DORMANCY IN APPLE TREE BUDS

B. G. Allen and M. Wann

Biomathematics Series No. 13
Institute of Statistics Mimeo Series No. 1631
North Carolina State University
Raleigh, North Carolina

September 1983
ACKNOWLEDGMENTS

This work was supported in part by USDA grant (Apple CIMP National Project No. 71-59-2481-1-2-039-1). Ann Ethridge typed this manuscript with efficiency and dispatch.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>i</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>iii</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A. General Remarks</td>
<td>1</td>
</tr>
<tr>
<td>B. A Note on Nomenclature</td>
<td>2</td>
</tr>
<tr>
<td>II. Factors Controlling Dormancy</td>
<td>2</td>
</tr>
<tr>
<td>A. Environmental Factors: Photoperiod and Temperature</td>
<td>3</td>
</tr>
<tr>
<td>B. Interaction of Environmental and Endogenous Factors</td>
<td>5</td>
</tr>
<tr>
<td>III. Phases of Dormancy</td>
<td>6</td>
</tr>
<tr>
<td>A. Induction of Dormant States</td>
<td>6</td>
</tr>
<tr>
<td>B. Effects of Temperature on Various Phases of Dormancy</td>
<td>7</td>
</tr>
<tr>
<td>C. Growth and Development in Dormant Period</td>
<td>9</td>
</tr>
<tr>
<td>D. Physiological Changes</td>
<td>10</td>
</tr>
<tr>
<td>IV. Hormonal Control of Dormancy</td>
<td>13</td>
</tr>
<tr>
<td>A. Origin of Endogenous Factors</td>
<td>13</td>
</tr>
<tr>
<td>B. Growth Inhibitors</td>
<td>15</td>
</tr>
<tr>
<td>C. Growth Promoters</td>
<td>17</td>
</tr>
<tr>
<td>D. Balance and Interaction of Inhibitors and Promoters</td>
<td>19</td>
</tr>
<tr>
<td>E. Mechanism of Control and Nature of Dormant States</td>
<td>20</td>
</tr>
<tr>
<td>V. Conclusions</td>
<td>24</td>
</tr>
<tr>
<td>VI. Bibliography</td>
<td>32</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Fig. 1. Diagrammatic representation of changes in temperature range for bud break at times of change in growth activity.

A. Narrowing and widening of the temperature range due to changes in the maximum temperature at which bud break can occur. (Adapted from Vegis (122)).

B. Narrowing and widening of the temperature range due to changes in the minimum temperature at which bud break can occur.

Fig. 2. A schematic representation of seed dormancy and germination involving three classes of phytohormones, gibberellins, cytokinin and inhibitors. "+" (or "-") indicates effective (or ineffective) levels of the hormone. (Adapted from Khan (70)).

Fig. 3. Possible pathway of action leading to bud break. (Adapted from Chandhry, Broyer and Young (30)).

Fig. 4. A postulated scheme for the action of GA. (Adapted from Jarvis, Frankland and Cherry (67)).

Fig. 5. Possible biochemical pathways in the termination of seed dormancy. (Adapted from Amen (5)).
I. Introduction

This report summarizes the results of a part of coordinated efforts led by Dr. H. J. Gold to develop apple orchard ecosystem models (144). One essential component of the system is apple tree buds. To integrate this component to the overall system, we must quantify the effects of environmental factors on bud growth and development. The difficulties in construction of such a model are mainly due to the complexity of the underlying biological mechanism and lack of detailed data. As a preliminary step of model development, we made a survey of current literatures on bud physiology emphasizing environmental effects.

A. General Remarks

In the summer, apple tree buds enter dormant period during which growth (dry matter accumulation) is sharply reduced. Normal growth will not resume until the next spring. It is known that changes in structure and internal function of the bud in dormant period can have profound effects not only on timing of bud break but also on bud growth (111).

The initiation, maintenance and release of dormancy may be viewed as a continuous process consisting of a sequence of stages (111). The morphological, physiological and molecular changes that occur during dormant period are thought to be under genetic control but may be modified by exogenous factors (57). From this point of view, the basic mechanism responsible for dormancy can only be revealed by simultaneous studies of changes in internal function of the bud and its relation to environmental conditions.
We report here a survey of literature on apple bud dormancy and, to a certain extent, some general aspects of dormancy in other fruit trees. Its purpose is only to draw together some basic knowledge in such a fashion that fundamental parts of this knowledge can be used to develop a framework for quantitative experimental studies into the bud development in relation to temperature and other environmental factors.

