Hydrogen cyanamide is effective in overcoming rest in dogwood (Cornus sericea L.) and 'Radiant' apple (Malus floribunda Sieb) throughout the rest period. The concentration required to overcome rest was dependent upon the growth stage. Highest concentrations were needed during rest development. Bud break was delayed when applied at quiescence. Sublethal freezing and high temperature can also overcome rest. These studies suggest that overcoming rest is related to sublethal stress and not specific to the treatment. Hydrogen cyanamide stimulates bud release and has little effect on shoot elongation.

The phytotoxicity of hydrogen cyanamide is dependent upon concentration and growth stage. Plants were most resistant to
hydrogen cyanamide during rest development, between 180 to 280°GS, and most susceptible before rest (160°GS) and during quiescence (315 to 360°GS).

Hydrogen cyanamide stimulated ethylene production when applied at sublethal dosages, and increased the leakage of electrolytes and specific ions at sublethal and lethal concentrations. Cellular damage caused by hydrogen cyanamide failed to stimulate ethane production. Alfalfa cell cultures treated with hydrogen cyanamide could be induced to produce ethane by removing the hydrogen cyanamide from the media. Ethane production was also inhibited by NEM, a potent binder of thiol groups, in freeze damaged dogwood shoots, and restored by treating the tissue with glutathione, a thiol containing compound.

Hydrogen cyanamide inhibited catalase in the bark tissue of 'Northern Spy' (Malus domestica Borkh) apple and lentil seeds (Lens culinaris Medic). Ethanol overcame rest in 'Radiant' crab-apple, stimulated catalase activity in lentil seeds, and had no effect on hydrogen cyanamide induced inhibition of catalase. These studies suggest that inhibition of catalase activity is not involved in the breaking of rest.

Hydrogen cyanamide conjugated with the thiol group of glutathione in a non-enzymatic cell-free system and thiol groups in mung bean (Phaseolus aureus Roxb) seedlings. Exogenous applications of glutathione reduced the electrolyte leakage caused by hydrogen cyanamide, suggesting that hydrogen cyanamide was detoxified by glutathione. The effect of hydrogen cyanamide in
overcoming rest was reduced by pretreatment with dithiotreitol (DTT).

These studies suggest that the mechanism of action of rest breaking agents involves the binding of thiol groups. A hypothesis on the role of glutathione on rest development, rest breaking, and stress resistance is proposed.
Overcoming Bud Dormancy with Hydrogen Cyanamide: Timing and Mechanism

by

Cheng-Chu Nee

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Overcoming Bud Dormancy with Hydrogen Cyanamide: Timing and Mechanism

Chapter 1
Introduction

Temperate woody plant species typically develop winter dormancy (rest) in response to short daylength. Plants in rest do not resume growth even when exposed to ideal growing environments. Under natural conditions, a chilling period of several weeks is required to "break" rest in most temperate plants. Satisfying the chilling requirement with chemical and/or physical treatments has been extensively researched (Doorenbos 1953, Erez and Lavee 1974). These studies have shown that numerous treatments can overcome rest, which have led researchers (Doorenbos 1953, Erez and Lavee 1974) to hypothesize that there are no specific dormancy breaking agents. Any treatment applied at sublethal dosages can overcome rest.

A major problem in using chemicals or other treatments to break rest is the varying results. At a recent meeting at Davis, California, on the "Potential and practical use of hydrogen cyanamide on grapevine" to overcome rest, this problem was expressed by several researchers (Bracho et al. 1984, Lavee et al. 1984, Luvisi 1984, Whiting and Coombe 1984, William and Smith 1984). Hydrogen cyanamide is capable of either stimulating bud break, delaying bud
break, having no effect on bud break, or causing bud and stem injury. Timing and environmental factors are speculated as causing this variability (Lavee 1984). Because of the uncertainty of knowing when and how much hydrogen cyanamide to use to break rest, the commercial usage of hydrogen cyanamide has been limited. Although these problems exist, the effectiveness of hydrogen cyanamide in overcoming rest in temperate plants has encouraged researchers to continue to determine ways to overcome these drawbacks.

In plants, calcium cyanamide was patented in 1910 for use as a fertilizer (Pranke 1913) in Germany. Prior to 1913, it was used as a herbicide against mustard weeds in oats. It was first noted, in 1938, to induce defoliation in soybean and nursery stocks (Baily 1945). Calcium cyanamide has been successfully used for overcoming dormancy in apple, pear and peach (Kuroi 1963, Morimoto and Kumashiro 1978), and grapevine (Bracho et al. 1984). Recently, Shullman et al. (1983) reported that hydrogen cyanamide was more effective in breaking grape dormancy than calcium cyanamide. They suggested that calcium cyanamide undergoes partial hydrolysis to hydrogen cyanamide and cyanamide ions which is believed to be the active form. In moist soils and plant tissues, hydrogen cyanamide is broken down into urea, ammonium, nitrate (Amberger 1984, Miller and Hall 1963) and possibly other intermediates (Amberger 1984). The metabolism of hydrogen cyanamide in plants is not well understood. Hofmann et al. 1954, first reported that cyanamide was
metabolized in soybean by an enzyme called cyanamidase. In barley and corn plants cultured with cyanamide-\( \text{C}^{14} \) part of the carbon dioxide expired was \( \text{C}^{14}\text{O}_2 \). In later studies, Hofmann et. al. (1954) found that sublethal cyanamide in oats, barley, wheat, and rye was converted to dicyanamide within two to three days after treatment. Alanine, tryptophane, leucine, phenylalanine, and valine were present in higher concentration in cyanamide-fed plants than in plants fed calcium nitrate as the nitrogen source. Alanine displayed the greatest activity when plants were fed barium cyanamide \( \text{C}^{14} \).

Latzko (1955) reported that Rathsack found dicyanamide, guanidine and guanyl compounds as intermediate decomposition products of cyanamide. In cotton, Miller and Hall (1963) found that sublethal doses of cyanamide were rapidly complexed or destroyed in the leaves and in the injected balls. The first product in the leaves was urea. Cyanamide was found to be metabolized rapidly, no cyanamide residue was found eight hours after application, and cyanamide appeared to be utilized as a substrate for growth rather than converted to dicyanamide, azide or cyanide. In addition to urea, another product of cyanamide decomposition in cotton was alanine or a closely related product.

Amberger (1984) reported that cyanamide rapidly penetrates plant tissues and is transported in both directions. He showed that barley seedlings treated with cyanamide produce high dry matter, total nitrogen, protein-N and amide-N content as compared
to another N-source. These studies suggest that hydrogen cyanamide is directly involved in nitrogen metabolism and protein production. Amberger (1984) further showed that arginine content of plants treated with cyanamide increased dramatically. This increase in arginine appears to be a direct synthesis from cyanamide via guanidine compounds.

Recently, Amberger (1961, 1963, 1984), and Nir et al. (1984) have found that cyanamide inhibits catalase activity. Nir (1984) associated the reduction in catalase activity with the breaking of bud dormancy. They reported that catalase activity in buds of grape plant increases markedly in autumn and decrease to a minimal level when the buds are ready to burst. A negative relationship in catalase activity was also found with rest breaking chemicals. The decrease in catalase activity is thought to cause an increase in peroxide content, thus creating conditions favoring the pentose phosphate pathway which is thought to be essential to dormancy breaking.

In animal systems, cyanamide is known to interfere with the metabolism of alcohol by decreasing the activity of aldehyde dehydrogenase in vivo, thereby resulting in an accumulation of acetaldehyde following the ingestion of ethanol. In man, the accumulation of acetaldehyde causes unpleasant symptoms (flushing, hypotension, palpitations, dizziness, etc.). For this reason, cyanamide has been available as an alcohol deterrent agent for the treatment of alcoholism in Canada, Europe, and Japan.
Kitson and Crow (1979) reported that cyanamide reacts under physiological conditions with amino and thiol groups, forming guanidine and isothiouronium compounds, respectively. They postulated that the inhibition of aldehyde dehydrogenase may involve thiourea (formed by breakdown of isothiouronium compounds) and formamidine disulfide (an oxidation product of thiourea). DeMaster et al. (1984) identified catalase as the enzyme that activates cyanamide to a reactive species that is responsible for the inhibition of aldehyde dehydrogenase in vivo. Shirota et al. (1984) reported that cyanamide irreversibly inhibits catalase by covalently binding to the apoprotein of the enzyme in a process requiring hydrogen peroxide.

As previously stated, researchers hypothesized that the effective treatment to overcome rest was related to sublethal stress (Doorenbos 1953, Erez and Lavee 1974). To date, however, there is no direct evidence to prove this relationship. Therefore, one of the objectives of this study was to prove or disprove this hypothesis. Previously, the reason for not being able to test this hypothesis was due to absence of an objective measure of sublethal stress. Recently Harber and Fuchigami (1986) and Kobayashi et al. (1982) reported that sublethal stress may be determined by measuring ethylene evolution. Tissues exposed to sublethal stresses, prior to cell membrane damage, produce high levels of ethylene and the amount of ethylene produced is a direct measure of stress. Upon membrane damage, electrolyte leakage
increases and ethylene production decreases accordingly. Another
gas, ethane, is also produced and correlates positively and nega-
tively with the increased leakage of electrolytes and the drop in
ethylene levels respectively. These tests were used to measure
sublethal and lethal stress and related to the breaking of rest.

A second objective was to explain the variable results
reported by others when hydrogen cyanamide is used to overcome
rest. These studies were based on the assumption that the plants' 
resistance to stresses was related to the physiological condition
of the plant. To determine whether the phytotoxicity and
effective rest breaking concentration were related to the growth
stage of the plants, hydrogen cyanamide levels were applied at
specific growth stage based on the Degree Growth Stage Model
(Fuchigami, et al. 1982).

Finally, this study was undertaken to determine the mechanism
of action of overcoming rest using hydrogen cyanamide. The
effects of hydrogen cyanamide on catalase activity and the conju-
gation of hydrogen cyanamide with glutathione in both in vivo and
in vitro system was studied.
CHAPTER 2
REVIEW OF LITERATURE

Definition and Characterization of Growth Stages

The terms and definitions reported in the literature to describe the period of winter dormancy (rest) in temperate plants are numerous and often confusing. Recently, Lang et al. (1985) reviewed some of the terms and definitions used by others and attempted to develop a universal terminology defining the stages of dormancy for horticulturists. They divided dormancy into three stages: 1) eco-dormancy, same as quiescence (Romberger 1963) or imposed dormancy (Doorenbos 1953) which is regulated by environmental factors; 2) ecto-dormancy, same as correlated dormancy (Romberger 1963), summer dormancy (Doorenbos 1953) and pre-dormancy (Wareing 1969) which is regulated by physiological factors outside the affected structure; and 3) endo-dormancy, same as rest (Wareing 1969), winter dormancy (Doorenbos 1953), innate dormancy (Wareing 1969), deep dormancy (Wareing 1969), and dormancy (Nooden and Weaver 1978) which is regulated by physiological factors inside the affected structure. Although the intention of developing a universal system of describing the various growth stages is good, the major drawback of all the proposed terminology and definition is their subjective nature in quantifying the various growth stages. To avoid confusion, the more established
terms, e.g. quiescence, rest, and correlative inhibition, will be used in this thesis to describe the general types of dormancy.

Attempts to describe the different physiological stages in the annual growth cycle of plants have been made (Smith and Keeford 1964, Brown 1967, Doorenbos 1953, Hanover 1980, Nienstaedt 1966). One of the earliest attempts to quantify the annual development of trees was made by Sarvas (1972, 1974). He divided the annual cycle of *Betula pubescens* into three major phases: the active period, autumn dormancy, and winter dormancy. In order to quantify each of these periods, he developed the concept of period units, chilling units, and dormancy units. The major problem he encountered was determining the beginning and ending of each period.

Recently, Fuchigami et al. (1982) developed a conceptual numerical procedure called the Degree Growth Stage (°GS) Model for quantifying the annual development of temperate zone woody species. The model consists of 360°GS and the cyclical function passes through five distinct sequential growth stages: 1) spring bud break (0°GS); 2) maturity induction point (90°GS); 3) vegetative maturity or onset of rest (180°GS); 4) maximum rest (270°GS); and 5) end of rest (315°GS) and end of quiescence or onset of spring bud break (0°GS). Fig. 1 (see appendix) is a diagram of the (°GS) Model describing the ontogenetic stages of development and its relationship to the development of cold hardiness in a temperate woody perennial. Table 1 (see appendix) describes and
defines the various point events (major growth stages), the bud
developmental status, the degree growth stage, and the segment
events.

The following discussion describes each of the point events:

**Spring Bud Break (0°GS, 360°GS):**

When the leaf or floral parts first appear from the
dormant bud. At this point, temperature is the dominant
factor regulating the growth rate. Short day length is not
effective in inhibiting bud growth from 0 to 90°GS. Plants
are not hardy to freezing stress and will not acclimate
between 0 and 90°GS.

**Maturity Induction Point (90°GS):**

When plant is first responsive to short daylength for
rest development. In dogwood, and a majority of the tem-
perate woody perennials, short days are perceived by the
leaves producing a translocatable promoter of rest and hardi-
ness. Exposure of plants to long days promotes growth.
Plants are not hardy to freezing temperature from 90 to
180°GS. However, some hardiness can be induced by subjecting
plants to stress conditions.

**Vegetative Maturity (180°GS):**

This period is synonymous with the onset of rest. At
this stage buds are no longer inhibited by correlative inhi-
bition which occurs between 0 and 180°GS. Defoliation or
removal of the terminal bud no longer stimulates dormant buds
to resume growth. After $180^\circ$GS, defoliation does not result in tissue injury (dieback) in overwintering plants. This stage starts the onset of cold acclimation (Nissila et al. 1978) and the acclimation to dehydration stress (Fuchigami unpublished results). Between $180$ and $270^\circ$GS, the rate of rest and hardiness development increases even at temperatures in excess of $20^\circ$C. Physiologically, chilling temperatures promote rest development between $180$ and $270^\circ$GS. Therefore, $180^\circ$GS indicates the beginning of the chilling requirement for overcoming rest in temperate species (Kobayashi et al. 1982).

**Maximum Rest ($270^\circ$GS):**

The point at which plants require the maximum time to resume bud growth when placed under an ideal long daylength, warm temperature growth regime. In a clone of dogwood, approximately 200 days of ideal growing conditions are required to stimulate terminal bud growth. At this stage, the rate of cold acclimation is at maximum. The level of plant hardiness, however, is not at maximum.

**End of Rest ($315^\circ$GS):**

The chilling requirement is fully satisfied and plants are no longer in rest. At this stage, favorable warm temperatures will stimulate the buds to grow. Between $315$ and $360^\circ$GS, plants are quiescent and buds are dormant because of low temperature. The optimum temperature required to stimu-
late growth widens from 315 to 360°GS. Plants are hardy at 315°GS. Between 315 and 360°GS, the range of temperature essential for hardiness promotion become narrower, while the temperature required for deacclimation widens. Hardiness during this period, therefore, is temperature-dependent and decreases as 360°GS approaches.

The amplitude of the sine curve is unimportant, and used only to illustrate whether growth is promoted or inhibited. Another method used to describe the annual cyclical nature of temperate plant development may be illustrated by a circle depicting 360°GS. Both methods suggest that the annual cycle is continuous throughout the year and from one year to the next.

Another important fact is that the stages do not naturally occur in a perfect sine function as depicted in Fig. 1. The rate of development and the promotion of each period of development is dependent upon the environment. Typically, the curve is skewed. The degree growth stages used to describe the °GS Model should not be confused with the day of the year. Each °GS is based on the physiological status of the plant.

