HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (PRUNUS CERASUS L.)

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
EUGENE ALBERT MIELKE
1974

This is to certify that the

thesis entitled

HORMONAL CONTROL OF FLOWER BUD DORMANCY
IN SOUR CHERRY (PRUNUS CERASUS L.).

presented by

Eugene Albert Mielke

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Frank G. Dennis Jr.
Major professor

Date August 8, 1974

O-7639

ABSTRACT

HORMONAL CONTROL OF FLOWER BUD DORMANCY
IN SOUR CHERRY (PRUNUS CERASUS L.).

By

Eugene Albert Mielke

Sour cherries (Prunus cerasus L. cv. Montmorency) are of great economic importance to Michigan orchardists. In the dormant state, the flower buds are well able to withstand most Michigan winter temperatures; however, every year a portion of the crop is lost to spring frosts. Occasionally, (e.g., two of the last five years) the loss is severe. Delay of bloom by 7 to 14 days would greatly reduce the chance of frost damage. An understanding of the mechanism controlling dormancy might provide a basis for programming anthesis.

The mechanism of dormancy is assumed to be partially, if not wholly, under hormonal control. Abscisic acid (ABA), an endogenously occurring growth substance, is suspected of being partially responsible for preventing bud growth, and elucidation of its role in dormancy was the purpose of this thesis.

Abscisic acid was isolated and positively identified by gas-liquid chromatography and mass spectrometry as one of the inhibitory materials present in both the acidic (free) and base hydrolyzable (bound) fractions of both aqueous and methanolic extracts of sour cherry buds. Free and bound ABA were found in both bud scales and floral primordia, total

quantities being greater in the scales, while concentrations were greater in the primordia. The bound form was assumed to be the 1'-glucose ester.

Levels of free and bound ABA in both the scales and floral primordia were measured by electron capture gas-liquid chromatography through three dormant periods from 1970 to 1973. In the scales, both free and bound ABA increased in late autumn, reached a maximum by early December, and slowly declined from their maximum levels in winter until just prior to bud swell, or remained fairly constant, then declined to a minimum just prior to bud swell. Free ABA increased again as the buds swelled, reaching a second maximum, lower than the first, at the time of full bloom, then declined through petal fall, while the bound ABA continued to drop, reaching its minimum at full bloom or petal fall.

Free ABA in the primordia rose rapidly in late autumn, reaching a maximum in November or December, then declined rapidly to or below the original level, where it remained until bud swell. As the buds swelled, ABA again rose, reaching a second maximum at the time of full bloom, then declined as the petals abscised. Bound ABA rose in a parallel fashion, but declined more slowly, reaching minimum values at full bloom or petal fall. Levels of bound ABA were two to five fold higher than those of free ABA.

The late autumn peak of ABA in the primordia coincided with the period of deepest dormancy in only two of the three years in which the levels were measured. The increase in autumn was coincident, not with the onset of dormancy, but with the onset of leaf abscission, maximum levels occur when about 90 to 95% of the leaves had abscised. Free ABA content returned to its original level 2 to 6 weeks prior to the termination of rest.

Mechanical defoliation of trees 2 to 6 weeks prior to the onset of natural leaf abscission prevented the increase in both free and bound ABA without affecting bud dormancy; however, the leaves may not be the source of this ABA, as leaves on forced cuttings inhibited neither bud break nor flower development, while ABA inhibited both.

ABA was applied under orchard conditions after bud swell had begun. Neither 50% ethanolic solutions applied to the bud scales nor aqueous solutions injected into limbs or buds delayed flower development. In the greenhouse, however, both ABA and Amo-1618 delayed bud break and flower development when applied to the bases of excised branches before the buds had begun to swell.

GA_3, GA_{4+7} or GA_{13} promoted bud break and flower development when applied to the bases of excised branches prior to the end of rest, and flower development after rest had been terminated. GA_3 or GA_{4+7} overcame Amo-1618-induced, but not ABA-induced, inhibition when used either in combination or sequentially, indicating that these inhibitors function in different manners, with Amo-1618 inhibiting GA synthesis and ABA at least partially inhibiting GA action. The effects of ABA were long-lived. The greatest inhibition occurred when ABA and Amo-1618 were used in combination, and the greatest promotion occurred when GA_3 and GA_{4+7} were used together. The fact that both bud break and flower development occur in the presence of Amo-1618 suggests that GA s are not the only hormones involved in these processes.

The data presented cast serious doubt on the role of ABA as a controlling factor in winter dormancy of sour cherry buds.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*PRUNUS CERASUS* L.).

By

Eugene Albert Mielke

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1974

ACKNOWLEDGMENTS

I wish to express my deep appreciation to Dr. F. G. Dennis for his constant support, assistance, counsel and encouragement.

I would like to especially thank Drs. M. J. Bukovac, D. R. Dilley, H. P. Rasmussen, J. A. D. Zeevaart and J. E. Varner for their guidance and encouragement.

Appreciation is extended to Dr. C. C. Sweeley for making the mass-spectrometer facilities available and to Mr. J. E. Harten for his technical assistance in performing the analysis, and to Mr. Donald Kenney and Abbott Laboratories for providing the gibberellins.

Finally, to my wife, Karen, who solved the problems no one else could.

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ABBREVIATIONS

The following abbreviations will be used in this thesis. Names given in parentheses are additional common or trade names.

ABA	abscisic acid (abscisin II, dormin)
Alar	succinic acid-2,2-dimethylhydrazide (SADH, B-9, B-995, daminozide)
Amo-1618	ammonium (5-hydroxycarvacryl)trimethyl chloride piperidine carboxylate (ACPC)
BA	N^6-benzyladenine
CCC	(2-chloroethyl)trimethyl ammonium chloride (Cycocel, chlormequat)
CEPA	(2-chloroethyl)phosphonic acid (Ethrel, ethephon)
Coumarin	1,2-benzopyrone
GA	denotes the series of gibberellins--use of a subscript denotes a specific gibberellin
GC-MS	combined gas-liquid chromatography and mass spectrometry
GLC	gas-liquid chromatography
IAA	indoleacetic acid
IBA	indolebutyric acid
Kinetin	6-furfuryl amino purine
Morphactin	2-chloro-9-hydroxyfluorene-9-carboxylic acid (Chlorflurenol, CF1)
NAA	1-naphthaleneacetic acid
NAD	1-naphthaleneacetamide
Naringenin	5,7,4'-trihydroxyflavanone

PBA	6-(benzylamino)-9-(2-tetrahydropyranyl)-9H purine
Prunin	naringenin-7-glucoside
TIBA	2,3,5-triiodobenzoic acid
TLC	thin-layer chromatography
Zeatin	6-(4-hydroxy-3-methyl-2-butenylamino) purine

INTRODUCTION

INTRODUCTION

Bud dormancy is a phase of temporary growth suppression in perennial plants which allows them to survive periods of environmental stress. During this time growth (defined as an irreversible increase in size) ceases while development (defined as anatomical, morphological or physiological change) continues.

Many investigators (Doorenbos, 1953; Samish, 1954; Wareing, 1956; Smith and Kefford, 1964; Vegis, 1964) have characterized the annual life cycle and divided it into separate phases, including: (1) the grand period of growth--that period of major growth and development from bud break to terminal bud formation, (2) the inductive phase--the period of cessation of growth during which growth and development may resume under suitable conditions, (3) true dormancy--that period in the cycle in which growth will not occur regardless of environment, (4) quiescence--that period following true dormancy in which the environment is unfavorable for growth, and (5) the growth initiation phase--that period during which the buds swell leading into the grand period of growth.

Dormancy has commonly been assumed to be under hormonal control, although other factors may be involved (Wareing, 1950, 1956; Nitsch, 1957a, 1957b; Vegis, 1964; Kawase, 1966). The known endogenous hormones involved in the control of different phases of growth and development include abscisic acid, auxins, cytokinins, gibberellins and ethylene (Paleg and West, 1972; Skoog and Schmitz, 1972; Thimann, 1972).

Abscisic acid is thought to be part of the promoter-inhibitor balance responsible for the control of dormancy (Khan, 1971; Wareing and Saunders, 1971); therefore, the major emphasis in this thesis will be to study the role of abscisic acid in the hormonal control of bud dormancy in sour cherry (Prunus cerasus L. cv. Montmorency).

LITERATURE REVIEW

LITERATURE REVIEW

Introduction

Although much work has been done on dormancy in other organs and species, this review will be concerned with the literature relating to dormancy of buds of woody plants.

Definitions

Dormancy is often separated into rest (also called constitutive dormancy, primary dormancy, innate dormancy, internal dormancy, true dormancy or endogenous dormancy) and quiescence (also called imposed dormancy, external dormancy, exogenous dormancy or false dormancy). Sussman and Halvorson (1960), considered rest to be "... a condition in which development is delayed due to an innate property of the dormant state such as a barrier to the penetration of nutrients, a metabolic block, or the production of a self-inhibitor." Rest will be defined as the stage in which growth will not occur under any conditions and quiescence as that stage in which growth will occur if plants are placed under suitable conditions.

Apical dominance (also called correlative inhibition) refers to the inhibitory effect of the terminal bud on the growth of lateral buds, while summer dormancy is a cessation of terminal growth in the summer, with the formation of an apical bud and bud scales (Nitsch, 1957a).

Secondary dormancy resembles rest in that growth will not occur under any conditions, and is an induced state which occurs after primary rest has been broken.

True dormancy may also be divided into three stages: predormancy, mid-dormancy and post-dormancy (Vegis, 1964). Predormancy may include summer dormancy and apical dominance. During this period of time, plants have not completely lost their ability to grow; however, the range of conditions under which growth may occur becomes narrower as predormancy progresses. Mid-dormancy is synonymous with true rest. Post-dormancy applies to the period of exit from true rest when plants can resume growth. As post-dormancy progresses, the range of conditions under which growth will occur becomes wider.

Chilling as used in this dissertation refers to the breaking of bud dormancy by exposure to low temperatures. Temperatures near or slightly above freezing, while not required to break dormancy, are more efficient than temperatures below freezing (Weinberger, 1950; Erez and Lavee, 1971). Temperatures much below freezing may cause serious injury to bud tissues.

Flower development in cherry has been divided into 10 stages from unswollen buds to petal fall (Ballard et al., 1971; Dennis and Howell, 1974). The following numerical values were used: 0, non-swollen; 1, first swelling; 2, green tip; 3, half green; 4, tight cluster; 5, open cluster; 6, first white; 7, balloon stage; 8, full bloom; and 9, petal fall. As used in this thesis, bud break refers to the attainment of numerical stage 2, while flower development refers to change between numerical stages 2 through 9.

Functions of rest

Bud dormancy allows the plant to survive during periods of environmental stress. In nature, chilling requirements prevent growth of those portions of the plant which would not survive winter temperatures until conditions are favorable for survival. Although growth is arrested, development continues.

Theories to explain rest

Many theories have been proposed to explain the cause of bud dormancy. One of the first was that auxins are limiting and growth resumes when the level of auxin reaches a threshold (Avery et al., 1937); however, auxin applications have generally not been effective in breaking dormancy (Mitchell and Cullinan, 1942; Sell et al., 1942, 1944; Hall, 1969; Singh and Singh, 1972). Growth promoters other than auxin, as well as growth inhibitors, are found in plants, and this led to theories that a single promoter was responsible for growth or that a single inhibitor could prevent growth. These single hormone theories persisted until no clear case for a single hormone could be established. They have now given way to the multiple hormone theory (promoter-inhibitor balance), which proposes that dormancy is a state caused by high levels of inhibitors together with low levels of promoters (Khan, 1971; Wareing and Saunders, 1971). Growth occurs when the balance shifts in favor of promoters. Support for this theory has come from exogenous application of growth regulators which have inhibited or promoted growth, and from studies of endogenous levels of hormones.

Both of these approaches probe the underlying mechanism of plant response to environment. The first approach is easier in terms of

time and expense, for one merely applies a material and measures a response; however, one is not sure how much of the applied material actually gets into the plant, or if the material which penetrates the plant remains in the original form, is conjugated to an inactive form, or is metabolized to other products. In addition, while it is possible to make zero applications, it is seldom possible to have zero levels due to the presence of the material endogenously. The second approach--the study of endogenous hormones--is the more difficult, and due to time limitations usually only one or two growth substances have been studied at any one time in any one species. Other difficulties with the latter approach include uncertainty as to what is actually being extracted, and interpretation of data, particularly if bioassays are used to measure levels. However, the method does provide some idea of what is actually occurring in the plant.

Exogenous Control of Summer Dormancy

Environmental factors

Light. Exogenous or environmental factors influencing dormancy in buds include temperature, light, moisture and nutrient availability. The induction of dormancy in buds of some plants is under photoperiodic control, and short photoperiods result in a stoppage of extension growth (Nitsch, 1957a, 1957b, 1966). The response is truly photoperiodic, as interruption of the long night with a short period of low intensity light prevents induction (Wareing, 1950). During the induction phase terminal growth ceases, usually accompained by the formation of a terminal bud; however, in Rhus typhina, Syringa vulgaris and Vitis

labrusca, short days cause the apical meristems to abort (Nitsch, 1957a). If a terminal bud develops, scales (modified leaves) may or may not be formed. In birch, the summer resting bud does not form scales (Nitsch, 1957a).

Nitsch (1957b) divided plants into four classes based on their photoperiodic reactions. These include: (1) long days prevent the onset of dormancy, causing continuous growth, and short days cause dormancy; (2) long days prevent the onset of dormancy, cause intermittent growth, and short days cause dormancy; (3) long days prevent the onset of dormancy, and short days do not cause dormancy; and (4) long days do not prevent the onset of dormancy.

Kawase (1961b) demonstrated in Betula pubescens that short days cause a stoppage of growth; however, if the long night were interrupted by a short period of low intensity light, the growth of the plants was intermediate between the short and long day treatments. Some cases of photoperiodically induced dormancy are reversible. Acer palmatum, Betula pubescens, Cercis canadensis, Fagus sylvatica, Larix europaea, Liquidambar styraciflua, Picea abies, Pinus spp., Populus tacamahaca, Quercus spp. and Tsuga canadensis, in which dormancy can be induced by short days, resume growth if they are placed under long photoperiods (Nitsch, 1957a, 1957b).

Some plants can be prevented from becoming dormant by keeping them under long day conditions. Several species of Pinus and Quercus respond to continuous long days by growing in flushes instead of continually (Nitsch, 1957a, 1957b). Liriodendron tulipifera and Robinia pseudacacia will continue to grow for about 13 months when exposed to continuous long days (Garner and Allard, 1923).

In some species, long days do not prevent the onset of dormancy. Acer pseudoplatanus, Phellodendron amurense, Pinus sylvestris and Syringa spp. became dormant in a short period of time after exposure to continuous long days (Wareing, 1956; Nitsch, 1957b). Little or no response to photoperiod is exhibited by Sorbus aucuparia, Syringa vulgaris and species of Fraxinus and Rosa (Wareing, 1956). Most cultivated species of fruit trees (Malus, Prunus and Pyrus) appear to fall into this latter group (Bradley and Crane, 1960; Erez et al., 1966; Wareing and Phillips, 1970).

Studies in which specific photoperiods were given to isolated portions of the plant show that the location of the photoperiodic receptor varies with the species under study. In actively growing Betula pubescens seedlings, the photoreceptor is located in the buds (Wareing, 1954) while in Quercus robur, a receptor is located in both the buds and the leaves (Wareing, 1954). In Acer pseudoplatanus and Robinia pseudacacia the receptor is located in the mature leaves (Wareing, 1954) while in Weigela florida, it is located in the young leaves (Downs and Borthwick, 1956b).

Photoperiod may interact with temperature. In Vitis labrusca, a night temperature of 17°C prevented, while a night temperature of 22°C was optimal for the induction of dormancy, and in Rhus typhina a night temperature of 5°C prevented the onset of dormancy (Nitsch, 1966). Nitsch (1966) concluded that a temperature-dependent mechanism necessary for the induction of dormancy operates during the dark period.

Decapitation, defoliation and drought. During the inductive phase, apical dominance is important, and removal of the apical bud, bud scales

or leaves, causes the lateral buds to grow (Reece et al., 1946; Fraser, 1962; Brown et al., 1967; Spiers, 1973). These treatments are most effective in causing regrowth when done in the summer, with the effect diminishing as winter approaches. Spiers (1973) noted that the removal of the bud scales promoted bud break in tung. If leaves were removed in mid-summer, regrowth occurred; however, fall defoliation delayed bud break the following spring in pecan and tung (Worley, 1971; Spiers, 1973).

Ramsay et al. (1970) demonstrated that in apricot, decapitation of the shoot in April, while the spurs were still growing and the leaves expanding, would induce axillary bud growth, while both decapitation and defoliation were required in May, after shoots and leaves had stopped growing. In June, neither treatment was effective.

Singh and Singh (1972) found that in mango, decapitation in early summer was not effective in inducing lateral bud growth; however, if the terminal and top 9 cm of the shoot (which included small fruits) were removed, lateral bud growth was induced. This treatment was not effective when fruits were fully grown and a terminal bud had formed. The authors imply that the fruit was partially responsible for inhibition of lateral bud growth, but did not test the effect of fruit removal.

Tinklin and Schwabe (1970) reported that removal of the bud scales promoted bud break in blackcurrant (Ribes nigrum). Lanolin or wax application did not substitute for scales, indicating that inhibition was chemical and not a result of the exclusion of oxygen as Vegis (1964) had suggested.

Severe water stress often stimulates bud break. If the soil in which coffee (Coffea arabica) was growing were allowed to dry until plants wilted, and then water applied, the buds opened readily (Alvim, 1960).

Alvim (1960) concluded that drought performs the same function in coffee buds that chilling performs in other plants.

Chemical Factors

Promoters. Auxins have been relatively ineffective in breaking summer dormancy. TIBA applied directly to the lateral buds in a lanolin paste, promoted lateral bud growth only slightly in mango (Singh and Singh, 1972). IBA as a 2% lanolin paste promoted lateral shoot development in normally unbranched stems of lowbush blueberry Vaccinium angustifolium) while IAA and NAA were not effective (Hall et al., 1969).

Cytokinins have been effective in some instances. BA and kinetin increased the number of lateral shoots forming on one-year-old seedling apple trees grown in the greenhouse (Kender and Carpenter, 1972). Under orchard conditions, BA stimulated bud growth of apple only on the current season's growth, and while shoots were still growing (Kender and Carpenter, 1972). Neither BA nor kinetin were able to stimulate lateral bud growth in apple or mango after formation of the terminal bud (Kender and Carpenter, 1972; Singh and Singh, 1972). Spray applications of a synthetic cytokinin (PBA) during the period of active shoot growth induced lateral bud growth in macadamia, while causing terminal buds to grow at a reduced rate (Boswell and Storey, 1974).