B. A Note on Nomenclature

In this survey, we will generally follow the nomenclature of Romberger (104). Thus, "dormancy" will refer to a period of time, or the state of the bud, during which that bud will show no extensive growth, for whatever reason. By "innate dormancy" or "rest" we mean that portion of the dormant period in which the cause of the lack of growth is due to endogenous factors; i.e., it is not imposed by the environment. The reduction or stoppage of growth due to environmental factors is called "quiescence" or "imposed dormancy". It must be remembered that the state of dormancy, its imposition and release, is a continuous process and that these terms are used only because they are sometimes useful. However, the distinctions are not completely adequate because they separate the internal and external influences in an artificial manner.

II. Factors Controlling Dormancy

It has long been noted that environmental factors influence the development, maintenance, and break of dormancy. Factors most studied are temperature and photoperiod (or light intensity) and to a lesser extent nutrient and water stress. Early theories of dormancy suggested that the resting state in buds results from a lack of nutrients in the
buds themselves. Now it is generally believed that dormancy is under the control of plant-produced chemicals, i.e., growth promoters and inhibitors (90). This does not imply that nutrient stress is not a factor in the induction of rest; it means merely that this stress acts, as do the other factors mentioned above, through the actions of hormones.

A. **Environmental Factors: Photoperiod and Temperature**

Changes in photoperiod have long been seen as a naturally occurring cyclic process that could account for the alternation between dormant and growth states (90, 122, 131). For the most part, short days are thought to induce dormancy; long days to end it. We will not comment upon this extensively since it has been shown (89, 128) that photoperiod changes do not induce dormancy in apples. Suffice it to say that, as in peaches (55), the intensity of apple bud break may be sensitive to light (56).

Temperature (i.e., air temperature) is another matter however. Cool temperatures (12.5°C) were found to be sufficient to halt growth of apple trees (89). Of course, the well-documented "chilling requirement" of many species is shown to be present in apples as well (44). There are indications, nonetheless, that the effect of temperature may be more complicated than simply a cool temperature induction and a chilling temperature break of dormancy.

Weinberger (134) suggests and Erez and his colleagues (51, 52) attempt to show that cycling temperatures (alternately high and low) affect the progression of dormancy in peaches. The important variables seem to be cycle length and maximum temperature. The longer the cycle (six or
nine days), the less the negative effect on rest progression. More interesting is the effect of maximum temperature: in a daily cycle (eight hours high, sixteen hours low) a high temperature of 21°C or higher completely negated the prior chilling. However, an alternation between 6°C and 15°C (and to a lesser extent between 6°C and 18°C) enhanced the chilling process; i.e., bud break was advanced. Thompson, et al. (115) noted the same trends in apple. Borkowska (22) showed that although chilling may be insufficient for bud burst it may be enough to promote bud elongation. Generally, he suggests that there may be two processes at work within the bud, one controlling burst and the other controlling cell elongation, and that each has its own environmentally optimum requirements.

Sparks (112) demonstrates that in pecan, subfreezing temperatures may also advance bud break. He attributes this to tissue injury or to prevention of the attainment of the deepest resting state. Indeed, it is generally believed that the optimum chilling temperatures are somewhat above freezing, roughly 2°C to 7°C (54, 100, 115), and that freezing retards bud development and dormancy break (25). A number of studies have suggested that the time of chilling, early or late in the winter, will affect both the number of buds which break and their subsequent growth (1, 72, 115). The rate of bud development in the spring was found to depend most heavily on the cold conditions of the preceding autumn or early winter (1). That is, warmer autumn conditions promote further bud development (delayed onset of quiescence), which facilitates renewal and completion of growth once dormancy has ended (1, 72). On the other hand, Abbott (1) suggests that warm temperatures in late winter or spring may allow for earlier bud break (i.e., earlier
removal of imposed dormancy). Abbott also hypothesizes that the temperature above which bud growth can resume in spring (i.e., above which dormancy is no longer imposed) is not constant; it depends upon the extent to which dormancy has been broken by cold treatment. Thus, those buds which receive more chilling can leave the quiescent state at lower temperatures than those which get less chilling. Eggert (44) suggests that there is a correlation, within the apple species, between the date of bloom and the chilling requirement: those varieties which bloom late need more chilling during winter.

The general effect of temperature on dormancy may be summarized as follows. Cooling air temperature promotes dormancy, which perhaps begins as quiescence and may therefore be reversed by subsequent warmer temperatures. Once the cooling has induced rest, that state is maintained until chilling brings it to an end. That the process is more complicated than this cursory summation allows is indicated by the facts noted above for temperature cycling and times of cold imposition. We later attempt to tie these observations to theories about the action of growth hormones.

