	100	23**
BA	1,000	22
IAA-BA	1,000-100	20
IAA-Sucrose	1,000-10%	39
IAA-BA-Sucrose	1,000-100-5%	20
IAA-GA-Sucrose	1,000-200-10%	23
IAA-GA-BA	1,000-200-100	19
NAA	500	116+
NAA-Sucrose	500-10%	47
NAA-GA	500-200	22
NAA-GA-Sucrose	500-200-5%	27
NAA-GA-Sucrose	500-200-10%	38
IPA	500	116+
IPA-GA	500-200	23
IBA	500	25**
IBA-GA	500-200	22

[a]Tables 8 and 6 contain data from the same experiment.

[b]BA=benzyladenine, IAA=indole acetic acid, GA=gibberellic acid, NAA=naphthalene acetic acid, IPA=indole proprionic acid, IBA=indole butyric acid. All compounds were incorporated in lanolin.

[c]Period of time elapsed until callus tissues completely closed the wounds. Means of 4 wounds (2 wounds/seedling). The number of asterisks indicates the number of wounds not closed after 116 days.

added, all three auxins promoted callus formation (Table 8). NAA and IPA caused a flattening of the stem similar to that observed with CEPA.

When 10% sucrose instead of GA was added to auxins, callus formation was promoted but not as much as with GA. Sucrose, added to either IAA and GA or NAA and GA, inhibited callus formation. BA, 100 ug/g, promoted callus formation more than sucrose in combination with either IAA or IAA and GA (Table 8).

Field Trial of Growth Substances on Red Maple

Callus formation. Callus formation was promoted, on low vigor trees only, by IBA in combination with either GA or zinc oxide, or both. NAA alone or in combination with GA, IAA, and IBA tended to inhibit callus formation, but the inhibition was evident only on high vigor trees. BA in combination with either IAA and GA or IBA and GA appeared to inhibit callus formation. Cysteine, GA, and BA had no effect on callus formation (Table 9).

Wounds closed faster on high vigor trees than they did on low vigor ones. On high vigor trees, none of the dressings containing growth substances promoted more callus formation than did lanolin. The wounds dressed with lanolin alone were almost closed after three months while nontreated wounds had not closed at all.

Bark dieback. Little bark dieback occurred with dressings containing lanolin compared to nontreated wounds (Table

Table 9. Effects of lanolin dressings containing growth substances and other compounds on callus formation, wood discoloration, and bark dieback three months after wounding red maples in May.

Dressings[a]	Wound closure (mm)[b]		Column length (cm)[c]		Bark dieback (mm)[d]	
	High vigor[e]	Low vigor[e]	High vigor	Low vigor	High vigor	Low vigor
None	0.5	1.0	26.0	8.0	12.5	17.5
Lanolin	12.0	5.0	9.8	9.5	1.5	14.0
L-C-H	10.5	3.0	6.0	4.4	0.0	2.5
GA	10.0	5.5	7.2	4.1	1.5	0.0
ZnO	13.0	4.5	7.4	5.3	0.0	0.0
BA	9.5	5.5	6.9	8.3	0.0	0.0
NAA	3.0	1.0	14.4	8.2	3.0	12.0
NAA-GA	2.5	1.5	22.3	10.3	4.5	8.0
IAA	7.0	7.5	11.3	7.3	0.0	0.0
IAA-GA	10.0	5.0	8.1	7.9	1.5	3.5
IAA-GA-BA	4.5	4.0	16.6	8.1	3.5	7.0
IBA	7.5	5.5	12.8	8.6	1.0	20.0
IBA-GA	8.0	11.5	9.6	6.5	4.5	0.0
IBA-ZnO	11.0	11.5	15.7	7.4	1.5	1.5
IBA-GA-ZnO	8.0	11.0	14.8	6.6	1.5	0.0
IBA-GA-BA	5.0	7.0	15.8	9.1	5.5	12.5

[a]L-C-H=L-cysteine - HCl (1%), GA=gibberellic acid (0.2%), ZnO= zinc oxide (2%), BA=benzyladenine (0.5%), NAA=naphthalene acetic acid (0.5%), IAA=indole acetic acid (0.5%), IBA=indole butyric acid (0.5%). All compounds were incorporated in lanolin.

[b]Size of the wounds at the time of wounding (15mm) minus the size of exposed wood measured horizontally at the largest point three months later. Means of 2 replications. LSD (P=0.05) = 5.7 mm.

Table 9. (Cont.)

[c]Length of columns of discolored wood measured along the longitudinal axis of the stem including the size of the wounds. Means of 2 replications. LSD (P=0.05) = 7.3 cm.

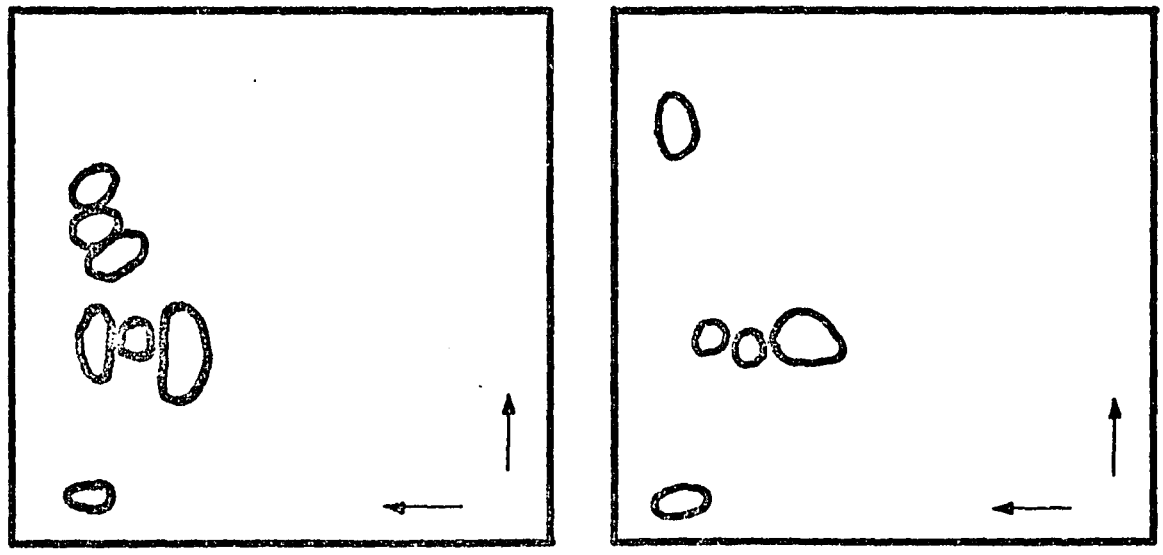
[d]Length of the zone of dead bark measured along the longitudinal axis of the stem without including the size of the wounds. Means of 2 replications. LSD (P=0.05) = 9.5 mm.