The effects of gibberellins in breaking summer dormancy have been variable. GA_3 was effective in promoting bud growth in Citrus once growth had ceased; however, it did not prevent cessation of growth (Cooper and Peynado, 1958). GA_3 prevented the onset of summer dormancy in Betula pubescens, B. lutea and Quercus borealis (Nitsch, 1957a; Kawase, 1961b), as well as the induction of dormancy in Betula by an

ABA-like inhibitor extracted from the leaves (Eagles and Wareing, 1963). GA_3 prevented both the onset of summer dormancy and ABA induction of dormancy in seedling apple (Robitaille and Carlson, 1971).

Inhibitors. Both ABA and ABA-like inhibitors extracted from leaves and buds induced dormancy in seedlings of Acer pseudoplatanus, Betula pubescens and Ribes nigrum (Eagles and Wareing, 1963, 1964; El-Antably et al., 1967). Powell and Seeley (1970) were able to induce terminal bud formation in apple seedlings with ABA, while Robitaille and Carlson (1971) inhibited growth and induced the formation of resting buds in dwarf apple trees with ABA, the effect being prevented by applications of GA_3.

Summary

Several environmental factors affect summer dormancy, photoperiod being one of the most important. Long days generally favor growth, short days, dormancy. In plants which are receptive, the photoreceptor may be located in either the leaves or apex or both. Apical dominance is important in many species, and removal of the apex allows lateral buds to develop, providing the treatment is performed in early summer.

Auxins are relatively inactive in affecting summer dormancy, while cytokinins, and particularly gibberellins, are capable of inducing growth of otherwise dormant plants. ABA inhibits or prevents growth of some species under otherwise favorable conditions, and GA and ABA are mutually antagonistic under such circumstances.

Endogenous Control of Summer Dormancy

Hormonal Factors

Role of auxin. A positive correlation was found in apple and horsechestnut shoots between auxin content and growth. Auxin was at a maximum in summer when shoot growth was most rapid, and then declined with the cessation of growth (Avery et al., 1937). Smith and Wareing (1972) found extremely low concentrations of a neutral auxin-like material in poplar (Populus x robusta) in March. This material reached a maximum in early June, then declined steadily to a low level by the end of November. Similar results were reported in mango during the induction of summer dormancy (Singh and Singh, 1972).

Role of cytokinins. In the leaves of birch, maple and poplar a zeatin-riboside-like material increased from May through September as leaves expanded, and then fell as they senesced (Engelbrecht, 1971). There was little or no zeatin-like material in the leaves and none of the zeatin-riboside-like material in the buds. Hewett and Wareing (1973a, 1973b) found that in poplar (Populus x robusta) cytokinins were highest in the xylem sap just before and during cessation of growth. A cytokinin-like substance in xylem sap of apple reached a maximum just after full bloom and declined with cessation of growth, reaching minimum values by early August (Luckwill and Whyte, 1968).

Role of gibberellins. GA_4 and GA_7 each have an Rf similar to ABA in many solvent systems and, moreover, reduce the inhibitory effect of ABA in the wheat coleoptile bioassay (Lenton et al., 1972). Lenton et al. (1972) noted that gibberellin-like activity from sycamore (Acer

pseudoplatanus) apices, as measured by the lettuce hypocotyl bioassay, decreased after five days in seedlings held under short photoperiods as compared to those maintained under long photoperiods.

Role of inhibitors. Tinklin and Schwabe (1970) measured the levels of substances in blackcurrant leaves (Ribes nigrum) which were transported basipetally and which inhibited the growth of lateral buds. Levels of both neutral and acidic inhibitors, as measured by bioassay, were low in May, rose to a maximum in October and then declined. There was more inhibitor in the neutral fraction than in the acidic fraction. Chel'-tsova and Lebedva (1971) reported that inhibitors in apple buds rose during late autumn, but not during the period when the growth rate slowed in the summer.

In apple, phloridzin content was low to non-existent during the period of active growth and increased as the days shortened, reaching a maximum during the period of deepest dormancy (Sarapuu, 1965). IAA was found to accelerate the decomposition of phloridzin to phenolic promoters in vitro. Sarapuu (1965) suggested that dormancy is caused by a blockage of the phloridzin-decomposing enzyme.

The induction of dormancy by short photoperiods has been correlated with the increase in inhibitors. Short days induce dormancy in Betula and long days cause the resumption of growth (Wareing, 1954). More growth inhibitor was extracted from leaves and buds of trees grown under short days than those grown under long days (Phillips and Wareing, 1958, 1959; Eagles and Wareing, 1963, 1964; Robinson et al., 1963; Robinson and Wareing, 1964; Ilyin, 1971). Phillips and Wareing (1959) observed that changes in the inhibitors preceded formation of the resting bud. Both β-inhibitor and the depth of dormancy increased in Betula with an

increasing number of short days and declined on subsequent exposure to long days (Kawase, 1961a, 1961b). Eagles and Wareing (1964) detected higher inhibitor levels in the mature leaves and stem apices of sycamore (Acer pseudoplatanus) trees two to five days after they were transferred from long to short days. This inhibitor increased during late summer and early autumn and inhibited growth of sycamore when extracted and reapplied to apices. ABA was isolated in crystalline form from birch and sycamore and characterized as the main inhibitor of the β-inhibitor zone (Cornforth et al., 1965, 1965b, 1966). Since this initial discovery, several workers have identified ABA in extracts of buds or leaves (Cornforth et al., 1966; Bonnet-Moseinbert, 1969; Badr et al., 1971; Lenton et al., 1972).

Saunders and Lenton (1969) using GLC, found higher levels of inhibitors in Betula under long days than under short days. This casts doubts on the identity of the ABA-like materials previously measured by bioassay, and emphasizes one of the shortcomings of bioassays--their lack of specificity.

Total acidic and ABA-like inhibitors in apricot and peach increased from low levels in early summer to maxima by early October (Corgan and Peyton, 1970; Ramsay and Martin, 1970b), and in apple terminal buds, levels of ABA and its 1'-glucose ester, as measured by GLC, increased from the end of June, reaching maxima by early October (Seeley, 1971).

Summary

Auxins, as measured by bioassay, appear correlated with growth, with the highest levels being found during the period of most rapid growth, after which they decrease. Cytokinin-like substances in leaves

increase during the summer to maximum just before senescence begins, while in xylem sap levels are highest just before cessation of terminal growth, suggesting that high levels of cytokinins may inhibit growth.

Inhibitors, including phloridzin and ABA-like substances measured by bioassay, are low in early summer, and increase with shortening days. In some instances bioassay results for ABA-like inhibitors are not supported by measurements made by GLC, a more precise method, while in other instances, GLC levels have paralleled bioassay levels. Caution is needed when interpreting bioassay data, for apparent increases in inhibitors may actually reflect decreasing levels of promoters.

Exogenous Control of Winter Dormancy

Environmental factors

Chilling. During the annual cycle transition from one phase to another is gradual. True dormancy or rest may be an intensification of summer dormancy, and an intensity curve may be represented by a bell-shaped curve (Hatch and Walker, 1969). Removal of dormancy in nature is usually accomplished by a period of low temperature. Some plants exhibit an obligate requirement for cold; the buds break rest after a threshold number of hours of chilling and additional chilling does not increase response. Others have a facultative requirement, and additional chilling beyond the threshold increases the amount of bud break (Darrow, 1942). Weinberger (1950) stated that temperature slightly above freezing broke dormancy faster than temperatures below freezing. Erez and Lavee (1971) reported a decrease in the dormancy breaking effect in stone fruits at temperatures approaching freezing with 6°C being optimum.

Eggert (1951) ranked various species according to the hours of chilling required to break dormancy as follows from the lowest to highest: red raspberry, black raspberry, prune, peach, currant, sweet cherry, pear, sour cherry, apple, grape and blueberry. This ordery may change with variety. For example, the chilling requirement for peach varieties varies from 200 hours to 1150 hours below 7°C (Samish, 1954). Eggert (1951) found that leaf buds needed more chilling than did flower buds and lateral buds more than terminal buds.

Common symptoms of insufficient chilling are erratic flowering over an extended period of time, reduced flower bud formation, abortion of shoots, and small, defored leaves. In pome fruits the flower primordia abscise, resulting in a small flower culster or only a leafy spur, and in stone fruits, entire buds abscise (Higdon, 1950; Samish, 1954).

In breaking rest in western red cedar (Thuja plicata), Pharis and Morf (1972) found low temperatures (3°C) to be necessary for the expansion of both ovulate and pistillate strobili. At warm temperatures (22°C), short days only partially replaced the chilling requirement, while at chilling temperatures, daylength had no effect on strobilus expansion.

Kester (1969) demonstrated that the chilling requirement is genetically inherited. When pollen from varieties of almond and peach with different chilling requirements was used on the same seed parent, a direct correlation was observed between the requirement of the male parent and the resulting progeny. Reciprocal crosses yielded seed populations with the same chilling requirements, demonstrating that the embryo genotype and not the maternal tissue of the seed controls this characteristic.

Secondary dormancy can be induced, or the required chilling period lengthened by high temperature (Overcash and Campbell, 1955; Vegis, 1964). Overcash and Campbell (1955) reported that peaches exposed to a diurnal thermoperiod of 16 hours at 4°C followed by 8 hours at 21°C required about 1000 hours at the 4°C temperature to break dormancy while only 750 hours were required at a constant 4°C.

Light. Photoperiod interacts with chilling temperatures in some cases. Betula pubescens, which responds to chilling, readily broke dormancy under either long or short days after chilling was fulfilled (Wareing, 1956). Long days were able to break dormancy in Pinus,but only after the chilling requirement had been partially completed, and buds of plants which did not respond to photoperiod before chilling (Acer pseudoplatanus and Robinia pseudacacia) broke faster after chilling when held under long days than under short days (Wareing, 1956).

Lavee and Erez (1969) demonstrated that peach leaf buds required light in order to open. Under constant illumination, leaf bud opening was promoted, while flower bud opening was promoted by continuous dark. Light quality also played a role. Green light or light of low intensity promoted, while red light or light of high intensity inhibited leaf bud opening. In contrast, flower bud opening was relatively independent of light quality or intensity (Erez et al., 1966, 1968).

Chemical factors

Auxin. Applications of auxin have generally not been effective in breaking dormancy and in fact has been suggested for delaying bloom (Mitchell and Cullinan, 1942; Sell et al., 1942, 1944). Flowering in

peach and tung was delayed 7 days by IAA, NAA or NAD, applied in February in a lanolin emulsion, 0.4% summer oil or light engine oil, with delay being correlated with bud injury (Mitchell and Cullinan, 1942; Sell et al., 1942, 1944); however, these materials had no effect on pear when applied in February (Mitchell and Cullinan, 1942). Applications of IAA and NAA promoted bud break and flower development in apple, peach and pear if applied after the buds had begun to swell (Bennett and Skoog, 1938; Mitchell and Cullinan, 1942).

Gibberellins. Gibberellins break dormancy in some species but not others. GA_3 promoted bud break in Douglas-fir (Pseudotsuga menziesii) (Lavender et al., 1973), and in partially chilled buds of basswood (Tilia americana) (Ashby, 1962), hydrangea (Hydrangea macrophylla) (Stuart, 1958), oak (Quercus borealis) (March et al., 1956), pear (Pyrus communis) (Brown et al., 1961) and red maple (Acer rubrum) (Mar h et al., 1956). Spring bud break was delayed for 1 to 3 weeks in Acer pseudoplatanus, Betula verricosa, Fagus sylvatica, Fraxinus excelsior, Sorbus aucuparia and Vitis vinifera by weekly applications of GA_3 between mid-August and late November (Brian et al., 1959; Weaver, 1959). These treatments had little effect on Acer rubrum, Castania sativa, Parthenocissus tricuspidata, Taxodium disticum and Ulmus procera (Brian et al., 1959).

In peach, GA_3 promoted bud break after partial fulfillment of the chilling requirement or delayed bloom the following spring if applied in the summer or fall. In two varieties of peach, Couvillion and Hendershott (1974) found that GA_3 was effective in promoting flowering only during restricted periods of time. In January, either exogenously applied GA_3 or additional chilling accelerated flowering, but by late

February neither treatment was effective. Stembridge and LaRue (1969) noted that application of GA_3 after partial fulfillment of the chilling requirement accelerated flowering; the longer the period of chilling, the greater the acceleration. Similar results were reported for almond (Hicks and Crane, 1968).

Exogenous applications of GA_3 were most effective in delaying bloom of peach during two periods (Corgan and Widmoyer, 1971; Painter and Stembridge, 1972). The first was in early summer at the time of flower bud initiation and the second, more effective, period was from August to early September when treatment delayed bloom up to 14 days. In addition to delaying bloom, September applications of GA_3 reduced bud hardiness (Stembridge and LaRue, 1969).

Cytokinins. BA has been used to break rest in dormant buds of grape (Vitis vinifera) (Weaver, 1973), apple (Malus sylvestris) (Chovjka et al., 1962; Pieniazek. 1964; Benes et al., 1965; Pieniazek and Jankiewicz, 1966) and pine (Pinus radiata) (Kummerow and Hoffman, 1963). Weinberger (1969) reported that a synthetic cytokinin, PBA, was effective in breaking rest of partially chilled peach buds, but could only substitute for a small portion of the chilling requirement, and Erez et al. (1971) noted that kinetin promoted bud opening in peach.

Ethylene and other gases. Vacha and Harvey (1927) broke the rest period in hardwood cuttings of apple, cherry, grape, pear and plum by treatments of 1000 ppm ethylene. Ethylene at 1000 and 10,000 ppm broke rest in flowering almond (Prunus triloba) and crabapple (Pyrus ioensis Bailey) (Denny and Stanton, 1928a). They found the locus of the gas's effect was in the bud (Denny and Stanton, 1928b).

Low levels of illuminating gas (ethylene) given for short periods of time promoted bud break in willow cuttings, while high levels resulted in death of elm, horsechestnut and red oak (Stone, 1913). Propylene at 1000 ppm broke rest in hardwood cuttings of apple, cherry, grape, pear and plum (Denny and Stanton, 1928a).

Bukovac et al. (1969) and Proebsting and Mills (1973) found September applications of CEPA, which releases ethylene in the tissue, delayed bloom of cherry (Prunus spp.) 3 to 5 days and decreased spring frost injury.

Ethylene chlorohydrin (25-100 ppm) effectively broke rest and promoted flowering in flowering almond and crabapple (Denny and Stanton, 1928a).

Other gases which have been effective in breaking dormancy include acetylene tetrachloride, ethylene dichloride, ethyl bromide, furfural, propylene chlorhydrin, and vinyl chloride, while chloroform and ethylene trichloride were less effective and ethyl iodide was injurious (Denny and Stanton, 1928a).

Other chemicals. Ballard (1914) reported a 10 to 14 day acceleration of flowering in 'Yellow Bellflower' apples after spraying with sodium nitrate and potassium hydroxide.

Erez et al. (1971) noted that while DNOC-mineral oil, thiourea, KNO_3 and kinetin each hastened flowering in peach, the best results were obtained when DNOC-mineral oil was used with thiourea, GA_3 or thiourea and KNO_3.

Inhibitors and growth retardants. Sullivan and Widmoyer (1970) applied high concentrations of Alar to 'Delicious' apples in September and thereby delayed bloom 4 to 5 days the following spring. In tung naphthalenic or paraffinic oil was effective in delaying bloom; however, the 1 to 50% oil sprays resulted in injury to the buds. This could be prevented by adding Alar to the oil mixture (Raese and Forrester, 1971). Raese (1971a, 1971b) reported that the naphthalenic oil was more effective than paraffinic oil with the most effective treatment being 50% oil with 2% Alar. January sprays were more effective than late-February sprays (Raese, 1971b). Alar itself was effective when used alone and only gave an additional 1 to 2 days delay of bloom when used with the oil. The concentration of oil in the spray was critical, with a 10% oil spray delaying bloom 3 to 4 days and a 50% oil spray delaying bloom 14 to 16 days (Raese, 1971a, 1971b).

Schneider (1970) used morphactin to delay bud break in apple and several other perennials. Slight toxicity was noted, with the first leaves being abnormal and misshapen, but the plants soon recovered. Phillips (1962) found that naringenin, a flavanone, completely antagonized the effect of GA_3 in breaking peach bud dormancy.

Little and Eidt (1968) reported that spring applications of ABA to cut stems delayed bud break 3 to 4 days in Acer rubrum, Abies balsamea, Fraxinus americana and Picea glauca. In addition, ABA inhibited cambial activity, cell division, and water uptake. Spray applications of ABA were ineffective in delaying bud break on forced cuttings of almond, apple, birch, blackcurrant, grape, peach, pear, plum and poplar, while applications to the cut bases of blackcurrant and willow almost completely inhibited bud development (El-Antably et al., 1967).

Summary

Removal of winter dormancy is usually accomplished by a period of low temperature, with temperatures slightly above freezing being optimum. Some plants have an obligate chilling requirement. The requirement varies greatly, both between and within species, and is genetically determined.

Auxins have generally not been effective in breaking dormancy and, in fact, delay bloom if applied in February, with applications at the time of bud swell promoting flower development, while cytokinins, ethylene and other gases have been effective in releasing dormancy in several species.

Gibberellins break rest and promote flower development in some species but not others, and their effectiveness is dependent on the time of application. Gibberellin generally hastens bud break and flower development if applied after partial fulfillment of the chilling requirement, but delays these processes if applied the previous summer or fall.

Alar, morphactin, abscisic acid and naringenin have been effective in inhibiting bud break and flower development, or antagonizing GA_3-promoted bud break.

Endogenous Control of Winter Dormancy

Hormonal factors

Role of auxin. Auxin was the first growth regulator which was proposed for the control of dormancy, and Hatcher (1959) found a positive

correlation between auxin content of apple and plum shoots and growth. Although little or no activity was found in peach buds during the dormant period, auxins were detectable in the spring as the buds began to swell (Blommaert, 1955). In Gingko, auxin was not detectable in unswollen buds, but increased rapidly as buds swelled, reached a maximum at the green tip stage, then declined with further opening (Gunckel and Thimann, 1949). In cherry and pear, no auxin was found in buds in November; however, auxin activity increased from minimum values in December, reaching a maximum in March in response to chilling, but not warm, temperatures (Bennett and Skoog, 1938).

Role of Gibberellins. Chailakhyan et al. (1965) detected an increase in GA-like substances in peach buds at the end of chilling. Eagles and Wareing (1964) correlated the end of rest with increases in one GA-like substance in Acer, but there was no relationship with a second, less polar GA-like substance. Ramsay and Martin (1970b) reported a large increase in GA-like activity near the end of rest in apricot buds; however, Luckwill and Whyte (1968) found only small amounts of GA in apple sap just prior to bud break and at other times of the year, suggesting that GA was not important in rest. Browning (1973a) found similar results for both xylem sap and buds of coffee (Coffea arabica) and concluded that the increase in GA in the buds was due to its liberation from a bound, storage form located there; however, Lavender et al. (1973) noted that GA-like materials in the sap of Douglas-fir, as measured by bioassay, increased with bud activity as soil temperature increased, suggesting that the gibberellins responsible for bud break come from the roots. Crozier et al. (1970) characterized GA_3 and three other gibberellins in the shoots of growing Douglas-fir trees but could

not detect them in dormant trees, adding support to the hypothesis that increasing gibberellin levels are responsible for growth.

