This investigation was undertaken to observe possible changes in growth substances in *Pyrus* throughout the dormant period. The primary objective was to determine if a correlation existed between growth substance content and the inherent chilling requirement of seeds and buds.

Samples of whole buds were taken at intervals from the onset of rest in September through full-bloom in March from three *Pyrus* types differing in chilling requirements. Single samples were also taken during rest from several additional *Pyrus* species of varying lengths of chilling requirements. Growth substances were removed with methanol and ether extractions. Seed and fruit samples were taken from several varieties of *P. communis* before and after subjection to periods of chilling temperatures. Individual seed samples were divided into three fractions—surface materials, seedcoats and embryos—prior to water and ether extractions. Separation of growth
substances was done with paper chromatography and active materials detected with an oat coleoptile straight-growth test.

A single growth inhibitor was found in all samples of Pyrus buds, seeds and fruit tissue. Based on both paper and thin-layer chromatographic separations and UV spectra, this material is tentatively identified as abscisic acid.

The inhibitor content in bud extracts of the three Pyrus types, while fluctuating from sample to sample, did not correlate with the amount of chilling that had accumulated at each sampling time. Inhibitor concentrations in whole buds remained relatively stable throughout rest. The inhibitor did not disappear from the buds when rest was broken or provide evidence of a consistent reduction with chilling. Pyrus species with longer inherent chilling requirements consistently showed higher inhibitor concentrations during rest than did species requiring short chilling periods to break rest.

No growth promoting materials were detected until full-bloom when a strong, unidentified promoter was found in samples of each of the three Pyrus types. Significantly higher concentrations of the material were present in the samples of the long chilling types than in the short chilling species.

In the fruit and seed samples, significantly higher concentrations of the growth inhibitor were present in fruit tissue, on the seed surface and in the seedcoats than in the embryo. After subjection to chilling temperatures the inhibitor content decreased significantly in
the embryo, but little change took place in the extracts of the fruit and seedcoats. No consistent growth promoters were found in fruit or seed tissues.

The presence of the growth inhibitor in each sample of whole buds throughout the dormant period indicates that rest may result in part from growth inhibitors. Growth inhibitor content appears to be related primarily to chilling requirement rather than to the amount of chilling the buds have received at any given time. Inhibitors may increase to functional levels during the period of rest induction, remaining at these levels until their effective concentration is exceeded by growth promoters prior to the resumption of growth.

The finding that only in the embryo did the inhibitor content respond significantly to chilling indicates that rest in seeds may be closely associated with the inhibitor concentration in the growing point. The embryo may be a site of inhibitor degradation rather than synthesis. Chilling may provide the conditions necessary for an enzymatic degradation of the inhibitor in active tissues.
A Study of the Physiology of Dormancy in the Genus *Pyrus*

by

Stanley Douglas Strausz

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1970
APPROVED:

Professor of Horticulture
in charge of major

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Date thesis is presented June 24, 1969

Typed by Gwendolyn Hansen for Stanley Douglas Strausz
ACKNOWLEDGMENT

I would like to express my gratitude to Dr. M. N. Westwood for the opportunity to undertake this project and for his guidance in conducting the research and preparing the manuscript.

I would also like to express my appreciation to the other members of my graduate committee, especially Dr. Ralph Garren, Jr. for his suggestions during the final thesis preparation.

The funds for this project were provided by a National Science Foundation research grant, GB-5182.
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A STUDY OF THE PHYSIOLOGY OF DORMANCY
IN THE GENUS PYRUS

INTRODUCTION

A period of cessation of visible growth prior to the onset of winter has long been observed in the seasonal growth sequence of deciduous species. The development of this condition is generally associated with the progressive senescence of certain plant parts. Beginning in late summer buds enter a dormant state in which they remain until the return of an environment favorable to the resumption of growth. Similarly, seeds do not usually germinate immediately upon being shed but remain dormant for an indefinite period.

Horticulturists refer to the state in which growth does not proceed whether as a result of an unsuitable environment or internal physiology as dormancy. Specifically, the period in which growth would not occur due to internal factors even under favorable conditions is termed rest, while a failure to grow because of adverse environmental conditions is designated quiescence.

Because the onset and termination of rest are gradual processes it is difficult to accurately define the limits of the rest period. During these transition periods confusion may arise in the usage of these terms. In many instances little or no distinction is made between dormancy and rest.
Chilling, the exposure of the plant to near freezing temperatures, is generally recognized as the means by which rest is broken. Winter chilling is naturally responsible for the termination of rest in buds as well as seeds of many deciduous species. The practice of subjecting seeds to low temperatures under moist conditions to break rest has long been known as seed stratification. The most effective temperatures are generally near 40°F, however both the amount of chilling and the optimum temperature vary with species (17, p. 49-63, 95).

This period of suspended growth is a means by which a species can maintain itself within its environment. Development is restricted during the time when particular environmental factors are limiting. In this condition the plant is able to withstand environmental extremes which could not be tolerated if the tissues remained actively growing. The mechanism(s) by which these factors are perceived and exert their influence has been the subject of intensive investigation in recent years. Several theories have been advanced which attempt to correlate temperature, moisture and light with the observable biological responses of bud break and seed germination.

Isolation of growth regulating substances from numerous plant organs preceded the development of the current hypothesis regarding dormancy induction. Experiments with species having a definite rest period led to the isolation of a growth inhibiting substance,
abscisic acid, common to both buds and seeds. Results of several trials showed this material to be present in measurably larger amounts in dormant than in nondormant tissues; thus the leading theory of rest suggests a balance between promoting and inhibiting materials with environment influences as the controlling mechanism.

Considering this theory of rest the genus Pyrus is well suited to its study. Within this genus are species varying extensively in the duration of their rest period. Pyrus koehnei, an evergreen, has no rest period while certain types of P. communis require 1500 or more hours of exposure to chilling temperatures to terminate rest; numerous species have rest periods of intermediate lengths. Recently the graftage of P. communis, a species requiring long chilling, to P. calleryana, which requires much less chilling, was reported to reduce the chilling time required to break rest in the P. communis buds (96). This apparent transfer of the chilling influence suggests that the dormancy factor might be a definite chemical agent.

This investigation was undertaken to follow the dormant period in several Pyrus species in terms of possible changes in growth substances. Using both simple and compound Pyrus systems correlation was sought between growth substance content and inherent chilling requirement in both seed and bud samples. The prime objective was first to determine if rest could be attributed to the growth inhibitor status of these tissues and, secondly, to determine if a particular
inhibitor system was common to Pyrus species in general. Finally, if such a common inhibitor was present, attempts would be made to identify it.
REVIEW OF LITERATURE

Early workers attempting to explain the nature of dormancy attributed it to an auxin shortage (10). Subsequently, a build-up of inhibitory materials, noted at the onset of rest, correlated with the shift from the long days of summer to the short days of fall. Further research, detecting the presence of both growth promoting and inhibiting substances, suggested the dormant state to be the result of a critical balance of these materials under photoperiodic control. Recently investigators have isolated from both dormant buds and seeds a growth inhibiting substance which is apparently involved in several physiological and biochemical processes associated with dormancy (7, 22, 23, 33).

Evidence from related fields of research indicated that additional materials may be involved. Some workers have found auxin action related to ethylene production while others have linked auxins, gibberellins and cytokinins to DNA, RNA, protein synthesis and enzyme systems (1, 3, 9, 18). Growth substances may exert ultimate control on the expression of genetic material.

Bud Dormancy

Avery et al. (10), studying growth hormones in Aesculus and Malus, detected auxins in increasing amounts during the period of
bud-swell. A new approach was offered by Hemberg (40, 41, 42) following work on potato tubers and *Fraxinus* in which he reported evidence for the existence of growth inhibiting materials of significance to the rest period. Large quantities of inhibitors were extracted from resting terminal buds, whereas extracts made prior to the resumption of growth showed much reduced inhibitor concentrations.

Several subsequent studies have also correlated endogenous inhibitors with the onset and breaking of rest in woody species. Extraction of resting peach flower buds yielded a specific inhibitor, naringenin, which, while present in large amounts between November and February, disappeared prior to bloom (43, 44, 90). Other workers have disputed the correlation of this material with rest itself (20, 26). Because the inhibitor was found primarily in bud scales its loss could be attributed to growth dilution and shedding of the bud scales as the flowers opened.

Extending the inhibitor concept, Blommaert (13) reported peach bud extracts to contain in addition to an acid inhibitor four growth promoting substances. During rest the disappearance of the inhibitor was accompanied by a corresponding increase in auxin. This suggested dormancy to be dependent on an auxin-inhibitor ratio. Similar results have been found by other workers in several different woody species (28, 51, 58, 61, 67).
Studying the rest periods in *Betula* and *Acer*, Wareing (75, 93) noted a direct photoperiodic influence on the induction and breaking of rest. Leaves had an inhibitory influence on buds under short day conditions. Investigations with *Acer* (73) showed the leaf inhibitor content to increase under decreasing daylengths paralleled by a similar inhibitor increase at the apex. Phillips and Wareing (74) were able to detect an inhibitor in leaves of *Acer* after only two cycles of short day treatments. A significant increase in the inhibitor concentration of the apical region was apparent after five such cycles, suggesting the production of the inhibitor in the leaves followed by translocation to the buds. Kawase (50, 52) noted in *Betula* that the short day stimulus was translocated from one portion of the tree to another. One branch of a birch seedling subjected to short days led to a measurable growth reduction in another held under long days.

Inhibitors were never found to disappear completely from the apical region but underwent quantitative changes presumably related to daylength (73). The reaction of the shoot apex was apparently determined by fluctuations of the inhibitor content above or below a specific threshold level (74).