There is a question about the reversible nature of plant development. Landsberg (1977) believes that under natural conditions the progression of bud growth and development are irreversible, while others disagree (Erez and Lavee 1971). During rest, Erez and Lavee (1971) reported that high temperature reverses rest
development. In addition, as stated previously, plant hardiness is also reversible.

The characterization of the growth stages of a clone of red-osier dogwood (Cornus sericea L., Wayland, Mass. clone) has been established (Renquist et al. 1978 and Kobayashi et al. 1982, 1983). In this thesis, the discussion will be directed toward the determination of the stages between 90 and 360°GS.

The maturity induction point, 90°GS, can be characterized by expressing plants growing under long daylengths (LD) to short daylength (SD) and observing the time required to develop 180°GS (Fuchigami et al. 1982). In the Massachusetts clone of dogwood, the critical photoperiod (approximately 14.2 hours) was found by using the mathematical model developed by Renquist et al. (1978). The rate of development between 90 and 180°GS under SD was found to be temperature-dependent with warm temperature between 15 and 20°C. being optimum for development. Under natural conditions, the critical daylength for this clone was determined by using the mathematical model for calculating the rate of development in response to the temperature occurring prior to this period. Growth chamber studies were then used to verify the critical photoperiod. In the Massachusetts clone of red-osier dogwood, about 40 days were required for buds to progress from 90°GS to 180°GS under SD at 21°C.

Under natural conditions, plants like red-osier dogwood obtain the 90°GS very early (several weeks after spring growth) and
remain in a transition state until the critical photoperiod is reached. Determination of the exact maturity induction point (90°GS) is difficult and, for most purposes, unnecessary.

It should be pointed out that not all plants develop rest in response to shortening photoperiod. Nitsch (1957) described other types of plants which respond by other means, including species that become dormant regardless of daylength. He notes, however, that plants which eventually obtain rest irrespective of daylength often become dormant faster under SD than LD. This suggests, possibly, that SD may play an important role in all plants even though other environmental factors play a role in rest development (Fuchigami et al. 1982).

The onset of rest, vegetative maturity (180°GS), is one of the essential point events to determine. Prior to this stage of development, buds are dormant because of correlative inhibition. Growing buds, e.g. terminal buds, and/or leaves, produce substances which prevent the lateral, and in some instances terminal, buds from growing. Removal of the growing buds and/or leaves eliminates the source of these growth inhibitors thereby releasing the bud from dormancy. This concept has been effective in determining the onset of rest, 180°GS, (Seibel and Fuchigami 1978).

Therefore, to experimentally determine 180°GS, plants are completely defoliated at timed intervals. Plants that sprout new bud growth are still controlled by correlative inhibition, while plants that do not sprout new bud growth are no longer under
correlative inhibition and are at rest (vegetative maturity). The
defoliation test, although useful for determining $180^\circ$GS, is not
useful in measuring the segment events between 180 and $315^\circ$GS.

Other studies to determine $180^\circ$GS have been made with limited
success. These include ethylene production of nodal stem sections
(Seibel and Fuchigami 1978), xylem water potential (Nissila and
Fuchigami 1978), electrical impedance and square-wave pattern
(Timmis et al. 1981), starch accumulation (Sarvas 1972), and
hardiness (Nissila and Fuchigami 1978).

Determination of the segment events between 180 and $270^\circ$GS is
accomplished experimentally by measuring the time it takes to
stimulate bud break under a LD and warm temperature (approximately
20-25$^\circ$C) environment. Under such conditions, resting buds will
eventually begin to grow even though they have not acquired suffi-
cient chilling. As the development progresses between 180 and
$270^\circ$GS, the amount of time (days) required to promote bud growth
increases to a maximum at $270^\circ$GS. The length of time required for
growth to resume is proportional to the depth of rest.

Kobayashi (1982) developed a mathematical model for determin-
ing the segment events between 180 and $270^\circ$GS in red-osier dog-
wood. This model considered the influence of temperature on rest
development and used the days to bud break under LD warm tempera-
ture method for assessing the $^\circ$GS. This study showed that the
rate of development from 180 to $270^\circ$GS increased with decreasing
temperature and with later growth stage. The degree growth stage
(°GS) throughout this period was determined by converting the days to bud break to °GS by the following equation:

\[ °GS = 90 \frac{(D-Dvm)}{(Dmr-Dvm)} + 180 \]

where D is days to bud break; Dvm is days to bud break at 180°GS; Dmr is days to bud break at maximum rest; 180 is °GS at vegetative maturity; and 90 is the total °GS required to develop maximum rest from vegetative maturity.

In developing this model the following assumptions were made. The optimum temperature and lower limits for maximum rest development were estimated based upon the data for Betula pubescens (Sarvas 1972). The optimum temperature was 3.5°C and lower limit was -3.5°C. The upper temperature limit (21°C) was taken from the data on peach (Erez and Lavee 1979a). Erez and Lavee (1979a) reported that temperatures above 21°C nullify some of the accumulated chilling units. The model developed by Kobayashi (1982) predicted maximum rest within two days for plants growing naturally at Corvallis, Oregon.

The rate of rest development and the degree of rest (days to bud break under LD, warm temperature regime) vary among different species and among different years for a single species (Amling and Amling 1980, Fuchigami et al. 1977, Kobayashi 1982). In 'Mahan' pecan only 16 to 25 days are required to break bud at maximum rest (Amling and Amling 1980), whereas in dogwood approximately 200 days are required to enhance bud growth.
The regrowth tests (days to bud break under LD, warm temperature) used to determine the growth stage for rest development were also used to determine the post-rest period between 270 and 315°GS (Kobayashi 1982). As with rest development, the post-rest period was stimulated by low temperature and the rate of development at each °GS changed throughout this period. The temperature optima (3.5°C) and lower and upper limits (-3.5°C and 21°C, respectively) used for rest development were also used for the post-rest period. Determination of °GS was made by converting the days to bud break to °GS by the following equation:

\[ °GS = 45(D-D_{ch})/(D_{mr}-D_{ch}) + 270 \]

where °GS is degree growth stage, D is days to bud break; D_{ch} is days to bud break at 180°GS; D_{mr} is days to bud break at maximum rest, 270°GS; 45 is the total °GS between 270 and 315°GS; and 270°GS is °GS at maximum rest. End of rest, 315°GS was defined as the stage when the time required for terminal bud break was equal to the time required for terminal bud break at 180°GS. The mathematical model developed by Kobayashi et al. (1983) predicted the end of rest within three days and predicted the developmental status of the buds for plants growing naturally at Corvallis, Oregon.

The growth stages during the quiescent phase of dormancy, 315 to 360°GS, were also determined by regrowth tests in response to standard temperature conditions (Kobayashi 1982). Throughout this period bud development increased when temperatures increased from
5 to 20°C. The rate of bud development increased with increasing plant development at all temperatures tested. The days required to promote growth at each growth stage was converted to °GS according to the equation:

\[ \text{°GS} = 45(D_{ch} - D)/D_{ch} + 315 \]

where °GS is degree growth stage; 45 is the total °GS required from end of rest (315°GS) to spring bud break (360°GS); Dch is days to bud break at end of rest; D is days to bud break; and 315 is °GS at the end of rest. Using bi-hourly temperatures, a mathematical model was proposed to predict development during the quiescent period and quite accurately predicted the beginning of spring bud break by three days and the °GS between 315°GS and 360°GS for plants growing outdoors at Corvallis, Oregon (Kobayashi et al., 1982).

Growth models of the annual cycle of temperate plants have been developed by others (Brown 1960, Smith and Kefford 1964, Sarvas 1972, 1974, Brierley and Landon 1946, Tompkins 1965, Aron 1974, Richardson et al. 1974, Gilreath and Lessman 1981, Wang 1963) but these models all suffer from one or more shortcomings. First, the models are not based on physiological processes; second, the models do not divide major periods into specific segments (°GS); and third, some of the models do not identify specific point events and, therefore, the starting and ending points are not known and are subjective.
Kobayashi et al. (1983) found a close relationship between rest development and the development of freezing stress in red-osier dogwood. Both cold acclimation and deacclimation were directly related to the °G, and temperature. Hardiness levels generally increased with decreasing temperature, and the rate of acclimation changed with °G. From 180 to 270 °G, the rate of acclimation increased to maximum at 270 °G and the plants did not deacclimate even at 20 °C. During the post-rest period (270-315 °G) the rate of acclimation decreases progressively, and the rate of deacclimation increased with increasing temperature. At the quiescent period (315-360 °G), the rate of acclimation continued to decrease, and only low temperature (less than 5 °C) is capable of promoting acclimation. In contrast, the rate of deacclimation increases at later growth stages. From these studies it can be concluded that from 270 to 360 °G the effective temperature range for acclimation decreases (e.g. more effective at lower temperature) while the range for deacclimation increases (Fuchigami 1982).

Methods of Overcoming Dormancy

Environmental Factors

In temperate woody perennials, rest is overcome by exposure to low temperatures (Brown et al. 1967, Doorenbos 1953, Nienstaedt 1967, Samish 1954, Wareing 1969, Weinberger 1950). The chilling requirement for breaking rest is dependent on the genetic makeup
of the plant (Bennett 1950, Erez et al. 1979, Doorenbos 1953, Gurdian and Biggs 1964, Nooden and Weber 1978, Nienstaedt 1966, Samish 1954) and possibly on the environment of the preceding season (Perry 1971). Westwood (1978) contends that the chilling requirement is an adaptive feature and depends on the plants native origin. Foresters have also found this to be true and their seedling reforestation practices depend upon seed origin. Generally, plants native to low latitudes and warm winters have shorter chilling requirements than those of higher latitudes away from bodies of water which have long cold winters (Westwood 1978). Plants from middle temperate regions with fluctuating warm and cold winter temperatures usually have longer chilling requirements to ensure the maintenance of cold hardiness throughout the freezing period (Westwood 1978).

Satisfaction of the chilling requirement in a resting plant is expressed by a rapid enhancement of bud growth (Eady and Eaton 1969, 1972, Hoyle 1960, Thompson et al. 1975). In red-osier dogwood, Kobayashi et al. (1982) found that the onset of bud growth for plants placed in a LD, warm temperature environment is a useful measure of the degree of rest and chilling.

Resting plants whose chilling requirement had not been satisfied grew slowly and poorly depending on the amount of chilling received (Kobayashi 1983). Coville (1921) found that trees and shrubs native to temperate regions remained dormant for as long as a year when placed in a continually warm environment. In addition
to delayed bud break, another major problem in inadequately chilled plants is the extent and position of bud break occurrence. Typically basal buds begin to grow while the rest of the buds remain dormant. The behavior of tropical tree species, e.g. mango (Mangifera indica L.), macadamia (Macadamia ternifolia F. Muell.), and litchi (Litchi chinensis Sonn) also behave like inadequately chilled temperate plants where parts of the same tree may be both dormant and actively growing simultaneously. Varied bud growth in plants can be explained by the difference in the bud development on a plant. Chilling temperature appears to play a key role in synchronizing the development of the buds on a plant.

The effective temperature range for chilling satisfaction appears to vary with different plants. Generally, low temperatures promote rest release (Lyr et al. 1970, Lamb 1948, Wareing 1969, Westwood 1978, Olmsted 1951). Bennett (1950) reported that temperatures between 0 and 7.2°C will break rest in numerous plants within two to three months. Sarvas (1972, 1974) reported that a range of -3.5 to 10°C was important for breaking rest in Betula pubescens. Samish (1950) suggested that below a threshold value, all temperatures will have a similar effect on breaking rest.

The optimum temperature for breaking rest also varies among plants. In most reported cases, 5°C, is thought to be the optimum temperature (Campbell and Sugano 1975, Erez 1971, Perry 1971, Timmis et al. 1981). Sarvas (1972) reported that 3.5°C was the
optimum temperature for breaking rest in _Betula pubescens_. In peaches (Erez 1971) and pear (Westwood and Bjornstad 1968) however, the optimum temperature was found to be 6°C and between 7 and 10°C, respectively.

Freezing and subfreezing temperatures in overcoming rest are not as effective as are temperatures just above freezing (Coville 1921, Nienstaedt 1967, Perry 1971, Nooden and Weber 1978, Olmsted 1951, Westwood and Bjornstad 1968, Westwood 1978, Samish and Lavee 1962, Sarvas 1972). Others claim that in the same species, temperatures below 0°C are not essential to rest breaking (Weinberger 1954, Brown 1960). Still others have found that subfreezing temperatures are quite effective in overcoming rest (Lyr et al. 1970). In some species, subfreezing temperatures greatly hasten the release from rest (Olmsted 1951, Samish and Lavee 1962). In pears, Sparks (1976) found that exposing plants to freezing temperatures between -4 and -13°C for one night caused an enhancement of bud break with decreasing temperature. Olmsted (1951) also reported that exposure of sugar maple to -8 and -12°C for 23 hours accelerated bud break by two weeks.

Warm temperature is not as effective as low temperature in overcoming rest (Perry 1971, Bennett 1950, Nichols et al. 1974, Erez 1971). Both Perry (1971) and Sarvas (1974) found that temperatures above 10°C were not effective in satisfying the chilling requirement. Others (Erez 1971, Nichols et al. 1974) have shown that temperatures above 10°C are effective. Erez and
Lavee (1971) reported that 10°C is only half as effective as 6°C in releasing rest in peach bud. In red-osier dogwood, Kobayashi et al. (1983) reported that temperatures up to 20°C were effective in meeting the chilling requirement.

Very high temperatures have been reported to overcome dormancy. A useful laboratory practice involves dipping leafless shrubs or cut branches into 30 to 40°C water for about 12 hours (Molisch 1908). Steam of the same temperature had a similar effect (Boresch 1924). Pouget (1963) reported that exposing grape cuttings to 50°C immediately broke dormancy. It is interesting that both high and subfreezing temperatures are capable of breaking rest. In both situations the dosage is of short duration and the response is relatively quick.

Fluctuating temperatures seem to be more effective than constant temperature for breaking rest (Lyr et al. 1977, Samish and Lavee 1962). In peach, gradual daily fluctuations between 6 and 14°C were more effective in breaking rest than either temperature held constant (Samish 1962). A daily cycle of 16 hours at 6°C and eight hours at 15°C was more effective than either temperature alone in breaking peach dormancy (Erez et al. 1979). In contrast, Sarvas (1974) found no difference in rest breaking by either fluctuating or constant temperatures.

Chilling negation or increasing the chilling requirement can occur during exposure to warm temperatures (Erez 1971, Vegis 1963). Vegis (1963) found that resting buds of Hyoscyamus can be
induced into secondary dormancy by exposure to temperatures either above or below an optimum range, thereby requiring additional chilling temperature to overcome rest. Weinberger (1954) also reported that during warm winters, more chilling temperature is required to overcome rest in peach. Similar conclusions have been found for *Liquidambar styraciflua* (Farmer 1968) and blueberry (Mainland et al. 1977). Nienstaedt (1966) reported that in a few North American forest tree species, warm temperature interruption can counteract the previous chilling temperatures. Erez and Lavee (1971) contend that the high temperature must occur within a few days after the chilling period to cause chilling negation. In time, a fixation process prevents the reversal of the chilling response.

The effects of high temperature on chilling negation depend on the stage of rest development and the temperature. Vegis (1963, 1964) reported that the temperature range for induction of secondary dormancy decreases progressively during the post-dormancy period. Romberger (1963) stated that warm temperatures applied during the early part of dormancy can cause chilling negation. In peach, warm temperatures during November and December counteracted chilling (Weinberger 1967). Above normal temperatures in December and January appear to be more effective in negating chilling than in November.

Even short periods of high temperature during a daily cycle can negate chilling. Erez et al. (1979) reported that exposure of
dormant peach to ten hours of 6°C or only six hours at 21 or 24°C causes chilling negation. As stated previously, the time of high temperature treatment following chilling appears to be critical. Thompson et al. (1975) also found that greater chilling negation occurs when interruption by high temperature occurs every two days rather than every four days.