Role of cytokinins. Luckwill and Whyte (1968) found, in apple xylem sap, a cytokinin which remained at a low level from August until just before bud swell in March, when it rose, reached a maximum at full bloom and then declined. Similar results were reported in coffee (Browning, 1973b). In both birch and poplar buds, cytokinin activity was absent during winter, but appeared after dormancy was broken, peaked just before bud opening and then declined (Domanski and Kozlowski, 1968).

Cytokinins increased in birch, maple and poplar buds from February through April (Engelbrecht, 1971). The principal material chromatographed with zeatin on TLC. Cytokinins were not found in buds of Populus x robusta in December and January, but increased in mid-March, then declined to a minimum prior to bud break in late May (Hewett and Wareing, 1973a). Cytokinin levels in buds on cuttings paralleled those in buds on intact trees, suggesting that they are synthesized in the shoots or in the buds themselves, rather than in the roots. Five cytokinin-like materials were demonstrated following Sephadex LH-20 column chromatography and bioassay, two of them co-chromatographed with zeatin and zeatin-riboside, respectively (Hewett and Wareing, 1973a, 1973b).

Role of inhibitors. Hemberg (1949, 1958) first related endogenous inhibitors to bud dormancy, finding higher levels of inhibitors in Fraxinus bud scales in October, when buds on forced cuttings were unable to grow, than in February when dormancy had been broken. Kawase (1966) reported a decrease in inhibitory activity during rest in leaf buds of Diospyros virginiana, Malus sylvestris, Prunus persica var. 'Eclipse'

and Ulmus americana. Davison (1965) observed a similar decrease during rest in xylem sap of willow. Biggs (1959) separated 3 inhibitors and 1 promoter from dormant peach buds and 2 inhibitors and 2 promoters from non-dormant buds. Total inhibitor content was less and total promoter greater in the non-dormant buds.

Inhibitor levels decreased in flower buds of peach during dormancy, even though the level was still high at the start of spring growth (Blommaert, 1955). The decrease was slightly more rapid in buds exposed to chilling temperatures as compared to the warm controls (Blommaert, 1959). Blommaert (1959) suggested that the inhibitor studied might be one or more phenolic acids. The Rf of his inhibitor was similar to that of the β-inhibitor of Bennet-Clark and Kefford (1958). Lane and Bailey (1964) made similar observations with regard to inhibitors found in silver maple buds.

Hendershott and Walker (1959b) observed a close relationship between the end of rest in peach buds and the decline of an inhibitor, although the level remained rather high even after the end of rest. Hendershott and Walker (1959a) identified the inhibitor as naringenin, a flavanone, and found its content was four-fold higher during rest than after rest was completed in 'Elberta' peach buds.

Dennis and Edgerton (1961) were unable to find a correlation between inhibitory activity of extracts from peach buds and rest, and most of the inhibitor was confined to the scales. Corgan (1965) observed high naringenin levels in peach flower buds throughout the dormant season, even 30 days or more following the end of rest. Erez and Lavee (1969) identified prunin in dormant peach buds and found it remained constant from December to March, except for a slight decrease at the end of December.

El-Mansy and Walker (1969) reported that total flavanone content was much higher in peach buds during rest than after rest. When expressed on a per bud basis, the lowest values occurred just prior to bloom.

Corgan and Peyton (1970) measured inhibitors in the total acid fraction of peach buds and in the purified ABA-like fraction. The ABA-like material increased from a low level in early summer to a plateau the first week in October, then remained fairly constant through the end of March, rest being broken by the end of January. However, inhibitory activity of the total acid fraction decreased during dormancy, possibly indicating an increase of growth promoting compounds or the decrease of another inhibitor. Corgan and Martin (1971) found that an ABA-like inhibitor in floral cups of the same variety fluctuated about a constant level, then declined at the end of rest.

Pieniazek (1964) identified phloridzin, another phenolic glucoside, in dormant leaf and flower buds of 'Antonovka' apple; however, she found no significant correlation between the content of phloridzin and rest. Pieniazek and Rudnicki (1971), using bioassay, found more ABA-like inhibitor in apple terminal buds as compared to lateral buds on both a fresh and dry weight basis. In the terminal buds, ABA disappeared completely by April, or may have been obscured by promoters, but remained unchanged in the lateral buds (Pieniazek and Rudnicki, 1971). No correlation between rest and inhibitor level was found by Strausz (1969) in buds of three species of Pyrus. Only one inhibitor, tentatively identified as ABA, was noted, and its level remained constant.

Dörffling (1963) and Phillips and Wareing (1958) bioassayed extracts of Acer pseudoplatanus buds. Inhibitor level increased in the fall to a high level, where it reamained until late winter, then decreased to a minimum before bud break.

In apricot buds, ABA-like inhibitors were high in October, remained constant through mid-December, fell to a low level by mid-January (end of rest), then increased slightly until anthesis (Ramsay and Martin, 1970). The inhibitors were located primarily in the scales but substantial amounts occurred in the floral primordia.

When dormancy in coffee flower buds was released by irrigation, ABA levels, as measured by GLC, remained constant prior to bud expansion, then increased as the buds swelled (Browning, 1973a).

Seeley (1971) used GlC to measure the levels of free and base hydrolyzable ABA (bound ABA) in apple terminal buds throughout the year. Both free and bound ABA began to increase in June, reaching a peak by the middle of September, at which time the free ABA fell slowly until it reached its lowest point and almost disappeared by May. The bound ABA fell about 30% from September to October, then began increasing again, reaching a second and higher peak in March, after which it fell rapidly. The increase in bound ABA appeared to occur at the same rate as the decrease in free ABA, suggesting interconversion.

A group of chemicals never explored in detail in relation to dormancy is the carotenoids. Xanthoxin, a breakdown product of carotenoids, occurs naturally in plants (Taylor and Burden, 1970a) and is as active as ABA in bioassay systems (Taylor and Burden, 1970b). Xanthoxin and its close analogs can be produced *in vitro* from carotenoids, primarily violaxanthin and neoxanthin (Taylor and Smith, 1967; Taylor, 1968; Taylor and Burden, 1970b; Burden *et al.*, 1972; Milborrow and Garmston, 1973), and can be converted to ABA (Burden and Taylor, 1970). To date this material has not been measured in relation to seed or bud dormancy; however, it may have been the neutral material observed in blackcurrant (Tinklin and Schwabe, 1970).

Summary

Auxin-, cytokinin- and gibberellin-like materials have been associated with periods of active growth. Auxin-like substances occur at low or neglible levels during the dormant period, and either increase just before bud swell, reaching a maximum at the green tip stage, or continue to rise during bud swell to a maximum as growth rate peaks. In some cases auxin-like materials increase in response to chilling, but not warm, temperatures. There are conflicting reports on the source of the auxin, with evidence for both synthesis in the roots and conversion from a bound form in the buds. Cytokinin-like compounds occur at low levels during rest. In some cases they increase just before bud swell; in others they rise earlier, declining to low levels before bud swell begins. Several gibberellin-like compounds have been reported to increase at the end of rest and before bud swell, while others do not change.

Inhibitors, including phenolics and ABA-like substances, are generally high in early stages of rest and decrease during rest; however, in several cases levels after rest are still relatively high, and/or do not fall until several weeks to months after the end of rest. In others no correlation exists between inhibitor levels and rest. If we assume the bioassay data published measured what was intended, then dormancy is not controlled by the same mechanism in all deciduous woody species, or even in closely related members.

In the past, measurement of growth substances has been dependent on the use of bioassays, which lack specificity. Some form of chromatography is needed for separation prior to bioassay, and, even with repeated chromatography, one cannot be sure if one is measuring the

response of one promoter or inhibitor, the effect of several promoters or inhibitors, or a combination of promoters and inhibitors. Therefore, one must exercise caution when interpreting bioassay results; an apparent drop in inhibitor may actually reflect an increase in promoters.

The technology now exists for measuring at least some of the growth substances by physical-chemical methods. Although these methods require preliminary chromatography in some cases, a substance may be measured precisely and accurately. Measurement of abscisic acid by this method in some cases provided results similar to bioassay data, but in others an entirely different picture has been obtained.

The role of growth substances in dormancy has been evaluated by measuring the levels occurring under natural conditions throughout the year, and then correlating them with rest and/or growth; however, little attempt has been made to alter natural processes which may influence dormancy, or environmental conditions (e.g. comparison of several temperature) to determine if the levels of the growth substances are actually correlated with dormancy per se.

INTRODUCTION TO SECTIONS ONE THROUGH FIVE

INTRODUCTION TO SECTIONS ONE THROUGH FIVE

Sour cherries (Prunus cerasus L. cv. Montmorency) are of great economic importance to Michigan orchardists. In the dormant state, the flower buds withstand most Michigan winter temperatures; however, most years a portion of the crop is lost to spring frosts. Occasionally (e.g., two of the last five years) the loss is severe. Delay of bloom by 7 to 14 days would greatly reduce the chance of crop loss due to spring frosts. An understanding of the mechanism controlling dormancy might lead to such a method for programming anthesis.

The mechanism of dormancy has been assumed to be partially if not wholly hormonal. Abscisic acid has been suspected of being partially responsible for preventing bud growth, and elucidation of its role was the purpose of this thesis.

The goals of the research were five fold; (1) to positively identify by physical-chemical means the presence of ABA in buds; (2) to measure by precise physical methods the levels of both free and base hydrolyzable ABA at various times throughout rest and quiescence; (3) to alter both naturally occurring processes and environmental conditions to determine their effects upon levels of ABA; (4) to determine if ABA applied exogenously under orchard conditions could delay bloom; and (5) to determine, by exogenous application, the effects of several growth substances on the growth of buds on excised branches.

Papers one and two are presented in a form suitable for submission to the Journal of the American Society for Horticultural Science, while

papers three through five are prepared in the format used by Physiologia Plantarum.

SECTION ONE

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). I. IDENTIFICATION OF ABSCISIC ACID.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (Prunus cerasus L.). I. IDENTIFICATION OF ABSCISIC ACID.

Abstract. We have demonstrated the presence of inhibitory substances in both the free and base-hydrolyzable fractions of methanol extracts of sour cherry buds. One of these, occurring in both fractions, was positively identified as abscisic acid by combined gas-liquid chromatography-mass spectrometry. The base hydrolyzable form of abscisic acid is assumed to be the 1'-glucose ester.

Introduction. Hemberg (8,9) first related endogenous inhibitors to bud dormancy, finding higher levels of inhibitory substances in Fraxinus bud scales in October, when buds on forced cuttings were unable to grow, than in February when rest had been broken. A similar inhibitory zone was later termed the β-inhibitor complex (2). Abscisic acid (ABA) was isolated in crystalline form from birch and sycamore leaves as the main inhibitor of this zone (6, 7, 12, 17) and has been identified as a component of the β-inhibitor complex from willow sap (11) and tung bud scales (19). It has been tentatively identified by TLC and bioassay in buds of apple (14) and peach (4,5), characterized by GLC in apple buds[1] and olive leaves (1), and positively identified in coffee flower buds by GC-MS (3). A water soluble form of ABA, the 1'-glucose ester, was characterized in apple buds by GLC (16).

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[1]Seeley, S. D. 1971. Electron capture gas chromatography of plant hormones with special reference to abscisic acid in apple bud dormancy. Ph.D. thesis, Cornell University, 128p.

The purpose of this study was to determine if ABA, in both the free and bound forms, was present in flower buds of sour cherry.

METHODS AND MATERIALS

Extraction. Fifty grams of dormant cherry, cv. Montmorency, buds were collected on March 28, 1970 from an orchard at East Lansing, frozen on dry ice and held at -18°C until analyzed.

The buds were ground in a Lourdes model MM-1A grinder for 6 min in 75 ml absolute methanol. The sample was transferred to a 500 ml Erlenmeyer flask, methanol added to give a final volume of 125 ml, and the flask shaken for 18 hr at room temperature. The sample was filtered through Whatman No. 1 filter paper and the filtrate centrifuged (Sorvall RC2-b centrifuge) at 25,000 x g for 30 min. The supernatant was evaporated in a flash evaporator at 40°C and resuspended in 100 ml distilled water.

The extract was adjusted to pH 7.3 with water saturated with $NaHCO_3$ and washed 3 times with ethyl acetate. The $NaHCO_3$ phase was adjusted to pH 3.0 with H_2SO_4 and partitioned 3 times with 25 ml ethyl acetate to obtain the acidic or "free" fraction. The water phase was adjusted to pH 11.0 with NaOH and heated for 1 hr at 60°C. The pH was then adjusted to 3.0 with H_2SO_4 and partitioned 3 times with 25 ml ethyl acetate to obtain the base hydrolyzable or "bound" fraction.

One-half of each fraction (25 gram equivalents) was evaporated on glass wool and placed on top of a 19 mm i.d. silica gel column (8 g Mallinkrodt 100 mesh silicic acid equilibrated with 4.5 ml 0.5M formic acid) and eluted with a gradient consisting of 160 ml hexane and 120 ml

ethyl acetate, both solvents having been redistilled and saturated with 0.5M formic acid (15). Fifty-two, 5 ml fractions were collected and 0.5 ml of each fraction was evaporated and analyzed by a wheat coleoptile bioassay.

Wheat coleoptile bioassay. 'Ionia' wheat seeds were soaked for 3 hr in water with aeration, sown on vermiculite moistened with one-half volume of water, and grown for 3 days in the dark at 25°C. A 4 mm section was removed 3 mm behind the tip and floated on distilled water approximately 3 hr until used. Each fraction to be analyzed was resuspended in 0.3 ml phosphate-citrate buffer (1.794 g K_2HPO_4 + 1.019 g citric acid/liter H_2O), pH 5.0 containing 2% sucrose. Five coleoptiles were placed in each tube and the tubes rotated in the dark for 20 hr in a clinostat at 25°C, after which the segments were measured utilizing a photographic enlarger.

Gas-liquid chromatography. The extract remaining in each fraction in the zone of inhibition (fractions 22-25) and the remaining one-half of the acidic and bound fractions were methylated with diazomethane (18), evaporated to dryness, resuspended in ethyl acetate, and injected into a Packard 7300 gas-liquid chromatograph equipped with a hydrogen flame ionization detector, and 2 mm i.d. x 1.83 m columns containing either 2% QF-1 on Chromosorb W 80/100 mesh, 2% OV-1 on Chromosorb W 100/120 mesh or 3% SE-30 on Supelcoport 60/80 mesh. The carrier was nitrogen at a flow rate of 40 ml/ min at 40 psig. Inlet, column, and detector temperatures were 250°C, 180°C and 250°C, respectively.

Mass spectrometry. Combined gas-liquid chromatography-mass spectrometry (GC-MS) was performed utilizing a LKB-9000 GC-MS, interfaced with a PDP 8/I computer. The column was 2 mm i.d. x 1.22 m containing either 3% SE-30 on Supelcoport 60/80 mesh or 2% DC-200 (12,500 cstk.) on Gas-Chrome Q 80/100 mesh. The carrier gas was helium at a flow rate of 30 ml/min. Temperatures of column, inlet flash heater, source, and molecular separator were 180°C, 230°C, 220°C and 230°C, respectively, and entrace and exit slit widths were 0.08 and 0.3 mm, respectively. The ion source was operated at 70.0 eV.

RESULTS AND DISCUSSION

Following column chromatography, five major zones of inhibition were evident (fractions 7-9, 15-18, 21-27, 37-40 and 48-51, labeled inhibitors I, II, III, IV and V, respectively) in both the acidic and bound fractions (Fig. 1). Two additional, minor inhibitory zones occurred, one at the origin and the other between inhibitors III and IV. More inhibitory activity,expressed as ABA-equivalents, was found in each case in the bound fraction as compared to the acid fraction (Table 1). The difference was slight with inhibitors I and V, over twice as much with inhibitor II, about three times as much with inhibitor III and almost six times as much with inhibitor IV; however, this experiment was not replicated. In addition these results may have been confounded by the presence of promoters.

(±)-ABA (R. J. Reynolds Tobacco Co.) co-chromatographed with inhibitor III; however, (±)-ABA was found only in fractions 22-25, while inhibitor III was found in fractions 21-27. This suggests that either

Figure 1. Growth response of wheat coleoptile segments to eluates from silica gel columns of the acidic (a) and bound (b) fractions of a metnanolic extract of sour cherry buds. A total of 25 g (f. w.) equivalents was chromatographed, and 0.25 g-eq. assayed. 5 ml of eluate was collected per fraction, using gradient elution with hexane-ethyl acetate. Roman numerals indicate major zones of inhibition.

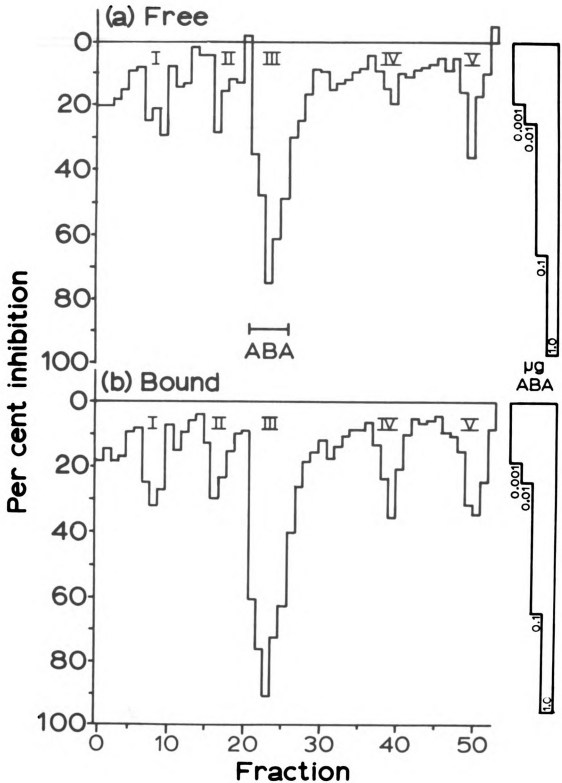
(a) Free
(b) Bound
Per cent inhibition
Fraction
ABA
μg ABA
I
II
III
IV
V
0
20
40
60
80
100
10
30
50
0.001
0.01
0.1
1.0

Figure 1.

Table 1. Amount of inhibitor activity, expressed as ABA-equivalents, in each of the five major inhibitory zones from silica gel columns of the acidic and bound fractions of a methanolic extract of sour cherry buds.

	μg ABA-equivalents[z]	
	Fraction	
Inhibitor	Acidic	Bound
I	0.020	0.023
II	0.008	0.018
III	0.502	1.413
IV	0.005	0.028
V	0.032	0.041

[z] In 2.5 gram equivalents (f.w.) of buds.

inhibitor III contained inhibitory materials in addition to ABA or that other materials in the extract interfered with resolution.