Eagles and Wareing (29) reported in 1963 the extraction of an inhibitor from the leaves of *Betula* under 14.5 hr photoperiods which, when applied to actively growing seedlings, completely inhibited apical growth. The treated apices closely resembled those of similar plants.
rendered dormant by short days. "Dormin" was proposed as a specific designation for the inhibitor functioning in dormancy regulation (30).

Research on the leaf abscission mechanism in cotton led to the isolation of a hormonal compound, Abscisin II, shown to accelerate abscission (60, 70). Subsequently Cornforth et al. (22, 23) showed that the growth inhibitory substance "dormin," previously isolated from Betula, was identical to Abscisin II. This material was recently designated abscisic acid (ABA) (7).

Several papers relating endogenous inhibitors to the dormant state noted gibberellic acid (GA) when applied to resting buds substituted for the normal cold treatment required to break rest. Donoho and Walker (27) were able to overcome dormancy with GA application in inadequately chilled buds of Elberta peach. Phillips (72) conducted several tests on peach to determine the possibility that dormancy and growth were under the combined control of GA and inhibitor(s). Naringenin inhibited the dormancy-breaking effect of GA—the extent of the inhibition being dependent on the relative concentration of the materials.

When Stuart (81) applied GA or IAA solutions to terminals of Hydrangea, the plants did not become dormant but flowered without exposure to low temperatures. Interruption of the dark period or GA application to Betula (52) counteracted the short day effect as well as
reduced inhibitor levels in growing points.

Eagles and Wareing (29, 30) found GA able to overcome induced growth inhibition with no toxic effects. They suggested that winter chilling may break dormancy by increasing endogenous GA rather than reducing the inhibitor content. Significant changes in GA levels in Acer noted between December and April indicated that a balance between GA and inhibitors may regulate dormancy.

Coleoptile tests suggested a competitive interaction between "dormin" and GA. "Dormin" inhibited growth but it could be restored with GA but not IAA (82). Dormancy induced in birch buds could also be overcome by GA applications but not with auxin. If GA and "dormin" were applied simultaneously, the inhibitor effect was nullified and growth maintained. Following the application of the inhibitor alone, both auxins and GA were markedly reduced in leaves and shoots. "Dormin" may therefore function as a GA antagonist.

Seed Dormancy

The last five-year period has produced most of the present knowledge of the phenomenon of seed dormancy. Several causes of seed dormancy have generally been recognized including rudimentary embryos, physiologically immature embryos, mechanically resistant or impermeable seedcoats and the presence of germination inhibitors (8). Despite some conflicting evidence, various interrelationships
have been shown to exist between these mechanisms which suggest a single system of biological regulation of seed dormancy (8). Many indications are that the dormancy mechanism for buds is similar to that in seeds (47, 93).

Currently the efforts of researchers have emphasized the role of growth regulating materials, particularly growth inhibitors, in controlling seed dormancy. Inhibitors have been extracted from dormant seeds of numerous species (12, 32, 47, 48, 59, 88, 89), but considerable variation is reported in the function of these inhibitors in the regulation of seed dormancy. Inhibitors have been isolated from all the important anatomical structures of the seed (32, 47, 59, 88, 89). Villiers (88) and Edwards (32) indicate the embryo to be the primary site of inhibitor synthesis with diffusion accounting for inhibitor concentrations in the external portions. Lipe and Crane (59) and Irving (47) reported obtaining 100 percent germination following the removal of seed coats from nonstratified seeds. Bioassay of peach seed extracts showed that the inhibitor disappeared during the sixth week of seed stratification (59). Villiers and Wareing (88, 89) found inhibitor removal not to be a prerequisite to seed germination in Fraxinus. Processes beneficial to germination taking place during after-ripening did not involve the destruction of inhibitory substances (12, 88, 89).

Several papers suggest that inhibitory materials are produced
within the embryo in response to low oxygen levels (12, 32, 88). High oxygen contents were shown to result in reduced inhibitor concentrations prior to germination in *Avena* (12). In addition to oxygen, water and its leaching influence may be of special importance to seed germination. While evidence suggests that growth inhibitors may be continually present in germinating seeds, it may be a function of imbibing seeds prior to germination to dilute inhibitors to levels sufficiently low to permit growth (12, 32, 89).

Gibberellins are reportedly a requirement for germination and growth. Naylor and Simpson (77) have published evidence that as seeds of *Avena* emerge from dormancy, a growth-stimulating substance suggestive of a gibberellin is formed. In dormant seeds of some eucalypt species the stratification and light requirements for germination were replaced by GA treatment (11). While Frankland (36) reports GA treatments ineffective in breaking dormancy in several species, presumably due to an inability to penetrate or otherwise overcome the retarding effect of seed coat structures, Jackson and Bleendell (48) found GA to overcome dormancy in seed of *Rosa*. GA treatment prior to chilling increased germination of some long chilling *Pyrus* species but had no effect on several short chilling types (95). Observing GA to counteract the inhibitor influence in breaking dormancy in seeds of *Fraxinus*, Villiers and Wareing (89) attribute dormancy control to a balance between a growth inhibitor and a
germination promotor requiring low temperatures for production. Several researchers suggest that natural leaching of dormant seeds may account for the reduction in inhibitors thereby increasing the promotive effect of endogenous gibberellins (47, 66). Westwood and Bjornstad (95) found both seedcoat removal and water soaking to enhance germination of pre-chilled seeds of P. communis. An inhibitor was extracted from unchilled Acer seeds corresponding in Rf values to ABA (47).

**Physiological Responses to Abscisic Acid**

ABA has been identified in extractions of some 20 different mono- and dicotyledonous species (21, 24, 37, 39, 62). It has been isolated from leaves, buds, fruits, seeds and twigs in amounts within the range of concentrations reported for other plant hormones. Fruits are the richest source, reportedly containing concentrations ten times the amount found in seeds. Rosa has yielded ABA in the greatest quantity for any species tested to date.

ABA appears active in several physiological processes. Leaf senescence, abscission, flowering as well as tuberization have been shown to be influenced (33).

**Buds**

Within 15 to 20 days following the ABA treatment of Betula,
Acer and Ribes seedlings grown under long days (18 hr), extension growth completely ceased with the formation of typical resting buds (33). Sprays of ABA applied to peach, pear and apple prior to the spring bud break had no effect on flowering. However, flowering in twigs placed in ABA solutions was completely inhibited, but could be partly overcome by the addition of GA. Continuous ABA applications led to fairly rapid senescence of leaves in all species tested.

Treatment of long day species during exposure to long days inhibited flowering; ABA applied to short day species induced a marked increase in flower initiation under long day conditions (33, 94). Reporting experiments on black currants, Wareing (94) found the general conditions favoring flowering to be reduced GA levels and increased inhibitor concentrations. Daily applications of 20 ppm solutions of ABA resulted in a cessation of growth and initiation of flowers in axillary buds. Similar conditions were observed in strawberry, but it was thought that flowering was due to a balance between growth promoters and inhibitors rather than to ABA itself (94). Evans (35) has indicated in the case of long day species, that the inhibitor acts against the long day processes in the leaves. Applications were highly inhibitory of flower induction even after the critical photo-inductive period had been exceeded and the flowering stimulus translocated out of the leaves.

Several authors suggest that the daylength stimulus is perceived
by leaves and subsequently transmitted to growing points, probably through the phloem (94). This was confirmed by Hoad (45) using an aphid honeydew technique to collect sieve tube sap from Salix. ABA was present in phloem exudate and in much higher concentrations under short days than long days.

Milborrow (62) pointed out that though ABA applications to young actively growing tissues elicit a response, that response may be quite different from the effect ABA exerts in mature tissue containing appreciable amounts of other growth regulating materials.

Seeds

ABA may be the major factor controlling seed dormancy, particularly in seeds possessing a biochemically regulated dormancy mechanism (62). Studies of Fraxinus indicate that both dormant and nondormant seeds possess growth inhibitory activity (78). A growth accelerating material antagonistic to the inhibitor was present in nondormant seeds. Applications of ABA inhibited germination as well as leaf development and chlorophyll synthesis. The germination inhibitor in Rosa was shown to be ABA (48, 62). Physical removal of the major source of ABA--the pericarp and testa--as well as leaching favored germination.

Bradbeer (14) suggests that dormancy is imposed on embryos of newly harvested hazel nut (Corylus) by the inward diffusion of
inhibitors from the pericarp and testa. ABA was, however, thought to account for less than one percent of the total inhibitor activity. No clear relation seemed to exist between the ABA level in the pericarp of *Fraxinus* seeds and the physiological state of the embryo (79). While a high ABA concentration was present, little was lost during cold treatment; a proportionally greater loss occurred from the embryo than the pericarp.

High ABA levels in seeds of *Fraxinus americana* were reduced through chilling to levels comparable to those present in the non-dormant type, *F. ornus* (79). The dormant state could be reimposed with ABA applications similar in quantity to that lost through chilling, but continuous exposure was required.

Sondheimer et al. (79) concluded that the loss of ABA during after-ripening was not the general control mechanism in seeds with a chilling requirement. Differences in the rate of loss of ABA in dormant and non-dormant embryos may result from differences in binding or permeability. Enzymatic destruction of ABA while required may be coupled with an increase in growth accelerating hormones to achieve dormancy control.

Both Sondheimer and Galson (78) and Bradbeer (14) report GA to be capable of overcoming seed dormancy. Bradbeer suggests chilling to be responsible for overcoming a metabolic block of gibberellin synthesis preventing germination. ABA may act as a
simple inhibitor of the promoting effects of auxin, gibberellins or cytokinin permitting a finer control of growth processes (62). Light and temperature regimes influencing seed dormancy may operate by stimulating the destruction of ABA or the production of growth promoting hormones.