Chemical Breaking Agents

Correlative Inhibition: Overcoming bud dormancy during summer dormancy (Doorenbos 1953) or correlative inhibition has been reported by several researchers. Hillman (1984) reported that fourteen treatments can overcome correlative inhibition of the lateral buds. In addition, five inhibitors of IAA transport have also effectively caused a release of correlative bud inhibition. These studies suggest possible interaction between auxin and ethylene in overcoming correlative bud inhibition (Hillman 1979).

Shigeura et al. (1975) reported that urea application to guava trees is effective in breaking correlative bud inhibition. Plants were first defoliated with urea. Within a few days, large numbers of axillary buds developed. This procedure is currently used by industry to successfully cycle and synchronize the production of guava fruits.

In a similar way, the forced production of apples has been successful in the tropics (Janick 1974). Plants are defoliated a few weeks after the harvest and before rest can occur in order to
overcome correlative bud inhibition and promote axillary floral and vegetative bud development. In contrast to guava, to be successful with apple, flower buds must already be formed at the time of defoliation. The same procedure has been successfully used to produce other fruit (Nee 1981, Weng et al. 1980).

Rest: There are numerous chemicals which break rest in temperate woody perennials (Doorenbos 1953). Possibly one of the earliest substances used in the field was mineral oils developed by Black (1936) in the mid-thirties. A year later, 1937, Chandler et al. found that the addition of dinitro cyclo hexylphenol, a respiration uncoupler, to mineral oil was considerably more effective in overcoming rest. This was later replaced by dinitro-ortho-cresol (DNOC) which is still used extensively (Jeffrey 1951). An extensive review of the work on oil-DNOC to break rest was done by Erez and Lavee (1974).

The narrow range oils (NRO), which were less phytotoxic than the winter oils and were introduced into the United States for pest control, were also used in breaking rest. Unfortunately, the NRO-DNOC combination showed no difference in phytotoxicity and caused significant yield reductions in Israel (Erez 1974). Alteration of the NRO:DNOC ratio (i.e. high NRO to low DNOC) could still overcome dormancy without risking yield. In addition, the promotion of vegetative bud break resulted in greater number of spurs, and thus greater fruit production in later years. The
phytotoxicity of this mixture appeared to relate to its concentra-
tion at time of application and to environmental conditions. Erez
and Lavee also substituted another uncoupler, dinitro-secondary-
buty-phenol (DNSBP), for DNOC and found it to be better than DNOC
for breaking rest.

Another uncoupling agent, pentachlorophenol (0.4%) in combi-
nation with lime-sulfur (5%), was found to be effective in over-
coming rest in oriental pears (Weng 1980). Use of this combina-
tion enabled the researchers to synchronize the flowering of
'Shinseiki' pear (a high chilling variety) and 'Heng-shan' pear (a
low chilling variety) at the same time. Normally, about two
months difference in flowering time occurs between both varieties.

The time of application is a critical factor in the success-
ful use of rest breaking chemicals such as DNOC-mineral oil mix-
tures. Application of DNOC-NRO mixture was best made close to
normal bud opening (Samish 1954). In South Africa, Strydon and
Skinner (1965) found that too late application of DNOC-mineral
oils on apple and pear trees caused reduced yields and higher
concentrations caused tissue death. Pavia and Robitaille (1978)
found that the effect of DNOC-GA₃ mixtures depended on the stage
of bud rest. On September 23, DNOC alone overcame rest and the
effect was amplified by addition of GA₃. At a later stage,
October 9 and 25, the only effective treatment was a combination
of DNOC and GA₃. None of the treatments were effective at maximum
rest. By mid-January, when rest was reduced, GA₃ alone or in combination with DNOC improved bud break.

A large number of nitrogen containing compounds are effective in breaking rest in plants. Since 1914, ammonia, potassium, and sodium nitrate have been known to break rest (Doorenbos 1953). Cracker and Barton (1957) found that potassium nitrate and thiourea broke seed dormancy. Blommaert (1965) reported that thiourea was effective in breaking rest in peaches.

As with uncoupling agents, combining nitrogen compounds with other compounds was more effective in breaking rest. Combining thiourea, potassium nitrate, oil, and DNOC emulsion was better than any lone treatment in breaking rest in 'Golden Delicious' apple in Brazil (Erez and Lavee 1974). In pecan, a combination of thiourea and potassium nitrate applied on two different days in September caused enhanced bud break in the early treatment only. In contrast to these findings, Shivashankava and Gowda (1980) obtained significant enhancement in bud break in grape with thiourea application, while Blaine and Allan (1979) obtained less bud break with a combination of thiourea and GA.

Because of the effectiveness of nitrogen compounds in breaking rest, others have been prompted to test other fertilizer compounds to break rest. In Peru, Puiggros, Franciosi and Solas (1982) used numerous fertilizer sprays to break rest in apple and found urea at 0.5 to 1.0% to be the most effective. Kuroi et al. (1963) reported that calcium cyanamide, patented in 1910 for use
as a fertilizer (Pranke 1913. "Cyanamide-Manufacture, Chemistry and Uses" The Chemical Publishing Co., Easton, Pa.), terminated rest in *Vitis vinifera* when applied as an aqueous suspension immediately after winter pruning. Similar results were obtained by others (Pereira 1978). Calcium cyanamide treatment also overcame rest in grapevine cuttings (Iwasaki and Weaver 1977). Morimoto and Kumashiro (1978) also found that winter application of calcium cyanamide broke rest in apple, pear, peach, and grapevine. In another study, they found that the effective compounds from a mixture of thiourea, potassium nitrate, calcium cyanamide, and Merit to break rest was calcium cyanamide.

The effective concentration of calcium cyanamide to break bud appears to vary with date of treatment and the plant genotype. Iwasaki (1980) reported that bud break in grape was induced with 20% calcium cyanamide when applied between August 30 and November 1. In Brazil, Miele et al. (1982) applied several concentrations of calcium cyanamide to 'Cabernet' grape and found that 30% gave the best results. Yang et al. (1982) found that 20% calcium cyanamide sprays, either singly or with Merit, enhanced bud break and reduced the tendency to develop apical dominance.

Calcium cyanamide ($\text{CaCN}_2$) in the presence of $\text{CO}_2$ and acidification (Macadam 1965) produce free hydrogen cyanamide ($\text{H}_2\text{CN}_2$). To test whether hydrogen cyanamide was the effective compound in breaking rest, Shulman et al. (1983) and Lin et al. (1983, 1984, 1985) independently treated grapevine with hydrogen cyanamide to
determine its effect on overcoming rest. Shulman et al. (1983) found that both calcium cyanamide and hydrogen cyanamide were effective at 0.25-1.25 M in overcoming rest in intact buds of grapevines. Hydrogen cyanamide was considerably more potent than calcium cyanamide. They suggested that calcium cyanamide undergoes partial hydrolysis to hydrogen cyanamide and cyanamide ions may be the active form of the chemical. In a similar study, Lin (1983) reported that hydrogen cyanamide was also effective in breaking rest in grape buds and intact grapevine plants. Crop yield from two applications of hydrogen cyanamide was larger than the controls and the fruits contained higher total soluble solids and titratable acids.

Like calcium cyanamide, the effectiveness of hydrogen cyanamide to overcome rest was dependent on the time of application and concentration (Bracho 1984). In California, Bracho et al. (1984) applied 1.25-6% hydrogen cyanamide to 'Cabernet Sauvignon.' The 28 May treatment date proved less effective than the 3 March treatment in promoting bud break. Hydrogen cyanamide was found to induce earlier bud break without increasing plant vigor. The higher concentration of hydrogen cyanamide caused a marked delay in bud break. Jensen and Bettiga (1984) reported that hydrogen cyanamide delayed and reduced bud break in 'Thompson Seedless' grapevine when applied on 2 March, only a week before normal budbreak. Others have also reported that the timing of hydrogen
cyanamide application is important in influencing bud break (Whiting 1984, Luvisi 1984, and Williams and Smith 1984).

At a recent international meeting held at Davis, California, on the "Potential and practical uses of cyanamide on grapevines," Wicks et al. (1984) concluded that hydrogen cyanamide will become a useful management tool in worldwide viticulture. In grapevines, hydrogen cyanamide has been shown to be effective in overcoming rest in the tropics, low desert regions, subtropics, and other world regions lacking adequate chilling temperatures for overcoming rest. In temperate and other regions, improving uniformity and timing of bud break could prove beneficial.

Hydrogen cyanamide applications are not limited to grapevines. Lavee et al. (1984) showed that "SK 83010," a commercial formulation of hydrogen cyanamide, was effective in breaking rest in various deciduous tree species including peach, almond, pecan, cherry, kiwi, etc. In warm regions, Lavee showed that somewhat higher concentrations of hydrogen cyanamide are needed for optimal enhancement of bud break.

In addition to mineral oils, uncoupler agents, and nitrogen-containing compounds, there are many other compounds which have been reported to overcome rest (Clutter 1978, Dutton 1924, Erez 1985, Doorenbos 1953). In general, these include:

1. Anesthetic-like compounds (Hosoki 1985), i.e. chloroform, ether, ethanol, acetone, methanol, formaldehyde, acetaldehyde, acetylene, etc. (Doorenbos 1953);
2. Toxic compounds, i.e. hydrogen peroxide, hydrogen cyanide, chloroethanol, strychnine nitrate, etc.;
3. Acids, i.e. acetic acid, hydrochloric acid, oxalic acid, sulfuric acid, tartaric acid, organic acids in general, etc.;
4. Bases, i.e. potassium hydroxide, etc.;
5. Salts, i.e. manganese salts, zinc sulfate, sodium nitrate, potassium chloride, copper sulfate, potassium nitrate, etc.;
6. Growth regulators, i.e. ethylene, IAA, IBA, NAA, GA, BAP;
7. Sulfur-containing compounds (Hosoki 1986), i.e. allyl sulfide, methyl disulfide, n-propyl disulfide, allyl isothiocyanate, M-propyl sulfide, glutathione, H₂S, SO₂, copper sulfate, sulfuric acid, thiocresol, methyl mercaptan.

Mechanism of Overcoming Rest

Doorenbos (1953) found that when one explores the methods reported to overcome rest, "one is confronted by a baffling variety of methods, that does more to complicate than clarify the problem. It seems hardly possible that cold, heat, light, injury, anesthetics, toxic substances, acids, and salts can all have the same effect." The nature of these treatments is not well understood and several authors have tried to explain this in one comprehensive theory involving the production of necro-hormones induced by the injury to tissue from the dormancy breaking treatment (Stuart 1910, Weber 1922a, 1922b, Dostal 1942, Erez and Lavee
1974). This hypothesis is quite attractive. However, there is no direct evidence that rest breaking agents damage the tissues or that necro-hormones stimulate the breaking of rest.

During the rest period the respiration rate is quite low (Samish 1954). Cold treatment and other dormancy breaking treatments have been reported to cause a rapid rise in respiration (Bachelard and Wightmann 1973, Hosoki 1984a, b) and this was thought to be directly involved in breaking rest. Further studies showed that although some treatments stimulated respiration, some did not (Doorenbos 1953). More recent studies have shown that an increase in respiration per se is not important, instead the stimulation of either the pentose phosphate pathway or cyanide resistant respiratory pathway is more important (Gosling 1980, Kikuta et al. 1971, Gassner 1925). The direct relationship of respiratory pathway and breaking rest is still not understood.

The effects of dormancy breaking agents have also been related to affecting enzymatic activity (Denny and Miller 1932). As early as 1915, Howard reported that in dormant apple twigs the oxidase and various hydrolases were stimulated by either injury, warm water bath, ether, or alcohol, etc. Coville (1921) reported that because starch was metabolized to sugar, amylase activity was stimulated. Later studies showed that the breakdown of starch to sugar was not important (Denny and Miller 1932). Lilac buds treated with ethylene chlorohydrin produced large increases in catalase and moderate increases in invertase instead of producing
amylase. In contrast, more recent studies by Nir et al. (1984) reported that the mechanism of action of dormancy breaking agents is via a reduction rather than an increase in catalase activity. They found that in grapevines, the activity of catalase in vivo increases markedly in autumn when rest is increasing and it begins to decrease toward the end of the rest period. Similar reduction in catalase activity was found when hydrogen cyanamide and thiourea were used to break rest. Other less effective rest breaking agents, such as DNOC, have no effect on catalase. Nir et al. (1984) suggested that the reduction in catalase activity caused an increase in hydrogen peroxide thus favoring the condition for the pentose phosphate pathway.

The action of dormancy breaking agents has also been related to an increase in membrane permeability. Coville (1921) was the first to suggest that chilling caused certain cell membranes to become more permeable. Taylorson and Hendricks (1979, 1980) working on seed dormancy, stated that dormancy is related to membrane function, including bud dormancy. They concluded that the anesthetic action in breaking seed dormancy and bud dormancy is due to membrane-modifying characteristics. The effective concentration is thought to distribute preferentially to lipid-solution interphase in cell membranes. Anesthetics, in general, have a dramatic effect on membranes (Richards 1978). Others (Atkins et al. 1984) believe that some anesthetics, i.e. ethanol and acetylide, may
serve as substrates for respiration rather than promote membrane permeability per se.

Hormones have also been associated with the breaking of rest. As early as 1935, Boysen and Jensen suggested that the onset of rest was due to a lack of auxin. It was later suggested by Zimmerman (1936) that sprouting buds contained auxin. Avery et al. (1937) found that dormant buds did not contain auxin. Although Boysen and Jensen could not force lilac stems with IAA solution, Amlong and Naundorf (1938) and others (Bennett and Skoog 1938, Borgstrom 1939) reported success in breaking rest by direct application of IAA and other compounds to the buds. In contrast, Gouwentak (1941) found that IAA only activated the cambium when the buds were no longer in rest and concluded that IAA had no effect on breaking rest. Similar results have also been found by Luckwill and Whyte (1968) on apple.

In the 1940's researchers believed there was a dormancy breaking hormone (Richter and Krasnosselskaya 1945). By injecting resting plants with extracts from actively growing buds, they were able to force them into growth. The forcing was not as effective as hot water treatments. However, they claimed the effect was not due to injury.

Cytokinins and gibberellins have also been reported to be related to rest removal in woody plants. In correlative studies, Lavee (1973) reported that as plants overcome rest the rise in cytokinins and gibberellins preceded the increase in respiration
and was followed by an increase in auxins. Similarly, Grochowska et al. (1984) found that dormant pruning of apple trees was accompanied by increased cytokinins in the tissues in early spring followed by increases in auxins and gibberellins as cell division and cell expansion progressed. Cytokinin-like substances also increased between quiescence and bud break in Populus tremula (Engelbrecht 1971), Populus balsamifera (Domanski and Kozlowski 1968), Populus × Robusto (Hewett and Wareing 1975). Zeatin riboside are considered to be the major components of the cytokinin peak. Since these cytokinins can regulate cell division and differentiation (Wareing and Phillips 1970), bud growth may be initiated or sustained by cytokinins because ABA, IAA, and gibberellin-like compounds are relatively low during this time. Gibberellin levels also were relatively low 27 days prior to budbreak, when ABA and IAA were relatively high. Their maximum occurred about 7-11 days after the peak in cytokinin levels and was associated with bud swelling. Levels were still high at budbreak, but very low immediately thereafter (Wood 1983). Exogenous application of cytokinins has also been effective in overcoming rest in apple (Shaltout and Unrath 1983), peaches (Erez and Lavee 1971), citrus (Nauer and Boswell 1981), and crabapple (Broome and Zimmerman 1976). Gibberellins, however, were not effective in breaking the rest of apples (Hatch and Walker 1969, Walker 1970) or peaches (Hull and Lewis 1959). In apples, combining GA and BA was similar to the effectiveness of BA alone in breaking rest (Shaltout and
Unrath 1983). In contrast to the increases in growth promoters, the levels of growth inhibitors, i.e. ABA, have been shown to increase prior to and during rest development and decrease as rest is broken (Wood 1983). Although good correlations of cytokinins, gibberellins, auxins, and ABA have been associated with rest, there is no direct evidence that they play a central role in overcoming rest.