GLC traces of methylated free and bound fractions, run on QF-1, both contained a peak with the retention time of approximately 2.3 min co-chromatographed with the cis-trans isomer of synthetic ABA (Fig. 2). Column chromatography reduced the number of compounds present in the extracts (Fig. 3). Particularly noteworthy is the absence of peaks with short retention times and the large peak with a retention time of about 6 min in the free fraction. GLC results were similar for samples run on either 2% OV-1 or 3% SE-30. Quantitative analysis indicated 0.223 and 0.787 mg ABA-equivalents/kg fresh weight--somewhat higher than the 0.201 and 0.565 mg/kg as measured by wheat coleoptile bioassay. This would suggest that either inhibitors with lower biological activity, or promoters, occur with ABA in inhibitors III.

Identical mass spectra were obtained for synthetic ABA and for the peaks at 2.3 min in the acidic and bound fractions (Fig. 4). Initial attempts to obtain a mass spectrum were hampered by the presence of oleic acid, which co-chromatographed with ABA. This fatty acid, which was separated on the 2% DC-200 column, is inhibitory in the wheat coleoptile bioassay, but is much less active than ABA. This may partially explain the discrepency between values obtained by bioassay vs. GLC and the broadened peak in column chromatography. These results are similar to those found in coffee flower buds, where ABA as measured by GLC did not equal the total inhibitory activity as measured by bioassay (3).

No attempt was made to determine the identity of the conjugated form of ABA, which is assumed to be the 1'-glucose ester (10, 13, 16).

Figure 2. GLC traces of the methylated crude acidic (a) and bound (b) fractions of a methanolic extract of sour cherry buds.

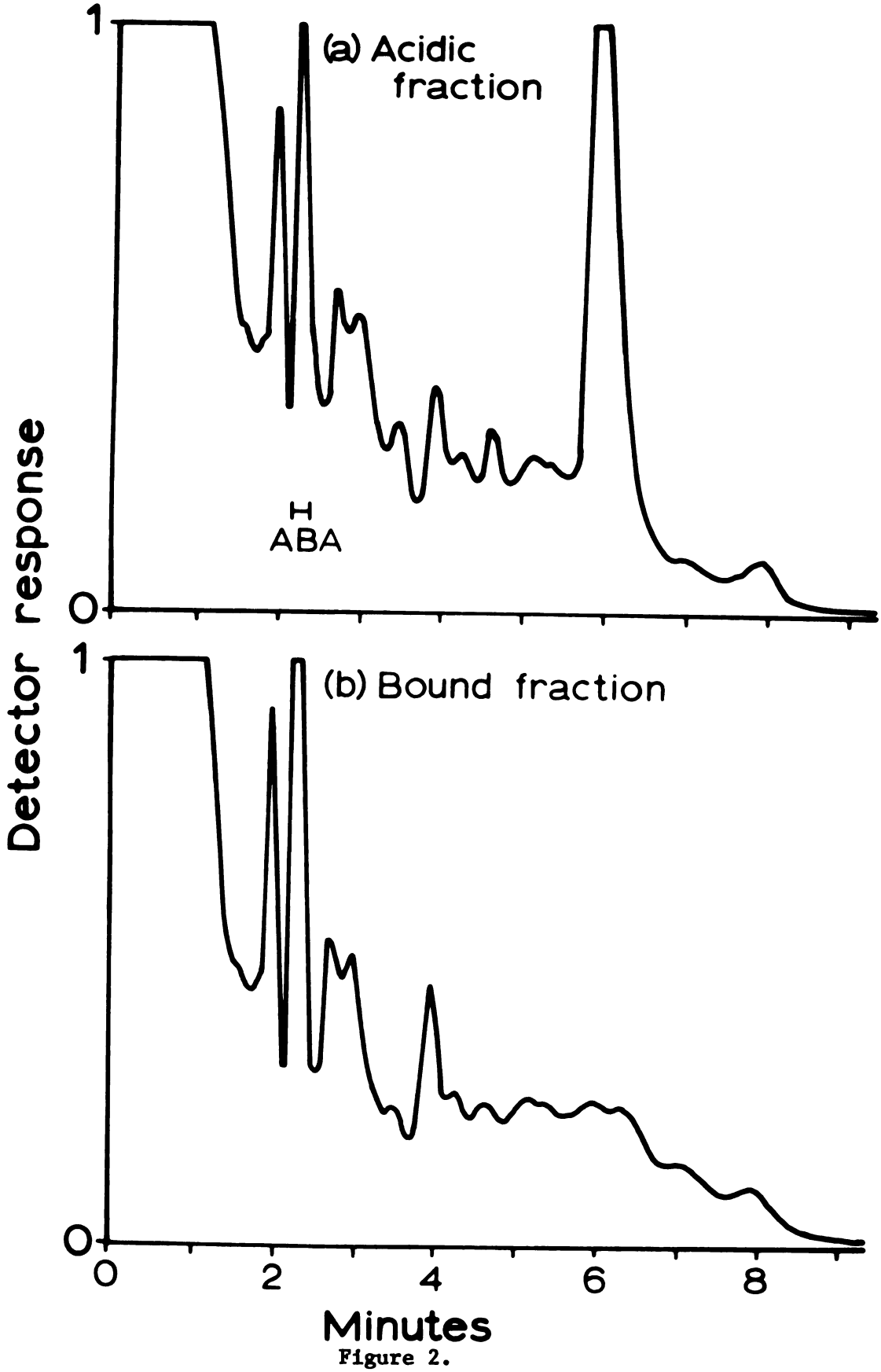
1
(a) Acidic
fraction
H
ABA
0
Detector response
1
(b) Bound fraction
0
0
2
4
6
8
Minutes

Figure 2.

Figure 3. GLC traces of methylated combined fractions 22-25 from column chromatograms of the acidic (a) and bound (b) fractions of a methanolic extract of sour cherry buds.

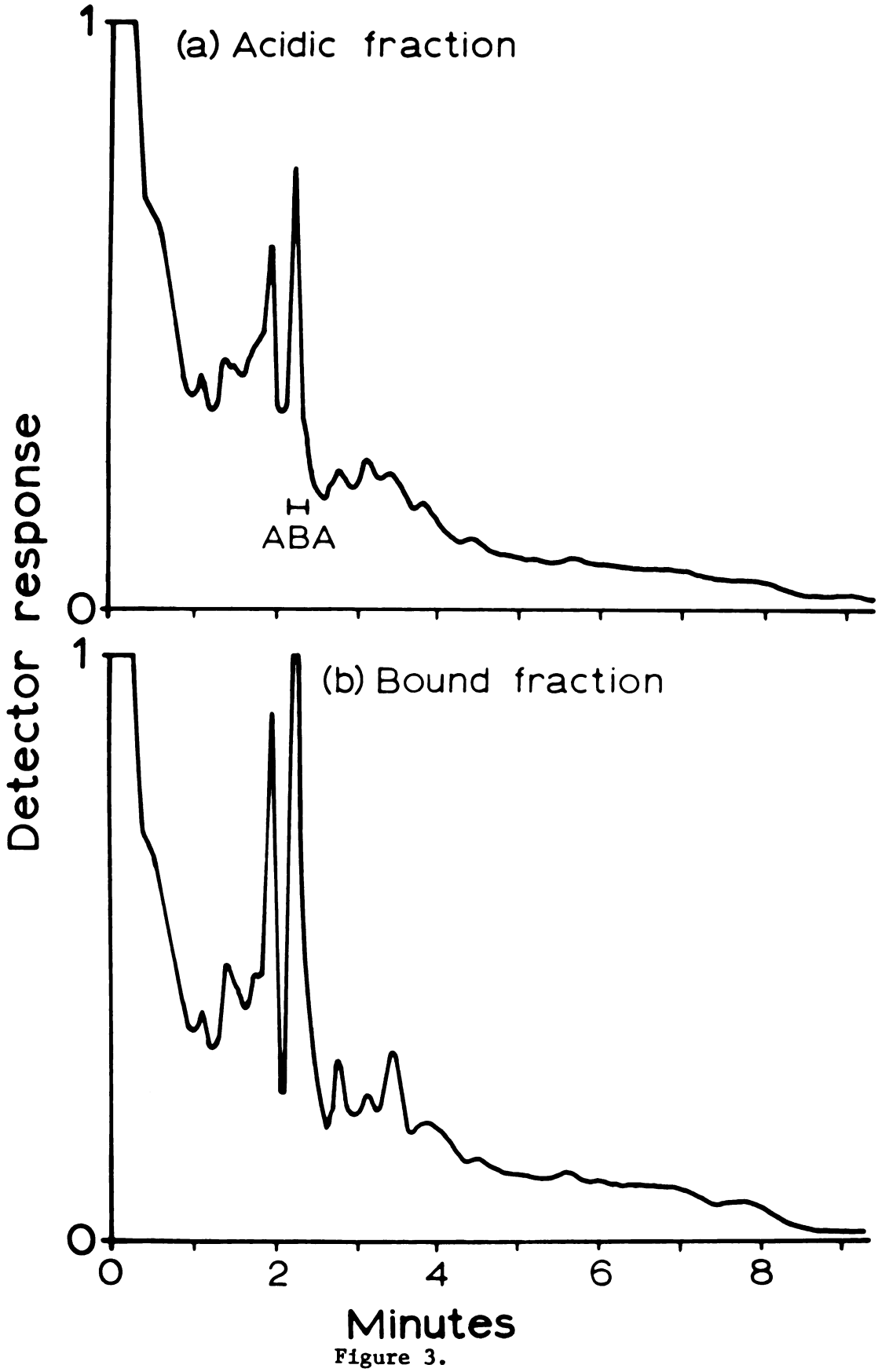
1
(a) Acidic fraction
ABA
0
1
(b) Bound fraction
0
Detector response
0
2
4
6
8
Minutes

Figure 3.

Figure 4. Mass spectrum at 70.0 eV of the peak co-chromatographing with synthetic ABA on GLC.

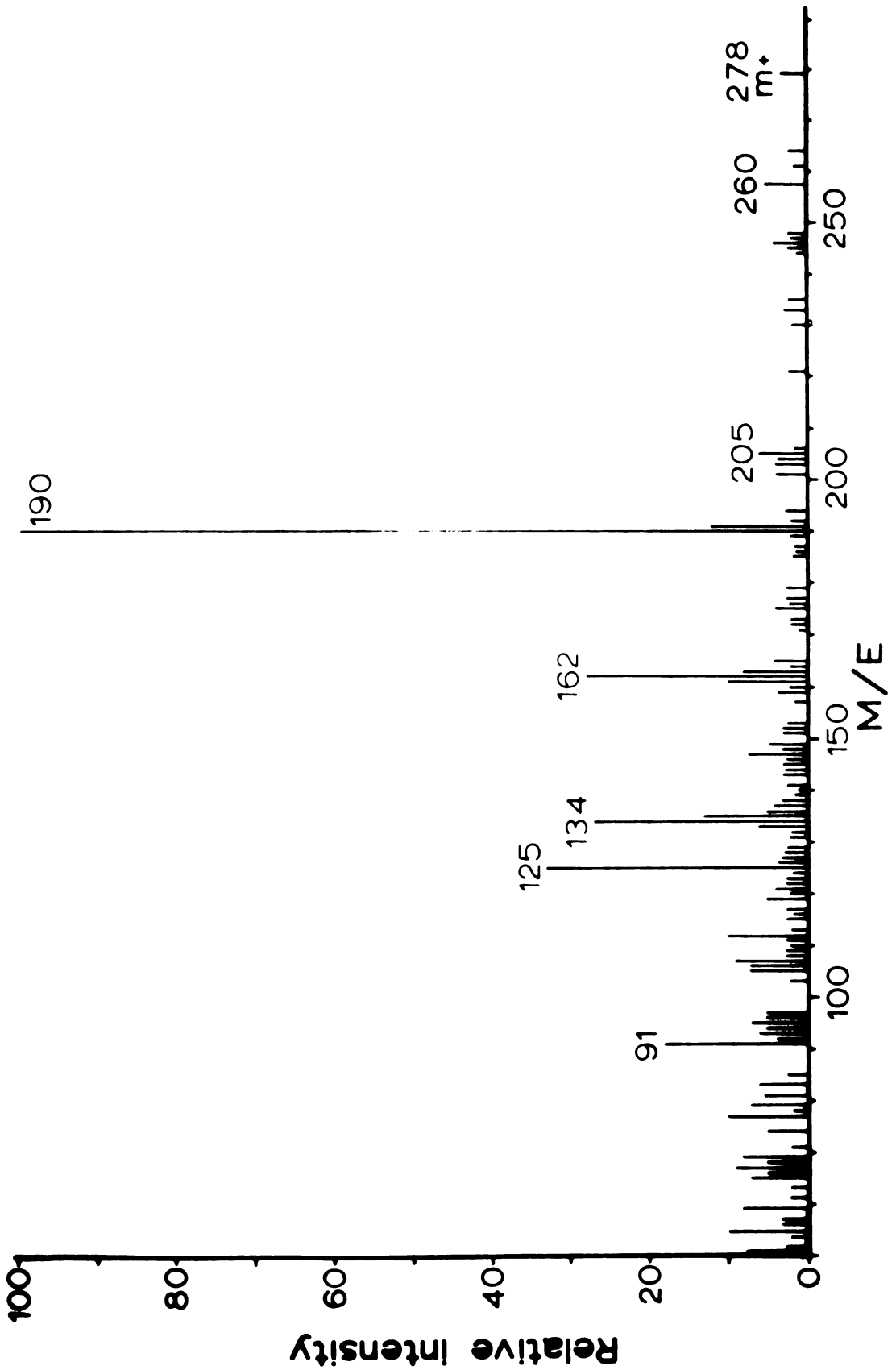
Relative intensity
100
80
60
40
20
0
M/E
100
150
200
250
260
278
m+
190
205
162
134
125
91

Figure 4.

The positive identification of endogenous abscisic acid, a growth substance suggested as a participant in the promoter-inhibitor balance control of dormancy, is significant. Using physical-chemical methods, we can now measure precisely the levels of this substance throughout the year to determine the involvement of ABA in the control of dormancy and/or flowering. The use of a physical method does not, of course, insure complete extration, nor does it provide assurance that the ABA extracted is present in the plant as the free acid.

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SECTION TWO

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). II. LEVELS OF ABSCISIC ACID AND ITS WATER SOLUBLE COMPLEX.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). II. LEVELS OF ABSCISIC ACID AND ITS WATER SOLUBLE COMPLEX.

Abstract. Abscisic acid (ABA) is thought to be partially responsible for the control of bud dormancy. Levels of both free ABA and its soluble complex, assumed to be the 1'-glucose ester ("bound" ABA), were measured by electron capture gas-liquid chromatography in the scales and floral primordia of sour cherry. On a per bud basis, more ABA was found in the scales than in the primordia, although the conc was greater in the primordia. Concentrations of bound ABA were greater than those of free ABA. The conc of free ABA changed more dramatically in the primordia than in the scales, rising rapidly in the fall, reaching a maximum in November or December, then declining rapidly (ca. 4 wk) to or below the original level. The autumn increase was coincident, not with the onset of dormancy, but with the onset of leaf abscission, maximum levels occurring when about 90 to 95% of the leaves had abscised. In addition, free ABA returned to its original level several weeks prior to the termination of rest. Levels of bound ABA paralleled those of free ABA, but declined more slowly during the early winter.

Introduction. We have identified ABA in both the free and the base hydrolyzable (bound) form, as one of the inhibitors present in sour cherry buds (14). Inhibitor levels have been found to be lower after rest than during rest in buds of apple (13), apricot (18), ash (11, 12) and peach (3, 4, 13); however, Dennis and Edgerton (9) were unable to correlate inhibitor levels with rest in peach buds.

Levels of ABA-like materials, as measured by bioassay, were the same after rest as during rest in apple flower buds (16), but did not decline until two months after the end of rest in 'Red Haven' peach flower buds (18); however, Corgan and Martin (5) using the floral cups of the same variety reported that such compounds declined at the end of rest. Seeley[1] noted that free ABA, as measured by GLC, declined during rest of apple leaf buds, being lowest just prior to the time of flowering. In addition he noted that the bound ABA increased during dormancy, and declined just prior to bloom.

Most of the inhibitory activity was found in the scales of apricot (19), blackcurrant (22), peach (8) and tung (21) buds, while Seeley[1] found the highest conc of ABA in the central portions of apple terminal buds.

The purposes of this study were to determine the distribution of ABA in sour cherry flower buds and its levels during and after rest.

Methods and Materials

Sample collection. In 1970-1971, flower buds were obtained from a mature, commercial Montmorency cherry orchard in Belding, Michigan. Four 25 g samples were collected at approximately 2 wk intervals from just prior to the onset of leaf abscission until just prior to bud swell, and every week thereafter until full bloom. The buds were frozen on dry ice, lyophilized, and stored in a freezer at -18°C. For extraction, 25 buds were selected at random from each of the 4 samples.

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[1]Seeley, S. D. 1971. Electron capture gas chromatography of plant hormones with special reference to abscisic acid in apple bud dormancy. Ph.D. thesis, Cornell University, 128p.

In 1971-1972, 25 flower buds were taken approximately every 2 wk from each of 3 trees planted in 1963, at East Lansing, Michigan, from 5 wk prior to the onset of leaf abscission through petal fall. In 1972-1973, similar samples were collected from the same orchard at 2 wk intervals from just prior to leaf abscission until bud swell began, then weekly until full bloom. All samples were collected and processed as in 1970-1971.

Evaluation of stage of rest. Periodically, branches 35 to 40 cm long were placed with their bases in distilled water for 14 days in a greenhouse at 21.4°C to determine when rest had been terminated. Mist was supplied for 6 sec each 3 min from 2 am to 12 pm during the last 2 sampling years. Rest was considered broken when 50% of the buds on the cuttings had reached a minimum of flowering stage 2 (green tip) (1, 10) within 14 days.

Extraction. Within years, one replicate from each sampling date was extracted at 1 time. After extraction, the samples were evaporated and stored at -18°C until all replicates had been processed.

Twenty-five buds of each sample were dissected into scale and floral primordia and the dry wt recorded. The primordia were ground in an ice bath in a 5 ml Knotes all glass tissue grinder with 2 ml of distilled water adjusted to pH 7.3 with $NaHCO_3$. The macerate was poured into a 25 ml Erlenmeyer flask and the grinder washed 3 times with 2 ml of the buffer; the washings were added to the macerate and the volume brought up to 10 ml. The scales were ground under liquid nitrogen in a mortar and pest . Liquid nitrogen was used to transfer the macerate to a 25 ml Erlenmeyer flask, and 10 ml buffer was added. All flasks were sealed

with 'Parafilm' and shaken for 3 days at 0°C. The samples were then transferred to 25 ml centrifuge tubes and centrifuged (Sorvall RC2-b) at 24,000 x g for 30 min at 0°C. The pellet was discarded.