**Dormancy Control**

Recent developments in dormancy research indicate that this process is under complex biological control. Growth regulating materials, specifically a promoter-inhibitor balance, may only be a single step in a series of reactions involving dormancy induction and control. Evidence has shown materials thought to be part of the dormancy reaction to be active in additional fundamental growth processes.

GA can cause marked changes in the activities of several specific enzymes including α-amylase, maltase, ribonuclease, nucleotidase and several proteases (77, 85). Synthesis of new amylase protein in the aleurone layer of barley seed has been attributed to the influence of GA (85). In studies of the seed of *Avena*, Naylor (65) found a loss of dormancy in the aleurone layer to coincide with changes in the response of aleurone cells to GA. After-ripening may induce a progressive increase in cells capable of autonomous enzyme synthesis. Bradbeer and Pinfield (15) state that GA could break dormancy through its induction of enzyme systems.
concerned with the mobilization of cotyledonary oil reserves. Because of reduced $^{14}$C-acetate incorporation, a switch from lipid formation in the maturing seed to lipid consumption in the germinating seed may take place. Thus it may be the prime function of GA to promote enzyme synthesis or activate preformed enzymes necessary to the utilization of endosperm for germination and growth (77).

Coleoptile tests have indicated a competitive interaction between ABA and GA (82). ABA apparently antagonizes the GA triggered production of $\alpha$-amylase in isolated aleurone layers (19, 91). Chrispeels and Varner (19) state that GA is also active in the formation of several proteases by aleurone layers with the release of large amounts of amino acids. ABA was found to inhibit GA-stimulated amino acid release. The pattern of the inhibition was similar to that involving $\alpha$-amylase--possibly resulting from the interaction of both hormones at similar or identical sites. The formation of the enzymes may however be several steps removed from the site of action of the hormones, implying an inherent involvement with the processes of protein synthesis and ultimately genetic material itself.

Recent evidence increasingly associates both GA and ABA with protein synthesizing mechanisms and the nuclear constituents--DNA and RNA (15, 16, 18, 34). Jarvis et al. (49) reported GA treatments of Corylus seeds to first increase RNA synthesis, then protein synthesis and finally produce changes in the embryonic axis and fresh
weight. Using various inhibiting materials Chrispeels and Varner (18) provided evidence that the expression of the GA effect requires the synthesis of enzyme-specific RNA. Abscisic acid may exert its action by inhibiting the synthesis of this RNA or preventing its incorporation into an active enzyme synthesizing unit. The ABA inhibition can, however, be overcome by the addition of a larger amount of GA (19, 82, 85).

From measurements of the incorporation of $^{32}$P into the soluble nucleic acids (RNA, DNA) of duckweed, van Overbeek (86, 87) found one of the earliest effects of ABA to be the inhibition of DNA synthesis possibly by blocking a portion of the DNA-polymerase system. Chrispeels and Varner (18) were unable to conclude that hormones affect messenger RNA synthesis directly but suggest some DNA directed synthesis of m-RNA must take place before the hormonal effect could occur at the level of protein synthesis. It is uncertain whether ABA and GA work at the level of transcription or translation in the synthesis of proteins. DNA synthesis of t-RNA, m-RNA or r-RNA may be required before the hormonal effect can take place (18).

The increased RNA synthesis from GA$_3$ treatment of Corylus seeds appeared to result from an increase in DNA template availability and chromatin RNA polymerase activity (49). The initiation of chromatin directed RNA synthesis may be a primary factor in breaking seed dormancy (34, 49). Work with potato tubers found dormant buds to
possess an exceedingly limited ability to incorporate \(^{14}\)C-uridine into RNA and DNA (83). Experiments show that dormant buds synthesize RNA at very low rates compared to growing nondormant buds. Among the causes of the lack of RNA synthesis is the possibility of genetic repression in the dormant bud. Chromatin from dormant buds was almost inactive in support of DNA-dependent RNA synthesis.

Data seems to indicate that GA exerts control at the gene level, bringing about a general derepression of the specific DNA required for the synthesis of certain proteins. Abscisic acid may inhibit the synthesis of such a specific RNA fraction. While they affect the synthesis of specific enzymes, GA and ABA appear to have no effect on the incorporation of amino acids into proteins (18).

In a series of papers Khan (54, 55, 56) presents evidence for the involvement of the cytokinins in the dormancy reaction. Kinetin was able to break dormancy in Xanthium by antagonizing the endogenous inhibitor while neither GA nor IAA were effective (54). Placing dormant embryos of several rosaceous species on moist blotters, Khan and Heit (57) found that the cotyledon in direct contact with the moisture turned green and increased in size while the upper cotyledon remained white. Application of kinetin induced the upper cotyledon to similar reactions. The photoreversible germination mechanism in lettuce seed was shown to be inhibited by ABA; reversal of the inhibition could be obtained by cytokinin treatment but not by an
excess of $\text{GA}_3$ (55, 56). Cytokinins were also found to induce RNA synthesis as well as form part of certain types of t-RNA (55).

The apparent kinetin induced dormancy release in rosaceous embryos appeared to be DNA-dependent (57). This led Khan to postulate that an interplay of cytokinins, inhibitors and other factors may regulate dormancy, germination and differentiation by repression and derepression of genetic sites (54). Such an influence on genetic material would allow the cell to synthesize specific m-RNA necessary for the synthesis of specific enzymes and proteins. Derepression of genetic sites sufficient to terminate rest could be brought about by several trigger reactions involving such factors as light, temperature, oxygen pressure or hormones (54).

Auxins may exert a further related influence. Spelsberg and Sarkissian (80) found differentiated tissues—judged by the quality and quantity of various proteins—to be dedifferentiated by IAA. Armstrong (9) states that the sensitivity of auxin induced growth to relatively specific inhibitors of RNA and protein synthesis suggests the primary action of auxin to involve nucleic acid metabolism. Auxin may function in the overall quantitative regulation of RNA synthesis rather than in the induction of specific proteins (53). Nooden and Thimann (69) suggest auxin may produce cell enlargement by promoting synthesis of a messenger RNA and some undefined unstable enzyme systems.
Such physiological processes as epinasty, root initiation, fruit ripening, leaf abscission and dormancy release, largely thought to be under the influence of growth regulating materials, have recently shown analogous responses to the action of ethylene (6, 64, 69). Abeles and Rubinstein (6) found IAA and NAA stimulated the production of ethylene from roots, stems and leaves of beans. Using bean explants, ethylene stimulated abscission and changes in ethylene evolution closely correlated with auxin levels (6, 76). Various abscission promoting agents, IAA, GA and ABA induced ethylene emission prior to tissue separation (4).

Morgan and Gausman (63) present data suggesting ethylene to influence the movement of auxin, possibly through either auxin binding or destruction. Abeles and Holm (2, 4) found short ethylene exposures to have no effect on auxin transport in bean, cotton or coleus, but found ethylene stimulated RNA synthesis as well as protein synthesis in the separation zone during abscission. Ethylene action was blocked by the protein synthesis inhibitors actinomycin D and cycloheximide (3). An initial short lag period occurring after the addition of ethylene before the enhancement of RNA synthesis was followed by another lag period prior to the stimulation of protein synthesis (1, 3). All classes of RNA are apparently influenced by ethylene (46). Since both IAA and ethylene stimulate similar protein syntheses, a difficulty may arise in distinguishing the effects of
growth regulators from those of ethylene (1, 3). Abeles et al. (5) recently suggested ethylene to be an intermediate between the phytochrome mechanism and the germination of photosensitive lettuce seed.

The study of dormancy control has indeed become complex. While initial evidence indicated the presence of a relatively simple control mechanism involving possibly a single factor—a lack of growth promotor or an excess of growth inhibitor—recent evidence suggests that ultimate control proceeds directly from the genetic material. The function of growth hormones may be to coordinate genetic expression with external environmental conditions. Such a coordinating influence would seem to involve a combination of all the important classes of growth regulating materials.

Several current papers have attempted to define a mechanism for dormancy control (8, 84). In an extensive review Amen (8) has set forth a four step model of seed dormancy including induction, maintenance, trigger and germination processes. While recent research has concentrated primarily on the dormant state in seeds, isolation of identical hormonal materials from both buds and seeds suggests a single mechanism common to both.
MATERIALS AND METHODS

Plant Materials

Bud Samples

To evaluate changes in growth regulating materials related to dormancy and rest monthly whole flower bud samples were taken from three Pyrus types of varying chilling requirements. Pyrus communis var. Bartlett on a P. communis rootstock represented a type requiring a long chilling period and P. calleryana, a species from south China, a short chilling type. The third type combining the long chilling scion species Bartlett with the short chilling rootstock P. calleryana was intended to evaluate the reduction in chilling requirement in the scion species of a similar combination reported in previous work (96). The Bartlett bud samples were taken from a selection of 20 trees of each rootstock type from the pear rootstock plot at the Lewis-Brown Horticultural farm in Corvallis, Oregon. The P. calleryana buds were obtained from a mature specimen tree in a Pyrus species collection. In each instance individual buds were removed randomly from that portion of the tree easily accessible from the ground.

During the 1966-1967 season buds of the three Pyrus types were obtained at three intervals during the dormant period--November 5
and 6, January 16 and March 8. No bud sample for *P. calleryana* was available on March 8 because bud-break for this short chilling species had occurred prior to this time. The sample for each type consisted of four individual 5 gm samples of whole buds (Table 1).

