Effect of Chilling, Hydrogen Cyanamide, Hot Water and Bud Scale Removal on Bud Break of 'Tifblue' Rabbiteye Blueberry

by

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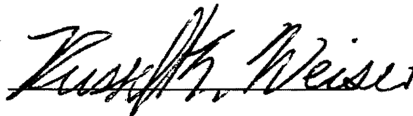

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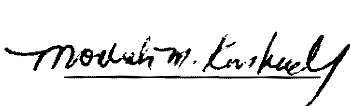

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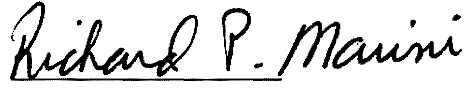

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(ABSTRACT)

Temperate deciduous fruit trees have poor and delayed bud break when they are grown in warm areas. The delay is due to a lack of the chilling which is required to break bud endodormancy. Bud endodormancy can be overcome in some species by treatments such as H_2CN_2, heat, and bud scale removal. We tested the effects of chilling, H_2CN_2, heat, and removing scales on bud break of floral and vegetative buds of 'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade).

Hydrogen cyanamide was effective in promoting floral bud break of 'Tifblue' only on whole plants, at chilling exposures between 300 to 500 hours. However, vegetative bud break was increased by H_2CN_2 at a wider range of chilling exposures than floral buds in both whole plants and cut shoots. Optimum vegetative bud break was induced by H_2CN_2 at 125 and 250 mM for whole plants and cut shoots, respectively. Hydrogen cyanamide was highly phytotoxic to floral buds compared to

vegetative buds. However, floral buds of whole plants became tolerant to H_2CN_2 as chilling increased. Injury to vegetative buds was significant only at 500 mM H_2CN_2.

The chilling requirement for 'Tifblue' floral buds of whole plants was 500 hours. In contrast, vegetative buds did not have a significant relationship with chilling exposure in either cut shoots or whole plants.

Heat treatment was effective in promoting floral bud break of cut shoots only at 190 chilling hours at 30 minutes heat exposure. Heat (47°C) for 1 hr was effective in promoting vegetative bud break, but the effectiveness varied with chilling level and depended on time of heat exposure. Bud scale removal did not promote floral bud break, but increased vegetative bud break, although not significant compared to control.

Finally, we discovered that vegetative buds remained dormant even after they had received more than adequate chilling. However, both H_2CN_2 treatment and floral bud removal resulted in increased vegetative bud break, although the effect of H_2CN_2 was less than floral bud removal. This suggests that vegetative buds were inhibited by floral buds and that H_2CN_2 could partially overcome this paradormant effect.

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Thanks Oh Allah, for the knowledge You have bestowed upon me, and may I use it for Your cause and satisfaction.

DEDICATION

This thesis is dedicated to my late father, who has devoted his life to the teaching profession. And this is also dedicated to my wife and family for their patience and support towards my success.

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Chapter One: Literature Review

Temperate woody plants generally undergo a dormant period during the winter season to ensure survival at freezing temperatures, and also to synchronize growth with favorable conditions (Vegis, 1964). Dormancy was recently defined as "a temporary suspension of visible growth of any plant structure containing a meristem" (Lang, 1987). Over the years many terms have been used to describe the different types of dormancy. *Summer* and *winter dormancies* denote the growth season. *Imposed dormancy* describes how dormancy is induced, while *deep dormancy* indicates the relative level of dormancy. *Quiescence* and *rest* on the other hand describe dormancy as lack of growth. As our understanding of dormancy grew, Lang (1987) was able, for the first time, to describe the types of dormancy in physiological terms. He divided dormancy into three general types: ecodormancy, paradormancy, and endodormancy.

Ecodormancy is synonymous with quiescence (Bewley and Black, 1985) or imposed dormancy, and is induced by stressful environmental extremes of temperature, water, nutrients, CO_2, and O_2.

Paradormancy is synonymous with correlated dormancy, summer dormancy, or pre-dormancy; and it is regulated by physiological or biochemical signals originating outside the affected structure. Apical dominance is a good example of paradormancy

(Martin, 1987). Paradormant buds will not grow in a favorable environment, but resume rapid growth if the inhibitory neighboring organs (such as leaves or buds) are removed.

Endodormancy is synonymous with rest, winter dormancy, innate dormancy, or deep dormancy; and it is regulated by physiological factors inside the affected structure itself. Internally inhibited buds normally require prolonged exposure to chilling temperatures before they can resume growth. Thus, plants exhibiting endodormancy are not generally suitable for growth in warm climates.

Chilling requirement. In regions where winter is mild, for example in the mediterranean, sub-tropical, and tropical highlands areas, insufficient chilling will result in poor and delayed bud break (Erez et. al, 1971).

The chilling requirement of endodormant buds appears to be genetically determined and is consistent from year to year, for a given genotype. However, a wide variation in chilling requirement exists among and between species. For example, the blueberry types evergreens, rabbiteyes, and Southern highbush; require 0, 400 to 600, and 800 to 1000 hours of chilling, respectively. Peaches, generally require 800 to 1200 hours of chilling. The most effective chilling temperatures for peaches and blueberries are between 0 to 7.2°C (Darrow, 1942). Temperatures below 0 and above 19°C are not considered effective (Richardson et. al, 1974).