Purification. The purification procedure was adapted from Seeley[1] and is shown in Figure 1.

The supernatant was transferred to a 25 ml Erlenmeyer flask, adjusted to pH 7.3 with $NaHCO_3$, and washed 3 times with 5 ml CH_2Cl_2. The CH_2Cl_2 was discarded. The pH was then adjusted to 2.5 with 1N HCl and the samples partitioned 3 times with 5 ml CH_2Cl_2 to obtain the acidic or "free" fraction. The pH of the water phase was raised to 11.0 with conc NH_4OH and the samples heated for 1 hr at 60°C. The pH was adjusted to 2.5 with 1N HCl and the samples partitioned 3 times with 5 ml CH_2Cl_2 to obtain the base hydrolyzable or "bound" fraction.

Water remaining in the acidic and bound fractions was removed by adding ca. 1 g of anhydrous Na_2SO_4 which had been purified by heating for 12 hr at 500°C. The CH_2Cl_2 was decanted into test tubes and evaporated to dryness under reduced pressure.

Analysis. The samples were resuspended in 1 ml of diethyl ether/methanol (9:1 v/v) and methylated with diazomethane according to Schlenk and Gellerman (20) as modified by Powell (17). The ether-methanol solution was evaporated and the samples stored at -18°C until analyzed by GLC.

The samples were resuspended in ethyl acetate and injected into a Packard 7300 gas-liquid chromatograph equipped with an electron capture detector. The column was 2 mm i.d. x 1.83 m, packed with 2% DC-200 (12,500 cstk.) of Gaschrome Q 80/100 mesh. Column, inlet, and detector

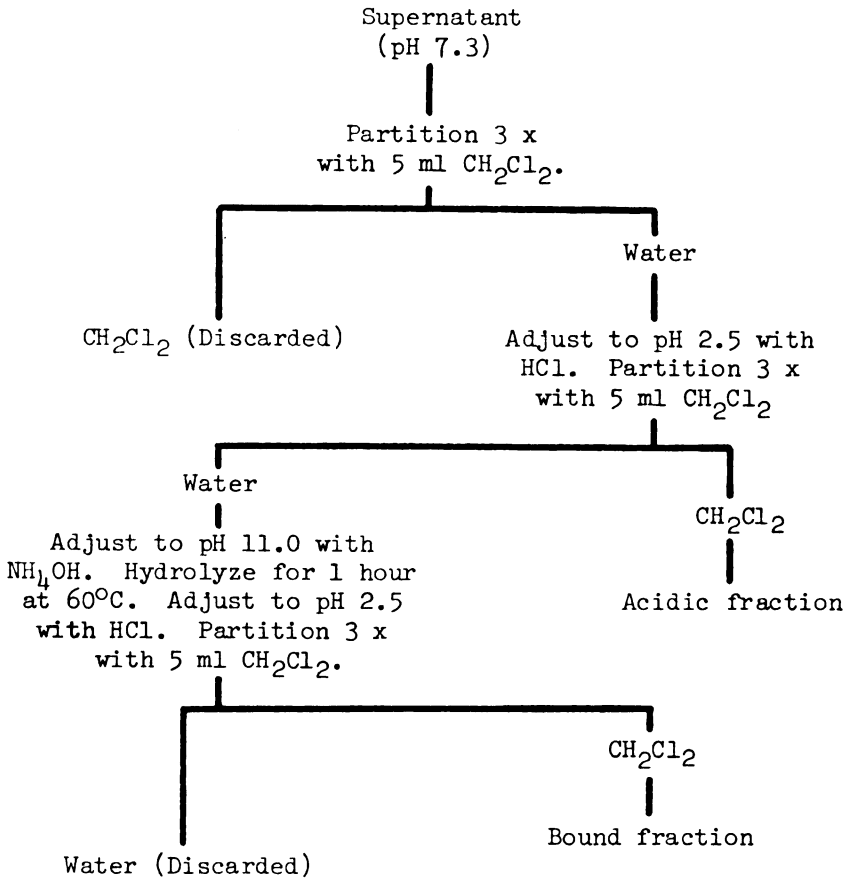

Figure 1. Flow diagram for the purification of plant extracts.

temperatures were 210°C, 250°C, and 270°C, respectively. The carrier gas was nitrogen at a flow rate of 40 ml/min at 40 psig. Nitrogen scavenger gas was supplied to the detector at 70 ml/min. The electron capture detector was equipped with ^{63}Ni foil and operated at 5 volts.

Quantitative measurements were made by measurement of the peak height of the cis-trans isomer only. Peak height was linear over a range of 10 to 800 pg/µl, with 0.5 pg/µl being the minimum detectable quantity.

Selected samples were subjected to GC-MS, under conditions described previously (13), to confirm the presence of ABA.

Results and Discussion

More ABA on a per bud basis was found in the scales than in the primordia, in agreement with findings in apricot (19), blackcurrant (22), peach (9) and tung (21); however, on a dry weight basis, more was found in the primordia (Fig. 2 and 3). Primordia account for only about 5% of the dry wt of the dormant bud; therefore, levels in the primordia have little effect on the level in the whole buds and statements made about the scales refer to whole buds as well.

Levels of ABA in sour cherry scales (Fig. 2) were at a maximum in late fall, then declined slowly but significantly (Tukey's test, 5% level) until early January when rest had been completed. In 1970-1971, the level rose slightly through early April, then fell and reached its lowest level just prior to bud swell. In 1971-1972 it continued to fall after rest had been broken, and reached its lowest level in late March just prior to bud swell. These findings agree with those reported previously in buds (3, 4, 13, 18) and in sap (8). Absolute levels

Figure 2. Levels of free abscisic acid in the scales and floral primordia of sour cherry buds for 1970-1971 (a) and 1971-1972 (b), as measured by electron capture gas-liquid chromatography.

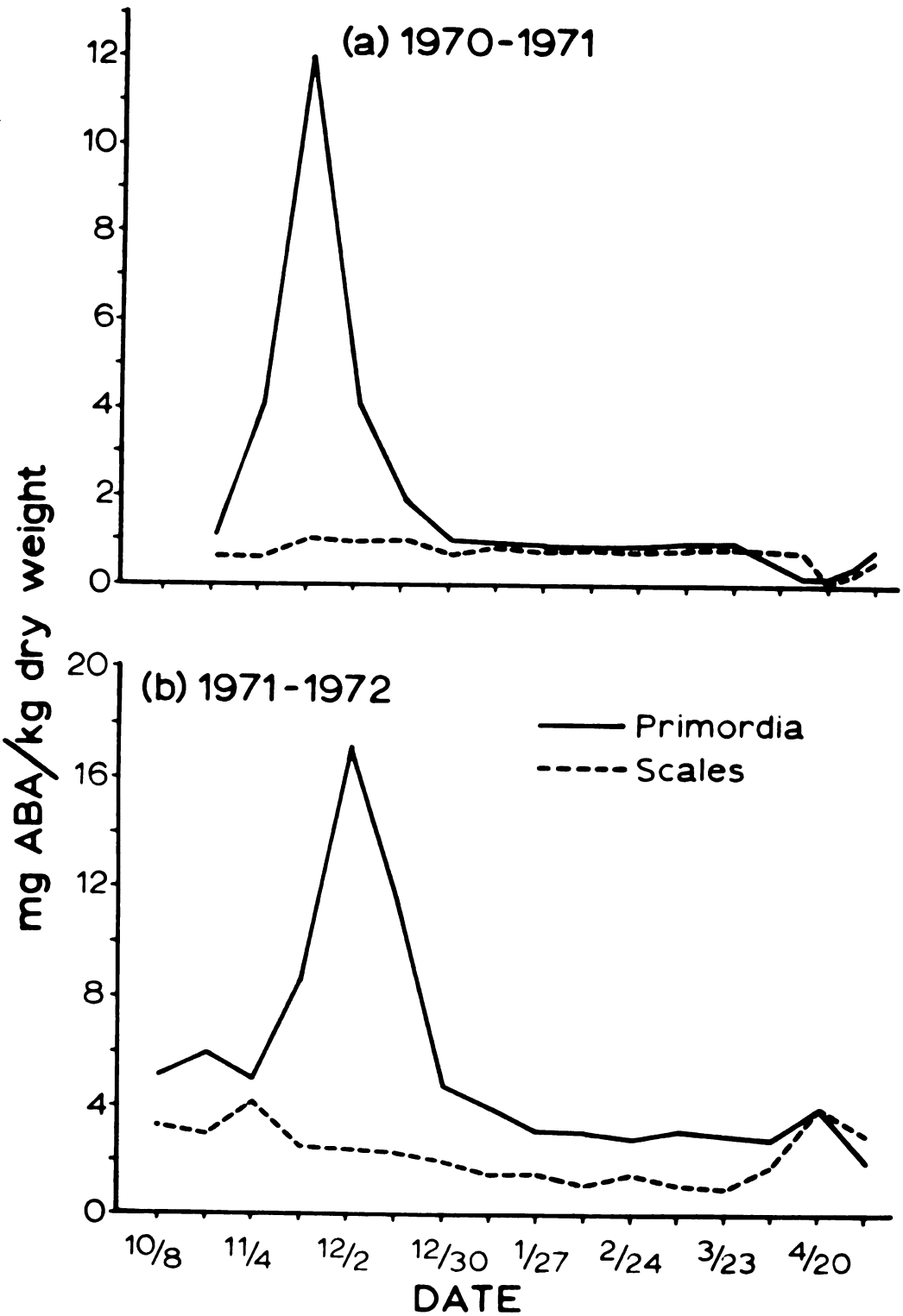
(a) 1970-1971
12
10
8
6
4
2
0
(b) 1971-1972
Primordia
Scales
mg ABA/kg dry weight
20
16
12
8
4
0
10/8
11/4
12/2
12/30
1/27
2/24
3/23
4/20
DATE

Figure 2.

in 1970-1971 were similar to those measured in apricot by bioassay (17), while in 1971-1972, they were approximately 3 fold higher. Many factors may be responsible for the difference, including location, climate, age of tree, vigor, previous crop load, soil type, nutrient and water availability, etc.

As the buds swelled, the level of free ABA in the scales increased, reached a maximum lower than the maximum in late autumn, then declined with petal fall. This phenomenon has been reported for an ABA-like compound in apricot buds (18).

ABA conc changed more dramatically in the primordia than in the scales (Fig. 2 and 3), rising rapidly in the fall, reaching a maximum in November or December, depending on year, then declining rapidly to a level at or below that in early fall. The levels then remained relatively constant, declining just prior to bud swell (1970-1971) or declined slowly and continuously to a minimum just prior to bud swell (1971-1972, 1972-1973). The levels again increased as the buds swelled, reaching a maximum at full bloom and declining again by the time the petals had abscised.

The results are similar to those for peach sap (7, 8) and willow-sap (2), in which ABA or a material similar to the β-inhibitor increased rapidly in the fall from a low level to a maximum just before full leaf abscission, approximately 4 wk later, after which it declined rapidly, reaching the original level about 6 wk before the end of rest.

Absolute levels of ABA in the primordia differed between seasons and rose 3 to 12 fold over the initial values. Note that the maximum values (9.95-16.66 mg/kg dry wt) are higher than those previously

Figure 3. Levels of free and bound abscisic acid in the floral primordia of sour cherry buds in 1972-1973, as measured by electron capture gas-liquid chromatography.

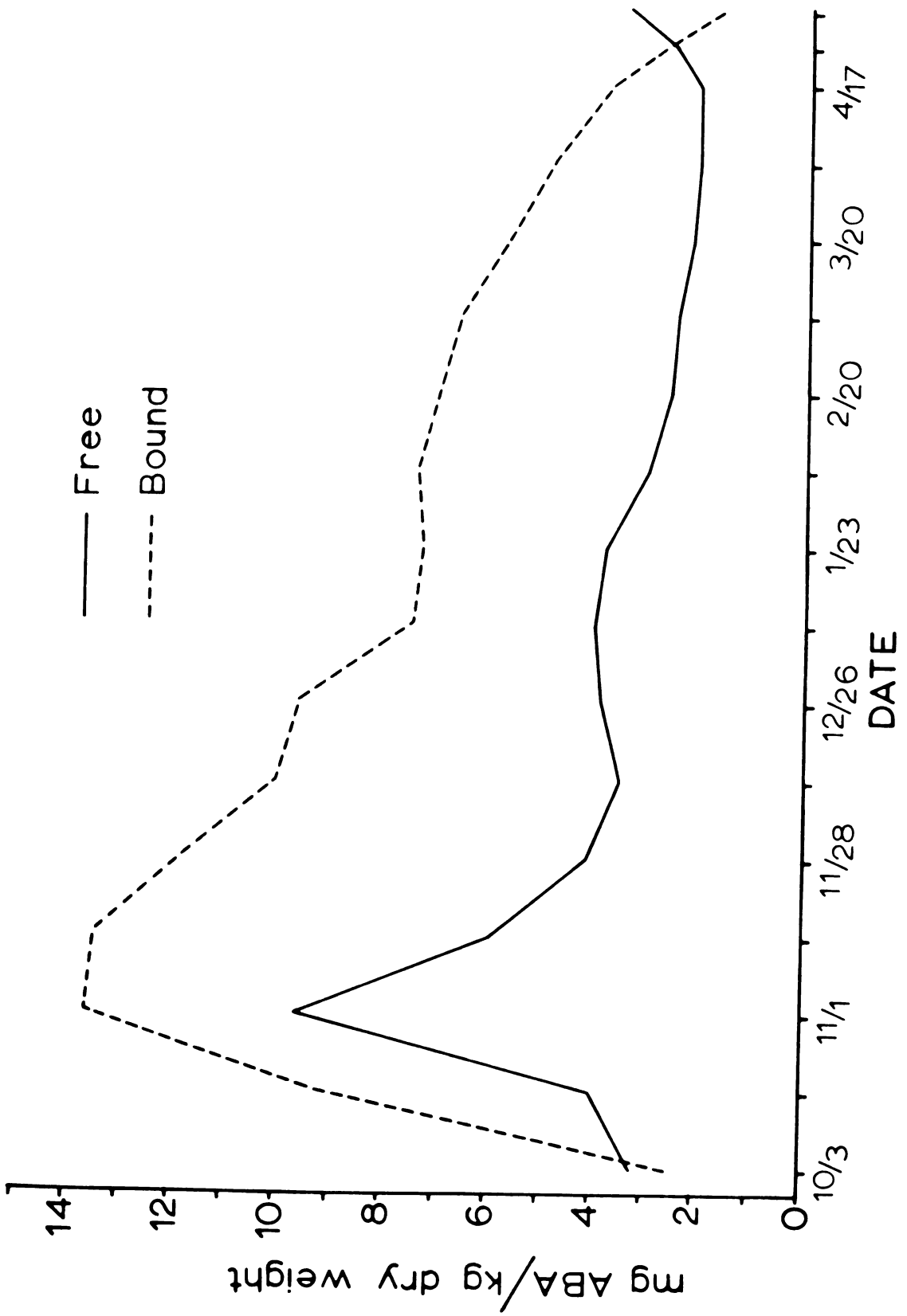
Free
Bound
mg ABA/kg dry weight
0
2
4
6
8
10
12
14
10/3
11/1
11/28
12/26
1/23
2/20
3/20
4/17
DATE

Figure 3.

reported for any tissue (7, 15). Differences between years may reflect differences in environmental factors.

In 1970-1971 and 1971-1972, the autumn peak of ABA was coincident with the period of deepest dormancy, as measured by the percentage of buds on excised branches attaining a minimum of stage 2(1, 10) within 2 wk of forcing in a mist bed at 21±3°C (Table 1). In 1972-1973, however, maximum levels occurred approximately 4 weeks before the time of deepest dormancy.

The level of bound ABA in the scales in 1970-1971 fluctuated, but remained relatively constant, from August until mid-February, then declined, reaching its lowest values at the time of full bloom, while in 1971-1972 the level was highest in early November and declined slowly and steadily through petal fall (Fig. 4). Levels did not increase during bud opening as did the free ABA. The scales were not analyzed in 1972-1973.

In all 3 years, bound ABA in the primordia rose in late autumn, and reached a maximum 1.5 to 5 fold higher than that of free ABA (Fig. 3 and 4). The maxima occurred about 1 to 2 wk later for the bound than for free ABA, then declined slowly and steadily (1970-1971, 1972-1973) or declined rapidly at first and then more slowly (1971-1972), reaching minima at full bloom or petal fall. These findings do not agree with the pattern found in apple[1], and maximum levels were 6 to 60 fold higher in cherry; however, this may be due to the difference in species and type of bud (vegetative bud in apple rather than floral). There may be conversion of the free to the bound form in cherry; however, this is unlikely due to the parallel variation. The free form appeared to be more readily metabolized, as it declined more rapidly.

Table 1. Percentage of buds attaining a minimum of stage 2 (green tip) within 14 days of forcing in a greenhouse without (1970-1971) or with mist (1971-1972 and 1972-1973).

Year					
1970-1971		1971-1972		1972-1973	
Date	%	Date	%	Date	%
10-23	25.9	10-08	23.1	10-03	24.3
11-20	15.4	11-04	25.5	10-17	20.0
12-19	37.6	12-02	8.6	11-01	23.4
1-16	95.8	12-30	34.1	11-14	6.6
2-13	100.0	1-13	57.4	11-28	0.0
---	---	1-27	95.1	12-12	35.2
---	---	2-10	100.0	12-28	43.4
---	---	---	---	1-09	81.7
---	---	---	---	1-23	90.0
---	---	---	---	2-06	100.0

Figure 4. Levels of bound abscisic acid in the scales and floral primordia of sour cherry buds for 1970-1971 (a) and 1971-1972 (b), as measured by electron capture gas-liquid chromatography.