B. Interaction of Environmental and Endogenous Factors

As mentioned above, there is some difficulty in a notion of dormancy which divides the dormant state into a rest (endogenous control) phase and a quiescent (environmental control) phase. For one thing, we have seen above that, even during rest, the environment (e.g., temperature) exerts an influence, shortening or prolonging rest. Furthermore, with a hormonal mechanism postulated for the control of
growth, and knowing that the environment can affect hormone levels in many ways, it is misleading to try to separate in time environmental and endogenous effects. Rather, we would think, as does Tyurina (119), of these effects as a dual control. This complements his notion that growth and dormancy are just parts of a continuous process and that in the course of one, the other is prepared for, actively. Thus there is an endogenous rhythm upon which environmental stimuli can operate. He states that if for some reason (e.g. because of a late growth start or under unfavorable growing conditions) the endogenous rhythm is not conducive to the imposition of rest, the environmental signals that the plant receives can compensate and initiate rest itself. This could also account for the fact that buds in a resting state will eventually leave that state, even if they do not receive their chilling "requirement." If they do get chilling, though, the endogenous and environmental factors work in harmony and vegetative growth can recommence much sooner.

III. Phases of Dormancy

A. Induction of Dormant States

There remains a question about the ability of plants to enter dormancy. Specifically, will the bud enter dormancy whenever a prolonged environmental stress is encountered, or does the bud have to be at a certain stage of development before dormancy is induced? There appears to be no resolution of this question as yet. Romberger (104) reports that cells which do not contain significant amount of lipids are not able to enter dormancy. In particular, O. Abbott (2) cites
Simon (1919), stating that buds will not enter a resting state until a certain degree of development has been attained. On the other hand, D. L. Abbott (1) supports the idea that mild autumns promote further growth and development, harsher falls inhibit this, but buds at any stage of development can enter a normal dormant state. Landsberg (72) confirms this. Brown and Abi-Fadel (26) state that buds at earlier developmental stages do not require more chilling to end rest, i.e., the bud developmental state seems to be no index of the chilling requirement. These reports are not conclusive; the requisite degree of development may be attained early in autumn or even in summer. The various stages of morphogenesis reported by these authors may correspond to development beyond the required minimum. More to the point is an investigation by Chandler et. al. (29) who state that apple leaf buds are quick to enter rest in summer if ever their shoots stop growing. Moreover, these authors found that a water stress of a few weeks duration, even in early summer, was enough to cause a resting state so deep that no growth was possible for the remainder of that summer.

B. Effects of Temperature on Various Phases of Dormancy

The realization that the onset of rest is not an instantaneous, but rather a gradual process probably led Vegis (122) to propose a classification scheme for dormancy consisting of three phases: predormancy, innate dormancy, and post-dormancy. During the predormant period, the temperatures at which the plant can grow become more restricted, until the plant can no longer grow at any temperature, the point which marks
the passage to innate dormancy. After some period of innate dormancy the plant becomes capable of growing in some small temperature range; as post-dormancy progresses this range becomes increasingly wider until it reaches the widest limits for vegetative growth. Vegis states that for fruit trees in the Rosaceae group the diagram would look like that shown in Fig. 1A. However, Tyurina (119) mentions a lowering of the growth temperature minimum as dormancy comes to an end (for apples) (Fig. 18). Vegis further states that a high temperature early in post-dormancy can actually induce a secondary dormancy. Thus he visualizes the widening of the range not as a monotonic process, but as one that can be reversed. His view of this widening phenomenon is supported by Rom and Arrington (103) who report that a stepwise increase in temperature produces a better bud break than a sudden jump to the final warm temperature.

This approach is more satisfactory for it does not try to separate endogenous and environmental factors. The new characterization of the process of dormancy uses forcing experiments to determine a depth of the dormant state. This generally results in a bell-shaped curve (49, 60) with the mode coinciding with innate dormancy. Such observations are consistent with the division of the dormancy period proposed by Samish (107). It consists of the following sequence: quiescence + preliminary rest + mid rest + after rest + quiescence. Here quiescence is a state of growth stoppage reversible by favorable conditions. Preliminary rest and after rest are characterized by a lack of growth, but an ability to be forced. Midrest corresponds roughly to innate dormancy, the state in which forcing is difficult or impossible.
This scheme, it seems, can be made to correspond to that of Vegis. The two characterizations may merely divide the continuous process of dormancy into different discrete portions. A probable correspondence is illustrated in Fig. 1A. We will see later if any such scheme is compatible with the internal mechanisms at work during dormancy.

C. Growth and Development in Dormant Period

It should not be thought that growth and development do not occur during the dormant period. Tyurina (119) and Romberger (104) state that the apical meristem cells remain active; the subapical cells are inhibited in their growth. Both growth and development in dormant buds proceed at slow but continuous rates (13, 27, 28, 134, 141). Flower buds not only increase in weight (33), they show an increase in flower clusters within the bud (27). Similarly, leaf buds demonstrate an increase in the number of primordia as dormancy progresses (104, 141). Even though Brown and Kotob (27) state that Zeller reports slow development of apple flower buds even during the coldest part of winter, it is generally believed that subfreezing temperatures may retard bud development (25, 119). Both Young (141) and Romberger (104) state that the bud parts developed during dormancy have a morphology different from those in vegetatively growing buds. Moreover, Young (141) has shown that the increase in size and in number of leaf primordia is more marked in March through June in buds with prolonged dormancy (given insufficient chilling, i.e., no temperatures below 15°C). This corresponds to the time when bud burst and rapid growth would occur had dormancy ended as usual. Perhaps there are two mechanisms, i.e.,
internal systems, one controlling growth and development within the bud, the other controlling the processes associated with bud burst. Indeed, Hewitt and Wareing (64) suggest that development of the bud during dormancy is mediated by regulators and that subsequent growth in spring results from a "well-defined metabolic pathway." The growth and development of apple leaf and flower buds during dormancy has been reported by Zlobina (143), Tyurina (119), Brown and Kotob (27).