[e]Based on the mean growth in diam for the last five years: 2.45 and 0.64 mm/yr for high and low vigor trees, respectively.

9). Bark dieback was more severe on low vigor trees than on high vigor ones. Independently of the vigor of the tree, bark dieback occurred especially with wounds that closed the least. In this trial, the wounds consisted of round holes, and cracking of the bark occurred only in one case among the 60 wounds treated with a lanolin dressings.

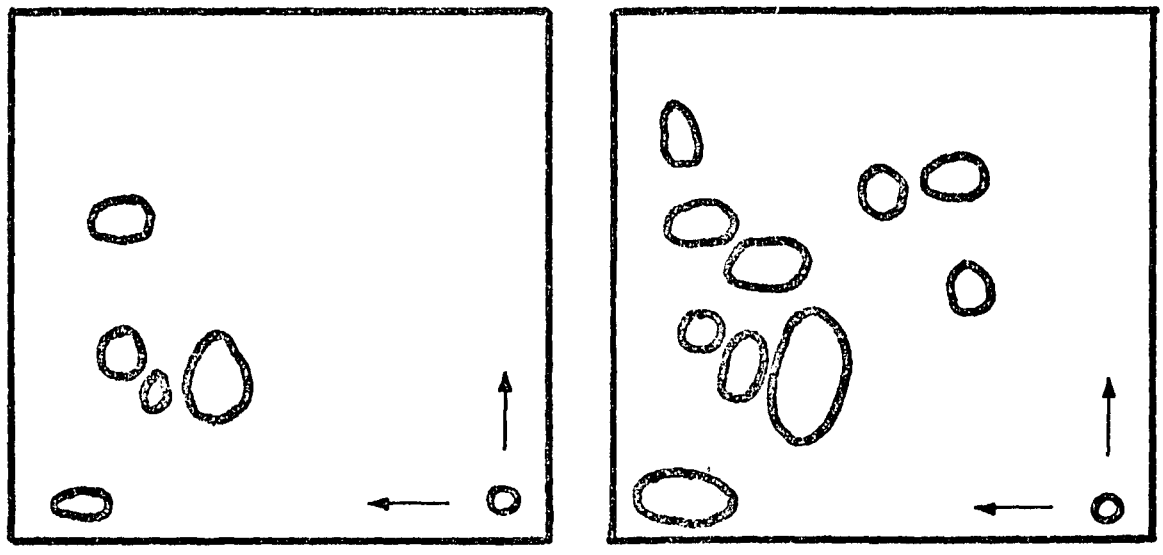
Discoloration of the wood. Discoloration was equally increased by dressings that promoted callus formation or inhibited it. All auxins increased discoloration. Except with NAA, the addition of GA to auxins annihilated their effect. As much discoloration occurred when BA was combined to auxins and GA as when auxins were used alone (Table 9). Discoloration was increased only on high vigor trees. Cysteine, GA, and zinc oxide slightly decreased discoloration. Lanolin decreased discoloration by about three times on high vigor trees but had no effect on low vigor trees. Independently of the dressings, less discoloration occurred on low vigor trees than on high vigor ones.

Chromatography of extractable phenols. Extracts from discolored tissues associated with lanolin or IAA-treated wounds were different (Fig. 3). Many compounds were detected only in the acid-hydrolyzed extract from IAA-treated wounds. There was little difference between water extracts. The discolored tissues from IAA-treated wounds contained a larger amount of non-hydrolyzed phenols than similar tissues from lanolin-treated wounds.



Water extract Acid-hydrolyzed extract

A



Water extract Acid-hydrolized extract

B

Fig. 3. Thin-layer chromatograms of extractable phenols from discolored tissues associated with wounds treated with A) lanolin alone, and B) lanolin plus IAA. The solvents are B:A:W 6:1:2 and 7% acetic acid:0.03% sodium acetate, horizontally and vertically, respectively.

Microorganisms. IAA, and NAA combined with GA, completely prevented fungi from invading the discolored tissues above and below the wounds. The NAA dressing and the dressings, IBA-GA, IBA-ZnO, and IBA-GA-ZnO, that promoted callus formation reduced invasion by fungi. Other dressings, whether they decreased discoloration or increased it, did not affect invasion of the discolored tissues by fungi (Table 10).

No dressing affected the presence of fungi immediately behind the wounds. No dressing prevented bacteria from invading the discolored tissues. More isolations yielded bacteria when the wounds were treated with dressings that contained lanolin than when the wounds received no dressing.

Field Trial of Growth Substances on White Pine, American Beech, Sugar Maple, and Red Maple

Callus formation. The only dressingsthat promoted callus formation more than lanolin contained IBA plus GA, on beech and red maple, and IBA plus GA and BA, on white pine and beech. A two-week delay in treating the wounds had no effect. Sugar maple responded to none of the dressings. Lanolin favored callus formation on all species (Table 11). IBA promoted callus formation on red maple two weeks after wounding but had no effect on the other species.

"Tree Aid" may have favored callus formation on red maple although not as much as lanolin. It inhibited callus formation on white pine and had no effect on beech and sugar maple. The dressings that contained BA, BA and IBA-GA-BA,

Table 10. Effect of lanolin dressings containing growth substances and other compounds on the presence of microorganisms in discolored tissues three months after wounding red maples in May.