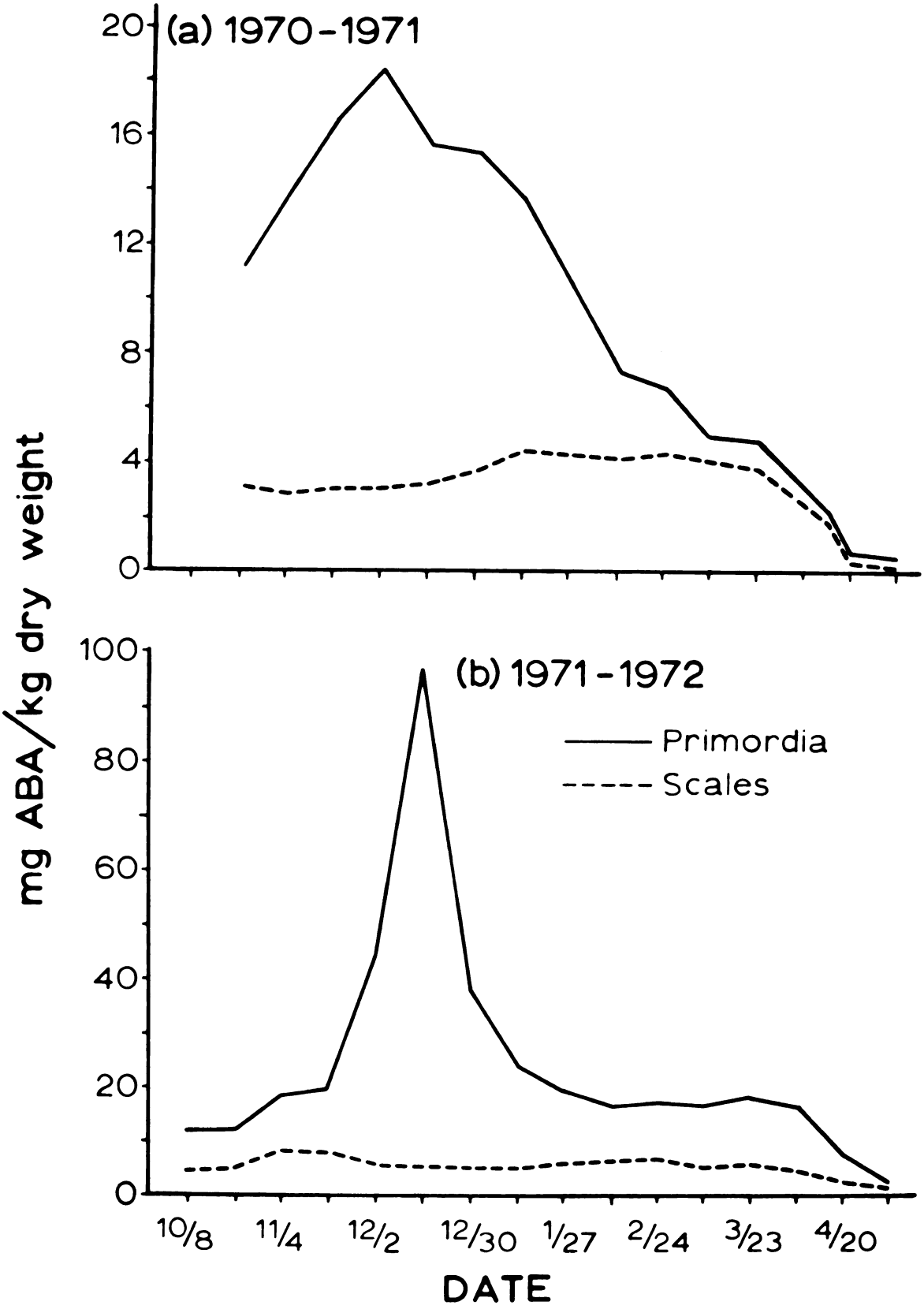
(a) 1970-1971
20
16
12
8
4
0
(b) 1971-1972
100
80
60
40
20
0
Primordia
Scales
mg ABA/kg dry weight
10/8
11/4
12/2
12/30
1/27
2/24
3/23
4/20
DATE

Figure 4.

Although the peak of free ABA in the primordia occurred during the period of deepest dormancy in 2 of the 3 years examined, the autumn increase coincided, not with the onset of dormancy, but with the onset of leaf abscission, maximum levels occurring when about 90 to 95% of the leaves and abscised. In addition, free ABA returned to its original level several weeks (6 wk in 1972-1973) prior to the termination of rest and was not correlated with the period of deepest dormancy in 1972-1973. Even if we assume that only a threshold level of ABA is required to maintain rest, the time between the decline in ABA and the end of rest is not consistent. The long, slow decline in bound ABA argues against its role in controlling rest.

Hormones in the classic sense are materials present in minute quantities which control physiological processes. At the time free ABA is at a maximum, the bud tissue is approximately 50% water. If ABA were uniformly distributed in the cellular fluid, the conc would be 10 to 17 ppm--too high to be considered hormonal.

The data presented argue against the role of ABA in controlling bud dormancy in sour cherry. Levels appear to more closely associated with the period of leaf abscission in the fall than with bud dormancy.

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SECTION THREE

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). III. EFFECTS OF LEAVES, DEFOLIATION AND TEMPERATURE ON LEVELS OF ABSCISIC ACID.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). III. EFFECTS OF LEAVES, DEFOLIATION AND TEMPERATURE ON LEVELS OF ABSCISIC ACID.

ABSTRACT

Levels of both free and bound abscisic acid (ABA), as measured by gas-liquid chromatography (GLC), increased during autumn leaf abscission. Mechanical defoliation of trees prior to the onset of leaf abscission prevented this increase in ABA without affecting rest; however, the leaves are probably not the source of this ABA, as the leaves on forced branches inhibited neither bud break nor flower development, while ABA inhibited both processes. Once the level of ABA had risen naturally, temperature had no effect on its disappearance. These results cast serious doubt on the involvement of ABA in the control of winter bud dormancy in sour cherry.

INTRODUCTION

The conc of both free and bound abscisic acid in flower primordia of sour cherry buds rise dramatically in autumn, then decline during natural chilling (Mielke and Dennis, 1974a, 1974b). Initial levels in September, when rest has already been induced, are relatively low, and the observed increase coincides with the period of leaf abscission (Mielke and Dennis, 1974b). These observations raised several questions, including the role of the leaf in the accumulation of ABA in the primordia, and the role of temperature in its subsequent decline.

Several workers have shown that levels of ABA or ABA-like inhibitors decline during chilling under field conditions (Blommaert, 1955; Biggs, 1959; Kawase, 1966; Ramsay and Martin, 1970b; Corgan and Martin, 1971; Seeley (unpublished); Mielke and Dennis, 1974b); however, none of these workers employed a warm temperature control. However, Blommaert (1959) reported that levels of a material in peach flower buds similar to the β-inhibitor (Bennet-Clark and Kefford, 1953) declined more rapidly at 7°C than at 16-26°C.

MATERIALS AND METHODS

Effect of defoliation

Eight-year-old sour cherry trees at East Lansing were used as a source of branches. In 1971-1972, one tree was hand defoliated on October 8 and a second on November 4, natural leaf abscission beginning about November 10. Additional trees were left as controls. Four replicates of 25 spur flower buds were taken from each tree at intervals from October 8 to March 9, and processed as previously described (Mielke and Dennis, 1974b).

In 1972-1973, 2 trees were manually defoliated on October 14, and 2 trees were designated as controls, natural leaf abscission beginning about October 22. Two branches 35 to 40 cm long were taken from each of the 4 trees approximately every 2 wk from November 14, 1972 to April 24, 1973, and placed with their bases in vermiculite in a greenhouse at 21±3°C. Mist was applied for 6 sec every 3 min from 2 a.m. to 12 p.m. Leaves on cuttings from the non-defoliated trees were removed when collected and flowering stages evaluated every other day for 14 days.

In 1973-1974, 4 trees were hand defoliated on October 20, and 4 trees designated as controls, leaf abscission beginning about November 15. Two branches from each of the 8 trees were taken at approximately monthly intervals from October 22 to February 4, and leaves were removed when present, and the cuttings forced in the greenhouse at 21±3°C under mist. Levels of ABA were not measured. The base of one branch was placed in 10 ml of distilled water, the base of the other in GA_3 (1000 ppm) in standard test tubes (18 x 150 mm). The branches were sealed to the tubes with 'Parafilm' to prevent the mist from diluting the solutions, which were brought up to volume as needed and changed after 14 days. Branches were forced for 28 days. Any attached leaves were removed when the cuttings were taken. The samples were placed in a greenhouse at 21±3°C and mist applied for 6 sec every 3 min from 2 a.m. to 12 p.m. The bases were recut and the flowering stages evaluated every other day for a 28 day period.

Effect of leaves and abscisic acid

Four branches 35 to 40 cm long were taken from each of 4 trees on 5 sampling dates from August 6 to November 13, 1973, the same 4 trees being used on each date. One branch with the leaves removed and one with the leaves intact from each tree were forced with their bases in either water or 100 ppm ABA.

Bud stage evaluation

Bud stages were evaluated every other day for a 14 or 28 day period, and values averaged for each branch. The following numerical values were used: 0, non-swollen; 1, first swelling; 2, green tip; 3, half green; 4, tight cluster; 5, open cluster; 6, first white;

7, balloon stage; 8, full bloom; and 9, petal fall (Ballard et al., 1971; Dennis and Howell, 1974).

Effect of chilling

Twenty-four, 6-year-old sour cherry trees, grown out-of-doors in 11 liter cans, were divided into 2 equal lots when approximately 95% of the leaves had abscised, and the remainder of the leaves were removed. One lot was placed in a greenhouse maintained at a minimum of 22°C (designated as warm). The other lot (designated as cold) was placed in cold storage at 4±2°C, 4 trees being transferred to the greenhouse after 8 wk, the remainder after 12 wk of chilling. Four trees growing in the field were designated as orchard controls.

Four replicates of 25 spur flower buds were collected at random from each lot of potted trees and 1 from each of the orchard controls at 2 wk intervals. The buds were handled and analyzed as described previously (Mielke and Dennis, 1974b).

RESULTS

As previously reported (Mielke and Dennis, 1974b) increases in levels of free ABA in the primordia coincided with the onset of leaf abscission, reaching a maximum when approximately 90 to 95% of the leaves had abscised. Mechanical defoliation on either October 8 or November 4 prevented this increase, and levels of ABA continued to decline throughout the sampling period (Fig. 1). Levels of ABA before November 18 and after December 16 were not significantly affected by defoliation. Similar results were found for bound ABA (Fig. 1),

Figure 1. Effect of defoliation on October 8 and November 4, 1971 on levels of free (a) and bound (b) abscisic acid in the floral primordia of sour cherry buds, as measured by electron capture gas-liquid chromatography. Significant differences for defoliation (Tukey's test): 5% = 2.39, 1% = 3.05.

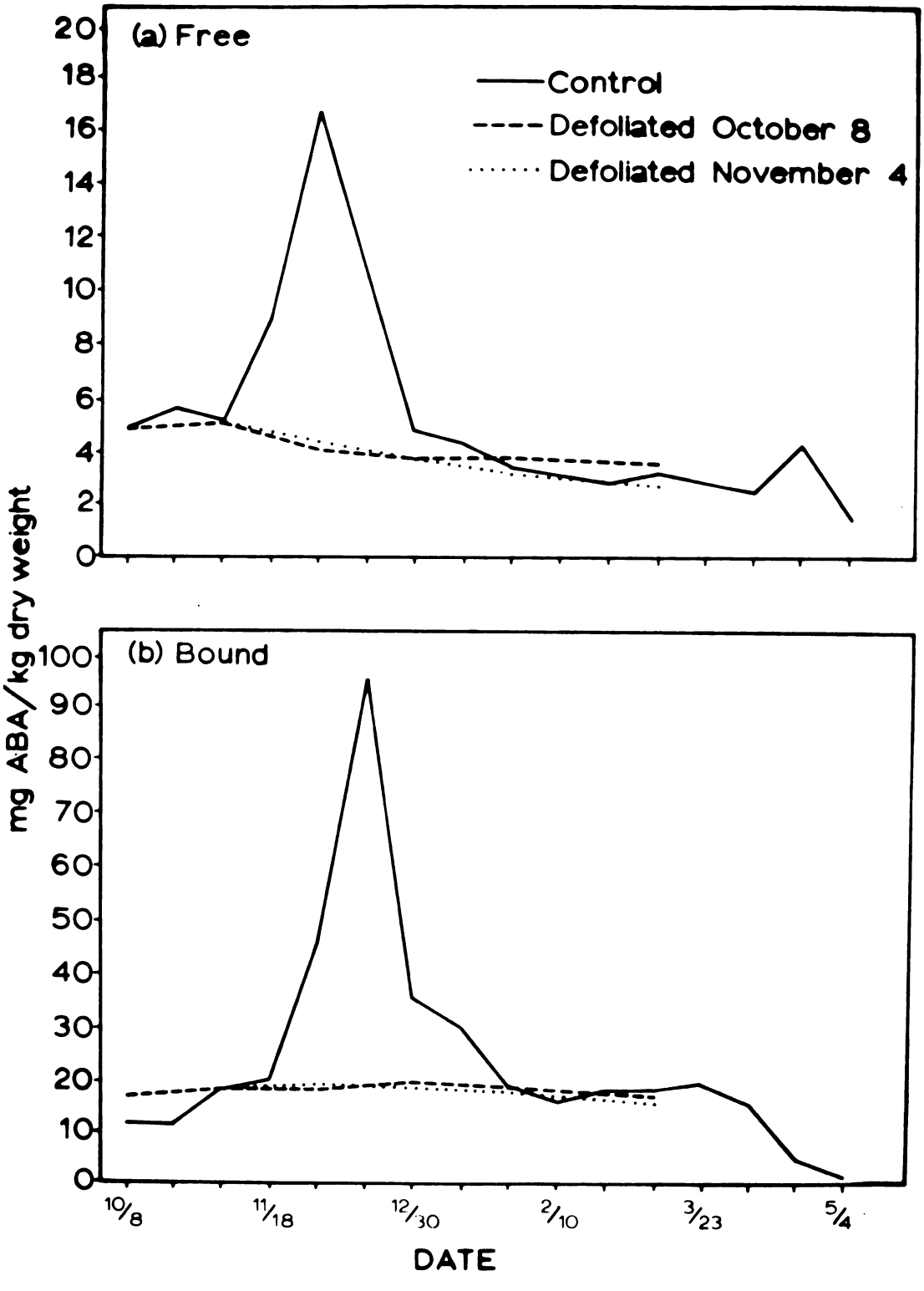
(a) Free
Control
Defoliated October 8
Defoliated November 4
(b) Bound
mg ABA/kg dry weight
DATE
10/8
11/18
12/30
2/10
3/23
5/4

Figure 1.

except that the level in the defoliated trees remained constant, rather than declining. Again, the levels of bound ABA in the defoliated trees were not significantly different from the controls except on December 2, 16, and 30.

Forcing experiments (Table 1) indicated no effect of late fall defoliation on ability of buds to grow, whether the cuttings were forced in distilled water or GA_3.

The presence of leaves reduced the mean flowering stage attained only in cuttings collected August 6 (Table 2). ABA significantly reduced the mean flowering stage attained by cuttings sampled on all dates except November 13, differences being significant at 1% for those sampled from August 6 to September 20 and 5% for those sampled October 22. There was no interaction between leaves and ABA.

Chilling had little effect on the decline of ABA in the flower primordia (Fig. 2). Levels of free ABA fell rapidly during cold storage and remained fairly constant for the remainder of the sampling period. Initially, ABA levels in buds of greenhouse trees fell more slowly than those in chilled trees and were significantly higher after 2 wk; however, there was no difference after 4 wk. Similar results were found for bound ABA (Fig. 2) with the exception that levels in greenhouse trees fell to a significantly lower level after 6 wk and remained significantly lower for the remainder of the sampling period.

DISCUSSION

The results (Fig. 1 and Table 1) indicate that leaf senescence and/or abscission results in an increase in ABA in the bud, while manual removal prevents the increase, and that high levels of endogenous ABA

Table 1. Effect of hand defoliation of trees and GA_3 on the mean flowering stage attained by cuttings after 14 days under mist at 25±3°C (1972-1973) or 28 days under mist at 21±3°C (1973-1974). Trees defoliated October 14, 1972 and October 20, 1973.

Sampling Date	Mean flowering stage[1]			
	GA_3 (ppm): 0		1000[2]	
	Defoliated	Non-defoliated	Defoliated	Non-defoliated
1972-1973				
11-14	1.05	1.03	--	--
11-18	1.00	1.00	--	--
12-12	1.21	1.46	--	--
12-28	1.23	1.26	--	--
1-09	1.86	1.88	--	--
1-23	2.18	1.95	--	--
2-06	2.52	2.56	--	--
2-20	2.77	2.86	--	--
3-20	3.05	3.07	--	--
4-03	3.72	3.80	--	--
4-17	4.39	4.28	--	--
4-24	9.00	9.00	--	--
1973-1974				
10-22	1.29	1.00	1.92	1.68
11-28	1.78	1.76	2.95	2.89
1-06	2.76	2.71	3.60	3.53
2-04	2.97	3.22	3.51	3.68

[1] Significant differences for defoliation (Tukey's test, 5% level): 1972-1973 = 0.38, 1973-1974 = 0.65.

[2] Treatment not made in 1972-1973.

are not required for rest. The data also indicate that if a promoter-inhibitor balance is controlling rest, ABA is not part of it, as branches from non-defoliated trees forced as readily as those from the defoliated trees in the presence of GA_3; however, another possible explanation is that GA_3 is not the natural promoter involved. The observed increase in ABA may result from its formation in the leaf in response to stress during leaf senescence and/or abscission, or it may arise elsewhere (e.g., in the roots). In either case, ABA may result from synthesis (Milborrow and Noddle, 1970; Noddle and Robinson, 1969), or from the breakdown of carotenoids (Taylor and Smith, 1967; Taylor and Burden, 1970; Burden et al., 1972; Milborrow and Garmston, 1973). However, the absence of an effect of the leaves in reducing the mean flowering stage after August 6, while applied ABA was effective for at least an additional 10 wk (Table 2), suggests that the ABA is not produced by the senescing leaves, or that the ABA is in an inactive pool or is converted to a bound, storage form. Although inhibitory activity in birch leaves as measured by bioassay (Kawase, 1961) increased in response to shortening days, ABA, as measured by GLC, declined (Saunders and Lenton, 1969). Grochowska and Lubinska (1973) reported the almost complete disappearance from apple leaves of an endogeous ABA-like material, as measured by bioassay, in early August, although the leaves did not abscise until the end of September. These observations argue against the senescing leaves as the source of the ABA which accumulates in the primordia, although the last data suggest that ABA might be responsible for the inhibition of cherry bud development in cuttings collected on August 6 (Table 2).

Temperature had little effect upon the disappearance of ABA from dormant buds of potted trees (Fig. 2). Abscission occurred approximately

Table 2. Effect of ABA and hand defoliation on mean flowering stage attained by cuttings forced for 28 days at 21±3°C.

	Leaves			
	Removed		Intact	
ABA (ppm):	0	100	0	100
1973-1974				
8-06	1.14	0.23**[1]	0.69[2]	0.15**
8-28	1.22	0.50**	1.39	0.64**
9-20	1.48	0.78**	1.50	0.83**
10-22	1.96	1.56*	1.88	1.48*
11-13	2.25	2.03	2.40	2.21

[1] Significantly different from the respective control at the 5 (*) or 1 (**) % level.

[2] Significantly different from the defoliated cuttings at the 5% level.

Figure 2. Effect of temperature on the decline of free (a) and bound (b) abscisic acid in the floral primordia of sour cherry buds as measured by electron capture gas-liquid chromatography. Potted trees: cold = cold storage at $4\pm2^{o}C$; warm = greenhouse at $22^{o}C$. Significant differences for temperature (Tukey's test); (a) 5% = 1.30, 1% = 1.65; (b) 5% = 1.20, 1% = 1.50.