During the 1967-1968 season whole bud samples were taken at monthly intervals from late September 1967 through late March 1968. Four 5 gm samples were obtained from each *Pyrus* type at each sampling time except following bud-swell in the spring (Table 1). The size of these final samples was based upon the number of buds per gram in the 5 gm samples of each type recorded during previous sampling periods. Both Bartlett types averaged approximately 12 buds per gram prior to bud-swell; thus final samplings consisted of four samples of 60 buds each. Samples of *P. calleryana* during the rest period averaged eight buds per gram; thus final samplings consisted of four 40-bud samples. The final samples were taken during full-bloom which occurred in late March for the Bartlett types. *Pyrus calleryana* bloomed in late February; thus no March sample was available. The full-bloom samples included the entire flower cluster that had emerged from each flower bud.

Processing of initial samples yielded evidence of a consistent growth inhibiting material in the buds of Bartlett on both *P. communis* and *P. calleryana* roots. A bud sample of approximately 500 gm was taken to provide a sufficient amount of this material for possible
Table 1. Composition of the samples of three *Pyrus* bud types at each sampling date during the 1966-1967 and 1967-1968 seasons.

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Bart. / <em>P. com.</em></th>
<th>Bart. / <em>P. call.</em></th>
<th>P. call.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>buds/gm</td>
<td>gm/sample</td>
<td>buds/sample</td>
</tr>
<tr>
<td>1966-1967</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 5</td>
<td>17.5</td>
<td>5*</td>
<td>85-90</td>
</tr>
<tr>
<td>Jan. 16</td>
<td>12</td>
<td>5*</td>
<td>60</td>
</tr>
<tr>
<td>Mar. 8</td>
<td>6</td>
<td>5*</td>
<td>30</td>
</tr>
<tr>
<td>1967-1968</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept. 26</td>
<td>16</td>
<td>5*</td>
<td>80</td>
</tr>
<tr>
<td>Oct. 31</td>
<td>13</td>
<td>5*</td>
<td>65</td>
</tr>
<tr>
<td>Nov. 26</td>
<td>13</td>
<td>5*</td>
<td>65</td>
</tr>
<tr>
<td>Dec. 30</td>
<td>14</td>
<td>5*</td>
<td>70</td>
</tr>
<tr>
<td>Feb. 1</td>
<td>13</td>
<td>5*</td>
<td>65</td>
</tr>
<tr>
<td>Mar. 2</td>
<td>2.5</td>
<td>24</td>
<td>60*</td>
</tr>
<tr>
<td>Mar. 30</td>
<td>F-B*</td>
<td>--</td>
<td>60*</td>
</tr>
</tbody>
</table>

*a* Full-bloom.

*Criterion upon which sample size was based.
identification or use in further experimentation. The sample was taken in mid-December 1967 from all available trees of Bartlett on P. communis root in the rootstock block at the horticulture farm.

To determine if the content of growth regulating materials in dormant buds reflected specific chilling requirements, buds were removed from several Pyrus species with varying lengths of rest periods. Samples were taken in late November and early December 1967 from the following seven species in the Pyrus species collection at the horticulture farm:

- P. betulaefolia Bunge
- P. calleryana Decn.
- P. communis L. (a Russian selection)
- P. fauriei Schneid.
- P. pashia Ham.
- P. serotina Rehd.
- P. ussuriensis Max.

Due to the limited size of the specimens of these species only one 5 gm bud sample was available.

Seed Samples

To evaluate reported changes in growth regulating materials in seeds in response to chilling, samples of seed were obtained from fruits of several commercial varieties of P. communis. Two lots of 380 seeds each of the variety Anjou were obtained, the first to be
processed prior to chilling and the second chilled at approximately 4°C under moist conditions from May to September 1968. A second set of samples consisted of 316 Bosc seeds processed in the unchilled condition and 410 seeds of the variety Eldorado processed after removal from fruits which had been stored at -1 to 0°C for approximately eight months.

**Expressed Juice**

Considering several reports of growth substances in fruit tissue, samples of juice were removed from pear varieties both before and after exposure to cold storage. Juice samples of 450 ml were obtained from pear fruits, var. Comice, following only a very short storage period and from the pear variety Eldorado following approximately eight months of storage at -1 to 0°C.

**Extraction and Chromatography Procedures**

**Bud Samples**

Immediately after removal from the tree, the samples of whole buds were weighed and stored at -18°C. The extraction procedure generally followed that outlined by Walker, Hendershott and Snedecor (90). Each 5 gm sample, both monthly and species samples, was ground in absolute methanol for 30 sec prior to extraction with 100 ml
absolute methanol at 0°C for 2 hr. Following extraction the solution was filtered, the residue washed with 25 ml cold methanol and the extract evaporated to dryness under reduced pressure at 40-45°C. The residue was taken up in acetonitrile and purified by partitioning in an acetonitrile-n-hexane mixture (100 ml : 100 ml). The hexane layer was separated and discarded while the acetonitrile fraction was evaporated as above.

The acetonitrile residue was taken up in 50 ml of 0.5 M NaHCO₃ (pH 8.75) which was then extracted with three 20 ml aliquots of anhydrous ether to give a neutral-alkaline (NA) fraction. The three ether extracts were combined (60 ml total), chilled at 0°C for 0.5 hr to aid in removing moisture and dried over 2 gm of anhydrous Na₂SO₄ at 0°C for at least 1 hr. After drying the ether was decanted and concentrated to 2.5 ml.

The pH of the remaining NaHCO₃ solution was then lowered to 2.8 with 2 N HCl and re-extracted with three 20 ml aliquots of anhydrous ether to give an acid (A) fraction. The ether extracts were combined and treated as described above for the NA fraction.

The growth substances present in each extract were separated by paper chromatography using Whatman No. 1 paper strips (1.5 in wide). Two-tenths ml of the concentrated ether extract was applied to each paper strip and developed approximately 20 cm in isopropanol/ammonia-28%/water, IpAW (8:1:1 v/v). Three strips were streaked
for each fraction of each extract for a total of six strips for every
5 gm sample.

To verify that the procedures used here were in fact removing
growth substances and to insure that active materials were not being
inadvertently discarded, samples of IAA and synthetic ABA were run
through the steps in extraction and chromatography. Twenty ml of
25 ppm solutions of IAA and ABA in absolute methanol were extracted
according to the procedures described for the bud samples and
chromatographed in each solvent system.

Seed Samples

Individual seed samples were separated into three fractions—a
preliminary wash of surface adhering material, seedcoats and
embryos—to correlate growth material content with anatomical
features of seed structure.

In pear two seedcoats can be distinguished (31, 38). The outer
coop is a somewhat hardened and thickened structure, usually brown in
color, derived from the integuments of the ovule and including some
fused nucellus tissue. The inner coat is a thin translucent layer made
up of remnants of endosperm and nucellus tissues. In this trial
reference to seedcoats includes both inner and outer structures.

Prior to removal of the seedcoats each seed sample was superf-
ficially washed 1 to 2 min with several changes of small volumes of
distilled water. These preliminary washes were combined, filtered and diluted to 250 ml for further extraction. Secondly, the seedcoats were extracted for two days with six changes of distilled water totaling 225 ml; the embryos were similarly extracted with a total of 175 ml of distilled water. The water extracts of both seedcoats and embryos were filtered and diluted to 250 ml.

The pH of the water extracts of each of the three seed fractions was lowered to pH 3 with 2 N HCl and the extracts divided into five 50 ml portions. Each 50 ml was extracted three times with 20 ml aliquots of anhydrous ether which were combined to give two 150 ml aliquots, chilled at 0°C for 0.5 hr and dried over 5 gm anhydrous Na₂SO₄ for at least 1 hr at 0°C. After drying the two 150 ml ether extracts were combined and concentrated to 2.5 ml.

Growth substances present in the concentrated ether extracts of each seed fraction were separated by paper chromatography on Whatman No. 1 paper strips (1.5 in). Two-tenths ml of the extract of each fraction was streaked on each of eight paper strips and developed 20 cm in IpAW.

Expressed Juice

The pH of the juice samples was lowered to pH 3 with 2 N HCl. Each 450 ml sample was then divided into four 100 ml portions which were extracted with 100 ml of anhydrous ether. The ether extracts
from two 100 ml portions of juice were combined, chilled 0.5 hr at 0°C, dried over 7 gm anhydrous Na$_2$SO$_4$ at 0°C and concentrated to 2.5 ml.

Two-tenths ml of the concentrated ether extract was streaked on each of four paper strips (Whatman No. 1, 1.5 in) and developed 20 cm in IpAW.

**Inhibitor Identification**

The bud sample intended for the identification of the growth inhibitor was processed according to a procedure outlined by Milborrow (62). Each 50 gm of the original 500 gm was ground 3 min in 80% methanol. Two such portions of ground sample (100 gm total buds) were extracted at 0°C in separate flasks for a period of ten days. Initially 1 l of 80% methanol was added to each flask; during the extraction period the solution was replaced twice, first with 1 l and then 0.5 l of fresh 80% methanol giving a final extract volume of 2.5 l per 100 gm of original bud sample. The methanol extracts were combined, filtered and concentrated to about 1.6 l under reduced pressure at 40-45°C.

The supernate (1.6 l) was extracted with five 175 ml aliquots of anhydrous ether following the adjustment of pH to 3.5 with 1 N H$_2$SO$_4$ (approximately 15 ml). Before discarding, the ether extract was extracted four times with alternate 25 ml aliquots of saturated
NaHCO$_3$ and distilled water to give a total volume of 100 ml.

The aqueous extract (100 ml) was adjusted to pH 7.5 with 1 N H$_2$SO$_4$ (10 ml) and extracted four times with 100 ml aliquots of anhydrous ether. These ether extracts were also discarded. The pH of the aqueous extract was lowered to 3.5 and re-extracted with four 150 ml aliquots of anhydrous ether--A fraction.