Dressings[a]	% of isolations yielding			
	Fungi		Bacteria	
	Above & below wound[b]	Behind wound[c]	Above & below wound[b]	Behind wound[c]
None	40	83	30	67
Lanolin	38	50	88	100
L-C-H	50	100	88	100
GA	50	75	50	100
ZnO	50	75	63	100
BA	38	50	38	75
NAA	15	50	63	100
NAA-GA	0	25	88	100
IAA	0	50	50	75
IAA-GA	50	100	50	100
IAA-GA-BA	38	75	75	100
IBA	25	100	38	100
IBA-GA	10	75	70	100
IBA-GA-ZnO	20	100	40	100
IBA-GA-BA	50	100	50	100

[a] L-C-H=L-cysteine-HCl (1%), GA=gibberellic acid (0.2%), ZnO=zinc oxide (2%), BA=benzyladenine (0.5%), NAA=naphthalene acetic acid (0.5%), IAA=indole acetic acid (0.5%), IBA=indole butyric acid (0.5%). All compounds were incorporated in lanolin.

[b] % based on 8 isolations (4 isolations/column of discolored tissues).

[c] % based on 4 isolations (1 isolation/column of discolored tissues).

Table 11. Effect of lanolin dressings containing growth substances on callus formation seven months after wounding various species of trees at the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Wound closure (mm)[a]							
	White pine [c]		American beech [d]		Sugar maple [e]		Red maple [f]	
	No delay	Delay	No delay	Delay	No delay	Delay	No delay	Delay
None	4.3	3.0	6.3	6.0	1.5	0.0	0.0	0.0
Lanolin	9.0	7.0	5.7	7.3	8.0	3.5	6.0	6.0
IBA	9.3	7.3	7.0	7.7	6.0	1.0	8.0	11.0
IBA-GA	9.7	8.3	10.0	10.3	7.5	5.0	13.0	12.0
BA	9.0	7.3	6.3	6.7	2.5	1.0	6.0	0.0
IBA-GA-BA	12.0	11.7	10.3	9.7	0.5	0.0	8.0	0.0
"Tree Aid"	0.7	1.7	6.3	6.0	2.5	0.5	4.0	5.0
CEPA	1.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0

[a]Size of the wounds at the time of wounding (15 mm) minus the size of exposed wood measured horizontally at the largest point seven months later.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 2.7 mm within and between sets.

[d]Means of 3 replications. LSD (P=0.05) = 1.5 and 2.4 mm within and between sets, respectively.

[e]Means of 2 replications. LSD (P=0.05) = 4.8 and 4.6 mm within and between sets, respectively.

[f]One tree only.

had an inhibitory effect on sugar maple, whether they were applied immediately after wounding or two weeks later. On red maple, the inhibitory effect was evident only after a two-week delay. CEPA strongly inhibited callus formation on all species (Table 11).

On sugar maple, callus formation was best early in the season (Table 12). A two-week delay in treating the wounds did not affect callus formation early in the season but it did after that. Lanolin favored callus formation on sugar maple at any time during the season. There was little difference between the lanolin, IBA, and IBA-GA dressings early in the season but the last two dressings inhibited callus formation later in the season. The dressings containing either BA, IBA plus GA and BA, or CEPA inhibited callus formation at any time during the season. "Tree Aid" had no effect early in the season. It may have slightly favored callus formation later in the season, but only when it was applied immediately after wounding (Table 12).

Bark dieback. When it occurred, bark dieback was associated with nontreated wounds, wounds treated with CEPA, and wounds treated with dressings, BA and IBA-GA-BA, that inhibited callus formation (Table 13). "Tree Aid" caused some bark dieback on sugar and red maple even though it did not inhibit callus formation. Very little bark dieback occurred on white pine and beech except when CEPA was used. Maples generally suffered more bark dieback than other species. Delaying the application of the dressings did not

Table 12. Effect of lanolin dressings containing growth substances on callus formation seven months after wounding sugar maples, before, during, and after the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Wound closure (mm)[a]					
	March 30[c]		May 7[d]		June 8[e]	
	No delay	Delay	No delay	Delay	No delay	Delay
None	5.0	5.7	1.5	0.0	0.0	0.0
Lanolin	8.3	10.7	8.0	3.5	6.0	3.0
IBA	9.3	9.0	6.0	1.0	2.0	0.3
IBA-GA	6.7	8.7	7.5	5.0	4.3	0.3
BA	6.0	5.3	2.5	1.0	4.7	0.0
IBA-GA-BA	2.0	0.7	0.5	0.0	4.0	0.0
"Tree Aid"	5.7	4.3	2.5	0.5	2.3	0.0
CEPA	0.0	0.7	0.0	0.0	0.3	0.0

[a]Size of the wounds at the time of wounding (15 mm) minus the size of exposed wood measured horizontally at the largest point seven months later.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 3.4 and 3.5 mm within and between sets, respectively.

[d]Means of 2 replications. LSD (P=0.05) = 4.8 and 4.6 mm within and between sets, respectively.

[e]Means of 3 replications. LSD (P=0.05) = 3.9 and 3.6 mm within and between sets, respectively.

Table 13. Effect of lanolin dressings containing growth substances on bark dieback associated with seven-month old wounds after wounding various species of trees at the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Bark dieback (mm)[a]							
	White pine [c]		American beech [d]		Sugar maple [e]		Red maple [f]	
	No delay	Delay	No delay	Delay	No delay	Delay	No delay	Delay
None	0.0	0.0	0.0	0.0	6.0	8.0	17.0	30.0
Lanolin	0.0	0.0	0.0	0.0	0.0	2.0	1.0	4.0
IBA	0.0	0.0	0.0	0.0	0.0	3.0	0.0	4.0
IBA-GA	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
BA	0.0	0.0	0.0	0.0	5.0	4.0	3.0	6.0
IBA-GA-BA	0.0	0.0	0.0	0.0	4.0	6.0	0.0	7.0
"Tree Aid"	0.1	0.1	0.0	0.0	7.0	7.0	7.0	5.0
CEPA	17.0	17.0	21.0	33.0	28.0	36.0	100.0	185.0

[a]Length of the zone of dead bark measured along the longitudinal axis of the stem without including the size of the wounds.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 1.3 and 1.4 mm within and between sets, respectively.

[d]Means of 3 replications. LSD (P=0.05) = 0.1 and 0.9 mm within and between sets, respectively.