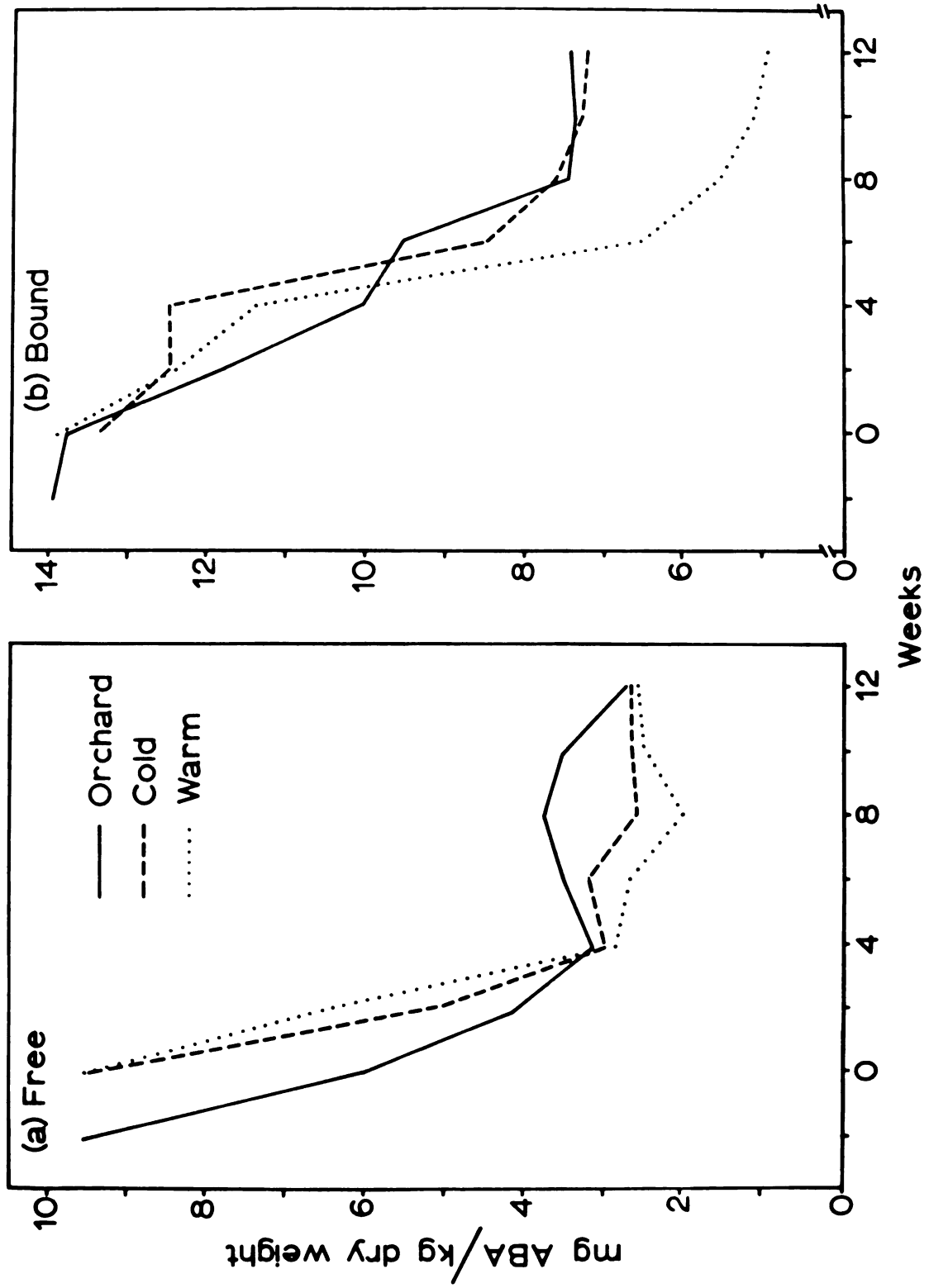
(a) Free
(b) Bound
Orchard
Cold
Warm
mg ABA/kg dry weight
Weeks
0
2
4
6
8
10
12
14

Figure 2.

2 wk later than in orchard trees, probably because the former were growing in a sheltered location. Buds on trees subjected to only 8 wk of cold opened slowly, indicating that chilling was insufficient; however, ABA conc had reached a minimum 4 wk earlier.

In cold storage the potted trees were kept in almost continuous darkness; however, the similarity in data for cold stored and orchard trees, particularly if the curves are shifted by 2 wk so that the times of leaf abscission coincide, suggests that light plays little role, if any, in the decline of ABA.

The lack of an effect of temperature on the disappearance of ABA, and the fact that defoliation prevents the increase in ABA without affecting rest, cast serious doubt on a regulating role of ABA in winter bud dormancy of sour cherry.

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SECTION FOUR

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). IV. EFFECTS OF ABSCISIC ACID APPLICATION UNDER ORCHARD CONDITIONS.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). IV. EFFECTS OF ABSCISIC ACID APPLICATION UNDER ORCHARD CONDITIONS.

ABSTRACT

Abscisic acid (ABA) injected into limbs or buds or applied to the flower bud scales of sour cherry trees, *Prunus cerasus* cv. Montmorency, delayed early stages of development up to 4 days; however, no treatment affected the time of full bloom. ABA (1000 ppm) applied to the bud surface in 50% aqueous ethanol was most effective, while bud injections of ethanolic solutions were toxic. Limb injections were effective only when repeated at 48 hr intervals.

ABA reduced the duration of full bloom, flower petal diameter, pedicel length and initial and final fruit set.

INTRODUCTION

Eagles and Wareing (1964) and Döffling (1963) extracted from *Acer pseudoplatanus* leaves an ABA-like substance which inhibited growth when reapplied to the apices. Similar results were obtained in birch (Kawase, 1961; Eagles and Wareing, 1964), while ABA applied continuously to birch via leaf feeding resulted in a cessation of growth (Eagles and Wareing, 1964). Spring applications of ABA through cut stems delayed bud break 3 to 4 days in *Acer ruburm*, *Abies balsamea*, *Fraxinus americana* and *Picea glauca* (Little and Eidt, 1968), while in *Olea* ABA inhibited either growth or flowering (Badr and Hartmann, 1972).

Spray applications of ABA have been ineffective in delaying bud break on forced cuttings of many species, while applications to the cut bases of blackcurrant and willow resulted in buds on stem cuttings remaining almost completely inhibited (El-Antably et al., 1967).

MATERIALS AND METHODS

Abscisic acid applications

Sour cherry trees 9 years of age were used throughout. Abscisic acid was applied as bud and limb injections in aqueous solution, and as bud injections or topical applications in 50% aqueous ethanol. Initial applications were made when buds had reached stage 3 (half green).

Limbs were injected with 4 conc of ABA (0, 1, 10 and 100 ppm), applied once, weekly (total of 4 applications), or every 48 hr (total of 14 applications). Each treatment, including a non-injected control, was applied to one limb on each of 4 replicate trees. A hole (1.14 mm in diameter) was made at right angles to the long axis, into the pith of branches 1.2 to 1.5 cm in diameter, and a 17 gauge flat pointed hypodermic needle inserted and attached to a 20 ml syringe which acted as a reservoir. Solutions were applied after sunset and left for 12 hr, more being added as required. Each subsequent injection was made approximately 2 cm more distal.

Buds were injected with 50 µl of 0, 10 or 100 ppm ABA in either water or 50% aqueous ethanol, using a 1 ml syringe fitted with a 24 gauge needle. As buds swelled and floral primordia emerged, injections were made directly into the floral cavity. Treatments were applied just before sunset at 48 hr intervals.

Fifty per-cent aqueous ethanol solutions containing 0, 100 and 1000 ppm ABA were painted on buds just after sunset at 48 hr intervals. The paint solutions were applied in a manner to simulate a spray application made to the runoff point.

Flower evaluation

Bud stages were evaluated every other day for a 28 day period, and values averaged for each branch. The following numerical values were used: 0, non-swollen; 1, first swelling; 2, green tip; 3, half green; 4, tight cluster; 5, open cluster; 6, first white; 7, balloon stage; 8, full bloom; and 9, petal fall (Ballard et al., 1971; Dennis and Howell, 1974).

Flowering stages were averaged for the terminal and basal halves of the treated branches. Flower petal diameter and pedicel length were measured at full bloom. Values for 10 flowers per replicate branch were averaged.

The numbers of swelling ovaries at shuck split were counted and divided by the number of flowers reaching anthesis to determine initial fruit set, with allowance for frost-damaged pistils. A second count was made following June drop to determine the effect of ABA on final fruit set. These last measurements had no direct bearing on delay of bloom, but were made to determine what, if any, effects ABA might have on fruit development.

Bud break was considered to be the attainment of flowering stage 2, while change between numerical stages 2 and 9 indicated flower development.

RESULTS AND DISCUSSION

Delay of flowering was proportional to ABA conc from days 10 through 16 only following limb injection at 48 hr intervals (Table 1); however, effects were not statistically significant (Tukey's test, 5% level). Single and weekly applications had no effect (data not shown). A gradient in flower development was observed, with the terminal half of the branch being slightly more advanced than the basal half; this was unaffected by ABA treatment.

Applications to buds of 1000 ppm ABA in 50% aqueous ethanol retarded flower development significantly on days 6, 10 and 14 (Table 2). Retardation was significantly greater than that caused by the 100 ppm limb injection on days 4 and 6. Maximum delay in reaching stage 5 was approximately 2 days with the 1000 ppm application; however, by day 16 (full bloom), there was no difference in flower development. By day 20, flower development was significantly promoted by the 1000 ppm treatment. When compared to the 100 ppm limb injection, promotion of flower development with 1000 ppm paint application was highly significant on day 18, and significant on day 20. This apparent promotion of development actually reflects a hastening of petal senescence, for treated flowers progressed from the balloon stage (stage 7) to petal fall (stage 9) in less than 24 hr, while the non-treated and ethanol-treated control flowers required approximately 4 days. As previously reported (Mielke and Dennis, 1974a) levels of endogenous ABA in flowers increase with bud swell, reach a maximum at full bloom and decline with petal abscission. A possible role for endogenous ABA at this time may be to either initiate or promote petal abscission.

Table 1. Effect of repeated injections of ABA into limbs of sour cherry trees at 48 hour intervals on the mean flowering stage attained under orchard conditions.

Mean flowering stage[1]

	ABA (ppm)			
Day	0	1	10	100
0	3.00	3.00	3.00	3.00
2	3.00	3.00	3.00	3.00
4	5.05	4.83	4.95	4.98
6	5.07	5.15	5.26	5.23
8	5.79	5.58	5.65	5.53
10	6.31	6.24	6.19	6.02
12	6.89	6.78	6.70	6.35
14	7.27	7.17	7.15	6.85
16	7.89	7.91	7.85	7.46
18	8.14	8.14	8.37	7.92
20	8.62	8.48	8.61	8.36
22	8.93	8.81	8.84	8.88
24	8.99	8.95	8.96	8.90
26	9.00	9.00	9.00	9.00

[1] Significant differences for ABA (Tukey's test): 5% = 0.64, 1% = 0.84.

Table 2. Effect of application of ABA at 48 hour intervals on the mean flowering stage[1] attained under orchard conditions.

	Method of application[1]				
	None	Limb injection	Bud surface		
ABA (ppm):	--	100	0	100	1000
Day					
0	3.00	3.00	3.00	3.00	3.00
2	3.00	3.00	3.00	3.00	3.00
4	5.00	5.00	4.75	4.88	4.58
6	5.31	5.16	5.31	5.19	4.71*[2]
8	5.94	5.73	5.75	5.70	5.50
10	6.50	6.23	6.56	6.32	6.16*
12	6.93	6.86	6.86	6.75	6.68
14	7.38	7.14	7.44	7.26	7.12*
16	7.88	7.59	7.88	7.76	7.89
18	8.14	8.01	8.24	8.22	8.50
20	8.42	8.60	8.56	8.76	9.00**
22	8.63	8.90	8.83	8.85	9.00
24	8.95	9.00	8.95	9.00	9.00
26	9.00	9.00	9.00	9.00	9.00

[1] Significant differences for ABA application (Tukey's test): 5% = 0.30, 1% = 0.39.

[2] Significantly different from treatment with 50% ethanol alone at 5 (*) or 1 (**) % level.

Delay of flowering by 1000 ppm ABA in 50% ethanol, when compared to the non-injected control, was significant on days 4, 6, 8 and 10. The additional days of significance are due to the combination effects of ABA and 50% aqueous ethanol, neither of which was significant by itself.

Bud injections of 50% aqueous ethanol were toxic, killing the buds within 24 hr (data not shown). Through day 20, aqueous injections of ABA into buds retarded flower development, while after day 20 there was apparently a slight promotion of flower development (Table 3). The retardation by the 100 ppm bud injection was significant on day 12 and highly significant on day 14. The promotion of development apparently is due to an acceleration of the petal abscission process. Maximum delay of flower development was approximately 2 days with approximately 1 day delay in reaching full bloom; however, the latter difference was not significant.

With limb injections, increasing conc of ABA reduced flower petal diameter at full bloom, the effect being highly significant at 100 ppm (Table 4). Increasing the frequency of injection reduced flower diameter slightly, but not significantly.

ABA also reduced pedicel length (Table 4). A slight reduction occurred with 100 ppm regardless of the frequency of application, while 10 ppm had a marked effect only when applied at 48 hr intervals. Pedicel length was inversely proportional to frequency of application, but the effect was not significant.

Initial fruit set (Table 4) was reduced only when 100 ppm ABA was applied at 48 hr intervals; however, both 10 and 100 ppm applications significantly reduced the number of fruit remaining after June drop. A similar effect of ABA in reducing fruit set was reported in grape (Weaver and Pool, 1969; Weaver, 1973).

Table 3. Effect of aqueous injection of ABA at 48 hour intervals on the mean flowering stage attained under orchard conditions.

	Method of application[1]				
	None	Limb injection	Bud injection		
ABA (ppm):	--	100	0	10	100
Day					
0	3.00	3.00	3.00	3.00	3.00
2	3.00	3.00	3.00	3.00	3.00
4	5.00	5.00	5.08	4.98	4.66
6	5.31	5.26	5.34	5.36	5.20
8	5.94	5.73	5.77	5.70	5.54
10	6.56	6.32	6.19	6.01	5.88
12	7.11	6.87	6.69	6.56	6.25*[2]
14	7.56	7.28	7.38	7.00	6.75**
16	8.00	7.87	7.93	7.66	7.67
18	8.19	8.24	8.30	8.10	7.85
20	8.51	8.72	8.71	8.41	8.75
22	8.78	8.89	8.96	8.85	9.00
24	8.95	9.00	9.00	9.00	9.00
26	9.00	9.00	9.00	9.00	9.00

[1] Significant differences for ABA application (Tukey's test): 5% = 0.44, 1% = 0.59.

[2] Significantly different from bud injection of water alone at 5 (*) or 1 (**) % level.

Table 4. Effect of concentration of ABA and frequency of limb injection on flower diameter and pedicel length at full bloom, and on fruit set of Montmorency sour cherry.

Frequency	ABA (ppm)	Flower diam. (mm)	Pedicel length (mm)	Fruit set (%) Initial	Fruit set (%) Final
Once	0	27.60	18.48	55.35	52.32
	1	26.62	17.75	53.50	55.08
	10	27.48	16.52	44.32	32.30
	100	21.02**[1]	13.00*	44.52	30.68*
Weekly	0	26.62	18.45	53.10	53.58
	1	26.58	17.76	44.58	43.88
	10	25.15	16.22	38.90	32.28*
	100	20.55**	12.62*	33.20	30.38*
Every 48 hrs.	0	27.22	18.30	55.35	54.58
	1	25.28	17.20	56.26	44.60
	10	23.48	12.20*	49.65	29.22*
	100	20.95**	10.15**	23.33*	19.61**
Main effects:					
Conc ABA[2]					
	0	27.15 a	18.47 a	54.60 a	53.49 a
	1	26.18 a	17.57 ab	51.38 a	47.85 ab
	10	25.37 a	14.98 b	44.29 ab	31.27 bc
	100	20.84 b	11.92 c	33.68 b	26.89 c
Frequency					
	Once	25.70	16.44	49.37	42.60
	Weekly	24.73	16.26	42.44	40.03
	Every 48 hrs.	24.23	14.71	46.15	37.00
Significant differences (Tukey's test):					
Treatment					
Conc ABA					
	5%	4.42	5.19	26.02	20.95
	1%	5.52	6.48	32.53	26.18
Frequency					
	5%	n.s.	n.s.	n.s.	n.s.
Main effects					
Conc ABA					
	5%	2.55	3.00	15.02	12.10
	1%	3.19	3.47	18.78	15.12
Frequency					
	5%	n.s.	n.s.	n.s.	n.s.

[1] Treatment means within columns and sets (frequency) are significantly different from injection of water alone at the 5 (*) or 1 (**) % level.

[2] Main effect means for ABA followed by the same letter are not significantly different at the 5% level.

Similar results on flower diameter, pedicel length and initial and final fruit set were obtained for both bud injection of ABA in aqueous solution or 50% aqueous ethanol applications to the scales (data not shown). Paint applications at 1000 ppm or bud injections of 100 ppm were identical in effect to the 100 ppm applications made by limb injection at 48 hour intervals.

These results indicate that exogenously applied ABA does not inhibit flower development in sour cherry, in contrast with the results obtained with cuttings of ash, fir, maple and spruce (Little and Eidt, 1968); however, El-Antably *et al.* (1967) found spray applications ineffective in delaying bud break on cuttings of almond, apple, birch, blackcurrant, grape, peach, pear, plum and poplar, while applications to the bases of blackcurrant and willow were effective in inhibiting bud break. The authors concluded that topical applications were ineffective because ABA failed to penetrate to the bud meristems.

One might postulate that in sour cherry, ABA is ineffective in delaying bloom because it does not penetrate to the primordia, is inactivated enzymatically, or is metabolized too rapidly to be effective; however, the reduction of flower diameter, pedicel length and final fruit set (Table 4) at lower conc and less frequent intervals of application than were effective in delaying flower development does not support the first explanation. The possibility cannot be dismissed that ABA is metabolized too rapidly to be effective in delaying bloom, while one of its metabolites is responsible for the observed side effects.

Since buds were in stage 3 (half green) when the first applications were made, ABA may prevent bud break, but once bud break has occurred and flower development begins, it is no longer effective.

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SECTION FIVE

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). V. EFFECTS OF APICES AND GROWTH SUBSTANCES.

HORMONAL CONTROL OF FLOWER BUD DORMANCY IN SOUR CHERRY (*Prunus cerasus* L.). V. EFFECTS OF APICES AND GROWTH SUBSTANCES.

ABSTRACT

Bud break and flower development in sour cherry, cv. Montmorency, branches was unaffected by decapitation or wounding of the branch below the apical bud.