The insoluble material removed upon filtration of the original methanol extract was triturated 5 min with 75 ml of saturated NaHCO$_3$ and centrifuged 15 min. The supernate was adjusted to pH 7.5 with 1 N H$_2$SO$_4$ and extracted with four 100 ml aliquots of anhydrous ether. These ether extracts were discarded and the aqueous NaHCO$_3$ adjusted to pH 3.5 and re-extracted four times with 150 ml aliquots of anhydrous ether. This ether extract was combined with that designated above as the A fraction.

The combined ether extracts (A fraction) were chilled at 0°C, dried over 20 gm anhydrous Na$_2$SO$_4$ and evaporated at 30-35°C under reduced pressure. The residue was taken up in absolute methanol-anhydrous ether (1:1) to give a final volume of 10 ml.

The growth inhibitor was separated by paper chromatography on Whatman No. 1 paper strips (1.5 in). Twelve strips were streaked with 0.2 ml of the concentrated ether extract; four strips were developed approximately 20 cm in each of the following solvents:
1. IpAW

2. n-butanol/95% ethanol/distilled water, n-BEW (7:1:2 v/v)

3. benzene/ethyl acetate/acetic acid, BEA (50:5:2 v/v)

To verify that the growth substances extracted from the other types of Pyrus samples corresponded in Rf values with those in the 500 gm sample, selected representative extracts of each type of sample were developed in the two additional solvents (n-BEW, BEA). Similar amounts of the remaining concentrated ether extracts were applied to each paper strip and a similar number of strips were streaked as originally described for the primary solvent (IpAW). Due to a lack of volume of extract not all samples could be run in each solvent system. Of the 1967-1968 monthly bud samples, extracts of the three Pyrus types taken in November were developed in n-BEW and extracts from the December sampling were developed in BEA. Portions of the extracts from unchilled Bosc seedcoats and the juice from unchilled pear fruits, var. Comice, were also run in the additional solvents.

Bioassay Procedures

An oat coleoptile straight growth test patterned after that of Nitsch and Nitsch (68) and including some techniques suggested by Crosby, Berthold and Spencer (25) was used to detect biological
activity in the chromatograms of all extracts. Fifty gm of oat seed, var. Forkdeer, were soaked 2 hr in 100 ml distilled water, seeded in plastic trays (11 x 15 x 3 in) on approximately 1.5 l of vermiculite, covered with an additional 0.75 l and the coleoptiles grown nearly four days in the dark at 24°C and 90-100% relative humidity. One l of water was added at planting and about 0.4 l added at two days. During the third day following planting the trays were given a 2 hr red light (15 watt red bulb) treatment to reduce internode growth. Except for the intervals for the red light treatment and watering, each tray remained covered with a sheet of black plastic throughout the growing period.

Approximately 90 hr after seeding, when the coleoptiles were between 1.5 and 2.5 cm in length, the coleoptile sections were cut; a 4 mm section was removed 3 mm below the tip. The sections were soaked in a 1 ppm MnSO$_4$ solution for 2 hr and transferred to vials (15.5 x 50 mm, shell vial) containing 1 ml of a citrate-phosphate buffer sucrose solution and a section of chromatograph strip. Two coleoptiles were added to each vial.

The citrate-phosphate buffer consisted of 1.794 gm K$_2$HPO$_4$ plus 1.019 gm citric acid monohydrate per liter of distilled water to which 2% sucrose by weight was added. Each chromatogram was divided into 20 equal segments plus the segment containing the original streak of tissue extract; an additional segment of equal width was
taken above the origin as a control.

The vials were arranged in order in trays, put on a horizontal shaker and shaken at about 120-150 cycles per minute in the dark. After 22 hr the coleoptiles were removed, placed in a darkroom photoenlarger, the coleoptile image enlarged by a factor of ten and measured to the nearest millimeter. The elongation of the two coleoptiles per vial was averaged and the average length of the coleoptiles in contact with each chromatograph section converted to a percent of the growth of the coleoptiles in contact with the control strip.

Subtraction of individual percentage growth measurements from 100 was used as a measure of relative inhibitor concentrations in the various tissue extracts. The relation between percent growth and percent inhibition measurements is shown in Table 2. Due to the tailing effect in paper chromatographic separation of increasing concentrations of a particular substance, each entry represents the average of the three sections with the lowest coleoptile growth response—greatest inhibition—from the area of the chromatogram in which ABA was detected. As indicated in Figure 1 a non-linear relation exists between the inhibitor concentration and the inhibition of coleoptile growth.
Table 2. Oat coleoptile growth response to abscisic acid.

<table>
<thead>
<tr>
<th>ABA ( \text{micrograms} )</th>
<th>Coleoptile Growth as Percent of Control(^b)</th>
<th>Percent Inhibition of Coleoptile Growth(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.275</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>0.55</td>
<td>64</td>
<td>36</td>
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<td>1.1</td>
<td>55</td>
<td>45</td>
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<tr>
<td>2.2</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>4.4</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>8.8</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

\(^a\) Micrograms (RS)-ABA spotted per chromatograph strip.

\(^b\) Average of three chromatograph sections with lowest growth response in area of chromatogram in which ABA was detected.

\(^c\) 100 minus percent coleoptile growth.
Figure 1. Oat coleoptile growth response to ABA.
RESULTS

Bud Trials

1966-1967 Season

No significant growth promoting substances were detected by measurements of coleoptile growth from chromatographs of the NA fractions of extracts of the 1966-1967 bud samples. Percentage growth measurements for each section of chromatogram remained relatively constant throughout the sampling period for each of the three Pyrus types. Coleoptile growth measurements from chromatographs of the A fraction of the bud extracts did give evidence of a constant zone of growth inhibition (Rf 0.80-0.90, IpAW). This inhibitor of oat coleoptile growth was present in each Pyrus sample at each sampling period. No other growth substances were noted in the remaining chromatograph sections of any sample.

The extent of this inhibition at the various sampling times for the three types of Pyrus bud samples is shown in Figure 2. In samples of each bud type a general decline in the inhibitor content took place during the sampling period. The inhibitor concentration, greatest in the November sample of Bartlett on P. communis, declined uniformly in subsequent samples. A lower inhibitor concentration was apparent in P. calleryana while intermediate inhibitor
Figure 3. Hours of chilling temperatures (32-46°F) accumulated during the 1966-1967 season.

Figure 2. Relative growth inhibitor (Rf 0.65-0.85, IpAW) levels in extracts (A fraction) of the 1966-1967 bud samples (5 gm/sample) of three Pyrus types.
levels were noted in initial samples of Bartlett on *P. calleryana*. The decline observed in later samples of these types was however not as consistent as in the case of Bartlett on *P. communis*.

The method used in obtaining the bud samples may contribute to the decline in inhibitor content in later samples. Because each sample was based on a weight measurement (5 gm) the samples taken following bud-swell contained significantly fewer buds than preceding ones (Table 1).

A general inverse relation appears to exist between the inhibition of growth (Figure 2) and the accumulated hours of chilling temperatures—the natural rest-breaking agent (Figure 3). The apparent decline in inhibitor levels corresponds to the accumulation of effective chilling temperatures. Fifteen hundred hr of temperatures at about 40°F is generally sufficient to break rest in *P. communis* var. Bartlett; thus rest would have been effectively ended in this bud type early in February. *Pyrus calleryana* requires only 400 hr of temperatures near 40°F to break rest (96). Sufficient chilling would have taken place by late November to break rest in this short chilling species.

1967-1968 Season

More extensive sampling was undertaken during the dormant season of 1967-1968. Because samples the previous year were based
on a weight measurement (5 gm), the samples taken following bud-swell contained significantly fewer buds than preceding ones (Table 1). The possibility existed that the decline in bud numbers per sample could contribute to the decline in inhibitor content. For this reason sampling procedures were altered in 1967-1968. Those samples taken after bud-swell, following the breaking of rest, were based on bud numbers rather than bud weights. This maintained bud numbers approximately equal in each sampling, minimizing any effect of growth dilution.

No growth substances were detected consistently in NA fractions of any samples during the dormant period. Again the A fraction of the extracts contained an inhibitor of oat coleoptile growth (Rf 0.65-0.85, IpAW). Coleoptile growth measurements from the A fraction of a representative sample are presented in Table 3. Histograms of the entire chromatogram for both A and NA fractions are shown in Figure 4. Table 3 and Figure 4 are included for the purpose of illustrating the fluctuation in results obtained from the bioassay of typical samples of the three *Pyrus* bud types. Despite several values which differ by amounts greater than the calculated least significant difference (LSD), only the inhibitor zone corresponding to (RS)-ABA is considered significant. The growth inhibition which appears at the solvent front in each chromatogram is thought to represent extraneous materials in the bud extracts plus materials possibly eluted
Table 3. Oat coleoptile growth response to individual chromatographic sections from the separation (IpAW) of extracts (A fraction) of buds (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of the October 31, 1967 samples of three Pyrus types.

<table>
<thead>
<tr>
<th>Chromatograph Section&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bud Type</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Coleoptile growth (% of control)</td>
<td>88</td>
<td>93</td>
<td>83</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>104</td>
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<td>2</td>
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<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>61</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>16</td>
<td>52</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>17</td>
<td>89</td>
<td>84</td>
<td>86</td>
</tr>
<tr>
<td>18</td>
<td>77</td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td>19</td>
<td>78</td>
<td>84</td>
<td>83</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each section represents 0.05 Rf.

<sup>b</sup> Inhibitor zone: sections 13-17, Rf 0.65-0.85, (corresponds to the Rf of synthetic ABA).