[e]Means of 2 replications. LSD (P=0.05) = 4.6 and 6.7 mm within and between sets, respectively.

[f]One tree only.

result in more bark dieback on white pine and beech while it did on sugar and red maple.

Cracking of the bark, above and below the wounds, did not occur on white pine, beech, and red maple. On sugar maple, cracks were observed around wounds dressed with "Tree Aid", IBA-GA-BA, and around nontreated wounds. CEPA caused a depression around the wounds on all species. The tissues at the periphery of the depression appeared swollen. On white pine, large amounts of resin exuded from the wounds treated with CEPA. Smaller amounts of resin exuded from nontreated wounds and wounds dressed with "Tree Aid" two weeks after wounding.

On sugar maple, bark dieback generally increased as the season advanced. Delaying the application of the dressings did not increase bark dieback early in the season while it did later. Lanolin, with or without growth substances, was most effective for preventing or reducing bark dieback at any time during the season (Table 14). Nontreated wounds and wounds treated with "Tree Aid" had equivalent amounts of bark dieback. CEPA caused the largest amounts of bark dieback and the trees appeared more susceptible early in the season.

Discoloration of the wood. Growth substances did not increase discoloration except in red maple. CEPA increased discoloration in all species and especially in red maple. Lanolin decreased discoloration in all species when it was applied immediately after wounding. It tended to increase discoloration when it was applied two weeks after wounding.

Table 14. Effect of lanolin dressings containing growth substances on bark dieback associated with seven-month old wounds after wounding sugar maples, before, during, and after the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Bark dieback (mm)[a]					
	March 30[c]		May 7[d]		June 8[e]	
	No delay	Delay	No delay	Delay	No delay	Delay
None	8.0	7.0	6.0	8.0	12.0	7.0
Lanolin	0.0	0.0	0.0	2.0	11.0	4.0
IBA	0.0	0.0	0.0	3.0	3.0	5.0
IBA-GA	0.0	0.0	0.0	2.0	1.0	7.0
BA	1.0	2.0	5.0	4.0	1.0	8.0
IBA-GA-BA	2.0	4.0	4.0	6.0	2.0	6.0
"Tree Aid"	7.0	15.0	7.0	7.0	4.0	13.0
CEPA	97.0	23.0	28.0	36.0	21.0	28.0

[a]Length of the zone of dead bark measured along the longitudinal axis of the stem without including the size of the wounds.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 13.4 and 15.1 mm within and between sets, respectively.

[d]Means of 2 replications. LSD (P=0.05) = 4.6 and 6.7 mm within and between sets, respectively.

[e]Means of 3 replications. LSD (P=0.05) = 12.8 and 13.1 mm within and between sets, respectively.

"Tree Aid" had no effect on discoloration (Table 15).

None of the dressings affected discoloration in sugar maple at any time during the season (Table 16). Independently of the dressings, trees wounded at the end of March had more discoloration than trees wounded in May or June. There was no difference in the amounts of discoloration between May and June.

Microorganisms. The results of isolations form all species of trees were combined. The growth substances tested had no effect on invasion of the discolored tissues by fungi. Lanolin reduced invasion of the tissues above and below the wounds when it was applied immediately after wounding (Table 17). When the application of the dressings was delayed, fungi were isolated more frequently from discolored tissues associated with treated wounds than nontreated wounds.

None of the dressings had any effect on the presence of bacteria in discolored tissues behind the wounds, or above and below the wounds, whether they were applied immediately after wounding or two weeks later (Table 18).

Table 15. Effect of lanolin dressings containing growth substances on discoloration of the tissues associated with seven-month old wounds after wounding various species of trees at the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Length of columns of discoloration (cm)[a]							
	White pine [c]		American beech [d]		Sugar maple [e]		Red maple [f]	
	No delay	Delay	No delay	Delay	No delay	Delay	No delay	Delay
None	3.3	4.2	14.2	9.6	5.0	5.9	7.4	4.5
Lanolin	4.0	3.8	3.5	12.1	4.1	6.4	4.8	18.0
IBA	5.1	4.3	3.7	7.5	3.4	5.3	13.1	11.3
IBA-GA	4.6	4.3	3.4	8.8	4.2	5.8	16.7	41.4
BA	3.7	3.3	6.0	5.0	3.4	8.6	5.5	18.0
IBA-GA-BA	4.2	4.4	3.3	10.5	5.2	6.3	20.0	21.0
"Tree Aid"	3.4	6.0	6.7	11.8	4.6	4.8	8.7	44.8
CEPA	7.9	7.5	8.0	13.6	6.9	9.1	21.5	19.4

[a]Length of columns of discolored wood measured along the longitudinal axis of the stem including the size of the wounds.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethyl-phosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 1.3 and 1.4 cm within and between sets, respectively.

[d]Means of 3 replications. LSD (P=0.05) = 9.3 and 7.9 cm within and between sets, respectively.

[e]Means of 2 replications. LSD (P=0.05) = 2.4 and 3.4 cm within and between sets, respectively

[f]One tree only.

Table 16. Effect of lanolin dressings containing growth substances on discoloration of the tissues associated with seven-month old wounds after wounding sugar maples, before, during, and after the time of leafing out. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	Length of columns of discoloration (cm)[a]					
	March 30[c]		May 7[d]		June 8[e]	
	No delay	Delay	No delay	Delay	No delay	Delay
None	8.7	12.9	5.0	5.9	5.0	4.9
Lanolin	8.3	10.3	4.1	6.4	4.0	3.7
IBA	11.3	11.4	3.4	5.3	3.9	6.0
IBA-GA	13.5	11.3	4.2	5.8	5.8	5.7
BA	13.8	11.8	3.4	8.6	3.5	5.3
IBA-GA-BA	12.3	15.2	5.2	6.3	5.1	4.5
"Tree Aid"	11.2	7.8	4.6	4.8	3.6	4.4
CEPA	14.5	12.3	6.9	9.1	5.7	5.8

[a]Length of columns of discolored wood measured along the longitudinal axis of the stem including the size of the wounds.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]Means of 3 replications. LSD (P=0.05) = 5.6 and 4.9 cm within and between sets, respectively.

[d]Means of 2 replications. LSD (P=0.05) = 2.4 and 3.4 cm within and between sets, respectively.