Ethylene is another hormone that has been related to rest development and rest removal. Seibel et al. (1978) reported a good relationship between ethylene level and rest in red-osier dogwood. During active growth the level of ethylene production is high and during rest it is low. In 1927, Vacha and Harvey reported that ethylene and propylene were effective in overcoming rest in tubers, bulbs, seeds, and cuttings of apple, plum, cherry, willow, red osier, lilac, alpine current, grape, cottonwood, cranberry, mock orange, and pear. More recently, others have also reported that ethylene can overcome rest in corms, bulbs (Hosoki 1984), seeds (Sinska 1984, Fu and Yang 1983, Adkins 1981), and cuttings (Paiva 1978, Vacha et al. 1927).

Wang et al. (1985) showed that the levels of 1-amino-cyclopropane-1-carboxylic acid (ACC), a precursor of ethylene synthesis, increased during the transition from dormancy to the active state in Prunus avium and Prunus serrulata. An inhibitor of ethylene biosynthesis, aminoethoxyvinylglycine (AVG) was found to delay bud break in blueberry (Dekazos 1979) and peaches
(personal communication, Carlos Crisosto). The role of ethylene in overcoming rest is not known.

In contrast to these findings, Paiva and Robitaille (1978) and Zimmerman et al. (1977) found no evidence that ethylene was involved in emergence from bud dormancy in 'Golden Delicious' apple and tea crabapple.

Polyamines have been described as growth promoters in plants and it was suggested that polyamines may act as a secondary message to the stimulation and regulation of nucleic acid and proteins (Bagni et al. 1980 and Galsch et al. 1980). Dormant Helianthus tuberosus was found to contain only trace amounts of polyamine but 10 to 20-fold increases occur upon dormancy removal (Bagni et al. 1980). Polyamines also increased rapidly during the breaking of dormancy and initiation of sprouting of potato tuber (Kaur-Sawhney et al. 1982). Polyamines were also found to increase flower bud formation, fruit-set, and yield in apple (Costa and Bag 1983, Costa et al. 1984). Wang et al. (1985) found that polyamines increased steadily during the transition from dormancy to active growth and following breaking dormancy by thidiazuron. The role of polyamines may be associated with the increased synthesis of DNA, RNA, and protein associated with rest removal (Wang 1985).

In reviewing the works of others on mechanisms of overcoming rest in plants, a major frustration arises in deciphering whether rest, quiescence, or correlative inhibition was being studied.
None of the previous studies related dormancy breaking results to specific stages of plant development. Because of this, some of the conflicting reports found in the literature may be due to a lack of understanding of the physiological stages of the plant.

**Hydrogen Cyanamide: Property and Metabolism**

Calcium cyanamide had its origin in the production of nitrogen fertilizer. It was produced by heating calcium carbide with nitrogen. The process was patented by Adolph Frank and N. Caro in 1898. Calcium cyanamide was later patented as a fertilizer in 1910, used as a herbicide before 1913, reported to defoliate cotton in 1938, and used as a defoliant for cotton, soybeans, and nursery stock in 1945. It was first reported to overcome rest of grapevine in 1963 by Kuroi *et al.*

Commercial calcium cyanamide, 65% CaCN₂, is also known by the trade name Cyanamid and lime nitrogen or L/N in the United States. With cold water, calcium cyanamide (CaCN₂) undergoes partial hydrolysis to the soluble hydrogen cyanamide (H₂CN₂) by the following reaction:

\[
2 \text{CaNCN} + 2 \text{H}_2\text{O} \rightarrow \text{Ca(HNCN)}_2 + \text{Ca(OH)}_2
\]

\[
\text{Ca(HNCN)}_2 \rightarrow \text{Ca}^{+2} + 2 \text{HNCN}^{-}
\]

Application of aqueous solutions of CaCN₂ produces free cyanamide. Depending on pH, further reaction of H₂CN₂ to urea or dicyandiamide may be synthesized. In moist soils, H₂CN₂ is the first product but is further metabolized to urea, ammonia, and
nitrate (Amberger 1984). Although this is the main route, a secondary pathway is possible yielding guanidine (Rathsack 1977, Amberger 1989).

In animals, cyanamide, in the form of its citrated calcium salt (trade names: Temposil, Dipsan, Abstem) or as a 1% aqueous solution, is used as an alcohol deterrent agent in the treatment of alcoholism in Canada, Europe, and Japan (Shirota et al. 1984). Its effect is similar to disulfiram (trade name: Antabuse).

Generally, these alcohol aversion agents block the metabolism of acetaldehyde, the product of alcohol (ethanol) oxidation, thereby triggering an unpleasant physiological reaction in animals and man. In vivo cyanamide is an inhibitor of aldehyde dehydrogenase isozyme causing the accumulation of acetaldehyde. Cyanamide, however, does not inhibit purified preparations of ALDH in vitro, suggesting that a metabolite of cyanamide is the active inhibitor of the enzyme (DeMaster et al. 1982). DeMaster et al. (1984) reported that the active inhibitor is catalyzed by a cyanamide-activating system present in liver mitochondria. Kitson and Crow (1979) suggested that the active inhibitor might be due to the products of cyanamide, amines, or thiols. Thiourea, a dormancy-breaking agent in plants, is a possible by-product of cyanamide containing thiols in animals. Shirota et al. (1984) and DeMaster et al. (1984) suggested that the inhibition of aldehyde dehydrogenase is dependent on catalase. Shirota et al. (1984) also reported that cyanamide irreversibly inhibits catalase by binding
to the apoproteins of catalase in a process requiring $H_2O_2$. In addition dimethylcyanamide, a product of cyanamide, caused a marked elevation in ethanol-derived acetaldehyde coincident with the depletion of hepatic glutathione levels. In disulfiram, its inhibition of aldehyde dehydrogenase may be overcome by sulfhydryl amino acids (Nagasawa 1977).

In plants, cyanamide can be readily taken up by the roots and above-ground plant parts (Amberger 1984) and transported in the vascular system in both directions. Cyanamide is metabolized, complexed, or destroyed in plant tissues. Miller and Hall (1963) reported that urea was the first product in cotton plant tissues. Within eight hours, no cyanamide was found in all tissues tested. They suggested that cyanamide may have been utilized for growth and did not produce dicyandiamide, azide, or cyanide. Hofmann et al. (1954) reported that in oats, barley, wheat, and rye cyanamide was converted to dicyanamide within two to three days and alanine, tryptophan, lysine, leucine, phenylalanine, and valine were produced at high levels. Rathrach (1955) also reported that cyanamide produced dicyandiamide, guanidine, and guonyl cyanide. Recently, Amberger (1984) reported that cyanamide stimulated the production of high levels of arginine. This synthesis is thought to be via guanino compounds as well as by inhibition of arginine activity. As with animals, cyanamide causes a decrease in catalase activity (Amberger 1984, Nir 1984). Amberger (1984) suggests that cyanide and cyanamide contain the very reactive cyanide group.
which reacts with the enzyme -Fe of catalase and promotes mitochondrial respiration (Taylorson and Hendricks 1977, Shulman et al. 1982). The promotive effects of cyanide or cyanamide on respiration is explained as favoring a shift from the Embden-Meyerhoff-Parmas (EMP) pathway to the pentose phosphate (PP) pathway leading to an increase in reduced nucleotide production essential for intensified metabolism. Catalase inhibition by cyanamide also causes an accumulation of H₂O₂ which is toxic to plant cells (Hendricks and Taylorson 1975). The resulting increase in H₂O₂ is also known to favor (PP) (Nir 1984) and higher levels of peroxide and glutathione. All these processes are thought to be involved in the control of rest either directly or through an increased activity of peroxide, specific oxidases, or ascorbate oxidase closely associated with the glutathione redox system (Amberger 1984). Amberger (1984) also noted that the reason for the high levels of arginine in cyanamide treated plants was due to the interaction of cyanamide with cysteine of the oxidized glutathione. Cyanamide's method of action remains unknown, however, Glaubach (1926) suggests that cyanamide may react with the sulfur group of glutathione. The relationship of arginine to rest-breaking mechanism was not speculated by Amberger (1984), but it is of particular interest because of its role as a precursor to polyamine biosynthesis (Smith, 1985).
Determination of Sublethal Stress

As stated previously, there is no specific rest-breaking agent. Because of the phytotoxic nature of many rest-breaking treatments, many researchers have stated that any treatment that is applied at sublethal dosages can break rest (Erez and Lavee 1974). Until now, however, proof of this hypothesis has not been possible primarily because it has been difficult to differentiate sublethal stress from death. Viability tests used to determine stress resistance are useful in determining whether or not a tissue is injured; however, they cannot measure sublethal stress (where tissue is not visibly injured). Nissila (MS thesis), Kobayashi et al. (1982), and Harber et al. (1986) found a procedure to differentiate between sublethal stress and lethal stress. They found that tissues exposed to sublethal stress produce high levels of ethylene that are directly correlated to the degree of stress. As soon as cell damage became evident, as measured by electrolyte leakage, the level of ethylene decreased in direct proportion to the increase in electrolyte leakage. In addition, they noted an increase in ethane gas which correlated positively with the electrolyte leakage and negatively with the drop in ethylene.

The Roles of Thiols in Sublethal Stress

Levitt (1980) suggested that the various types of injury caused by radiation (growth disturbance and metabolic disruption) are due to the effects of the initial products produced by the
cellular constituents in response to ionizing radiation. The best clue as to the nature of at least some of these initial damaging products is from observation of the chemical nature of the radioprotectants that prevent or reduce injurious effects. Levitt (1980) found the most successful groups of radioprotectants to be the thiols. An understanding of their mode of protection should reveal the mechanism of at least one type of radiation injury.

Four main hypothesis have been proposed to explain the protective effects of the thiols (Eldjarn 1967):

1. Radical scavenging:
   
   Preventing cellular oxidation normally initiated by radiation. In addition, the scavenging of Cu or Fe ions have been suggested (Foye and Mickels 1965).

2. Repair of the ionized target molecules by H transfer:
   
   The small-molecular protective thiols form mixed disulfides with the target (protein) molecules by combining with the SH and SS groups of these larger molecules.

3. Anoxia:
   
   Whereby most of the early results of injury may be due to the oxidation of the thiols, leading to a reduced oxygen tension and decreased radiosensitivity (Dewey 1965).

4. Mixed disulfide formation:
   
   To test the latter theory, Pihl and Sanner (1963) investigated papain, the most radiosensitive enzyme studied, which contained only a single essential -SH group. By con-
verting the -SH group to a mixed disulfide with cysteamine, the enzyme became more resistant to the ionizing irradiation treatment.

Shapiro et al. (1966) suggested that the protein SH groups on the surface of the cell and inside the cell are the target for ionizing radiation damage. Levitt (1980), therefore, arrived at the following conclusions: 1) that the radiation-induced increase in cell permeability is a result of a loss of membrane SH; 2) that the loss of membrane SH is due to the oxidation of SH to SS groups; 3) that these SS groups form intermolecularly, inducing aggregation of membrane proteins; and 4) that the repair of the radiation-induced damage of the membrane is due to the reduction of these intermolecular SS groups to SH. Levitt (1980) has also related these conclusions to explain the mechanism of resistance and injury in the other stresses.

In heat stress, Lin et al. (1985) found that the electrolyte leakage caused by lethal stress in soybean seedlings can be reduced to a minimum by pre-exposing the seedlings to 40°C. A 15 kD heat shock protein (HSP) was found to be induced at 40°C which is believed to protect the membrane proteins. Although SH groups were not implicated in these studies, recent studies of heat stress by Nieto-Sotelo et al. (1986) showed that heat stress causes an increase in glutathione concentration and a decrease in cysteine levels after only one hour of treatment. The synthesis of the HSP was also suppressed by elevated levels of glutathione.
CHAPTER 3

OVERCOMING REST AT DIFFERENT GROWTH STAGES WITH

WITH HYDROGEN CYANAMIDE

ABSTRACT

Hydrogen cyanamide was effective in overcoming rest in red-osier dogwood (Cornus sericea L.) and 'Radiant' crabapple (Malus floribunda Sieb.) at specific growth stages. The concentration of hydrogen cyanamide to overcome rest was dependent on the growth stage. Plants in maximum rest required the highest concentrations. During the quiescent period, hydrogen cyanamide at the higher concentration delayed bud break.

Although hydrogen cyanamide overcame rest, it did not stimulate stem elongation. Hydrogen cyanamide applied at the onset of rest (180°GS) to just beyond maximum rest (280°GS) produced short-spur type growth. Applications during the post rest phase (335°GS) had little or no effect on shoot elongation. These studies suggest that hydrogen cyanamide overcame bud rest but other unknown factors are required for stem elongation.

INTRODUCTION

Temperate fruit and nursery plants typically develop rest (winter dormancy) in response to short daylength. Plants in rest do not resume growth even when exposed to ideal growing environments. Under natural conditions, a chilling period of several
weeks is required to "break" rest. This limits the production of temperate perennials to areas which have adequate chilling temperatures. Chemical and/or physical treatments to overcome rest have been extensively studied (Doorenbos 1953, Erez and Lavee 1974). Studies have shown that numerous treatments are capable of overcoming rest. Doorenbos (1953) and Erez and Lavee (1974) hypothesized that there is no specific dormancy breaking agent. Treatments which cause sublethal stress can overcome rest.

Calcium cyanamide has been used as a fertilizer, herbicide, fungicide (Dannenhauer, 1923), a defoliant (Miller 1965, Hall and Harrell 1942) and recently as a dormancy breaking agent in grape (Kuroi, et al. 1963, Pereira and Oliveira 1978; Yeng, et al. 1982), raspberry (Snir 1983) and other deciduous trees (Morimoto and Kumashiro 1978). In the presence of water and carbon dioxide, calcium cyanamide rapidly forms hydrogen cyanamide. Recently, researchers found that hydrogen cyanamide is more effective than calcium cyanamide in overcoming dormancy (Lin 1983, 1985; Shulman et al. 1983; Bracho 1984).

Although effective as a dormancy breaking agent, the resultant effects of hydrogen cyanamide on plants is not predictable. In grapevines, variable results were obtained depending on the variety, timing of treatment, latitude and severity of weather. On the same variety, hydrogen cyanamide can either have no effect on bud development or promote, delay and kill buds depending on
the concentration and time of application (Lavee 1984; Bracho 1984; Whiting 1984).

The objective of this study was to evaluate and describe the effects of hydrogen cyanamide on overcoming rest at specific growth stages in 'Radiant' crabapple and red-osier dogwood; to test the hypothesis that the hydrogen cyanamide's dormancy breaking effectiveness is strongly dependent on the developmental stage of the plant. If the hypothesis proved correct the predictability of plant response could be greatly increased by proper timing of rest breaking treatment. The degree growth stage model (0°GS) developed by Fuchigami et al. (1982) for red-osier dogwood was used to characterize specific growth stages before and during the rest period.