Since growth and development do occur during dormancy, albeit slowly, how is dormancy different from vegetative growth? As mentioned above, Tyurina (119) and Romberger (104) state that growth inhibition occurs in the subapical meristem cells, and the former author stresses that it is the transition to dormancy that initiates apical meristem cell activity. Thus, there is no shoot elongation. But there are more fundamental and important distinctions between dormant and non-dormant states. Tyurina (120) hypothesizes that the advantage to temperate-climate plants of a dormant phase is that such a phase allows the plant to separate the energy-requiring processes of growth and of preparation for cold hardiness. This indeed appears to be the major function of dormancy: reducing susceptibility to environmental stresses.

D. Physiological Changes

Whatever is responsible for the maintenance of dormancy, the external differences that are seen must reflect cellular changes occurring in the buds. These changes not only result in the dormant state but also prepare the cells for the subsequent phases of their development (120). The internal changes are a direct result of the
mechanisms of dormancy control. Thus an examination of the changes may reveal the nature of those mechanisms.

During the latter part of summer, perhaps after the onset of quiescence, photosynthesis builds up a reserve of carbohydrates and other compounds (2, 119, 120). Also noted is the synthesis of high-molecular-weight, acid-insoluble phosphorus compounds. As dormancy progresses, this ceases as acid-soluble phosphorus compounds (apparently for storage) are manufactured (119). Phosphorus uptake (109), and, correspondingly, phosphorus metabolism (119) cease almost entirely during the deepest part of rest. The DNA-RNA-protein system has been working actively in autumn (119) and by the time rest passes to imposed dormancy the metabolic activity is completed through DNA transcription. The fact that RNA and protein synthesis occurs during dormancy is confirmed by many investigators (13, 37). Ekelund (46) notes the slow pace of RNA and protein synthesis, rRNA being the primary nucleic acid produced. However, Yeh Feng et. al. (140) note that later in dormancy (January to March) changes in the nucleus occur and more protein and RNA are produced. Although transcription may be complete by end of rest, the mRNA cannot enter the cytoplasm because of a double membrane around the nucleus and the absence of nucleopores (140). Other changes associated with the onset of dormancy include a decrease in amino acids (48), perhaps due to active protein synthesis and increase in the protein content of the ribosomal and mitochondrial fractions (119). Also reported is a rapid hydorlysis of polysaccharides and the appearance of large quantities of oligosaccharides (118). Samish (107) reports that the protoplasts in the cell contract and assume a convex shape;
they withdraw from the cell wall, rupturing the plasmodesmata, and become covered with a lipoid layer. This is most evident in apple and pear. Tyurina (119) states that upon entry into dormancy there is a coincident increase in nuclear dimensions and protoplasmic content.

During the course of the dormant period, various changes in cell activity occur. There is an increased metabolic activity of cell wall formation and lipid droplets are at a maximum in early December; these comprise the source for membranes of new organelles formed later (140). Ekelund (46) notes the increase of ribonuclease activity from autumn to spring, with much greater increase in March and April. An early article by Abbott (2) reports that hydrogen ion concentration decreases through winter in apple bud cells, a fact that he claims affects the activity of certain plant enzymes. Bachelard and Wightman (10) note that Q10 absorption increases through dormancy; however Hatch and Walker (60) saw no significant difference in respiration rates until late in the dormant period, February or March.

As dormancy is coming to a close, many of the changes seen at the inception of dormancy are reversed. Amino acid content increases, due to the rapid breakdown of the protein found in large quantities up to this time (10, 48). Nucleopores reappear, allowing mRNA to enter the cytoplasm (140). Carbohydrates are markedly decreased (10), especially starch content in apples (65). Organic acid content increases just prior to break (48). There is a net loss of nitrogenous material (10) and phosphorus uptake is resumed (109). Bachelard and Wightman (10) report that the respiratory quotient decreases from about 3 (indicating anaerobiosis) to between 1 and 1.5. They state that metabolic activity
changes from catabolism to anabolism about two weeks prior to renewed growth. Before this occurs, the carpel size of the Golgi bodies increases, an important event because of the extra cell surface area provided. At about the same time, the number of mitochondria increases, indicating that more energy is needed for cell reactions. Also smooth endoplasmic reticulum (ER) is transformed into rough ER in preparation for synthesis of proteins to be transported (140). Samish (107) reports that the protoplasts swell and reestablish plasmodesmotic connections. Total sugars increase significantly about two weeks after the completion of rest (48), particularly the monosaccharides glucose and fructose (10). A number of investigators (10, 114, 128) report the increased water content of cells as they end dormancy. Taylor and Dumbroff (114) cite a change from a low of 38% of total fresh weight to 71% at burst in sugar maple buds.