[e]Means of 3 replications. LSD (P=0.05) = 3.4 and 3.1 cm within and between sets, respectively.

Table 17. Effect of lanolin dressings containing growth substances on the presence of fungi in discolored tissues associated with seven-month old wounds. One set of wounds was treated immediately after wounding and the other, two weeks later.

Dressings[b]	% of positive isolations[a]			
	Behind wound[c]		Above & below wound[d]	
	No delay	Delay	No delay	Delay
None	87	87	39	19
Lanolin	80	93	15	34
IBA	100	93	26	25
IBA-GA	93	94	21	32
BA	87	94	15	31
IBA-GA-BA	73	100	18	28
"Tree Aid"	100	94	35	32
CEPA	94	93	17	23

[a]Pooled results from 3 white pines wounded on May 8, 3 American beeches wounded on May 4, 8 sugar maples: 3 trees wounded on March 30, 2 trees on May 7, and 3 trees on June 8, and 1 red maple wounded on May 7.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]% based on 15 isolations (1 isolation/column of discolored tissues).

[d]% based on 60 isolations (4 isolations/column of discolored tissues).

Table 18. Effect of lanolin dressings containing growth substances on the presence of bacteria in discolored tissues associated with seven-month old wounds. One set of wounds was treated immediately after wounding, and the other, two weeks later.

Dressings[b]	% positive isolations[a]			
	Behind wound[c]		Above & below wound[d]	
	No delay	Delay	No delay	Delay
None	100	93	63	54
Lanolin	93	100	47	68
IBA	100	87	53	59
IBA-GA	93	88	60	58
BA	80	88	48	58
IBA-GA-BA	93	100	60	65
"Tree Aid"	100	93	43	57
CEPA	94	80	66	60

[a]Pooled results from 3 white pines wounded on May 8, 3 American beeches wounded on May 4, 8 sugar maples: 3 trees wounded on March 30, 2 trees on May 7, and 3 trees on June 8, and 1 red maple wounded on May 7.

[b]IBA=indole butyric acid (0.5%), GA=gibberellic acid (0.2%), BA=benzyladenine (0.5%), "Tree Aid"=commercial aerosol spray dressing from Heritage House, CEPA=2-chloroethylphosphonic acid (8%): Amchem Products Inc., Ethrel formulation 68-240. All compounds were incorporated in lanolin.

[c]% based on 15 isolations (1 isolation/column of discolored tissues).

[d]% based on 60 isolations (4 isolations/column of discolored tissues).

DISCUSSION AND CONCLUSIONS

Lanolin

Lanolin favored callus formation but the amount of callus tissues produced was correlated with the vigor of the tree. In a strict sense, lanolin did not promote callus formation as growth substances did. The beneficial effect of lanolin has been known for at least 40 years (44,67). It is surprising that it did not become a major wound dressing material.

As advanced by McQuilkin (43), its "healing properties" are probably of a physical nature. The living tissues exposed by wounding are subjected to very drastic changes. The loss of moisture is probably the most harmful of these changes. The cells closest to the edges of the wound die. They temporarily play the role of the missing bark in preventing water losses. The amount of moisture at the wound site seems to be the most important factor affecting bark dieback. Wounds covered with lanolin always stayed very moist while nontreated wounds dried out.

The main property of lanolin is to prevent bark dieback by reducing water losses, thus allowing callus formation to start without delay. Prevention of bark dieback by lanolin may not be equally successful with wounds of any sizes or shapes. Long cracks often developed in the bark above and below large elliptical wounds.

The effect of lanolin was annihilated when the compounds it carried had an adverse effect on callus formation as was the case with CEPA. The vigor of the tree had relatively little effect on the ability of lanolin to prevent bark dieback. Delaying the application of lanolin caused a slight increase in bark dieback, but all species were not equally susceptible to a two-week delay. Sugar and red maple were more susceptible to bark dieback than were white pine and beech.

When applied immediately after wounding, lanolin generally decreased discoloration. Probably this effect is physical in nature. Lanolin most certainly affects free gas exchange that goes on when the wound is directly open to the atmosphere. The chemical oxidations occuring immediately after wounding (71) are impeded by lanolin. Glue and methocel had the same effect and Houston (30) attributed their effect to the fact that they effectively prevented air from entering the wound. Lanolin did not reduce discoloration when it was applied two weeks after wounding.

Some results show that lanolin reduced invasion by fungi. The results were not consistent and are probably due to random variation. In the first case (Table 5), lanolin had an effect only on large wounds. In the second case (Table 17), the percentage of positive isolations varied from 19% to 39%, but nothing can account for that difference.

Lanolin has advantageous physical properties. Its plasticity allows callus formation without the dressing

cracking or falling off. It does not dry out quickly and thus can stay for a long time on wounds. Lanolin performed well under the climatic conditions that prevailed during the trials, but it could not be of great practical use. The trials were carried out in a forest situation where the trunks are shaded most of the time. Lanolin has a low melting point, around 40 C, and it cannot withstand very long exposure to the sun without running off. McQuilkin and Showalter (44) have developed many different formulations of lanolin having a higher melting point and thus being more suited for general use as wound dressing.

Ethylene

Apple seedlings responded very erratically to treatments with CEPA. On older trees, the results were more consistent. All concentrations of CEPA, 0.01% to 8%, inhibited callus formation. Larger wounds may have been affected more than small wounds because of the larger amount of dressing they received. When the dressings contained 8% CEPA, not only callus formation was inhibited, but the living tissues around the wounds were killed as evidenced by the large amounts of bark dieback associated with these dressings. Red maple was the most sensitive, and white pine, the least affected. Senescence in plants is often associated with abnormally high levels of ethylene (51) which have an adverse effect on some growth processes (10).

CEPA has been reported to stimulate general plant growth (1), but according to Burg, Apelbaum, and Kang (10) ethylene inhibits cell division and differentiation while favoring cell expansion. They maintain that stimulation of growth by ethylene can occur only through cell expansion. The effect of ethylene on cell expansion was evident by the swelling of the tissues observed around the wounds treated with 8% CEPA. The growth ring that formed during the time the dressings were on the trees was disproportionately wider at the edges of the depression than anywhere else around the stem.