LSD (5% level) = 16, comparison of chromatograph sections for a single bud type.
Figure 4. Histograms of the oat coleoptile growth response to A and NA fractions of extracts of buds (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of the October 31, 1967 samples of three Pyrus types.
from the chromatographic paper by the solvent.

No differences in coleoptile growth response in the inhibitor zone were apparent between samples of the two Bartlett types (Table 4). Differences were evident, however, between several of the monthly samples. A marked difference in response was noted between P. calleryana and Bartlett buds. Inhibitor levels in each of the bud types generally increased during the sampling period (Figure 5). Samples of Bartlett on P. communis had significantly higher inhibitor concentrations in February and March samples taken at the termination of rest than in September and October samples taken during the onset of rest. The inhibitor content of buds of Bartlett on P. calleryana fluctuated from month to month but remained somewhat higher than those on the long chilling root P. communis.

The short chilling type P. calleryana contained a significantly lower inhibitor concentration than the longer chilling species. No marked changes occurred during the dormant period but the inhibitor level increased significantly in the full-bloom samples. In each Pyrus type the samples taken after bud-swell contained the highest inhibitor contents. Fluctuations in inhibitor levels in P. calleryana generally paralleled those in Bartlett on P. communis. The inhibitor content rose to some extent from the onset of rest in September, reaching a maximum during the period of deepest rest in November to subsequently decline toward the end of the rest period.
Figure 6. Hours of chilling temperatures (32-46°F) accumulated during the 1967-1968 season.

Figure 5. Relative growth inhibitor (Rf 0.65-0.85, IpAW) levels in extracts (A fraction) of the 1967-1968 bud samples (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of three Pyrus types.
Table 4. Oat coleoptile growth response in the inhibitor zone (Rf 0.65-0.85, IpAW) of extracts (A fraction) of the 1967-1968 bud samples (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of three Pyrus types.

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Bud Type</th>
<th>Bud Type</th>
<th>Bud Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967-1968</td>
<td>coleoptile growth (% of control)</td>
<td>mean</td>
<td>mean</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59</td>
<td>81</td>
</tr>
<tr>
<td>Oct. 31</td>
<td>64</td>
<td>44</td>
<td>78</td>
</tr>
<tr>
<td>Nov. 26</td>
<td>56</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>Dec. 30</td>
<td>62</td>
<td>44</td>
<td>82</td>
</tr>
<tr>
<td>Feb. 1</td>
<td>50</td>
<td>58</td>
<td>74</td>
</tr>
<tr>
<td>Mar. 2</td>
<td>44</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Mar. 30</td>
<td>44</td>
<td>39</td>
<td>___</td>
</tr>
<tr>
<td>mean 56</td>
<td>48</td>
<td>70</td>
<td>___</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average of three chromatographic sections with lowest coleoptile growth response.

LSD (5% level) = 9, comparison of bud types.
6, comparison of sampling dates.
11, comparison between sampling dates for a single bud type.
12, comparison between bud types for a single sampling date.

No characteristic relationship was evident from the comparison of the inhibitor content (Figure 5) with the accumulated hours of chilling temperatures (Figure 6). Fewer hours of chilling were recorded during the 1967-1968 season than in 1966-1967. The accumulation of the 1500 hr normally needed to break rest in the Bartlett variety did not take place until mid-March, a point reached
in early February the previous year. The chilling requirement for
P. calleryana was exceeded by mid-December (Figure 6). A sub-
sequent decline in coleoptile growth inhibition was also evident in the
December bud sample of this species.

The final samples of each Pyrus type were taken at full-bloom.
Acid fraction extracts from these samples of both Bartlett types
showed only slight change from previous samples, but the inhibitor
content was generally higher. A marked increase in the inhibitor
level of P. calleryana was evident. These full-bloom samples may,
however, not be representative. Prior to processing these samples
thawed as did the entire set of Bartlett on P. calleryana samples, due
to an accidental unplugging of the freezer in which they had been
stored.

No consistent growth promotion was observed in either fraction
in any sample of three Pyrus types during the dormant period, however
a marked zone of promotion (Rf 0.85-0.95, IpAW) was present in the
NA fractions of the full-bloom samples (Figure 7). Coleoptile growth
in this zone reached a maximum of 250% of the controls in the extracts
from the two Bartlett types and a maximum of 150% in the sample from
P. calleryana.

A second but much less prominent zone of growth promotion
was noted in A fraction extracts (Rf 0.45, IpAW) of all the Pyrus types
(Figure 8), but again only in samples taken at full-bloom. From
Figure 7. Growth promoter activity (Rf 0.85-0.95, IpAW) in extracts (NA fraction) of the 1967-1968 bud samples (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of three Pyrus types.
Figure 8. Growth promoter activity (Rf 0.45, IpAW; chromatograph section 9) in extracts (A fraction) of the 1967-1968 bud samples (Bart., 60-70 buds/sample; P. call., 40-45 buds/sample) of three Pyrus types, corresponding in Rf value to IAA.
initial tests of the extraction and chromatography procedures it was found that this zone of promotion corresponded in its Rf value to that of pure IAA.

Bioassay of bud extracts from additional Pyrus species taken in November-December 1967 provided results not unlike those previously reported. The NA fractions contained no evidence of growth promoting materials while a similar inhibitor (Rf 0.65-0.85, IpAW) was present in the A fraction. These species ranged in chilling requirements from very short, as represented by _P. pashia_, to a long chilling Russian selection of _P. communis_. Included were _P. fauriei_, a short chilling species, _P. ussuriensis_, moderate chilling, _P. betulaefolia_, a moderate to long chilling type, and two selections of _P. serotina_, in general a short to moderate chilling species but a type which has reportedly shown considerable variation in chilling requirements (71).

The inhibitor content reflected the inherent chilling requirement of the particular species (Figure 9). Species with longer chilling requirements generally contained more inhibitor. _Pyrus betulaefolia_, a moderate chilling species apparently contained a slightly larger quantity of the inhibitor than the Russian selection of _P. communis_, the reportedly long chilling type. Bud extracts from the two samples of _P. serotina_ varied considerably in inhibitor content; in the first the inhibitor concentration was negligible while the content of the second
Figure 9. Relative growth inhibitor (Rf 0.65-0.85, IpAW) levels in extracts (A fraction) of November-December 1967 bud samples (5 gm/sample) of several *Pyrus* species.
suggested a moderate chilling selection. The geographic origin of these *P. serotina* trees is unknown.

**Seed Trials**

No consistent growth promotion was noted in the extracts of the several varieties of *Pyrus* seeds tested. A growth inhibiting material was present in the extracts of each seed variety corresponding to that found in bud extracts (Rf 0.65-0.85, IpAW). Coleoptile growth was reduced to varying degrees by each fraction of both unchilled and chilled seeds (Table 5). The greatest proportion of the material was present in the external structures of the seed—nearly equally divided between the preliminary wash and seed coat extracts (Figure 10). Inhibitor levels of preliminary wash and seedcoat extracts varied only slightly with chilling. Unchilled embryo extracts had inhibitor concentrations less than half that of the external structures and in each case definitely decreased with chilling. Variation in inhibitor content in the variety Anjou was similar to that in the unchilled Bosc and chilled Eldorado seeds, especially in embryo extracts, in which chilled embryos contained less inhibitor than unchilled ones.

**Expressed Juice**

Juice samples from fruits of both pear varieties contained a zone of growth inhibition corresponding to that found in both
Table 5. Oat coleoptile growth response in the inhibitor zone (Rf 0.65-0.85, IpAW) of extracts of seeds (Anjou, 380 seeds/sample; Bosc, 316; Eldorado, 410) of several varieties of *P. communis*.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Unchilled Anjou</th>
<th>Chilled Anjou</th>
<th>Unchilled Bosc</th>
<th>Chilled Eldorado</th>
<th>mean</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary wash</td>
<td>27^a</td>
<td>36</td>
<td>38</td>
<td>45</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>Seedcoats</td>
<td>31</td>
<td>40</td>
<td>36</td>
<td>29</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Embryos</td>
<td>63</td>
<td>94</td>
<td>79</td>
<td>76</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>mean</td>
<td>40</td>
<td>57</td>
<td>50</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Average of three chromatographic sections with lowest coleoptile growth response.

Anjou variety:

LSD (5% level) = 8, comparison of chilling levels.
11, comparison of fractions.
15, comparison of fractions within a single chilling level.
14, comparison of chilling levels for a single fraction.

Bosc-Eldorado varieties:

LSD (5% level) = Chilling levels: NS
8, comparison of fractions.
12, comparison of fractions within a single chilling level.
16, comparison of chilling levels for a single fraction.
Figure 10. Relative growth inhibitor (Rf 0.65-0.85, IpAW) levels in extracts of seeds (Anjou, 380 seeds/sample; Bosc, 316; Eldorado, 410) of several varieties of *P. communis*.

Figure 11. Relative growth inhibitor (Rf 0.65-0.85, IpAW) levels in juice samples (450 ml) of two varieties of *P. communis*. 
bud and seed samples (Rf 0.65-0.85, IpAW). Little change was evident in juice from chilled pear fruits compared to that from unchilled tissue (Figure 11).

**Inhibitor Identification**

Rf values of the growth inhibitor, apparently common to the various plant parts and *Pyrus* species extracted, compare favorably with those found for synthetic ABA (Table 6). A close correlation existed in each of the three types of solvent systems--alkaline (IpAW), neutral (n-BEW) and acidic (BEA).