The 0°GS model has been used to describe and quantify plant development (Kobayashi 1983) and has proven to be a good predictor of cold hardiness (Kobayashi et al. 1982). The model uses numerical units to describe developmental stages. Major phenological events of particular interest are: (1) 0°GS, Spring bud break, rapid plant growth in response to warming temperature; (2) 90°GS, maturity induction point, when plants become responsive to shortening daylength; (3) 180°GS, vegetative maturity, when rest, hardiness, and chilling requirements begin; (4) 270°GS, maximum rest, plants in deepest rest and still require chilling temperatures; (5) 315°GS, end of rest and onset of quiescence, plant
chilling requirement satisfied; (6) 360°GS, spring bud break, when growth begins indicating the start of another annual growth cycle.

MATERIALS AND METHODS

Single-node stem cuttings of a clone of red-osier dogwood (Cornus sericea L.) native to Wayland, Massachusetts were propagated in a greenhouse mistroom in the spring of 1985 in Corvallis, Oregon. Rooted cutting were transplanted into a 1:1:1 (V/V/V) soil:sand:bark mixture in 15 cm pots and grown in a lath-house (20% shade) exposed to natural conditions. Lath-house air temperature was recorded by a Weather Measure Corp. Model H311 hygro-thermograph placed in a instrument shelter located 1.1 m above the ground.

At two week intervals, starting 5 September 1985, 34 uniform plants were randomly selected from the lath-house. Six plants (3 were defoliated) were placed in a 20/15°C + 3°C day/night greenhouse under 16 hr photoperiod to test the growth stage. The day-length was extended from 0600 to 2200 hr with General Electric Lucalux LU400 lamps. Average luminous flux density at plant height was 36.4 Klx. Degree of rest (°GS) was determined by observing days to terminal vegetative bud break as previously described (Fuchigami et al. 1977). Bud break was based on the first appearance of green leaves. The other 28 plants were defoliated, the entire stem painted with either 0, 0.5, 1.0, or 2.0 M hydrogen cyanamide solution, and placed in a 20/15 °C ± 3 °C
day/night greenhouse under natural short day conditions during the winter. Time and percent of bud break was determined at the green-tip stage. Shoot length was determined at the end of the first flush of terminal bud growth.

A clone of 'Radiant' crabapple plants generated from tissue culture by Microplant Nursery, Gervais, OR, was also tested to determine the effect of hydrogen cyanamide on rest. The regenerated plants were grown in a 1:1:1 (V/V/V) soil:sand:bark mixture in 5 cm pots in a lath-house as described previously. The OGS was determined by placing 3 defoliated and 3 foliated plant at 2 weeks intervals beginning in 5 September, 1985 under the 16 hr. photo-period greenhouse environment. At the same dates and timed intervals as in the previous study of dogwood, 20 uniform plants were randomly selected, divided equally, and painted with either 0, 0.25, 0.5, or 1.0 M hydrogen cyanamide, and placed in the 20/15 °C + 3°C day/night greenhouse under natural light condition.

To compare the effects of hydrogen cyanamide and cytokinins on rest, 20 red-osier dogwood plants during the quiescent phase of development (330°GS) were randomly selected from the lath-house and placed in the 20/15 °C + 3 °C greenhouse under natural light condition. The plants were divided equally into 4 groups. The stems evenly painted with either H2O (control), 0.1 mM 6-benzylaminopurine (BA), 0.5 M hydrogen cyanamide or 1.0 M hydrogen cyanamide. The plants were checked daily for bud break.
Significant Studentized Ranges at the 5% and 1% level, new Multiple-Range Test (Duncan 1955), and standard deviation were used to analyze data.

RESULTS AND DISCUSSION

The effective concentrations of hydrogen cyanamide to enhance bud break was dependent on physiological status of the plants (Fig 3.1). Before rest development (160 GS), when dormant buds were correlative inhibited, hydrogen cyanamide did not promote bud break and was phytotoxic at concentration above 1 M. At the onset of rest, 180 GS, all concentrations of hydrogen cyanamide were effective in overcoming rest in dogwood (Fig. 3.1, 3.2). Bud break increased with increasing concentration (Fig. 3.2). From 200 GS to 270 GS, the rate (Fig. 3.1) and percent bud break (Fig. 3.2) increased with increasing concentrations. Thus, higher concentrations of hydrogen cyanamide were required to break rest as the intensity of the rest increased. During post-rest (280 to 310 GS), the concentrations of chemical required to stimulate bud release decreased progressively with advancing growth stage (Fig. 3.1, 3.2). During quiescence, after 315 GS, hydrogen cyanamide inhibited bud growth (Table 3.1, 3.2) or severely injured the quiescent buds and stems.

Although bud break could be induced at all growth stages with hydrogen cyanamide, the extent of shoot growth (stem elongation) was dependent on the physiological growth stage of the plant.
(Fig. 3.3). From the onset of dormancy (180°GS) to just beyond the maximum rest phase (280°GS), shoot elongation was short and spur-like with short internodes and a full complement of buds (data not shown). During the post rest phase and quiescent phase, (290 - 330°GS), stem elongation induced by hydrogen cyanamide was considerably greater than stem elongation induced at the earlier growth stages. Stem elongation induced during quiescence by hydrogen cyanamide was either slightly more or the same as the controls (Fig. 3.3). Applications of hydrogen cyanamide during the later phase of quiescence, after 330°GS, inhibited bud break and stem elongation (Fig. 3.3).

As previously reported (Nee, 1986), increasing concentrations of hydrogen cyanamide delayed bud break in red-osier dogwood during quiescence (Fig. 3.4). In contrast, the 6-benzylaminopurine stimulated buds to break earlier than the controls and the hydrogen cyanamide treatments.

The mechanism involved in overcoming rest may involve at least two steps: bud release and shoot elongation. Hydrogen cyanamide stimulates or triggers bud release but has little or no influence in stimulating shoot elongation. Although hydrogen cyanamide overcomes rest, it has little or no effect on the shoot elongation during rest (Fig. 3.1, 3.2, 3.3).

Chilling temperature stimulates the synthesis of substances essential for both bud break and shoot elongation. In the latter situation, chilling enhances elongation even after the chilling
requirement for bud break had been satisfied (Fig. 3.3). Similar results were obtained on 'Radiant' crabapple plants (results not shown). The mechanism of overcoming rest with hydrogen cyanamide is not known. In plants and animals hydrogen cyanamide is known to inhibit catalase activity (Nee 1986, DeMaster et al. 1984, Shirota et al. 1985, Nir 1984). Nir (1984) hypothesized that the inhibition of catalase causes an increase in hydrogen peroxide which stimulates the pentose phosphate pathway thereby overcoming dormancy. Chilling of dormant plants was also found to reduce the activity of catalase. In contrast to this, Guthrie (1931) and Nee (1986) reported that rest breaking chemicals stimulate catalase activity.

In a companion study (Nee 1986), hydrogen cyanamide was found to conjugate with the thiol groups of glutathione in a non-enzymatic system, and with the thiol groups in germinating lentil seeds. Chloroethanol, a rest breaking agent, was also found to bind with the thiol groups of glutathione (Johnson 1965). Because of the non-specific nature of rest breaking agents and because of their effects at sublethal dosages, it is hypothesized that the mechanism of action of rest breaking agents is via the conjugation of thiol groups. The reason hydrogen cyanamide reacts differently at the various growth stages may be related to the presence or absence of thiol-containing compounds such as glutathione and/or thiol containing protein such as catalase.
Glutathione is an effective detoxifying agent (Johnson 1965, Smith 1984, 1985) and the conjugation with hydrogen cyanamide suggests that glutathione may serve in detoxifying hydrogen cyanamide. The variable levels of glutathione or thiol/disulfide compound in plants at different growth stages (Guy et al. 1982), may explain the differences in hydrogen cyanamide induced phytotoxicity at various stages of plant development.

Another possible mode of action of the hydrogen cyanamide-glutathione conjugation in overcoming rest is a more direct one. Application of glutathione and yeast extracts (containing glutathione) have been shown by Guthrie in 1941 to overcome rest in several plant species. In addition, Hosoki (1986) recently showed that sulfur containing compounds are effective rest breaking agents. It is possible that the breakdown of the glutathione-hydrogen cyanamide conjugation to other sulfur containing compounds and/or to polyamine via synthesis of arginine from glutamic acid may be involved in overcoming rest. Amberger (1984) showed that hydrogen cyanamide is rapidly metabolized to arginine in several plants. Polyamines were also reported to increase at the termination of dormancy in apple plants under natural conditions, and following thiodiazuron-induced dormancy breaking (Wang et al. 1985).

These studies demonstrate why there is a variable shoot response to hydrogen cyanamide treatments. From the present results, hydrogen cyanamide can be used to reliably and predict-
ably break rest in buds between 180 and 315°GS. The concentration of hydrogen cyanamide should be adjusted depending on the growth stages. The variation of response can be greatly reduced by proper timing and concentration of treatment.
Fig. 3.1. The effects of hydrogen cyanamide on the time of bud break at different growth stages in red-osier dogwood (*Cornus sericea* L.).
Fig. 3.2. The effects of hydrogen cyanamide on percent bud break at various growth stages in red-osier dogwood.
Fig. 3.3. The effects of hydrogen cyanamide on shoot elongation at various growth stages in red-osier dogwood.
Fig. 3.4. Effects of hydrogen cyanamide and 6-benzylaminopurine on bud break (inhibition) during the quiescence stage.
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CHAPTER 4

THE PHYTOTOXICITY OF HYDROGEN CYANAMIDE DURING THE REST PERIOD AND ITS RELATIONSHIP TO OVERCOMING REST

ABSTRACT

The phytotoxicity of hydrogen cyanamide throughout the rest period in red-osier dogwood (*Cornus sericea* L.) and 'Radiant' crabapple (*Malus floribunda* Sieb.) was determined. Phytotoxicity to hydrogen cyanamide was dependent on the concentration and growth stage. Plants were most resistant to hydrogen cyanamide during rest development, between 180° to 280°GS and most susceptible before rest (160°GS) and during quiescence (315°-360°GS). Hydrogen cyanamide stimulated ethylene production when applied at sublethal dosages, and increased the leakage of electrolytes and specific ions at sublethal and lethal concentrations. High correlations between ethylene production and the stimulation of bud release during the rest period, 180 to 315°GS, were found. This provides direct evidence that sublethal dosages are important for overcoming rest. In contrast to this, during quiescence, high negative correlation between ethylene production and bud release were found indicating that hydrogen cyanamide at sublethal dosage were inhibiting to bud release. Sublethal freezing and high temperature treatments to resting 'Radiant' crabapple plants also confirmed the theory that sublethal stress overcomes rest.
Cellular damage caused by hydrogen cyanamide failed to stimulate ethane production. Alfalfa cell cultures treated with hydrogen cyanamide could be induced to produce ethane by removing the hydrogen cyanamide from the media. Ethane production in freeze damaged dogwood shoots was also inhibited by NEM, a binder of thiol groups and restored by treating the tissue with glutathione, a thiol containing compound. The relationship of sublethal stress as a mechanism of overcoming rest is discussed in this paper.

INTRODUCTION

Doorenbos (1953) reported in a review of bud dormancy that a great diversity of chemicals were capable of overcoming rest in woody plants. Since then other researchers in pursuit of a universal and effective rest breaking agent, have continued to find new ways of overcoming rest by either physical (Hosoki 1983, 1983a, 1984, Cathy 1956), chemical (Adkins 1984, Blommet 1965, Kepczynski 1977, Paiva 1978, Sińska 1984, Hosoki 1986, Kuroi 1963, Lavee 1984) or biological (Paiva 1978, Couvillon 1984) means. In 'Radiant' crabapple, a total of 9 chemicals and freezing and high temperatures treatments caused the breaking of bud rest (Nee 1986). The diversity of the factors that is capable of breaking rest suggests that the treatments per se is not the cause of overcoming rest. Instead, the causal agents seem to reside in the plant itself and may be stimulated by the rest breaking treatments. Several researchers (Doorenbos 1953, Erez and Lavee 1974,
Levitt 1980) suggested that there is no specific dormancy-breaking agent. Any treatment applied at sublethal dosages can overcome rest.

A major problem in the use of chemicals to break rest is the variable results obtained. At a recent meeting at Davis, California on the "Potential and practical uses of hydrogen cyanamide on grapevines" to overcome rest, this problem was expressed by several researchers (Bracho et al. 1984, Lavee et al. 1984, Luvisi 1984, Whiting and Coombe 1984, Williams and Smith 1984). Hydrogen cyanamide is capable of stimulating bud break, delaying bud break, having no effect on bud break, and causing bud and stem injury. Timing and environmental factors are speculated as the cause of this variability (Lavee 1984). Because of the uncertainty of knowing when and how much hydrogen cyanamide to use to overcome rest, the commercial usage of hydrogen cyanamide has been limited.

The mechanism of action of hydrogen cyanamide in overcoming rest is not well understood. Nir et al. (1984) believes that the inhibition of catalase activity by hydrogen cyanamide causes an increase in hydrogen peroxide which stimulate the pentose phosphate pathway thereby overcoming dormancy. The inhibition of catalase by hydrogen cyanamide was also reported in other plants (Amberger 1984) and in animals (DeMaster 1984, Shirota 1985). In contrast to this, however, others have found that rest breaking agents stimulate catalase activity (Guthrie 1931, Nee 1986).
In a companion study (Nee 1986), hydrogen cyanamide was found to bind with thiol groups. It is thought that the binding of thiol groups in proteins and other thiol containing compounds may have a bearing on the activity of hydrogen cyanamide. Glutathione, a thiol containing tripeptide found in large quantities in both animals (Johnson 1965) and plants (Rennenberg 1982, Smith 1984), conjugates nonenzymatically to hydrogen cyanamide in an in vitro system (Nee 1986). The presence, or absence, of glutathione in vivo may have a bearing on the effects of hydrogen cyanamide in overcoming rest and causing injury. Its role in regulating bud break, bud delay and phytotoxicity will be discussed.

Previous studies by Harber et al. (1986) and Kobayashi et al. (1981) have reported that sublethal stress imposed by freezing and dehydration, respectively, can be determined by measuring ethylene evolution. Tissue exposed to sublethal stress, prior to cell membrane damage, produces high levels of ethylene. With increasing membrane damage the amount of ethylene evolved decreases inversely with the increase in electrolyte leakage. In addition ethane production was found to correlate positively with the electrolyte leakage and is thought to be a good indicator of cell injury. In this study, the objectives were to determine the phytotoxicity of hydrogen cyanamide at various growth stages, the relationship of sublethal stress to overcoming rest and the relationship of thiol groups to ethane production.
METHODS AND MATERIALS

Single-node stem cuttings of a clone of red-osier dogwood native to Wayland, Massachusetts were propagated in a greenhouse mistroom in the spring of 1985 in Corvallis, Oregon. Rooted cuttings were transplanted into a 1:1:1 (by volume) soil:sand:bark mixture in 15 cm pots and grown in a lath-house exposed to natural conditions. Lath-house air temperature was recorded by a Weather Measure Corp. Model H311 hygrothermograph placed in a instrument shelter located 1.1 m above the ground.

At two week intervals starting 5 September 1985, 34 uniform plants were randomly selected from the lath-house. Six plants (three defoliated) were placed in a 20/15°C day/night greenhouse under 16 hr photoperiod (LD) to determine its dormancy status (Fuchigami 1983). The remaining plants were hand defoliated, divided into 4 equal groups. The entire stem from each group was painted with either distilled water, 0.5M, 1.0M or 2.0M of hydrogen cyanamide. The plants were then placed in a 20/15°C ± 3°C day/night greenhouse under natural short-day conditions. The percentage of stem die back, and lateral, terminal and serial bud growth was measured after treatments. The data were then compared with the bud dormancy status as measured by the time for regrowth in the LD environment.