IV. Hormonal Control of Dormancy

A. Origin of Endogenous Factors

We have seen that environment is one influence on the dormancy status of woody plants. It remains to be considered by what internal mechanisms the changes in this status are affected. As mentioned previously, a hormonal control system is generally accepted (see Wareing and Sanders (131) for a review). In particular, a balance between inhibitor(s) and promoter(s) is hypothesized (131). An early work (41) showed that dormancy is a property of the buds alone, i.e., no other tissues are dormant. In fact Corgan and Martin (33) suggest that in peach flower buds rest is localized within the floral cup.
Semin and Madis (109) confirmed that the roots do not become dormant by measuring uptake of labeled phosphorus. It is not at all clear however that the place of origin of the inhibitors or promotors is within the bud. Mielke and Dennis (86, 87) suggest that the senescing leaves may be responsible for abscisic acid accumulation in the bud in autumn. Mauget's work (84) would support such a conclusion. Davison and Young (38) report a quick rise of abscisic acid (ABA) concentration in xylem sap after leaf fall. Along the same lines, Corgan and Peyton (34) and Chandler and Tufts (28) state that later leaf retention corresponds to later bloom. On the other hand, Hodgson (65) reports no relationship between leaf retention and rest. Promoters too have been associated with the leaves: Eady and Eaton (43) note that in cranberry, gibberellic acid is transported from leaves to terminal buds prior to elongation. Luckwill and Whyte (80) suppose that cytokinins, another group of promotors, may be translocated from the roots to the buds in spring since the authors could detect none of these substances in the xylem sap from September to January. However, Borokowska (21) found that in apples, the distal buds had consistently high cytokinin activity throughtout dormancy. Hewitt and Wareing (64) claim that the increase is independent of the roots, but dependent on the sap. Romberger (104) considers any root influence on growth hormone levels unlikely, an opinion based primarily on the fact that break of buds can occur normally in excised shoots.

The possibility of influences on the buds by other tissues seems to be supported by a number of investigators who note that the dormant state of buds can vary depending on their position on a shoot. In
peaches, a difference was found in the optimal chilling temperatures (54); terminal buds had the highest optimum (were easiest to break), lateral buds on lateral shoots had the lowest. Eggert (44) suggests that lateral buds lag behind terminal buds and spur buds because of a resumption of apical dominance. Weinberger (132) notes one result of prolonged dormancy in peaches to be bloom only near the tips of shoots, perhaps followed sometime later by basal bud bloom. This would seem to corroborate Borkowska and Powell's hypothesis (21, 22) of a "rest influence" that moves from base to apex as dormancy commences; thus distal buds tend to be less dormant. In a somewhat related experiment, Robitaille (102) reports that on shoots placed in a horizontal position, the buds on the lower side exhibited dormant characteristics longer. He attributes this to an auxin effect. In contrast, a group of French authors report strikingly different results. Mauget (84), working with almond trees, found that the basal ends of shoots are normally least dormant. Aires and Crabbe (8) and Barnola et al. (12) confirm this and go on to speak of a dormancy resisting (growth stimulating) factor at the basal end, an "inertia" (growth inhibiting) factor at the distal end. Mauget (84) also describes the effect of mechanical defoliation, i.e., reduction of the dormant state as being strongest on the distal buds.

B. Growth Inhibitors

Repeated reference has been made to the importance of inhibitors and/or promoters in the control of dormancy. Many investigators have reported correlations between endogenous levels of these substances and the onset or lift of dormancy. For instance, El-Mansy and Walker (49)
have investigated the inhibitor naringenin in peach flower buds; they found its levels to coincide with the bell-shaped rest intensity curve. A substance of particular interest has been abscisic acid (ABA) found to be a constituent of the β-inhibitor complex, very active in inhibiting growth. Reports of its accumulation in late summer/early fall (63, 87, 119), presence during dormancy (21, 33, 63, 69, 72, 77, 82, 98, 99) and diminution in spring (4, 63, 87, 88, 119) are plentiful. Some researchers have found, however, that its levels do not correspond to rest intensity (40, 86, 114). In particular, Dennis and Edgerton (40) state that ABA levels correspond not to rest but to dormancy, a view that appears to fit with the schemes of Vegis or Samish noted above, i.e., the gradual development of rest, which perhaps then corresponds to ABA above some threshold of irreversibility. However, Mielke and Dennis (87) report that in mechanically defoliated trees with low ABA levels, the resting state is as intense as in buds with high ABA levels. It has been suggested that the relative concentrations of a bound and a free form of ABA are important in determining dormancy status: free ABA, causing dormancy, decreases through winter with a concomitant increase in bound ABA, an inactive form which allows bud burst (15, 77, 86).