Bud break was inhibited by 100 ppm abscisic acid (ABA) applied to the cut bases prior to the end of rest. After the termination of rest, ABA delayed flower development. Amo-1618 was as effective as ABA in delaying flower development, and when used in combination their effects were additive.

GA_3, GA_{4+7}, and GA_{13} applied to the bases of excised branches promoted bud break and flower development prior to the end of rest and flower development after rest was terminated. The greatest promotion of flower development occurred when GA_3 and GA_{4+7} were used in combination.

GA_3 or GA_{4+7} was able to overcome Amo-1618-induced, but not ABA-induced, inhibition, suggesting that ABA inhibits the action of GA s while Amo-1618 inhibited their synthesis.

INTRODUCTION

ABA-like inhibitors extracted from the leaves of *Acer* and *Betula* inhibited growth when reapplied to the apices (Kawase, 1961; Eagles and Wareing, 1964). Application of ABA to the bases of excised branches

inhibited or delayed bud break in ash, blackcurrant, fir, maple, spruce and willow (El-Antably et al., 1967; Little and Eidt, 1968). Badr and Hartmann (1972) noted that ABA either inhibited growth of olive or caused flower buds to grow vegetatively. Under orchard conditions, ABA did not delay flower development in sour cherry once bud break had occurred (Mielke and Dennis, 1974b).

The ability of gibberellins to promote bud break varies with time of application. After partial fulfillment of the chilling requirement, GA_3 promotes bud break (Marth et al., 1965; Donoho and Walker, 1956; Stuart, 1958; Brian et al., 1959; Walker and Donoho, 1959; Brown et al., 1961; Ashby, 1962; Couvillion and Hendershott, 1974), while late summer to autumn applications in the field delay bud break the following spring (Brian et al., 1959; Weaver, 1959; Hicks and Crane, 1968; Stembridge and LaRue, 1969; Corgan and Widmoyer, 1971; Kachru et al., 1971; Badr and Hartmann, 1972; Painter and Stembridge, 1972).

The purpose of this study was to determine the effects of gibberellins, ABA and Amo-1618, alone and in combination, on bud break and flower development in sour cherry.

MATERIALS AND METHODS

Sour cherry branches 35 to 40 cm long were collected from 10-year-old trees at intervals from August 6, 1973 to February 4, 1974. There were 4 branches per treatment. In each experiment the same 4 replicate trees were used for all sampling dates. All chemicals were applied in aqueous solutions.

Wounding was performed by removal of a 15 x 3 mm strip of bark, 5 mm below the apical bud.

All branches were placed with their bases in 10 ml of the test solutions in standard test tubes (18 x 150 mm). The cuttings were sealed to the tops of tubes with 'Parafilm' to prevent the solutions from being diluted by the mist. The tubes were placed upright in test tube racks in a greenhouse at 21±3°C. Mist was supplied for 6 sec every 3 min from 2 a.m. to 12 p.m. Solutions were added as required and changed after 14 days. Based of the branches were recut every other day, and the treatments continued for 28 days.

Bud stages were evaluated every other day for a 28 day period, and values averaged for each cutting. The following numerical values were used: 0, non-swollen; 1, first swelling; 2, green tip; 3, half green; 4, tight cluster; 5, open cluster; 6, first white; 7, balloon stage; 8, full bloom; and 9, petal-fall (Ballard et al., 1971; Dennis and howell, 1974).

Rest was considered broken when 50% of the buds on the cuttings attained flowering stage 2 within 14 days. Change between numerical stages 2 and 9 was considered flower development.

RESULTS AND DISCUSSION

Neither removal of apical buds nor wounding was effective in promoting bud break after growth had ceased (Table 1). These results agree with those of Ramsay et al. (1970) who found that decapitation of apricot shoots when growth had ceased (after June) was ineffective in promoting lateral bud break.

Cuttings collected from August 6 to February 4 were subjected to the following treatments: water control; ABA (1, 10 and 100 ppm); GA_3

Table 1. Effect of apical bud removal, wounding and sampling date on the mean flowering stage attained by cuttings forced for 28 days at 21±3°C.

	Flower bud development[1]		
Apical bud:	Intact	Wounded	Removed
1973-1974			
8-06	0.96	—[2]	1.16
8-28	1.52	--	1.58
9-20	1.73	—	1.89
10-22	1.96	1.85	2.11
11-13	2.25	2.09	2.23
11-28	2.31	2.15	2.21
1-06	3.02	2.93	3.03
2-04	2.98	2.88	3.04

[1] Significant differences for treatments (Tukey's test): 2 means: 5% = 0.31, 1% = 0.47; 3 means: 5% = 0.39, 1% = 0.57.

[2] Treatment not made.

(1, 10, 100 and 1000 ppm); GA_{4+7} (1, 10 and 100 ppm); GA_{13} (1, 10, 100 and 1000 ppm); Amo-1618 (10 and 1000 ppm on Feb. 4 only).

Control cuttings collected August 6 and August 28 exhibited little growth (Fig. 1). The flowering stage attained after 28 days increased 75% on September 20 and October 22, then decreased by one-third by November 28. Buds on cuttings collected January 6, when rest had been broken, developed much more rapidly than in previous samples, with a slight additional increase in flower development on February 4.

ABA at 100 ppm significantly inhibited bud break in the first 3 samples, but its effects were non-significant from October 22 to November 28 as the ability of the control branches to grow decreased. After rest had been broken, ABA at conc higher than 10 ppm significantly delayed flower development. Ten ppm had no significant effect at any sampling date. These results agree with those for blackcurrant and willow (El-Antably et al., 1967), where application of ABA to the bases of branches resulted in a delay of bud break.

Amo-1618 at 1000 ppm, used only on February 4, was slightly more effective than ABA in retarding flower development. Amo-1618 is one of a group of compounds which are potent inhibitors of kaurene synthetase, the enzyme responsible for converting trans-geranylgeranylpyrophosphate to ent-kaurene in the synthesis of gibberellin (Kessler and Kaplan, 1972). Their growth-retarding ability has been attributed to inhibition of gibberellin synthesis (Paleg et al., 1965; West and Upper, 1969; Kuo and Pharis, 1973). The effectiveness of Amo-1618 in retarding bud opening in cherry suggests that breaking of rest is due in part at least to acquisition of the ability to synthesize gibberellins.

Figure 1. Effects of ABA, Amo-1618 and GAs on flower bud development in cuttings forced for 28 days at 21±3°C in a mist bed. Significant differences for treatments (Tukey's test): within sampling dates, 5% = 0.50, 1% = 0.59; between sampling dates, 5% = 0.59, 1% = 0.69. Asterisks indicate treatments which are significantly different from the respective control at the 5 (*) or 1 (**) % level.

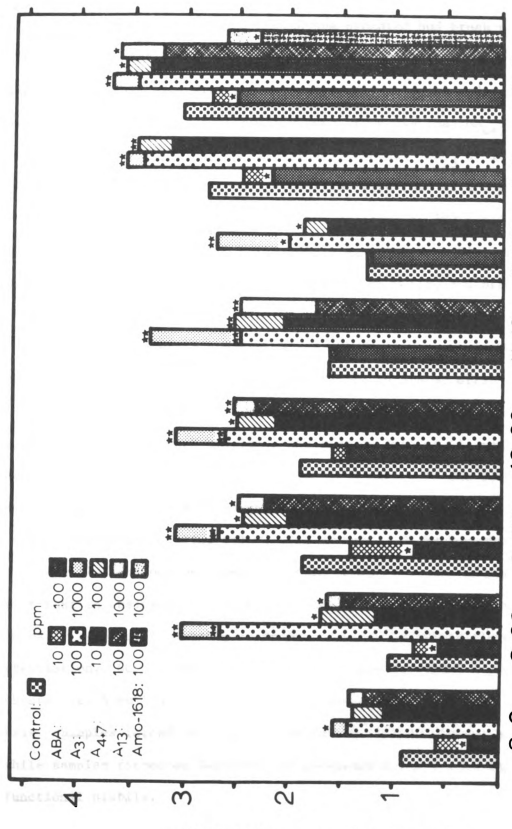
Mean flowering stage
1
2
3
4
8-6
8-28
9-20
10-22
11-13
11-28
1-6
2-4
1973-1974
Control:
ABA:
A3:
A4,7
A13:
Amo-1618:
ppm
10
100
100
1000
10
100
100
1000
100
1000

Figure 1.

GA_3 at 1000 ppm and GA_{4+7} at 100 ppm promoted bud break on all sampling dates (Fig. 1), the difference being non-significant in only one case (GA_{4+7}, Aug. 6). With the exception of treatments made on August 6, GA_3 at 1000 ppm was significantly more effective than GA_{4+7} at 100 ppm in promoting bud break and flower development. GA_{13} at 1000 ppm and GA_3 at 100 ppm produced responses identical with that of 100 ppm GA_{4+7}, with the exception of August 28 when the response to 100 ppm GA_3 was significantly higher than to either GA_{4+7} at 100 ppm or GA_{13} at 1000 ppm (Fig. 1). The other levels of GA, while promoting bud break and flower development, were not significantly different from the control.

The lower flowering stage attained by all treatments on November 28 as compared with samples taken immediately before or after suggests that dormancy was deepest on this date. Hatch and Walker (1969) found similar results in peach utilizing the minimum conc of GA_3 necessary to cause growth as a measure of the degree of dormancy in the buds, and Mielke and Dennis (1974a) reported the percentage of buds attaining stage 2 was minimal during this period (November 20 to December 2).

The fluctuations in flowering stage attained by the controls suggest that intensity of rest varies during the year; however, response in early samples is probably confounded due to incomplete flower differentiation. Some flower buds on cuttings sampled August 6 produced shoots 3 to 6 cm long with scales and floral primordia spaced along the axis. Samples forced on August 28 exhibited flowers without a pistil, while samples forced on September 20 possessed flowers with apparently functional pistils.

Amo-1618 (0, 10 and 1000 ppm) and ABA (0, 10 and 100 ppm) were applied in a factorial design to branches collected on February 4. When

used alone, both ABA at 10 and 100 ppm and Amo-1618 at 1000 ppm significantly reduced the mean flowering stage attained (Table 2). When used in combination, the effects of the two chemicals were additive.

Amo-1618 and GA_3 (1000 ppm each) and ABA and GA_{4+7} (100 ppm each) were applied in all possible combinations of pairs on February 4 to determine their interactions. Amo-1618 was ineffective in inhibiting GA-stimulated bud development (TAble 3). These data agree with those of Kessler and Kaplan (1972) who found that Amo-1618 inhibited GA synthesis in barley seeds without affecting GA action. On the other hand, ABA negated the effects of both GAs, as was shown for blackcurrant (El-Antably et al., 1967). Amo-1618 and ABA therefore function in different manners. Apparently Amo-1618 inhibits GA synthesis without inhibiting GA action, while ABA at least partially inhibits GA action. The greatest promotion of flower development occurred when GA_3 and GA_{4+7} were used in combination, but their combined effects were less than additive. This suggest that both GAs act at the same site, or are readily interconverted.

To determine if the effects of the inhibitors were reversible, sequential application of growth substances was made to cuttings collected November 28, January 6 and February 4. Either ABA (100 ppm) or Amo-1618 (1000 ppm) was applied to cuttings for a 14 day period. They were then transferred to gibberellin solutions for the final 14 days of forcing.

Neither GA_3 at 1000 ppm nor GA_{4+7} at 100 ppm overcame the inhibition induced by a 2 week application of ABA (Fig. 2a), indicating that the effect of ABA in inhibiting the action of GA is persistent. Similar results were obtained with cuttings collected on November 28 and January 6.

Table 2. Effects of ABA and Amo-1618 on the mean flowering stage attained by cuttings sampled February 4, 1974, and forced for 28 days in a mist bed at 21±3°C.

ABA (ppm)	Flower bud development: Amo-1618 (ppm) 0	10	1000
0	3.04 a[1]	2.88 ab	2.59 bc
10	2.58 bc	2.52 bc	2.13 de
100	2.46 cd	2.30 cde	1.98 e

[1] Means followed by the same letter are not significantly different at the 5% level (Tukey's test).

Table 3. Effects of ABA (100 ppm), Amo-1618 (1000 ppm), GA_3 (1000 ppm) and GA_{4+7} (100 ppm) on the mean flowering stage attained by cuttings sampled on February 4, 1974, and forced for 28 days in a mist bed at 21±3°C.

	Mean flowering stage[1]			
	Gibberellin			
Inhibitor	None	GA_3	GA_{4+7}	GA_3+GA_{4+7}
None	2.86 c[2]	3.40 ab	3.20 b	3.52 a
Amo-1618	2.50 de	3.42 ab	3.23 b	--[3]
ABA	2.56 de	2.82 cd	2.76 cde	--
Amo-1618+ABA	2.20 f	--	--	--

[1] Significant differences for treatments (Tukey's test): 5% = 0.28, 1% = 0.33.

[2] Means followed by the same letter are not significantly different at the 5% level.

[3] Treatment not made.

Figure 2. Effect of gibberellin application following either ABA (100 ppm) or Amo-1618 (1000 ppm) on the mean flowering stage attained by cuttings sampled February 4, 1974, and forced for 28 days in a greenhouse at 21±3°C. Treatment for the first 14 days was either ABA (a) or Amo-1618 (b). Control was held in water for the entire 28 day period. Significant differences for treatments (Tukey's test): (a) 5% = 0.36, 1% = 0.49; (b) 5% = 0.42, 1% = 0.58.

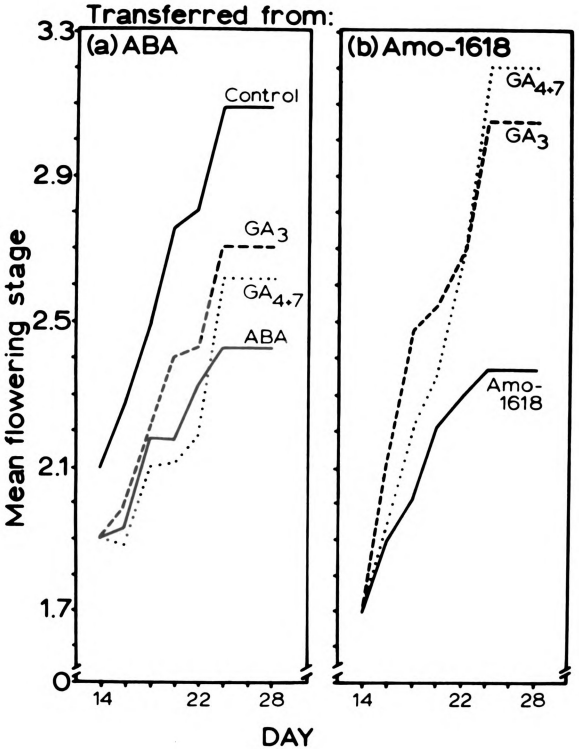
Transferred from:
(a) ABA
(b) Amo-1618
Control
GA3
GA4+7
ABA
GA4+7
GA3
Amo-1618
Mean flowering stage
3.3
2.9
2.5
2.1
1.7
0
14
22
28
14
22
28
DAY

Figure 2.

Inhibited buds on Amo-1618 treated cuttings grew rapidly on transfer to GA_3 or GA_{4+7} (Fig. 2b), GA_3 being slightly more effective initially. Response was significantly greater for the GA treatments after 4 to 6 days in comparison with that of cuttings left in Amo-1618. The response on transfer to ABA did not differ from that of continued growth in Amo-1618.

These data plus the ability of GAs to promote bud break (Fig. 1) suggest that endogenous GAs are involved in both bud break and flower development; however, the fact that both processes can occur even in the presence of Amo-1618 indicates that GAs are not the only hormones involved.

Exogenously applied ABA inhibits both bud break prior to the end of rest and flower development thereafter when applied before growth begins. GAs promote bud break and/or flower development both before and after the end of rest. Amo-1618 inhibits flower development after rest has ended. This compound appears to inhibit flower expansion by inhibiting GA synthesis without affecting GA action, while ABA inhibits GA action, with the effects being long lived. The greatest inhibition occurred when ABA and Amo-1618 were used in combination, and the greatest promotion when GA_3 and GA_{4+7} were used together.

Although GAs promote bud break and flower development prior to the end of rest and promote flower development following the termination of rest, and ABA and Amo-1618 inhibit these processes, they are not in complete control of dormancy, and other hormones probably play a role in the complex mechanism controlling dormancy.

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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Abscisic acid (ABA) was identified by GC-MS as one of the five inhibitory zones in both the free and bound fractions of extracts from sour cherry buds.

Although concentrations of ABA in the primordia rose rapidly in late autumn to peak in late November to early December, and then fell rapidly to intial levels, the increase coincided with the onset, not of bud dormancy, but of leaf abscission, with the maximum levels occurring when about 90 to 95% of the leaves had abscised. This peak coincided with the period of deepest dormancy in two of the three years in which the levels were measured. In addition, the concentration of ABA returned to a low level two to six weeks prior to the termination of rest. Even if one assumes that a threshold level of ABA is required to maintain rest, the time between the drop in ABA and the end of rest is not consistent.

Mechanical defoliation of trees prior to the onset of natural leaf abscission prevented an increase in endogenous ABA without affecting the intensity of rest. Forced cuttings from defoliated trees (low ABA) achieved the same stage of flowering as did cuttings from the non-defoliated trees (high ABA) even in the presence of gibberellins. If we assume that a promoter-inhibitor balance is controlling bud dormancy, then either ABA is not the inhibitor or GA is not the promoter.

Once ABA levels had increased, chilling temperatures were not required for their decline. Initially, ABA declined more slowly under warm conditions than under cold; however, no differences were apparent after 4 weeks, and levels remained low for the duration of the experiment. If ABA controls dormancy, its decline should occur only under chilling conditions.

Exogenous applications of ABA were ineffective in delaying bud development under orchard conditions once swelling had occurred, while in the greenhouse, ABA and Amo-1618 inhibited bud break and flower development when applied before swelling began. This suggests that ABA might be ineffective in delaying bloom once bud swell has begun.

GA_3, GA_{4+7}, and GA_{13} promoted bud break and flower development prior to the end of rest, and flower development once rest had been terminated. Gibberellin overcame Amo-1618-induced, but not ABA-induced, inhibition, suggesting that Amo-1618 inhibits by blocking GA synthesis rather than its action, while ABA inhibits, at least in part, GA action. The data suggest that during the breaking of rest, buds acquire the ability to synthesize gibberellins; however, the fact that bud break and flower development occur even in the presence of Amo-1618 implies that gibberellins are not the only hormones involved.

Further knowledge of the role of growth substances in dormancy is required. At least four other acidic inhibitors which occur in extracts of sour cherry buds should be identified and measured in relation to dormancy, as well as auxins, gibberellins and cytokinins.

Still to be determined are the cause of the rise in ABA in the primorida coincident with leaf abscission, and the effect of ABA and Amo-1618 on flower development when applied prior to bud swell under orchard conditions.

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