To verify the results obtained on dogwood, a tissue cultured clone of 'Radiant' crabapple (Malus syvestris L.) plants produced by Microplant Nursery, Gervais, OR, were transplanted into a 1:1:1
(by volume) soil:sand:bark mixture in 5 cm pots and grown in the lath-house condition described previously. As with the dogwood, the growth stage GS was determined by placing 3 defoliated and 3 foliated plants at 2-week intervals beginning on 5 September 1985 under the 16 hr. photoperiod greenhouse environment and observing bud break as before. At the same dates and timed intervals, 20 uniform plants were randomly selected, divided equally, and painted with either 0, 0.25, 0.5, or 1.0 m hydrogen cyanamide, and placed in the 20/15°C + 3°C day/night greenhouse under natural light conditions.

**Ethylene, ethane and electrical conductivity measurement**

Twenty-four hours after the hydrogen cyanamide treatment, the stems of the dogwood plants were cut into 2 cm sections, weighed and placed in separate 20-ml vials and sealed with rubber serum stoppers. The vials were placed in the dark at 23°C for 24 hours. To determine ethylene and ethane levels a 1-ml sample of the gas was drawn from the vial and injected into a Carle 211 analytical gas chromatograph equipped with a 1.22 m x 3.8 mm 80/100 mesh activated alumina column and a flame ionization detector. The column and detector temperature was 80°C, and the flow rate was 20 ml/min.

Following the above test, each vial containing stems was filled with 15 ml of deionized distilled water and placed on a shaker for 24 hrs. Conductivity of the solution was measured with a Markson pH/conductivity meter, Model 604. The samples were then
killed at 80°C for 20 mins, shaken for 24 hrs, and total conductivity measured. Percent leakage was calculated by dividing the initial conductivity by the total conductivity and multiplying by 100.

**Sublethal freezing and high temperature study**

Thirty-six crabapple plants in rest were defoliated and frozen to -4, -7 or -10°C or heated to 42, 47 or 52°C for 1 hour. Three plants from each treatment were placed in a 21°C controlled environment room under 16 hr. photoperiod (4 Klx) and evaluated daily for growth and dieback. Stems from 3 plants per treatment were sectioned into 1 cm sections to determine ethylene and electrolyte leakage (Harber 1986).

**Specific ion leakage**

'Radiant' crabapple plants produced from tissue culture at Microplant Nursery, Gervais, OR, were transplanted to 5 cm plastic pots containing a 1:1:1 (by volume) of sand:soil:bark mixture in June 1985, and then grown in 20/15°C+3°C day/night greenhouse under natural daylength. On November 25, 1985, 20 uniform plants were randomly selected. Four plants each were painted with either 0, 0.25, 0.5, or 2.0 M hydrogen cyanamide. Forty eight hours after treatment, one gram (fresh weight) of internode stem section from each plant was placed in vials containing 15 mls of deionized distilled water and shaken for 24 hrs. Electrical conductivity of the solution was measured as before. The solutions were then
assayed for specific elements (Table 4.1) with a Jarrell Ash ICAP 9000.

**Ethane release**

Three studies were conducted to determine the effects of hydrogen cyanamide on ethane production. In the first study, 30 250 ml Erlenmeyer flasks of alfalfa (*Medicago sativa* L. (Seranac) cell suspension cultivars growing on B5 medium) were treated with 400 mM hydrogen cyanamide at 23°C ± 1°C. After 3 hrs. the media from 10 of the flasks were discarded, the cells rinsed with media minus hydrogen cyanamide, and then the cells were incubated in the media minus hydrogen cyanamide. Ethane production was measured as stated previously by sealing the flasks with serum stoppers after 0, 3, 6 and 9 hrs. following the hydrogen cyanamide treatment, incubating the samples in the dark for 3 hrs. and injecting a 1 ml sample into the gas chromatograph.

In the second study, 70 dormant sheets of "Twentieth Century" pear (*Pyrus pyrifolia* (Burmif.) Nakai) obtained from trees growing at the Lewis-Brown horticultural farm at Corvallis, OR, were painted with 50 mM hydrogen cyanamide. The cut ends of the stems were placed in 250 ml Erlenmeyer flasks containing distilled water. The stems were incubated at 23°C ± 1°C under 16 hr. day-length. Light intensity provided by cool white fluorescent tubes at the top of the branches was 237W/cm². Eighty hours after the hydrogen cyanamide application, 10 branches were completely painted with 20 mM glutathione, GSH, and re-incubated. Ethane
production was determined as before after 0, 20, 40, 60, 80, 100 and 120 hours following the hydrogen cyanamide treatment.

In the third study, maximum rest (270\textdegree GS), dogwood stems were cut into 1 mm sections. One gram of the stem pieces was treated with 2 ml of either distilled water, 2 mM N-ethylmaleimide (NEM) or 20 mM of glutathione (GSH). The stems were then frozen at -15\textdegree C for 24 hours, thawed and then incubated in sealed vials at 23\textdegree C for 24 hours. Ethylene and ethane were measured with the gas chromatograph described previously. Each treatment was replicated four times.

STATISTICS: Significant Studentized Ranges at the 5% and 1% confidence level, New Multiple-Range Test (Duncan 1955) and standard deviations were used to analyze the data.

RESULTS AND DISCUSSION

The phytotoxicity of hydrogen cyanamide was dependent on the growth status of red-osier dogwood (Fig. 4.1) and 'Radiant' crab-apple (data not shown). Prior to the onset of rest, 160\textdegree GS, the plants were most susceptible to hydrogen cyanamide, causing dieback at all concentrations and complete stem dieback at 2.0 M. From the onset of rest (180\textdegree GS) to immediately beyond maximum rest (280\textdegree GS), the plants were the most tolerant to hydrogen cyanamide treatments. Throughout this period less than 20\% dieback occurred even at the 2.0 M concentration. During the post-rest period (280-310\textdegree GS) and the quiescent period (320-360\textdegree GS) the plants were
increasingly more susceptible to the chemical. During this period approximately 80% of the stems were killed at the 2.0 M level and at the 1.0 M concentration the dieback progressively increased with later stages of development.

In a companion study (Nee 1986), the effective concentration of hydrogen cyanamide to overcome rest in red-osier dogwood and 'Radiant' crabapple was also found to depend on the growth status of the plant. Generally, as plants develop rest (180-280°GS) higher concentrations of hydrogen cyanamide were necessary to break rest, whereas during the post-rest period (290-320°GS) considerably lower concentrations were sufficient in breaking rest, and during the quiescent period (320-360°GS), hydrogen cyanamide treatments delayed bud break.

Previously, researchers (Doorenbos 1953, Erez and Lavee 1974, and Levitt 1980) hypothesized that any sublethal stress can break rest. Direct support of this hypothesis is shown in Table 4.1. During the rest period (180-310°GS) a high correlation between the production of ethylene induced by hydrogen cyanamide treatments and percent of bud break was found (Table 4.1). In contrast to this, before rest, 160°GS, and immediately after the chilling requirement for rest was satisfied, 320°GS, the correlation was much less. During quiescence, 330-335°GS, a high negative correlation was found (Table 4.1) indicating that hydrogen cyanamide treatments that stimulated ethylene production delayed the percentage of bud break. Harber et al. (1986) and Kobayashi et al.
(1981) reported that the production of ethylene is a good measure of sublethal stress. Exposing plant tissues to increasing levels of stress stimulates ethylene production to a maximum level immediately before an increase in electrolyte leakage becomes evident. After this point, further stress causes a decrease in ethylene levels which correlates negatively with electrolyte leakage and ethane production. High correlation between the extent of damage and electrolyte leakage was found (Table 4.1) throughout the test period, and at all sampling dates no ethane was produced by the cyanamide treatments (data not shown). Similar results were also obtained for 'Radiant' crabapple (data not shown).

Further evidence that sublethal stress can overcome rest is shown in Table 4.2. Both sublethal freezing and high temperatures were able to break rest in 'Radiant' crabapple plants. The plants subjected to the sublethal temperature treatments (-7°C and 47°C) broke buds uniformly 8 days after treatment, whereas the treatments before the sublethal stress (-4°C and 42°C) did not show any bud growth 30 days after treatment. Electrical conductivity and percent of buds killed per plant data support the fact that sublethal stress was important for overcoming rest.

The close relationship between sublethal stress, ethylene production, and the break of rest suggests that ethylene may be involved in overcoming rest. Previous researchers (Doorenbos 1953) speculated that a "necro-hormone" stimulated by sublethal
stress was responsible for breaking rest. No such hormone had been found to date. The fact that ethylene is a universal hormone induced by many stresses and the close relationship between sublethal stresses and breaking rest suggests that ethylene may be the "necro-hormone."

Further support of this hypothesis is the fact that ethephon, or ethylene releasing compound, was effective in breaking rest in 'Radiant' crabapples (data not shown). Others have also reported that exogenous ethylene applications can overcome rest in numerous plant species (Adkins and Ross, 1981; Hosoki, 1984; Sinska and Gladon, 1984).

During the development of rest, Seibel et al. (1978) and Kobayashi et al. (1981) found that as plants develop rest, from 90°GS to 180°GS, the stem and bud tissues decrease production of ethylene. During rest, between 180 to 315°GS, the production of ethylene is low. After the chilling requirement is satisfied and before any growth is visible the production of ethylene begins to increase and continues to increase as the tissues grow, between 315 and 90°GS.

The increase in ethylene produced in tissues exposed to sublethal dosages of hydrogen cyanamide (Fig. 4.2) may be due to an alteration of the cell membranes. This may account for the increase in electrolyte leakage observed (Tables 4.1, 4.2; Fig. 4.3). Previous researchers have also noted that an increase in membrane permeability may be the cause of breaking rest (Doorenbos
The increased ethylene produced may be due to the activation of the ethylene forming enzyme (EFE) that is reported to be associated with membrane (Mayak 1981). EFE is required for the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene.

There is good evidence that hydrogen cyanamide may be increasing membrane permeability without causing cellular death (Table 4.3). Treatment of dormant 'Radiant' crabapple plants with increasing concentrations of hydrogen cyanamide resulted in a progressive increase in the leakage of several readily mobile elements (K, Mg, Mn, Na, P, S, and Zn). Visual observations of the treated plants (data not shown) indicated that the leakage of electrolyte and specific elements occurred before any visual evidence of phytotoxicity. Others have also shown that stresses can induce electrolyte leakage without killing the tissue (Harber et al. 1986, Kobayashi et al. 1986, Palta et al. 1979). These results thus suggest that another possible mechanism of overcoming rest is via an increase in membrane permeability. This could explain the reason for the non-specificity of dormancy breaking agents.

The reasons for the increase in membrane permeability is not well understood. In a companion study (Nee 1986) hydrogen cyanamide was found to conjugate non-enzymatically with the thiol group of glutathione and possibly with other thiol containing compounds. The conjugation of thiol containing compounds, including thiol
containing proteins by hydrogen cyanamide, could have a dramatic effect on membrane permeability and other biological processes (Fahey 1975).

The close relationship between the effectiveness of rest breaking treatments and cellular damage, and the reason for the varying effects of the chemical treatments at different growth stages may be related to the glutathione thiol-disulfide status of the cells. Glutathione (GSH), a tripeptide, is the predominant low-molecular weight thiol present in most living cells (Fahey et al. 1975). One of its major roles is to maintain the protein thiol groups in the reduced state (Baron 1943, Fahey 1975).

In *Neurospora crassa*, Fahey et al. (1975) found that the glutathione thiol-disulfide status was related to the dormancy status of the conidia. Oxidized (GSSG) and protein-bound glutathione (PSSG) content increased dramatically with dormancy. During active growth the ratio of GSH to GSSG increases to about 1,000, primarily through changes in GSSG levels, whereas GSH levels vary by a factor of only about two. The level of PSSG was found to generally parallel that of GSSG. When dormancy occurs the levels of both GSSG and PSSG increases dramatically and the GSH:GSSG ratio decreases to around six. Fahey et al. (1975) postulated that glutathione thiol-disulfide reactions is a mechanism for controlling dormancy and that the GSSG levels plays a role in the regulation of protein synthesis through control of polysome formation (Kosower et al. 1972).
In plants, Esterbauer et al. (1978) and Guy et al. (1982) also reported that the GSH:GSSG ratio changes during the rest period. Although Guy et al. (1982) work on GSH:GSSG with red-osier dogwood related to cold acclimation, we speculated based on our background on rest and hardiness of this species, that the GSH:GSSG was low during rest and increased after rest was broken. These results support the work by Fahey et al. (1975) on *Neurospora*.

The glutathione thiol-disulfide status during the various stages of development should have a major effect on the plants sensitivity to hydrogen cyanamide. It is hypothesized that the action of rest breaking agents and the reason for the variable results at different growth stages is due to the glutathione thiol-disulfide status. During periods of active growth (160°GS) and during quiescence (315-360°GS) the ratio of GSH:GSSG and PSH:PSSG is high, therefore, the thiol binding agents, e.g. cyanamide, can react readily with the free thiol groups, thus inactivating or denaturing them. Depending on the quantity or quality of thiol substances that are bound, the plant can be inhibited or killed. When the plants are at rest, between 180-315°GS), the ratio of GSH of GSH:GSSG and PSH:PSSG is very low due to the increased production of GSSG and PSSG. In this condition, glutathione protects the proteins from inactivation or denaturation thereby making the plants more resistant to thiol binding agents, e.g. cyanamide.
The mechanism of how the binding of thiol groups overcome
rest is not known. Johnson (1965) found that chloroethanol (ethylene chlorohydrin), a potent rest breaking agent, also conjugated with glutathione. The conjugation leads to production of other metabolites including increased production of high levels of GSH and to the breakdown of the tripeptide to glycine, glutamic acid, and S-carboxymethylcysteine. Preliminary, GC-Mass spectroscopy and nuclear-magnetic resonance (NMR) analysis of the glutathione-cyanamide conjugate also suggests a similar breakdown of the conjugate.

Although there is no data to support this, the high production of arginine noted by Amberger (1984) following hydrogen cyanamide induced rest breaking in grapevines and the increased levels of polyamines noted by Wang et al. (1985) in apple following chilling satisfaction and thidiazuron (Wang 1985) induced rest breaking could be due to the breakdown of glutathione. The glutamic acid formed by the breakdown of GSH could be rapidly metabolized to arginine and polyamines.

Another possible way of stimulating growth, breaking rest, is due to the increased production of GSH as noted by Johnson (1967) following chloroethanol application. Guthrie (1941) reported that direct applications of yeast extracts (containing glutathione) and GSH to various plant species can break their rest period. It is possible that the increased GSH could stimulate growth by regulat-
ing new protein synthesis through the control of monosome formation (Fahey et al. 1975).

As stated previously, the production of ethane in stressed tissue has been shown to correlate positively with the increase in electrolyte leakage and is a good indicator of membrane damage and cell injury (Harber et al. 1986, Koyabashi et al. 1981). The production of ethane is thought to be due to the peroxidation of membrane lipids (Toppan et al. 1982). Earlier it was noted that ethane production was not observed following cellular damage and increase electrolyte leakage induced by hydrogen cyanamide. To explain why ethane was not produced, three experiments were conducted. In the first study (Fig. 4.4) the alfalfa cell cultures treated with hydrogen cyanamide did not produce ethane. Washing the hydrogen cyanamide treated cells with sterile hydrogen cyanamide free media enabled the cells to produce ethane. This suggests that the continued presence of hydrogen cyanamide in the media was needed to prevent ethane production. This may also indicate that the damage caused by hydrogen cyanamide was reversible. Cells that are severely damaged by stresses lose their ability to produce ethane (Kobayashi et al. 1982).