Specifically, Lesham et al. (77) working with almonds report that one stereoisomeric form, trans-trans-ABA, is of overriding importance in the inactivation, and that the binding is glycosidic in nature. Bardle and Bulard (15) propose that the change from a free to a bound form is due to chilling, but Mielke and Dennis (87) claim that chilling is not necessary for a decrease in ABA activity in cherry tree buds. Corgan and Martin (33) suggest that ABA breakdown is a secondary result due to enzymatic changes brought about by chilling. A somewhat different finding
is reported by Kawase (69) for peach and *Malus sylvestris*: dormancy lasted as long as the inhibitor activity increased; as soon as it started to decrease, the bud breaking process commenced, even though activity was as high as levels which earlier maintained dormancy. It appears (40, 82) that the inhibitor resides for the most part in the bud scales, one report (40) stating that it did not enter the primordia until after they have expanded. Mielke and Dennis (86) point out that the concentration of ABA is highest in the primordia, especially in November and early December, coincident with the deepest rest in some years. This is consistent with the theory that ABA is transported from leaves to scales (where it is stored), then to primordia. It is also consistent with the thoughts of Corgan and Martin (33) that rest is a property of the floral cup.

C. Growth Promotors

Growth promoters have also received a great deal of attention. But since vegetative growth was thought to be the normal state for plants, promoters were at first studied only in relation to inhibitors or to an inhibitor/promoter balance. Nevertheless, Taylor and Dumboff (114) now claim that it is variation in cytokinins, not ABA, that is responsible for dormancy break in sugar maple. Gibberellins have been studied extensively and high levels of their activity (perhaps with simultaneous decrease in inhibitor) have often been found to promote resumption of vegetative growth (4, 66, 111, 119, 138). In relation to the dormancy period, Jarvis et al. (67) propose that chilling "potentiates" gibberellic acid (GA) synthesis, which then proceeds when
higher temperatures occur. Hull and Lewis (66) and Walker and Donoho (125) claim that GA has no effect on apple tree growth or dormancy. Auxins have been implicated in dormancy control less frequently (4, 20). Blommaert (20) postulates that increase in auxin levels by cold treatment functions to inactivate the inhibitor. Eggert (45) has proposed a model for auxin control of apple spur bud growth. As long as cells enlarge, auxin does not build up; it is present in medium concentrations and so promotes growth. However, when environmental factors slow or stop growth, auxin concentration increases to inhibitory levels. This continues and buds enter "fore-rest". Auxin accumulates further, unless there is some feedback or diminution of production due to the senescence of the leaves.

Cytokinins are generally recognized as being important in dormancy control (14, 21, 64, 98, 119), and are known to be active in apple fruitlets and shoots (78). In fact, Khan (70) states that Piendazek (1970) found cytokinins capable of reversing ABA inhibition in apple explants, but not so for GA or auxins. Hewitt and Wareing (64) emphasize the enhancement of cytokinin activity is dependent upon cold treatment. The dynamics claimed by Borkowska (21), who proposed ABA and cytokinins as the inhibitor/promoter pair in apples, are as follows: in the period from December to February, cytokinin activity is low (but detectable) with an increase in March and subsequent disappearance just before burst. Hewitt and Wareing (64) corroborate this and add that cytokinin activity again increases with shoot elongation. Barthe (14), working with apple seed embryos, maintains that cytokinins exist in free or bound form, similar to ABA, and that free-form cytokinin increases during afterripening leading to germination.
D. **Balance and Interaction of Inhibitors and Promoters**

Corgan and Martin (33), noting the fluctuating levels of ABA during dormancy, conclude that rest is a more complex phenomenon than a simple accumulation and maintenance of an inhibitor at a concentration high enough to stop growth. This view is supported by the findings of Kawase (69), as stated above, and Ramsey and Martin (99), who show that both the promoter and the inhibitor levels increase during dormancy. They found however that the increase in promoter just prior to break was much greater than that of inhibitor, so that the ratio of promoter to inhibitor increased. These and other findings support the hypothesis that the relative amounts (or concentrations) of promoter and inhibitor are important in determining the dormancy status: a promoter-inhibitor balance. The manner in which the effects of these substances are balanced will be discussed later.