While causing extensive bark dieback, 8% CEPA drastically increased discoloration in the wood. Discoloration resulted either directly from the action of ethylene on phenol metabolism or indirectly from the killing of the living tissues by CEPA, or from both. Living tissues were probably killed by a drastic change in pH caused by CEPA itself or the products resulting from its breakdown. The CEPA formulation used had a pH of approximately 2. In the plant, it breaks down to ethylene, phosphate, and chloride ions.

Increased resistance to invasion by microorganisms is often related to faster production and higher levels of ethylene whether it is naturally produced by the plant (64) or artificially applied to plant tissues (76). While it had no effect on fungi, CEPA, 0.01% and 0.1%, reduced invasion by bacteria. The effect of CEPA on bacteria is questionable because it occurred only on large wounds. It is possible

that CEPA affected bacteria by lowering the pH enough to make the exposed wood unsuited for bacterial growth. Large wounds received three to four times more dressing than small wounds. At least on large wounds, 1% CEPA should have been more effective but it was not and neither was 8% CEPA on other types of wounds.

Even though exposure of plant tissues to ethylene generally results in increased PAL activity (12), CEPA in lanolin did not affect the activity of that enzyme except when it inhibited it. Daily applications of 10 ug/ml CEPA in propylene glycol slightly promoted PAL activity. Only very low ethylene concentrations, 0.1-10 uliters/liter, promote enzyme activity, and constant exposure is necessary (13,32). Furthermore, the range of concentrations where ethylene is effective is narrow. Chalutz (13) and Hyodo and Yang (32) reported maximum PAL activity at 10 uliters/liter followed by a sharp decrease at 100 uliters/liter ethylene. The drastic decrease in PAL activity observed with 1% CEPA was probably due to the injurious effects which that compound had on living tissues as discussed earlier.

It is possible that CEPA increased PAL activity, even when it was incorporated in lanolin, but that the large variation between the samples masked the difference due to the treatments. The variation between samples was smaller when CEPA was in propylene glycol. Although it is commonly used for growth substances, lanolin may not be the best of carriers for CEPA especially when a very narrow range of small

concentrations is critical. Ethylene may have been released either too slowly, too quickly, or not at all when it was incorporated in lanolin. No references to the use of CEPA in a lanolin carrier were found. Total phenol contents were measured from seedlings treated with CEPA in lanolin. The absence of change in the amounts of phenols present in the tissues tends to corroborate the results obtained for PAL activity when lanolin dressings were used.

Auxins

Under field conditions, auxins either had no effect or inhibited callus formation. Tilford (79) reported that 0.2% IBA in lanolin was injurious to red maple, but IBA was applied only at the edges of the wound while the exposed wood received varnish. Injury may have resulted from the application of varhish rather than from IBA. In this study, IBA was never injurious, and 0.5% IBA inhibited callus formation on sugar maple only when it was applied in June. Davis (21) obtained similar results with 1% IBA.

Lavee and Haskal (39) reported that 0.5% IBA or IAA promoted callus formation on fruit trees. Although they conducted their tests in the field, they treated pruning wounds on branches that were smaller and more actively growing than stems of trees. IAA and possibly IBA promoted callus formation on apple seedlings. Seedlings responded better than older trees to many growth substances.

The inhibitory effect of IAA and NAA on red maple probably resulted from using excessive amounts. It is common for auxins to cause growth inhibition when they are used at concentrations far beyond the optimum needed for growth promotion (78). The optimum concentration for growth promotion is smaller for NAA than for IAA (46,55) or IBA (78). On apple seedlings, IAA did not inhibit callus formation while IBA caused some inhibition and NAA resulted in even more inhibition.

Growth inhibition caused by high concentrations of auxins has been attributed to the effect of auxins on ethylene production (11,47). High auxin levels promote ethylene production which in turn acts as a feedback mechanism by inhibiting growth.

Lanolin partly circumvented the inhibitory effect of auxins. Wounds dressed with auxins in a lanolin dressing formed more callus tissues than nontreated wounds; and except for NAA, auxins did not cause any more bark dieback than lanolin alone. Thus, it is possible that after the effect of auxins on ethylene production is exhausted, callus formation will resume at the same rate as with lanolin alone.

Compared to lanolin alone, auxins generally increased discoloration but not all auxins caused as much discoloration. NAA and IAA caused more discoloration than IBA. It is possible that lanolin partly circumvented the effect of auxins on discoloration. There was often less discoloration associated with auxin-treated wounds than with nontreated wounds.

As suggested by Rhodes and Wooltorton (52), the effect of auxins on discoloration is probably related to increased endogenous ethylene production as was discussed earlier. Increased discoloration would result from the stimulatory effect of ethylene on the metabolism of phenols. The findings (2) that NAA and IAA are more effective for stimulating ethylene production than IBA tend to support this conclusion.

No special precautions were taken to aseptically wound the trees so that usual wounding conditions would be simulated. Microorganisms were most certainly introduced at random into the wounds as the tools were often left on the ground. Consequently, it was not unusual to obtain positive isolations from discolored tissues immediately behind the wounds. For the same reason, it is impossible to categorically state that a dressing had fungistatic effects when few positive isolations were obtained behind the wounds as the wounds may have remained sterile. The results obtained in discolored tissues above or below the wounds are of much greater importance as they indicate how successful the tree was in resisting further invasion from microorganisms already present behind the wounds.

Some auxins reduced invasion by fungi but did not affect bacteria. IBA had no effect except on red maple. NAA reduced invasion and IAA totally prevented it on red maple. The increased discoloration caused by auxins was not by itself responsible for the increased resistance to invasion by fungi. Nontreated wounds and wounds treated with other dressings that

did not prevent invasion: CEPA, IAA-GA-BA, and IBA-GA-BA, were associated with as much or more discoloration than wounds dressed with auxins. The discoloration associated with dressings that contained auxins, especially IAA and NAA, was different from the discoloration usually encountered. It was a deeper brown and the greenish-colored zone often present at the outer edges of the columns was absent.

Increased resistance to invasion seemed related to the fate of the tissues immediately surrounding the wound, whether or not the tissues not killed by the action of wounding itself, stayed alive. IAA caused no bark dieback while NAA did; IAA prevented invasion while NAA only reduced it. CEPA, which caused a discoloration similar to that of auxins but at the same time caused much bark dieback, did not affect invasion by fungi.