In further attempting to identify the growth inhibitor, portions of the concentrated bud extract (500 gm) were separated on thin-layer chromatographic plates, eluted and ultra-violet spectra determined. Four ml aliquots of the bud extract were spotted on thin-layer plates (silica gel G) as were 4.0 ml aliquots of a 25 ppm methanol solution of synthetic ABA. Each plate containing both the known and unknown materials was developed twice to a distance of 15 cm in either of the following solvents:

- n-butanol/propanol/ammonia-28%/water, n-BPAW (2:6:1:2 v/v)
- isopropanol/ammonia-28%/water, IpAW (8:1:1 v/v)

The zones containing the applied materials on each plate were divided into sections representing 0.1 Rf unit, eluted for 30 min, centrifuged and a UV spectrum made for both the known and unknown
Table 6. Rf values of growth inhibitor in extracts of different organs of several *Pyrus* species.

<table>
<thead>
<tr>
<th>Kind of Tissue and Species Extracted</th>
<th>Solvent System</th>
<th>Rf Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IpAW</td>
<td>n-BEW</td>
</tr>
<tr>
<td>Synthetic ABA</td>
<td>0.65-0.80</td>
<td>0.85-0.95</td>
</tr>
</tbody>
</table>

**Flower Buds:**

<table>
<thead>
<tr>
<th>Species</th>
<th>(gm)</th>
<th>IpAW</th>
<th>n-BEW</th>
<th>BEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bart. / <em>P. communis</em> (%)</td>
<td></td>
<td>0.65-0.75</td>
<td>0.85-0.95</td>
<td>0.00-0.05</td>
</tr>
<tr>
<td>Bart. / <em>P. communis</em> (5 gm)</td>
<td></td>
<td>0.85-0.90</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>1966-1967</td>
<td>0.65-0.85</td>
<td>0.90-0.95</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>1967-1968</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bart. / <em>P. call. 1</em></td>
<td></td>
<td>0.80-0.90</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>1966-1967</td>
<td>0.65-0.75</td>
<td>0.85-0.95</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>1967-1968</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. communis</em></td>
<td></td>
<td>0.75-0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. communis</em></td>
<td></td>
<td>0.80-0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. pashia</em></td>
<td></td>
<td>0.65-0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. fauriei</em></td>
<td></td>
<td>0.65-0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. usurienesis</em></td>
<td></td>
<td>0.70-0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. communi</em></td>
<td></td>
<td>0.70-0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. betulaefolia</em></td>
<td></td>
<td>0.75-0.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Seeds: (P. communis)**

<table>
<thead>
<tr>
<th>Variety</th>
<th>(Unchilled)</th>
<th>Pre-Wash</th>
<th>Seedcoats</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anjou</td>
<td>Unchilled</td>
<td>0.70-0.80</td>
<td>0.70-0.75</td>
<td>0.70-0.75</td>
</tr>
<tr>
<td></td>
<td>Chilled</td>
<td>0.70-0.75</td>
<td>0.70-0.75</td>
<td>0.70-0.75</td>
</tr>
<tr>
<td>Bosc</td>
<td>Unchilled</td>
<td>0.70-0.80</td>
<td>0.75-0.80</td>
<td>0.90-0.95</td>
</tr>
<tr>
<td></td>
<td>Embryo</td>
<td>0.75-0.80</td>
<td>0.75-0.80</td>
<td></td>
</tr>
<tr>
<td>Eldorado</td>
<td>Chilled</td>
<td>0.75-0.80</td>
<td>0.70-0.80</td>
<td>0.75-0.80</td>
</tr>
</tbody>
</table>

**Fruit: (P. communis)**

<table>
<thead>
<tr>
<th>Variety</th>
<th>(Unchilled)</th>
<th>Pre-Wash</th>
<th>Seedcoats</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornice</td>
<td>Unchilled</td>
<td>0.65-0.75</td>
<td>0.80-0.95</td>
<td>0.00</td>
</tr>
<tr>
<td>Eldorado</td>
<td>Chilled</td>
<td>0.70-0.75</td>
<td>0.80-0.75</td>
<td>0.00</td>
</tr>
</tbody>
</table>
materials. The plate developed in n-BPAW was eluted in acidic 95% ethanol and the plate developed in IpAW was eluted with acidic absolute methanol.

A UV spectrum for a 10 ppm solution of synthetic ABA in methanol is shown in Figure 12A. While maximum absorbance occurs at the same wavelength, chromatographing synthetic ABA alters its characteristic spectrum considerably. Figure 12B shows the absorbance spectra for both synthetic ABA and the bud extract developed in n-BPAW and eluted with acidic 95% ethanol; Figure 12C shows the spectra of the same materials developed in IpAW and eluted with acidic methanol. Characteristics of the spectra and the Rf values of the growth inhibitor from the bud extract approximate those of the known material separated by thin-layer chromatography. The wavelength of maximum absorbance for synthetic ABA is 260 millimicrons in each solvent system while the unknown material shows a maximum absorbance at 270 millimicrons.

The Rf values in the various solvent systems and the UV absorbance spectra suggest that the growth inhibitor found in these various organs of *Pyrus* species is similar to but may differ slightly from synthetic ABA.
Figure 12. UV spectra of synthetic abscisic acid (ABA) and growth inhibitor from Pyrus bud extracts (A fraction). A. UV spectrum of ABA; B. UV spectra of ABA (Rf 0.80) and bud extract (Rf 0.80), n-BPAW; C. UV spectra of ABA (Rf 0.90) and bud extract (Rf 0.85), IpAW.
DISCUSSION

An inhibitor of oat coleoptile growth is apparently common to several species of the genus *Pyrus*. This same material was also found to be present in fruit and seed tissues as well as in flower buds. Isolation of the inhibitor from the tissues of different organs of a single species corresponds to other recent instances in which growth inhibitors were found common to different plant parts (30). If this material is indeed abscisic acid, its presence in *Pyrus* adds to a growing list of recent reports of the isolation of ABA from diverse plant species and organs.

**Bud Trials**

Extracts of the bud samples taken monthly during the 1967-1968 season gave no indication of a decline in inhibitor levels during the various stages in the development of rest (Figure 5). This contrasts to several reports which indicated inhibitor concentrations to undergo a quantitative decline (40, 41, 73). The inhibitor level in each of the three *Pyrus* bud types showed a slight overall increase between the initial sampling in late September and the resumption of growth. However aside from those samples taken after bud-swell, which may not accurately reflect inhibitor levels, little significant change took place in the concentration of the growth inhibitor during the dormant
period. This is particularly evident in samples of Bartlett on *P. calleryana* and ungrafted *P. calleryana*. Bartlett on *P. communis* showed a gradual accumulation of inhibitor throughout the dormant period.

Table 1 shows that throughout the dormant period of 1967-1968 the number of buds per sample of each bud type remained essentially unchanged. Comparison of the uniform sample size and overall stability of inhibitor content in each sample during rest suggests that the inhibitor content remains essentially constant in each bud.

Upon initial examination, the level of inhibitor in 1966-1967 samples of the three *Pyrus* bud types (Figure 2) appears to decline, particularly in Bartlett on *P. communis*. Further consideration of the size of individual samples (Table 1) shows a continual decline in the number of buds extracted at each period for all bud types, particularly Bartlett on *P. communis*. While the measured inhibitor content appeared to decline, the actual amount of inhibitor per individual bud possibly remained essentially unchanged during rest. If the inhibitor level in the whole bud remained relatively unchanged throughout rest and the period of bud-swell, determination of the concentration in the various substructures of the bud might have shown the quantitative changes reported by other authors (40, 41, 73).

While accumulated hours of chilling temperatures is the generally accepted measure for the determination of the length of rest,
no consistent relationship was apparent between chilling and the inhibitor content. In *P. communis* 1500 hr of accumulated temperatures at about 40°F practically signals the termination of rest; no corresponding change in inhibitor content was observed in the monthly bud samples during 1967-1968. A notable difference was observed in the levels of the inhibitor in each bud type. Of the three types of buds, extracts of *P. calleryana* consistently reduced coleoptile growth less than those of the remaining types. *Pyrus calleryana*, native to areas of mild winter climates in central, south China, also has the shortest chilling requirement (95).

A reduction in chilling requirement previously had been observed following the grafting of a long chilling scion on a short chilling rootstock (96). This however did not correlate with the inhibitor contents of buds of Bartlett on *P. communis* and Bartlett on *P. calleryana* during 1967-1968. No significant differences were observed. The short chilling rootstock had no apparent influence on the levels of the specific growth inhibitor found in the buds of the stock and scion species.

Extracts of the additional *Pyrus* species of varying length of chilling requirements showed a corresponding variation in inhibitor levels (Figure 9). While numerous articles report the existence of ABA in different species, few if any indicate the content among species of a single genus. *Pyrus pashia*, native to areas in western
China, India and Pakistan with warm winters and very few chilling hours, requires one of the shortest periods of chilling to break rest of any *Pyrus* species. Of the species of known geographic origin extracted, it contained the lowest level of growth inhibitor. A measurably higher inhibitor content was evident in buds of *P. fauriei*, which while native to South Korea also requires a short chilling period but is reportedly hardier than other short chilling types.

*Pyrus ussuriensis*, native to areas of severe winter conditions in Siberia, is among the hardiest of tree fruits (95). *Pyrus betulaefolia*, native to temperate areas of China, has a moderate chilling requirement. The *P. communis* represented in Figure 9 is a long chilling Russian selection adapted to temperate areas of the Caucasus mountains of southern Russia.

In temperate regions temperatures fluctuate considerably during the dormant period. Species native to these areas characteristically require long chilling periods to break rest. To survive, growth must not resume until there is little danger of the recurrence of damaging freezes. If inhibitor levels reflect chilling requirements, it is consistent that the temperate species *P. betulaefolia* and *P. communis* contain the highest concentrations of inhibitor.