In an investigation on apple buds, Borkowska and Powell (22) suggest that the processes of bud burst and shoot elongation are under the control of different hormones, probably cytokinins and gibberellins respectively. Khan (70) too, suggests different roles for these hormones, as specified in his model for see dormancy. Noting that ABA levels increase and cytokinin levels decrease during stress, and that cytokinins can modify the effects of other hormones without having a marked effect by themselves (49), he proposes that gibberellin, cytokinin, and ABA play primary, permissive, and preventive roles respectively. That is, gibberellins generally promote seed germination whereas cytokinins and ABA act, and interact with each other, to either allow or prevent gibberelin from carrying out its role. A schematic representation is
shown in Fig. 2. Khan also proposed a similar scheme for bud dormancy, but allowing for auxins to play a role, perhaps even prior to or in conjunction with gibberellin. This later suggestion is consistent with the enhancing capabilities of IAA on GA found by Ahmed and Mathew (4). If it is allowed that varying concentrations of all four hormones have effects more subtle than an effective-ineffective dichotomy, the picture of bud dormancy becomes quite complicated. Moreover, Corgan and Peyton (34) suggest possible interactions with inhibitors other than ABA.

Some such model may be appropriate. It can go a long way toward explaining certain anomalous results. For example, Mielke and Dennis (85, 87) note that temperature had no effect on ABA levels. The model might then propose that the chilling treatment affected the cytokinin levels in this species (cherry), negating ABA, and producing normal break of dormancy.

E. Mechanism of Control and Nature of Dormant States

The most important question still remaining concerns the mode of operation of the inhibitors and the promoters. It is still a largely unanswered question, various investigators proposing various mechanisms. It is now generally accepted that dormancy does not represent a total inhibition of cellular activity, but rather a "change in direction" of the growth process (119). Young et. al. (141) speak of "certain morphological manifestations inherently induced by the environment." Thus, strictly speaking, the terms "inhibitor" and "promoter" are inaccurate. They derive from the earlier belief that the inhibitor acts as a hormone which stops all growth and development. We have seen though that the cells still respire, grow, and develop.
Further evidence that dormancy is not a simple shutdown of cellular activity is provided by Esashi and Leiopold (57). In fact, they show that dormancy is a programmed part of the plant's life cycle. When a total gene repressor is applied before dormancy, a dormant state is not attained. This result is not to be expected if dormancy is due to a lack of essential elements in the metabolic activity of the cell. It suggests that active transcription of genetic material is necessary for the initiation of rest.

Given that the onset of dormancy is an active process, how is it accomplished? There are two general categories into which suggestions fall. First, the inhibitor/promoter pair affects the availability of substances necessary for growth. Alternately, the pair is hypothesized to interact with substances already present. Note, however, that if we assume ABA to be the inhibitor, then, as Wareing et al. (130) suggest, its simple structure may enable it to act in more than one enzyme system. No single mechanism need be hypothesized, even for a single inhibitor.

The inhibitor/promoter pair may act as hormones or as participants in intermediate metabolic reactions (104). Such theories suggest that ABA, as the inhibitor, interferes with the metabolic reactions of growth or that promoter is necessary for such reactions to continue. On the other hand the promoter may interfere with dormant metabolism, ABA may enhance it. Specific suggestions include

(a) allosteric binding of inhibitor and promoter on a key growth enzyme (91, 130).
(b) enzyme activation roles of the inhibitor and promoter
(65, 75) and
(c) interaction with cell membranes, controlling the availability
of enzymes, substrates, etc. (75).

Any of these means alone does not seem capable of explaining the "change
in direction" of metabolic activity seen upon entry to dormancy.

Zelniker (142) proposed that the lack of growth is due to a
deficiency in high energy phosphate bonds. Whether this is due to a
speculated effect of the β complex (i.e., the uncoupling of phosphorylation
and the electron transport system) (81) or due to the utilization of
the tyrosinase pathway rather than the cytochrome oxidase system
(Todd, 1953, in (30)), the result is less ATP production. Chaudhry
et. al. (30) suggest that high temperature variation in spring causes
renewed production of the high energy bonds. Lack of energy may be
sufficient to explain the cessation of visible growth and may thus occur
in the subapical meristem cells, but it cannot account for the active
metabolism of the apical meristem cells. Some other mechanism(s) must
be involved.

For the reasons mentioned above, many authors have focused
attention on promoter/inhibitor control of protein synthesis. Control
of protein synthesis can arise from action at the transcription or
translation stages or after translation into polypeptides. Some
investigations (3, 31, 126) indicate that control may occur after
transcription, probably at the translation stage. Most, however,
concentrate on the block to transcription hypothesized as a function of
the inhibitor. Some possibilities include:
(a) action of the inhibitor/promoter as an inactivator/activator pair for DNA or RNA polymerase (91, 128), or
(b) effect of the inhibitor on the availability of ribonucleoside triphosphates (117).