The dressings that prevented fungi from invading discolored tissues also inhibited callus formation and increased discoloration. I suggest that auxins can promote endogenous ethylene production to a level that affects the general metabolism of phenols without killing the tissues. As a result, the tree develops greater resistance to invasion by microorganisms.

Auxins could have inhibited fungi directly. It has been reported (18) that 4% 2,4-D inhibited the growth of several bacteria and fungi, in culture. Others (15) reported that 5,000 ug/ml NAA and other auxins inhibited the growth of many fungi. Fungistatic effects should be more evident

immediately behind the wounds than away from them, where dilution of the auxin would be the greatest. This was not observed.

Although the inhibitory effect of auxins on fungi may have contributed to the results, their effects on callus formation, discoloration of the wood, and the patterns exhibited by extractable phenols on thin-layer chromatography plates, indicate that auxins affected some physiological processes in the trees and very likely stimulated natural defense mechanisms.

Gibberellin

GA alone had no effect on callus formation; it decreased discoloration, but did not affect invasion of the discolored tissues by microorganisms. Lavee and Haskal (39) also reported that GA had no effect on callus formation. They showed that 2% zinc oxide promoted callus formation but they obtained much better results when zinc oxide was added to IBA. They did not try adding GA to IBA. On seedlings, GA acted as if it reversed the inhibition caused by auxins. On older trees, inhibition was evident only with NAA and GA did not reverse it.

GA promoted callus formation only when it was added to IBA. As determined on apple seedlings, a 5:2 ratio of auxin to gibberellin was most suited. Digby and Wareing (22) reported that a 5:1 ratio was best but they did not try the 5:2 ratio. The addition of either zinc oxide or both zinc oxide and GA

to IBA produced the same effect as adding GA. These three dressings were the only ones that promoted callus formation significantly more than lanolin on red maple. Zinc oxide was not tested on other species but the dressing containing IBA plus GA was also effective on beech.

In addition to being required for normal wood formation (22), the combination of auxin and gibberellin could have other favorable effects on callus formation. Simultaneous treatment of pea stumps with auxin and either gibberellin or cytokinin, or both, produces results supporting the theory of hormone-directed transport of nutrients (78). Seth and Wareing (60) showed that GA or kinetin, when added to IAA, caused a greater accumulation of radioactive phosphorus in pea stumps than IAA alone. There is also some evidence showing that the application of either gibberellin or cytokinin along with auxin results in increased levels of endogenous auxin in the stem.

The effect of the dressing containing IBA plus GA on callus formation could be due to its effect on the transport of endogenous auxin and other metabolites to the wound site. This could explain why zinc oxide had the same effect as GA. Zinc is thought to have a regulatory role on auxin metabolism via its effects on enzymes involved in the metabolic pathway from tryptophan to auxin (24).

The addition of GA to auxins had a variable effect on discoloration compared to auxins alone. The effect depended on the auxin that was combined with GA. The effect on

fungi of the dressings containing auxin and GA was also variable. The dressings containing either NAA plus GA or IAA were the only ones to drastically limit invasion by fungi.

Cytokinin

Although BA promoted callus formation on seedlings, it had no effect or inhibited callus formation on older trees. Its effects on discoloration and invasion of the discolored tissues by microorganisms were similar to those of lanolin. In combination with either IAA and GA or IBA and GA, it inhibited callus formation on red and sugar maple and promoted it on white pine and beech. Because the dressing containing IBA plus GA also promoted callus formation on beech, as much as the dressing containing IBA plus GA and BA, it is possible that BA had no effect on beech.

Lavee and Haskal (39) tested dressings containing either IBA plus kinetin or IAA plus kinetin in a 10:1 ratio and reported that these dressings had no effect or slightly inhibited callus formation. The same dressings promoted callus formation on apple seedlings, but on older trees a 1:1 ratio was used and it is possible that the inhibitory effect was due to an excessive amount of BA.

There are some indications that growth substances involved in cambium activation may differ in gymnosperms and angiosperms. Zajackowski (85) mentions that auxin and kinetin have a synergistic effect on Scots pine while GA has very little effect on conifers compared to its effect on angiosperms.

This could explain why the dressing containing IBA plus GA did not affect callus formation on white pine while the dressing containing IBA plus GA and BA did.

Factors Influencing the Effects of Growth Substances

The major problem encountered in this study was the wide range of responses that characterized some of the treatments. Consequently it was often impossible to statistically detect differences between some of the dressings. The types of variations encountered could be classified into three groups: 1) variation within the same tree, 2) variation within trees of the same species, and 3) variation between species, and possibly between angiosperms and gymnosperms. Such factors as the time of wounding in relation to the growing season and delayed treatment were examined because of their practical significance for tree wound treatment.

Variation Within the Same Tree

Growth substances may not be equally effective during any periods of the year. This is a very important factor in trying to promote callus formation because wounds occur at any time. One experiment on sugar maple was designed to determine if the effectiveness of growth substances would change during different periods of the growing season. Sugar maple did not respond to any of the dressings containing growth substances. The results obtained in this study are valid only for a short period of time in the spring. Based on other findings, it is

possible to extrapolate the effects of growth substances to other periods of the growing season.

The results obtained from wounding sugar maple trees before, during, and after the time of leafing out support previous findings (7,17,83). Healing is best early during the growing season because the cambium is more active and there is a longer period of time left for callus tissues to grow. On apple trees, the main period of callus development follows dormancy by a few weeks (17).

Callus formation was already drastically affected when sugar maples were wounded early in May and did not occur at all that year when the wounds were made in early June. Lanolin extended the period during which callus formation could still occur until early June: the latest time at which wounds were treated. On apple trees, callus tissues developed until September when lanolin was applied (17). The small difference between wound closure of wounds made in May and June was probably due to the one-month shorter period which the latter had to form callus tissues. Such a difference is usually eliminated during the following year (28).