In the second study (Fig. 4.5) the dormant shoots of "Twentieth Century" (Pyrus pyrifolia) pear treated with 50 mM hydrogen cyanamide did not produce much ethane throughout the treatment period. Application of glutathione to the hydrogen cyanamide treated shoots 80 hrs. later caused the tissues to produce high
levels of ethane only 20 hours after the glutathione treatment (Fig. 4.5). The stimulation of ethane by glutathione application suggests that the binding of the thiol groups by hydrogen cyanamide may either be affecting lipid peroxidation of membranes or severely injuring the cells, therefore, preventing ethane production. Wendel et al. (1979) reported that the level of GSH is important for lipid peroxidation, low levels promoting peroxidation. The enhancement of ethane following the GSH treatment suggests that the damaged caused by hydrogen cyanamide may be reversible. Fahey et al. (1975) has shown that the oxidized sulphydryl groups on protein can be reduced with GSH. Therefore, addition of GSH could reverse the damage to membrane proteins caused by the oxidation of thiol groups on proteins by hydrogen cyanamide. To further test the importance of thiol compounds on ethane production, dormant red-osier dogwood plants were subjected to freezing temperatures (-15°C) that damaged but not completely killing the tissue. Fig.4.6 shows that the frozen controls produced high levels of ethylene and ethane confirming that the tissues were damaged and not killed (Harber et al. 1986). Treatment of the stems with either NEM, a thiol binding compound, or GSH reduced ethylene production. NEM also reduced ethane production, while GSH enhanced ethane production as compared to the frozen controls. Like hydrogen cyanamide, the binding of thiol groups by NEM may be the reason for the reduced production of ethane in damaged tissues. In contrast, the addition of thiol groups, e.g. GSH
application, enhanced ethane production. In conclusion, these studies indicate that the phytotoxic effect of hydrogen cyanamide is dependent on the dormancy status of the plants. Higher concentration of hydrogen cyanamide is required between 180 and 280^\text{GS} when plants are developing rest. Hydrogen cyanamide was also found to stimulate ethylene production when applied at sublethal concentration (Fig. 4.2), and enhances the leakage of electrolytes (Fig. 4.3) and specific ions, therefore, increasing membrane permeability at sublethal and lethal concentration. These studies provide good evidence that the mechanism of overcoming bud dormancy is not dependent on the treatment used per se, but on the sublethal nature of the treatment. The increase production of ethylene and membrane permeability may be involved in overcoming rest. Hydrogen cyanamide binds non-enzymatically with the thiol groups of GSH and possibly other components. The glutathione thiol-disulfide and protein thiol-disulfide status of the cells may be related to the variable effects of hydrogen cyanamide at the various growth stages. It is hypothesized that during rest the GSH/GSSG and GSH/PSSG ratio is very low and increases as rest is broken. When the ratio is low, the cells are more resistant to cyanamide and other stresses. Ethane production is inhibited by hydrogen cyanamide and NEM. The production of ethane appears to be dependent on the presence of thiol groups. Restoration of ethane production may be induced by washing away the thiol binding compound or adding GSH, a thiol containing compound.
Table 4.1. Correlation coefficients between percent of bud break and ethylene production, and percent of stem dieback and electrical conductivity (E.C.) in red-osier dogwood at different growth stages (°GS).

<table>
<thead>
<tr>
<th>°GS @ RXY</th>
<th>160</th>
<th>180</th>
<th>220</th>
<th>230</th>
<th>270</th>
<th>280</th>
<th>290</th>
<th>310</th>
<th>320</th>
<th>330</th>
<th>335</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Bud Break</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene</td>
<td>+.29</td>
<td>+.94</td>
<td>+.97</td>
<td>--</td>
<td>+.46</td>
<td>+.96</td>
<td>+.61</td>
<td>+.56</td>
<td>+.23</td>
<td>-.83</td>
<td>-.97</td>
<td>+.02</td>
</tr>
<tr>
<td>Dieback E.C.</td>
<td>+.92</td>
<td>+.85</td>
<td>+.93</td>
<td>+.80</td>
<td>+.69</td>
<td>+.65</td>
<td>+.58</td>
<td>+.75</td>
<td>+.60</td>
<td>+.68</td>
<td>--</td>
<td>+.91</td>
</tr>
</tbody>
</table>

@: RXY = Correlation coefficient
Table 4.2. The effects of freezing and high temperatures on overcoming 'Radiant' crabapple rest and electrical conductivity. (E.C.)

<table>
<thead>
<tr>
<th>Temperature $^\circ$C</th>
<th>% Buds Dormant</th>
<th>% Buds Sprouting</th>
<th>% Buds Killed</th>
<th>E.C. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>-7</td>
<td>45</td>
<td>40</td>
<td>15</td>
<td>13.5</td>
</tr>
<tr>
<td>-10</td>
<td>0</td>
<td>15</td>
<td>85</td>
<td>28.8</td>
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<tr>
<td>42</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>9.0</td>
</tr>
<tr>
<td>47</td>
<td>42</td>
<td>42</td>
<td>16</td>
<td>9.0</td>
</tr>
<tr>
<td>52</td>
<td>0</td>
<td>18</td>
<td>82</td>
<td>30.2</td>
</tr>
</tbody>
</table>
Table 4.3. Influence of hydrogen cyanamide on (1). Percent of bud break. (2). Electrolyte leakage (E.L.) (3). Specific elements leakage in relationship to dormancy breaking of crabapple. (JARRELL-ASH ICAP 9000 has been used to measure leakage)

<table>
<thead>
<tr>
<th>Treatments (M)</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bud Break (%)</td>
<td>10</td>
<td>30</td>
<td>45</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>E.L.(u hmos)</td>
<td>17</td>
<td>32</td>
<td>39</td>
<td>125</td>
<td>304</td>
</tr>
</tbody>
</table>

Element leakage: (Unit: ppm)

<table>
<thead>
<tr>
<th>Element</th>
<th>0.0274</th>
<th>.0141</th>
<th>.0086</th>
<th>.0086</th>
<th>&lt;0</th>
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</thead>
<tbody>
<tr>
<td>Al</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>.0202</td>
<td>.0845</td>
<td>.101</td>
<td>.1469</td>
<td>.1763</td>
</tr>
<tr>
<td>B</td>
<td>.0281</td>
<td>.456</td>
<td>.0597</td>
<td>.0281</td>
<td>.0702</td>
</tr>
<tr>
<td>Ba</td>
<td>.004</td>
<td>.0098</td>
<td>.0094</td>
<td>.0094</td>
<td>.0015</td>
</tr>
<tr>
<td>Ca</td>
<td>4.008</td>
<td>4.597</td>
<td>4.362</td>
<td>4.126</td>
<td>5.187</td>
</tr>
<tr>
<td>Co</td>
<td>.0004</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Cu</td>
<td>.0028</td>
<td>.0011</td>
<td>.0011</td>
<td>.0092</td>
<td>.0256</td>
</tr>
<tr>
<td>K</td>
<td>3.681</td>
<td>6.191</td>
<td>6.936</td>
<td>18.77</td>
<td>35.08</td>
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<td>Li</td>
<td>.0086</td>
<td>.008</td>
<td>.0075</td>
<td>.0062</td>
<td>.0048</td>
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<tr>
<td>Mg</td>
<td>.2959</td>
<td>.4438</td>
<td>.4438</td>
<td>.4734</td>
<td>2.604</td>
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<tr>
<td>Mn</td>
<td>.0081</td>
<td>.039</td>
<td>.0356</td>
<td>.0443</td>
<td>.0994</td>
</tr>
<tr>
<td>Mo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Na</td>
<td>.0744</td>
<td>.1155</td>
<td>.2141</td>
<td>.2059</td>
<td>.2575</td>
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<tr>
<td>Ni</td>
<td>.0007</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.0007</td>
</tr>
<tr>
<td>P</td>
<td>.2289</td>
<td>.3412</td>
<td>.541</td>
<td>1.236</td>
<td>5.191</td>
</tr>
<tr>
<td>S</td>
<td>.482</td>
<td>.17</td>
<td>.1947</td>
<td>.4801</td>
<td>2.979</td>
</tr>
<tr>
<td>Se</td>
<td>0</td>
<td>0</td>
<td>.0117</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sr</td>
<td>.0036</td>
<td>.0093</td>
<td>.0096</td>
<td>.0083</td>
<td>.0168</td>
</tr>
<tr>
<td>Zn</td>
<td>0</td>
<td>0</td>
<td>.0024</td>
<td>.0126</td>
<td>.1291</td>
</tr>
</tbody>
</table>

* JARRELL-ASH ICAP 9000 has been used to measure leakage.
Fig. 4.1. Effects of hydrogen cyanamide concentration on stem dieback at various growth stages in dogwood.
Fig. 4.2. Relationship of growth stages to ethylene production in dogwood treated with hydrogen cyanamide.
Fig. 4.3. Relationship of growth stages to electrical conductivity in dogwood treated with hydrogen cyanamide.
Fig. 4.4. The effects of hydrogen cyanamide on the inhibition of ethane in alfalfa suspension cultured cells.
Fig. 4.5. The effects of glutathione on the release of ethane in 'Twentieth Century' pear shoots.
Fig. 4.6. The effects of N-ethylmaleimide (NEM) and glutathione on the release of ethylene and ethane in damaged dogwood stems.
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Guthrie, J.D. 1941. Sprays that break the rest period of peach buds. Boyce Thompsom Inst. 12:45-47.


Miller, L.P. 1933. Effect of sulphur compounds in breaking the dormancy of potato tubers and inducing changes in the enzyme activities of the treated tubers. Contrib. Boyce Thompson Insti.


CHAPTER 5

POSSIBLE MECHANISMS OF OVERCOMING REST BY HYDROGEN CYANAMIDE

ABSTRACT

The inhibition of catalase by hydrogen cyanamide was found in the bark tissues of 'Northern Spy' apple shoots (*Malus domestica* Borkh.) and lentil seeds. Ethanol stimulated catalase activity in lentil seeds (*Lens culinaris* Medic) and had no effect on hydrogen cyanamide-induced inhibition of catalase.

*In vitro* and *in vivo* studies indicate that hydrogen cyanamide binds with thiol groups. Hydrogen cyanamide conjugated with the thiol groups of glutathione in a nonenzymatic cell-free system and of mung bean seedlings. In 'Spartlett' (*Pyrus communis* L.) pear shoots, exogenous applications of glutathione reduced the electrolyte leakage caused by hydrogen cyanamide suggesting that hydrogen cyanamide was detoxified by glutathione. The effectiveness of hydrogen cyanamide in overcoming dormancy was also reduced when pretreated with dithiothreitol (DTT). The role of the binding of thiol groups in overcoming rest is discussed.

INTRODUCTION

In plants, calcium cyanamide was patented in 1910 for use as a fertilizer (Pranke, 1913) in Germany. Prior to 1913 it was used as a herbicide against mustard weeds in oat, and subsequently as a defoliant in cotton (Hall and Harrell 1942), soybeans, and nursery
stocks (Bailey 1945). Calcium cyanamide has been used successfully for overcoming dormancy in apple, pear, peach (Kuroi et al. 1963, Morimoto and Kumashiro 1978), and grape (Braco et al. 1984). More recently, Shulman et al. (1983) reported that hydrogen cyanamide was more effective in breaking grape dormancy than calcium cyanamide. They suggested that calcium cyanamide undergoes partial hydrolysis to hydrogen cyanamide and cyanamide ions which are believed to be the active form of the compound. In moist soils and plant tissues, hydrogen cyanamide is broken into urea, ammonium, nitrate (Amberger 1984, Miller and Hall 1963), and possibly other intermediates (Amberger, 1984).

The metabolism of hydrogen cyanamide in plants is not well understood. Hofmann et al. 1954, first reported that cyanamide was metabolized in soybean by an enzyme called cyanamidase. In barley and corn plants cultured with cyanamide-\textsuperscript{14}C, part of the carbon dioxide expired was \textsuperscript{14}CO\textsubscript{2}. In later studies, Hofmann et al. (1954a) found that within two to three days cyanamide was converted to dicyanamide in oats, barley, wheat, and rye. Cyanamide fed plants contained higher levels of alanine, tryptophane, leucine, phenylalanine, and valine than plants fed calcium nitrate as the nitrogen source. Alanine displayed the greatest activity when plants were fed barium cyanamide \textsuperscript{14}C.

Latzko (1955) reported that Rathsack found dicyanamide, guanidine, and guanyl compounds as intermediate decomposition products of cyanamide. In cotton, Miller and Hall (1963) found that
sublethal dosages of cyanamide were rapidly complexed or destroyed in the leaves and injected balls. The first product in the leaves was urea. Cyanamide was found to rapidly metabolize leaving no residue eight hours after application. Cyanamide appeared to be utilized as a substrate for growth rather than converted to dicyanamide, azide, or cyanide. In addition to urea, another product of cyanamide decomposition in cotton was alanine or a closely related product.

Amberger (1984) reported that cyanamide rapidly penetrates plant tissues and is transported in both directions. He showed that barley seedlings treated with cyanamide produced high dry matter, total nitrogen, protein-N and amide-N content as compared to another N-source. These studies suggested that hydrogen cyanamide was directly involved in nitrogen metabolism and protein production. Amberger (1984) further showed that arginine content of plants treated with cyanamide increased dramatically. This increase in arginine appears to be from a direct synthesis of cyanamide via guanidine compounds.

Amberger (1961, 1963, 1984) and Nir et al. (1984) found that cyanamide inhibits catalase activity. Nir et al. (1984) associated the reduction in catalase activity with the breaking of rest. They reported that catalase activity in buds of grapevines increases markedly in autumn and decreases to a minimal level when the buds are ready to burst. A positive relationship in catalase activity was also found with rest breaking chemicals. The
decrease in catalase activity is thought to cause an increase in peroxide content, thus creating conditions favoring the pentose phosphate pathway which may be essential to dormancy breaking. Conversely, Guthrie (1941) reported that rest breaking agents promoted catalase activity.

In animal systems, cyanamide interferes with alcohol metabolism by inhibiting aldehyde dehydrogenase activity in vivo, thereby resulting in an accumulation of acetaldehyde following the ingestion of ethanol. In man, the accumulation of acetaldehyde causes unpleasant symptoms (flushing, hypotension, palpitations, dizziness, etc.). For this reason, cyanamide has been available as an alcohol deterrent in the treatment of alcoholism in Canada, Europe, and Japan.

Kitson and Crow (1979) reported that under physiological conditions cyanamide reacts with amino and thiol groups, forming guanidine and isothiouronium compounds, respectively. They postulated that the inhibition of aldehyde dehydrogenase may involve thiourea (formed by breakdown of isothiouronium compounds) and formamidine disulphide (an oxidation product of thiourea). DeMaster et al. (1984, 1985) identified catalase as the enzyme that activates cyanamide to a reactive species that is responsible for the inhibition of aldehyde dehydrogenase in vivo. Shirota et al. (1984) reported that cyanamide irreversibly inhibits catalase by covalently binding to the apoprotein of the enzyme in a process requiring hydrogen peroxide.
The mechanism by which hydrogen cyanamide breaks bud dormancy in plants is still unknown. The objective of this study is to investigate the effects of hydrogen cyanamide on catalase activity and the conjugation of thiol groups.

MATERIALS AND METHODS

Catalase Activity

The bark tissues of detached current season quiescent 'Northern Spy' apple shoot (Malus domestica Brokh) were completely painted with either 0, 0.5, 1.0, or 2.0 M. of hydrogen cyanamide. After 24 hours of incubation at 23.5°C, the bark was removed, frozen rapidly with liquid nitrogen, and ground to a powder with mortar and pestle. One gram of the ground bark was mixed with 20 mls. of deionized distilled water and 1 ml. of 3% hydrogen peroxide at 23.5°C. The mixture was stirred with a magnetic stirring bar and the time to release 1 ml. oxygen was measured as catalase activity (Appleman 1951). The gas evolved from this mixture was verified to be oxygen by gas chromatography.