But again, to reflect the selective change in metabolic activity, most investigators have hypothesized that ABA and the promoter function only on specific genes or RNA's (3, 31, 67, 119, 121, 123, 128). Chaudhry et al. (30) have reviewed a number of proposals, specifically for Pyrus Communis, and have concluded that the primary factor in dormancy control is that identified by Tuan and Bonner (117), repression of DNA and consequent lack of protein synthesis. Such repression may be due to histones (67) or to other phosphoproteins (30) (see Fig. 3). In neither of these cases is it clear how an inhibitor fits in. On the other hand, Jarvis (67) postulates an operon system (Fig. 4).

Indeed, the manner in which the inhibitor-promoter pair interact is a matter of some speculation. Apart from the opposing effects of allosteric binding mentioned above, there have been a number of suggestions. We have already touched upon the ideas of Khan (70). He states that studies indicate that ABA and cytokinins act at the same level (RNA transcription, for example) but that this is not known for certain. It has been suggested (130) that ABA and a promoter (perhaps cytokinins in the case of apples) affect the biosynthesis of one another. It has even been proposed that GA and ABA arise from the same precursor (3). A fact about cytokinins that may be important is that many tRNA's actually have cytokinins incorporated into them (58). Any interaction between ABA and cytokinins would then manifest itself in
blockage of tRNA formation and subsequent lack of protein synthesis. The observation (114) that cytokinin levels, not ABA levels, are crucial to break of dormancy is perhaps tied to this point.

Other observations about inhibitor-promoter interactions are numerous. Chrispeels and Varner (31) suggest that GA and ABA act antagonistically (but neither competitively nor noncompetitively). They postulate the promotive effect of GA on the transcription of specific mRNA's and the inhibitory effect of ABA to stop that synthesis. Kawase (69) notes that application of GA decreases inhibitor levels in vivo in *Malus sylvestris*. This may be accomplished via hydrolytic and proteolytic enzymes (which are affected by GA (138)), increasing the permeability of cell membranes. Increased water content dilutes the ABA. Zelniker (142) noted that for rapid synthesis of nucleic acids, the cell must reach certain levels of hydration: at 65% hydration RNA content increases (coinciding with initiation of growth), at 71% DNA content increases (coinciding with growth maximum).

In a note of caution, Lang (75) and Chrispeels and Varner (31) state that all the results indicating action by inhibitors or promoters on the protein-synthesizing system are open to reinterpretation. These authors recognize the fact that the most that can safely be said now is that nucleic acid and protein synthesis must proceed at certain rates for the hormones to exert an influence.

V. Conclusions

Given our present knowledge, and the lack of conclusive evidence for any particular theory of dormancy regulation, it is certainly not
possible to propose a general correspondence between the phases of dormancy as espoused by Vegis (122) or Samish (107) and the endogenous mechanisms of control. It is possible that observations of empirical behavior will allow one to decide between likely proposals. That is, any promising candidate among models of internal, biochemical action must at least be amenable to such tests.

Little work has been done to try to reconcile environmental influences and endogenous controls. Those that have are sketchy. For instance, Moustafa (88), working with peach seeds, states that cold (stratification) inactivates auxin oxidase, causing an increase in auxin concentration, more RNA synthesis and consequent de novo synthesis of lipase. Also hypothesized is a rather more vague effect of cold on the removal of an inhibitor of \( \beta \)-oxidation of fatty acids. As cited above Jarvis (67) suggests that chilling "potentiates" GA synthesis, which proceeds later with increased temperatures. More generally, El-Mansy and Walker (48) state that cold inactivates some inhibitors and stimulates synthesis and release of enzymes necessary for the degradation of metabolites. Much more work needs to be done in this area. Any modeling attempt will necessarily be quite speculative on the point of environmental-endogenous control interaction.

Several empirical models have been proposed (72, 100, 101). They deal with the action of chilling in the break of dormancy and subsequent growth. Amen (5) has proposed a model of seed dormancy; it deals only superficially with external influences. If his ideas can be fleshed out, a useful basis for a model of bud dormancy may be available. His basic scheme is as pictured in Fig. 5.
In closing, we would like to point out those areas or ideas that we believe to be most worth further investigation. The modeling attempt by Amen (5) should be expanded and experimental results obtained to corroborate his ideas. The work of Khan (70) on the interaction of three or more hormones all involved in dormancy control appears to have much promise. A point that does not seem to be adequately emphasized is the distinction between apical and subapical meristem cells. It may turn out that these two areas have distinct hormonal control mechanisms. The major weak point of course is the absence of a link between environment and biochemical workings. Work here is needed.
Fig. 1.
Fig. 2
$P_i$, SUGAR, AMINO ACIDS, ETC. 

DNA-PHOSPHOPROTEIN COMPLEX

TEMPERATURE (CRITICAL VARIATION)

$ATP$

$DNA + Protein + P_i$

$RNA$

$PROTEIN$

RENEWED PLANT GROWTH & DEVELOPMENT

Fig. 3
VI. BIBLIOGRAPHY