Differences in inhibitor content noted between the samples of *P. serotina* suggest that the specimens from which these buds were taken may not have been of identical parentage. As previously
mentioned, selections of this species are known to vary in chilling requirement (70).

Among the species extracted the bud size varied extensively. *Pyrus fauriei* possesses extremely small buds—approximately 90 per gram; only ten buds of *P. serotina* weigh a gram. Subsequent differences in the number of buds per sample could influence the inhibitor content.

Inhibitor levels did not correlate with the amount of chilling in the three *Pyrus* bud types (Figures 2, 3, 5, 6), but seemed to correspond to the inherent chilling requirement of the various species (Figure 9). Lower inhibitor levels were characteristic of short chilling species. Thus growth inhibitor content appears to be related primarily to chilling requirement rather than to the amount of chilling the buds have received at any given time.

At the onset of rest, the inhibitor may accumulate to levels characteristic of each species and remain at a relatively stable concentration in individual buds throughout rest. In *Pyrus* the initiation of flower buds occurs in the months of June and July; extension of the sampling period further into the summer growing season might have proved beneficial in detecting any initial changes in inhibitor levels at the onset of rest. Sampling may have begun too late to detect a possible rise in the inhibitor content but no decline corresponded to the accumulation of sufficient hours of chilling required to break rest.
The presence of the prominent growth promoter only in the 1967-1968 full-bloom samples (Figure 7), if representative, suggests that growth promoting materials undergo greater quantitative changes in Pyrus than growth inhibitors. Emergence from rest may depend more upon growth promoters exceeding the effective concentration of the growth inhibitor than a specific decline in inhibitor levels. Threshold levels may then govern the effective action of growth promoters as well as inhibitors (74). The longer chilling species in addition to higher inhibitor contents during rest possessed significantly greater growth promoter activity than shorter chilling species (Figure 7).

**Seed Trials**

Data from the seed extracts of this trial generally supports current reports regarding the occurrence of inhibitors in seed tissue (12, 32, 47, 48, 59, 88, 89). The inhibitor was present in the major anatomical structures of the seed, the greatest concentration being external to the embryo itself--on the seed surface or in the seedcoats. A reduction was observed in the seed inhibitor content in response to chilling, however only in embryo tissues. No significant change took place in inhibitor levels in either the gelatinous surface material or seedcoats following a chilling period sufficiently long to permit seed germination. Sondheimer and Galson (78) report little loss of ABA
from the pericarp of seeds of *Fraxinus* during cold treatment but considerably greater loss from inside the seed did occur.

Considering the overall concentration of the inhibitor in outer seed structures versus embryo tissue, it appears doubtful that any marked change would have been observed had whole seeds been extracted. This appears to contradict reports of the disappearance of seed inhibitors prior to germination, but lends support to others indicating that the removal of inhibitors was not a prerequisite to seed germination. Lipe and Crane (59) found growth inhibitors to disappear during the sixth week of stratification of peach seed. Westwood and Bjornstad (95) found washing of chilled *P. communis* seeds to greatly increase germination. Processes occurring during after-ripening of *Fraxinus* were not considered by Villiers and Wareing (12, 88, 89) to involve the destruction of inhibitor substances.

Comparison of seed and bud structures suggests a correlation of function between bud scales and seedcoats. In most species the bud scales are considered modified leaves while seedcoats are derived principally from the integuments; both surround a growing point as protective tissue. The distribution of inhibitor in the bud may also be analogous to that in the seed. Because the major concentration of the seed inhibitor was in the seedcoats, the failure to detect a decline in whole bud inhibitor contents during rest may result from a masking effect of the bud scales. In each instance the growing point may
possess an effective but low inhibitor level compared to the accumulations in external structures.

Several researchers found the peach bud inhibitor, naringenin, primarily in the bud scales (20, 26). Progressive shedding of these scales was thought to account for the reported loss of the inhibitor prior to bloom. In samples of the Bartlett variety taken after bud-swell (Figure 5), the inhibitor content remained relatively stable. Determination of the distribution of the bud inhibitor would have been useful.

It has been suggested that the site of synthesis of growth inhibitors in the seed lies in the embryo (32, 88). As a site of synthesis, the embryo must necessarily produce large quantities of the inhibitor to account for the high concentrations present in the seed-coats and in the flesh of the fruit. The inhibitor level in each case was nearly two to three times that in embryo tissues (Figures 10, 11). Bradbeer (14) suggests that inward diffusion from the pericarp and testa imposes dormancy on the embryo of Corylus. ABA was however thought to account for only a fraction of the inhibitor activity.

In deciduous species it is generally accepted that leaves synthesize ABA in response to decreasing photoperiods (73, 75, 93, 94). In certain instances inhibitors have been detected after exposure to only two cycles of short day treatment (74). Following synthesis this material must enter the vascular system prior to translocation
to the apical meristem. Hoad (45), using an aphid honeydew technique, has shown ABA to be present in the sieve tube sap of Salix. Once having entered conductive tissue, the inhibitor can be translocated to the fruit as well as the bud.

It appears unlikely that the embryo could supply the inhibitor to seed and fruit tissues in the quantities observed while it is itself a developing organ throughout much of the growing season. Upon maturation it enters a dormant state in which it is incapable of renewing growth until subjected to some form of after-ripening. These conditions would seemingly limit the capacity of the embryo to synthesize large quantities of growth substances. If, however, the leaf were the ultimate source of inhibitor for the seed as well as the bud, sufficient synthetic capacity would exist to provide the quantities of inhibitor extracted.

Chilling, regarded as the agent responsible for breaking rest in seeds as well as buds, produced a significant change in the inhibitor content only in embryo extracts (Figure 10). The decrease in concentration would generally be attributed to some form of enzymatic breakdown. Sondheimer and Galson (78) suggest enzymatic breakdown may account for the reported decrease in ABA from Fraxinus seeds during chilling. A physical breakdown of the inhibitor could be excluded since significant changes did not take place in seed surface or seedcoat inhibitor levels. The embryo, containing the active
meristematic tissue, could reasonably develop such enzymatic reactions as required to degrade a growth inhibitor. The effect of chilling in breaking rest in seeds may then be to initiate the enzymatic breakdown of growth inhibitory materials in the embryo.

Because the inhibitor content of the embryo did not disappear completely upon the resumption of growth, the possibility exists, as suggested previously (74), that threshold levels govern the effective concentration of growth substances. These materials presumably are present continuously in various plant organs but only control growth when their concentrations rise above or fall below specific levels.
SUMMARY AND CONCLUSIONS

Extracts of buds from several species of the genus *Pyrus* as well as extracts of fruits and seeds of *P. communis* made before and after subjection to periods of chilling temperatures yielded a common inhibitor of oat coleoptile growth. Based on Rf values in several solvent systems from paper and thin-layer chromatographic methods and ultra-violet spectra, the inhibitory material is tentatively identified as abscisic acid.

Exposure to chilling temperatures normally responsible for breaking rest in deciduous species did not substantially alter the inhibitor content in whole bud samples taken monthly throughout the dormant period from three *Pyrus* types—Bartlett on *P. communis* root, Bartlett on *P. calleryana* root and ungrafted *P. calleryana*. No differences in the inhibitor levels were evident between bud samples from the variety Bartlett on either the rootstock requiring long chilling, *P. communis*, or on *P. calleryana* a short chilling type. A difference was observed in the inhibitor content between samples of *P. calleryana* and those from the long chilling Bartlett variety, a *P. communis* type. Samples of *P. calleryana* had a significantly lower inhibitor content during winter than did the *P. communis* types. Differences also were noted in extracts of other *Pyrus* species. Species requiring a shorter exposure to chilling temperatures to
break rest contained less of the inhibitor than longer chilling species.

No consistent growth promotion was observed in any sample except those taken at full-bloom. In these samples a strong unknown promoter was present in NA extracts from each of the three *Pyrus* types. Considerably greater growth promotion was observed in the long chilling *P. communis* types than in the short chilling species, *P. calleryana*. These results may, however, not be representative because the samples were accidentally thawed prior to processing.

From extracts of seeds of *Pyrus* varieties it was found that the embryo contained the lowest level of the growth inhibitor. The inhibitor content of seedcoat and fruit tissue was two to three times that of the embryo. A significant decline in inhibitor concentration in response to chilling of sufficient duration to break rest was noted in embryo tissues but little change took place in seedcoat or fruit tissue extracts.

The presence of a growth inhibitor in each bud sample throughout the dormant period indicated that rest may, at least in part, result from growth inhibitors. The evidence from the species trials suggests the growth inhibitor content to be a better estimate of inherent chilling requirements in *Pyrus* than of the amount of chilling the buds have received at any given time. Each species representative of a particular length of chilling requirement may have a characteristic inhibitor level; however, the inhibitor did not disappear
from whole buds when rest was broken or provide evidence of a consistent reduction during chilling. Significant promoting materials did not appear until rest was broken and growth had resumed. No gradual changes in inhibitor or promoter levels took place. This suggests that the growth promoter-inhibitor balance as a factor in bud dormancy control may function only during a limited period. Inhibitors may increase to functional levels during the period of rest induction, remaining at these levels until their effective concentration is exceeded by growth promoters prior to the resumption of growth.

The finding that only in the embryo did the inhibitor content respond significantly to chilling indicates that rest in seeds may be closely associated with the inhibitor concentration in the growing point. The embryo may be a site of inhibitor degradation rather than synthesis. Leaves are the probable source of inhibitor for both buds and seeds.

Chilling apparently provides the conditions necessary for the enzymatic degradation of the inhibitor in active tissues. A physical breakdown did not take place because little change in inhibitor concentration was observed in seedcoats following chilling.
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