In a second study, the effect of cyanamide on catalase activity was compared with sodium azide, a potent inhibitor of catalase, and ethanol (0.5%). One gram of lentil seeds was soaked for 12 hours with the first set of test solutions, rinsed with deionized distilled water, and then soaked for another 12 hours with the second set of test solutions. The treatments studied were as follows:
<table>
<thead>
<tr>
<th>TREATMENT NUMBER</th>
<th>FIRST 12 HRS</th>
<th>SECOND 12 HRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5% ethanol(EOH)</td>
<td>distilled water(DW)</td>
</tr>
<tr>
<td>2</td>
<td>0.5% EOH</td>
<td>1 mM. cyanamide(CY)</td>
</tr>
<tr>
<td>3</td>
<td>DW</td>
<td>0.5% EOH</td>
</tr>
<tr>
<td>4</td>
<td>1 mM. CY</td>
<td>0.5% EOH</td>
</tr>
<tr>
<td>5</td>
<td>DW</td>
<td>DW</td>
</tr>
<tr>
<td>6</td>
<td>DW</td>
<td>1 mM. CY</td>
</tr>
<tr>
<td>7</td>
<td>1 mM. CY</td>
<td>DW</td>
</tr>
<tr>
<td>8</td>
<td>DW</td>
<td>0.5% mM. sodium azide(SA)</td>
</tr>
<tr>
<td>9</td>
<td>0.5% EOH</td>
<td>0.5% mM. SA</td>
</tr>
</tbody>
</table>

After the second application, catalase activity was measured by the gasometric procedures previously outlined.

**Reaction with Thiol Groups**

**IN VITRO:** In order to study the possible conjugation of hydrogen cyanamide with thiol groups, 1 mM. glutathione was added to 0, 0.5, 1.0, and 2.0 M. hydrogen cyanamide solutions for 12 hours at 23.5°C. After this time, the free (unbound) thiol groups remaining in each test solution was determined by stopping the reaction with 2 mM. N-ethylmaleimide and measuring the absorption at 300 mu. with a Beckman DU 2400 spectrophotometer (Roberts and Rouser 1958). The same treatments were repeated four times to verify the results.
IN VIVO: Twenty dried viable mung bean seeds were soaked in 50 mls. of 0, 50, 250, and 500 mM. hydrogen cyanamide for 48 hours on a shaker (90 rpm) at 23.5°C in the dark. The seeds were then rinsed with distilled water and ground with a mortar and pestle in 0.15% (W/V) sodium ascorbate solution at 0°C. The homogenate was then centrifuged at 2777 g. for 15 minutes at 0°C. One ml. of the clear supernatant was added to 1 ml. of 0.2 M. potassium phosphate buffer at pH 8.0 and 0.2 ml. 5,5-dithio(2-nitrobenzoic acid) (DTNB) (10 mM. in 0.02 M. potassium phosphate at pH 7.0). The absorption was measured with a Beckman DU 2400 spectrophotometer at 415 mu. and this was corrected for the color of the supernatant and DTNB. The above procedure was modified from De Kok et al. (1981).

Detoxification of Hydrogen Cyanamide with Thiol Compounds

Three studies were made to determine the effects of thiol-containing compounds on detoxifying hydrogen cyanamide treated plant tissues. In the first study, excised shoots of 'Spartlett' pear, obtained from the OSU Lewis Brown Horticultural farm, were thoroughly painted with one of the following treatments: 1) deionized distilled water; 2) 10 mM. GSH; 3) 0.5 M. hydrogen cyanamide; or 4) 10 mM. GSH + 0.5 M. hydrogen cyanamide; or the shoots were cut into 2.0-2.5 cm. pieces and soaked in one of these solutions: 5) deionized distilled water; 6) 10 mM. GSH; 7) 25 mM. hydrogen cyanamide; or 8) 10 mM. GSH + 25 mM. hydrogen cyanamide.
Forty-eight hours after treatment, the tissues were placed in distilled water. The percent of conductivity was determined as described previously (Nee 1986).

In the second study the shoots of dormant ‘Radiant’ crabapple (Malus domestica Borkh) were painted with two sets of treatment solutions. The second treatment was made four hours after the initial treatment.

<table>
<thead>
<tr>
<th>TREATMENT NUMBER</th>
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<th>SECOND APPLICATION NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
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<td>DW</td>
<td>DW</td>
</tr>
<tr>
<td>2</td>
<td>0.5 M. DTT</td>
<td>DW</td>
</tr>
<tr>
<td>3</td>
<td>0.5 M. CY</td>
<td>DW</td>
</tr>
<tr>
<td>4</td>
<td>1.0 M. CY</td>
<td>DW</td>
</tr>
<tr>
<td>5</td>
<td>0.5 M. DTT</td>
<td>0.5 M. CY</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
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<td>1.0 M. CY</td>
</tr>
<tr>
<td>8</td>
<td>1.0 M. CY</td>
<td>0.5 M. DTT</td>
</tr>
</tbody>
</table>

The plants were maintained in a greenhouse at 20°C during the day and 15°C at night (+/- .3°C) under natural photoperiod and light conditions. Percent bud break was determined 30 days after treatment.

Statistics

Significant Studentized Ranges at the 5% and 1% level, new Multiple-range Test, and standard deviation were used to analyze the data.
RESULTS AND DISCUSSION

Hydrogen cyanamide inhibited catalase activity in apple bark tissue (Fig. 5.1). These results confirm previous reports by Amberger (1961, 1963), Nir et al. (1984), and DeMaster et al. (1985). Nir et al. (1984) state that reduction of catalase activity is associated with overcoming rest. They found that resting plants had higher catalase activity than chilling satisfied plants and the decrease in catalase activity was related to the effectiveness of the rest breaking chemicals. The decrease in catalase activity is thought to cause a rise in hydrogen peroxide which then favors the pentose phosphate pathway which is believed to be involved in the breaking of rest.

In animals, cyanamide is used as an effective alcohol deterrent agent by inhibiting aldehyde dehydrogenase (AlDH) isozymes in vivo systems. In in vitro systems it has no effect on AlDH activity. When cyanamide is administered before ethanol, it causes a rapid increase in acetaldehyde levels in animals and man. Recently, DeMaster et al. (1985) found that the inactivation of aldehyde dehydrogenase by cyanamide is dependent on its effect on catalase, which is responsible for the conversion of cyanamide to an inhibitor of aldehyde dehydrogenase. The active metabolite involved in this inhibition is unknown. Cyanamide is an inhibitor of catalase (DeMaster et al. 1985). Whether cyanamide inhibits catalase directly or indirectly by product inhibition remains to be elucidated.
The pathway of catalase inhibition in animals can be inhibited in vivo by pretreating with ethanol or 3-amine-1,2,4-trizole (3-AT). To determine whether this system also operates in plants, lentil seeds were either pretreated or not pretreated with ethanol prior to cyanamide application (Fig. 5.2). The results suggest that a different system may occur in plants. Ethanol stimulated catalase activity and cyanamide continued to inhibit catalase activity regardless of when the ethanol application was made. The inhibition of catalase activity by 3-AT was considerably more effective than cyanamide, and ethanol had no effect on 3-AT inhibition of catalase. This suggests that in plants the inhibition of catalase activity by cyanamide is not a complete blockage as with 3-AT. Amberger (1984) reported that the inhibition of catalase by cyanamide is quite specific and a reversible process.

In another study (unpublished results) ethanol overcame the rest period of 'Radiant' crabapple. Others have also reported that ethanol can break rest in plants (Hosoki, 1985). These results, coupled with the results on increasing catalase activity with ethanol, are in contrast with the findings of Nir et al. 1984. Guthrie (1941) also reported that rest breaking agents can stimulate catalase activity.

The resulting increase in hydrogen peroxide caused by the inhibition of catalase activity of cyanamide is believed to increase GSH levels which is thought to be involved in the breaking
of rest (Amberger, 1984). This enhanced GSH level is thought to be involved in the sharp rise in arginine levels in plants treated with cyanamide because of its reaction with the cysteine molecule found in GSH (Amberger, 1984). The relationship between arginine and overcoming rest was not explained.

In plants (Rennenberg 1982, Smith et al. 1984) and animals (Johnson, 1965), glutathione is a detoxifying agent. Hydrogen cyanamide, at the concentrations needed to break rest, is sublethal to plant tissues causing an increase in electrolyte leakage and an increase in ethylene production (Nee 1986). High concentrations result in tissue death. The concentration required to overcome rest and cause injury is dependent on the growth stage of the plant (Nee 1986). Researchers (Doorenbos 1953, Levitt 1980, Erez and Lavee 1974) hypothesized that dormancy breaking agents and environmental stresses which break dormancy are effective only at sublethal dosages.

Fig. 5.3 shows that hydrogen cyanamide binds with thiol groups of GSH in a cell free, in vitro system. Similar results were obtained by treating germinating mung bean seedlings with hydrogen cyanamide (Fig. 5.4). With increasing concentrations of hydrogen cyanamide, the level of thiol groups decreases after 48 hours of incubation. These results indicate that hydrogen cyanamide binds with free thiol groups and is detoxified by conjugating with GSH. In detoxification of herbicide, Adams (personal communication) found that herbicides form a conjugate with the
The herbicide-GSH conjugate is no longer toxic. The "detoxified" effect of GSH is confirmed by treating 'Spartlet't pear shoots with hydrogen cyanamide and GSH (Fig. 5). In both methods of application, i.e. painting and soaking, GSH significantly reduced the electrolyte leakage caused by hydrogen cyanamide, thus suggesting that GSH reduced the injury caused by hydrogen cyanamide.

In another study (Fig. 5.6), dormant 'Radiant' crabapple plants were treated with dithiotreitol (DTT) either before or after hydrogen cyanamide applications to determine whether the addition of more thiol groups had any effect on cyanamide's role in overcoming rest and/or causing injury. When plants were pretreated with DTT four hours before hydrogen cyanamide treatment, the amount of budbreak was significantly reduced and similar to the DTT control treatment. In contrast, application of hydrogen cyanamide four hours before DTT treatment caused a stimulation of bud break similar to the hydrogen cyanamide control treatment. These studies suggest that DTT pretreatment "protected" the plant from cyanamide with additional thiol groups. Posttreatment of DTT was ineffective and indicates that the hydrogen cyanamide penetrates and translocates rapidly, binding with the natural thiol groups before DTT can have an effect.

The inhibition of catalase and dehydrogenase activity by hydrogen cyanamide could be attributed to the binding of the free thiol groups found on the enzymes. Both enzymes are known to
contain significant amounts of thiol groups (Friedman 1973, Levitt 1966).

These results on the binding of hydrogen cyanamide by the thiol groups may explain the variable and inconsistent results in breaking bud rest reported by other researchers (Bracho et al. 1984, Luvisi 1984, Whiting and Coombe 1984, Williams and Smith 1984). It is known that the ratio of oxidized (GSSG) to reduced (GSH) forms of glutathione varies with the stage of plant development (Hopkins 1943, Guy et al. 1984) and possibly among the plant genotype. As the chilling requirement for overcoming rest is being satisfied, the ratio of GSH:GSSG increases rapidly (Esterbauer and Grill 1978, Fahey et al. 1975). This may explain the results obtained in a companion study, when hydrogen cyanamide was applied at various growth stages to 'Radiant' crabapple and red-osier dogwood plants (Nee 1986). In both species, the concentration of hydrogen cyanamide required to overcome rest increased during rest development (180-270°GS) (Fuchigami et al. 1982) and decreased during post rest development (270-315°GS). The effective concentrations required to overcome rest were related to the sublethal dosages of hydrogen cyanamide (Nee 1986). This suggests that the level of glutathione thiol-disulfide status in plants varies with the stage of development and an understanding of its composition may be important in the proper use of rest breaking agents for safely and effectively overcoming rest.
In *Neurospora crassa*, Fahey et al. (1975) found that the glutathione thiol-disulfide status was related to the dormancy status of the conidia. Oxidized (GSSG) and protein-bound glutathione (PSSG) content increased dramatically with dormancy. During active growth the ratio of GSH to GSSG increases to about 1000, primarily through changes in GSSG levels, whereas GSH levels vary by a factor of only two. The levels of PSSG generally paralleled those of GSSG. When dormancy increases, the level of both GSSG and PSSG increases dramatically and the GSH:GSSG ratio decreases to around six. Fahey et al. (1975) postulated that the glutathione thiol-disulfide reaction is a mechanism for controlling dormancy and that the GSSG levels play a role in the regulation of protein synthesis through control of polysome formations (Kosower et al. 1972).

The method of rest breaking agents is still not understood. This study and others (Nee 1986) confirm earlier reports that dormancy breaking agents are effective only at sublethal dosages. At sublethal levels, resting plants produce high concentrations of ethylene (Nee 1986). Others have shown that ethylene is able to break rest in some plants (Kepczynski and Rudnicki 1975, 1977). Exogenous applications of ethephon, a generating compound, also overcame rest in 'Radiant' crabapple; however, the effect was not as good as hydrogen cyanamide or other dormancy breaking agents (Nee 1986). This suggests that ethylene may not act directly in
breaking rest but possibly acts indirectly by its sublethal effect on plant tissues.

To confirm that the conjugation of hydrogen cyanamide binds with glutathione in the *in vitro* system reported previously (Fig. 5.4), the solution was lyophilized, the sulfur containing compounds were separated by HPLC, and the sulfur containing compounds were characterized by $^{14}$C-NMR and GC-MASS Spectrometry. These analyses, although not yet definitive, suggest that two sulfur containing compounds, other than glutathione, are formed when hydrogen cyanamide conjugates with the thiol groups of glutathione. One of the compounds, which appears to be quite stable, was identified as a glutathione-cyanamide conjugate. The conjugation appears to occur between the thiol group of glutathione and the carbon end of cyanamide. It is not known whether these sulfur containing compounds are involved in breaking rest. Recently, Hosoki *et al.* (1986) reported that several sulfur containing compounds and natural plant materials (i.e. garlic paste) containing high levels of sulfur containing compounds are effective dormancy breaking agents. Studies are continuing to identify the sulfur containing compound from glutathione-cyanamide conjugation.
Fig. 5.1. Effects of hydrogen cyanamide on catalase activity in excised stems of apple.
Fig. 5.2. The effects of ethanol, hydrogen cyanamide, sodium azide, and distilled water pretreatment and posttreatment on the activity of catalase of lentil seeds.
Fig. 5.3. Nonenzymatic effects of hydrogen cyanamide on SH group of glutathione.
Fig. 5.4. Effects of hydrogen cyanamide on the SH groups in mung bean seeds.
Fig. 5.5. Interactions between hydrogen cyanamide and glutathione on electrical conductivity of 'Spartlett' pear shoots.
Fig. 5.6. The interactions between hydrogen cyanamide and dithiolthreitol (DTT) on bud break and stem dieback of 'Radiant' crabapple plants.
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A.1. Diagram of degree growth stage (°GS) model.
A.2. Bud growth, growth status, and terminology for point events and segment events during the annual cycle of development.

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<thead>
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<th>Developmental Status</th>
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<th>Degree Growth Stage (°GS)</th>
<th>Segment Events</th>
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<td>Spring Bud Break</td>
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<tr>
<td>Growing</td>
<td></td>
<td>0°-90°</td>
<td>Rapid Growth Phase</td>
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<td>Maturity Induction Point</td>
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<td>90°-180°</td>
<td>Slow Growth/Correlative Inhibition Phase</td>
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<td>Growth stops/End of Correlative inhibition phase</td>
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<td>180-270</td>
<td>Deepening Rest Phase</td>
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<td>270-315</td>
<td>Decreasing Rest Phase</td>
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## APPENDIX

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<td>Bud growth, growth status, and terminology for point events and segment events during the annual cycle of development.</td>
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