If it is true that cambial activity throughout the growing and dormant season is controlled by a balance between growth promotors and inhibitors, and there is strong evidence to support that theory (78), growth substances may be less effective in promoting callus formation as the growing season advances (7). Late in the season, higher concentrations of growth substances could be needed to promote callus formation

vigor trees. This could be due to an interaction between different dressings. On these trees, two wounds opposite each other received different dressings, and the dressings that promoted callus formation the most on low vigor trees were often opposite to dressings that inhibited callus formation the most. Wound responses can be detected on the opposite side of the tree after only 14 days (54).

The effects of auxins on discoloration of the wood and invasion by microorganisms were not modified by the vigor of the trees as much as callus formation was. Compared to lanolin in each group, IAA proportionally caused more discoloration on low vigor trees than on high vigor ones. When isolations from auxin-treated wounds yielded fungi, there was no difference between low and high vigor trees.

Variation Between Different Species

Wounds on different species or varieties close at different rates. Tilford (79) reported that callus formation was fastest on white oak and progressively decreased on American elm, red maple, and hickory. In this study, sugar maple was the slowest to form callus tissues and the least responsive to any growth substances.

The nature or the amounts of endogenous auxin could vary between species and different amounts of growth substances could be required to promote callus formation on different species. There could also be some inhibitors present in different amounts in some species. As mentioned earlier, it is possible

that gymnosperms and angiosperms require different growth substances for cambial activation. The results obtained on white pine tend to support that hypothesis.

Practical Application

The ultimate objective in using a wound dressing should be to prevent decay. Thus it would be desirable to stimulate callus formation only if the wound closes quickly enough so that wood-rotting fungi cannot enter the wounded tissues. Decay may be best prevented by promoting defense mechanisms immediately after wounding. The decay process could be stopped at an early stage by effectively preventing pioneer microorganisms from penetrating the discolored tissues.

If the dressings that promoted callus formation or increased resistance to invasion by fungi have the same effects on a broader spectrum of species and conditions, they could become very useful for tree wound treatment. Although growth substances can promote callus formation and increase resistance to invasion, they probably cannot effectively stimulate both processes at the same time.

The way we treat wounds would probably have to be changed if growth substances were to be used. Wounds could be treated in two stages to assure maximum effectiveness of the dressings. Whenever a wound occurs, it should be treated as soon as possible with an auxin alone to stimulate defense mechanisms. If callus formation has not occurred satisfactorily

before the following growing season, a combination of auxin and gibberellin could be applied at the beginning of the growing season when it appears to be most effective.

Growth substances are expensive. This factor could seriously limit their practical uses. The costs of some of the dressings tested was approximately $17/gal for lanolin, $110/gal for the IBA-GA dressing, and $60/gal for the IAA dressing compared to $1.50/gal for "Treeheal" and $7/gal for "Tanglefoot Tree Paint".

Lanolin seems a better choice for promoting callus formation than the commercial dressing tested in this study. As suggested before (44), antibacterial and fungicidal compounds could be incorporated in lanolin to produce an inexpensive dressing that could effectively prevent decay.

In summary, within the range of concentrations tested, CEPA did not stimulate defense mechanisms at least to the point that resistance to invasion by microorganisms was affected. Because it is physiologically active only when continuously present at very low concentrations and within a narrow range of concentrations, direct application of ethylene to stimulate defense mechanisms proved to be impracticable, with lanolin as a carrier.

The addition of gibberellin to at least one auxin, IBA, in a lanolin carrier proved to be superior to auxin alone for promoting callus formation. It was the only dressing that significantly increased callus formation in red maple and American beech. Gibberellin could be replaced by zinc oxide

when it was tried on red maple. On white pine, the presence of benzyladenine with IBA was necessary to promote callus formation.

This is the first time to my knowledge that callus formation has been promoted by the use of growth substances following wounding on stems of shade or forest trees. The effectiveness of growth substances for promoting callus formation is affected by many variables which cannot be controlled. Many questions need to be answered before such compounds can be used to stimulate callus formation.

Auxins, especially IAA, inhibited callus formation. This "injurious" effect has been reported for many auxins by everybody who tried them for promoting callus formation. Although auxins inhibited callus formation, they probably stimulated natural defense mechanisms by stimulating endogenous ethylene production. If their effect on the invasion of the discolored tissues by pioneer fungi can be demonstrated on a larger scale, it could supercede the adverse effect they have on callus formation. Even though auxins have always been considered as "injurious" from men's point of view, they may be most beneficial for the tree.

SUMMARY

1. Many growth substances stimulated callus formation on apple seedlings, but the combination of auxins with GA, in a 5:2 ratio, was most effective to promote well organized callus tissues. Sucrose or BA did not enhance the effect of auxins. Compounds with sulfhydryl groups, as well as ascorbic acid either did not affect or inhibited callus formation.
2. CEPA in lanolin did not affect phenol content or PAL activity in tissues in the vicinity of wounds on stems of apple seedlings. CEPA (10 ug/ml) in propylene glycol slightly promoted PAL activity when it was applied daily.
3. Wounds treated with the commercial dressing "Tree Aid" closed no more than nontreated wounds, and had as much bark dieback. This dressing had no effect on discoloration of the wood or on invasion of the discolored tissues by microorganisms.
4. Lanolin prevented bark dieback around the wounds and favored callus formation. It extended, by at least two months, the period during which callus formation could still occur during the same growing season sugar maples were wounded. It reduced the amount of discoloration in the wood but did not prevent microorganisms from invading the discolored tissues.

5. The presence of both IBA (0.5%) and BA (0.5%) on white pine, and IBA (0.5%) and GA (0.2%) on red maple and beech, was necessary to stimulate callus formation. Sugar maple did not respond to any growth substances. Zinc oxide (2%) could replace GA when it was tested on red maple. The dressings that promoted callus formation had little effect on discoloration of the wood, and did not prevent microorganisms from invading the discolored tissues.
6. When they were tested on red maple, IAA (0.5%), and NAA (0.5%) in combination with GA (0.2%), completely prevented fungi from invading the discolored tissues; they increased the amount of extractable phenols in that wood; and they inhibited callus formation.
7. Many factors influenced the effectiveness of the growth substances that promoted callus formation. Some factors were inherent to individual trees, species, or group of species. Other factors, such as a delay in treating the wounds and the period of the growing season during which the wounds were made, were accidental.

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