Overcoming endodormancy. The mechanism of bud endodormancy is poorly understood, nevertheless there are numerous ways to overcome endodormancy.

Inadequate chilling can be overcome with chemicals (Erez et. al, 1971; Erez, 1987; Hosoki et. al, 1986), high temperature (Hosoki, 1984; Nee, 1986), and bud scale removal (Abbot, 1969; Spiers, 1972). Erez and Lavee (1974), and Levitt (1980) hypothesized that any treatment applied at sub-lethal dosages can overcome endodormancy. Nee (1986) supported the hypothesis that sub-lethal stress can break bud endodormancy by showing that hot water (47°C for 1 hr) and Hydrogen cyanamide (H_2CN_2) (125 mM) induced ethylene synthesis prior to vegetative bud break of 'Radiant' crabapple. However, ethylene is thought not to be directly related to endodormancy release. Nee (1986) showed that, applying ethephon (an ethylene generating chemical) to red-osier dogwood and 'Radiant' crabapple did not break vegetative buds better than H_2CN_2.

There are many chemicals that can break endodormancy. Fuchigami and Nee (1987) reviewed chemicals that are capable of breaking dormancy in plants. They reported that H_2CN_2 was more effective than mineral oils, narrow range oils, uncoupling agents, nitrogen-containing compounds, sulphur-containing compounds, salts, acids, toxic compounds, anaesthetic-type compounds, and growth regulators, in promoting vegetative bud break of temperate woody plants. Shulman et al. (1986) also reported that H_2CN_2 was a very effective dormancy breaking agent. Sprays of 1-5% commercial cyanamide induced early, uniform and full vegetative and floral bud break of almond, apple, actinidia, fig, grapevine, peach, persimmon, and plum (Shulman et al., 1986). Treatment of 1% H_2CN_2 induced germination of grape seeds, and

substituted for chilling requirements. Shulman et. al (1983) discovered H_2CN_2 was more effective than calcium cyanamate ($CaCN_2$). They suggested that $CaCN_2$ is less effective since it undergoes only partial hydrolysis to

H_2CN_2 (the active form).

Nee (1986) found that in 'Red-osier' dogwood and 'Radiant' crabapple the concentration of H_2CN_2 required to overcome vegetative bud dormancy was dependent on the growth stage. Plants with endodormant buds required the highest concentration. However, although H_2CN_2 overcame endodormancy in dogwood and crabapple, the stem did not elongate. Nee (1986) suggested that some unknown factors were required for stem elongation. Similarly, Sieler et. al (1991) found that, in 'Red Haven' peach, H_2CN_2 concentration and physiological stage of vegetative buds were critical for successful bud break. Maximum percentage of vegetative bud break was observed when 0.25 M cyanamide was applied between endodormancy and ecodormancy. Concentrations higher than 0.5 M were highly phytotoxic to peach vegetative buds at all dormancy stages.

Beside chemical and hot water treatment, bud scale removal can also break bud endodormancy (Iwasaki and Weaver, 1977; and Spiers, 1972). Spiers (1973) showed that bud scale extract from tung exhibited similar characteristics to ABA by inhibiting lettuce seed germination. The extract also showed equal retention time compared to ABA when its methylated derivatives were separated by gas-liquid chromatography. In

contrast to Spiers's findings, Iwasaki and Weaver (1977) found that extractable ABA content did not correlate with dormancy.

Abbott (1969) suggested that scales are inert hormonally, but are physiologically active- increasing both in size and weight as dormancy progresses. One function of bud scales is to provide protective covering to the bud. In addition, until senescence scales ensure against premature bud break by competitive resorption of nutrients. These nutrients, stored in the scales, provide a reserve which may sustain the early growth of the bud. Thus scales also act as a buffer against a resumption of growth (Abbot, 1969). Abbott (1969) showed that removing scales from dormant apple trees hastened flowering, reduced flower abortion, and produced a more uniform growth of clusters and better fruit set. Abbot (1969) also hypothesized that spraying Dinitro-O- Cresol (DNOC) killed the scales so prevented competitive resorption of nutrients.

ABA and Bud dormancy. Abscisic acid (ABA) has often been associated with chill-related dormancy. Many reports have indicated a role for ABA in dormancy of deciduous trees (Addicot and Lyon, 1969). During and Bachmann (1975) used High Pressure Liquid Chromatography (HPLC) to quantify ABA in *Vitis vinifera* during endodormancy. They found that ABA content in the buds and nodes increased twelve-fold in buds harvested in mid-October compared to buds harvested in early September. Emmerson and Powell (1978) found that removing bud scales in three varieties of *Vitis vinifera* did hastened bud break. However, although ABA content was higher in scales

than in primordia, changes in ABA level during chilling and bud break occurred only in primordia. Powell (1987) concluded that although the evidence supports a role for ABA in the early stages of dormancy, it has not been proven. He argued that, in all cases of past research work, a control under non-dormant conditions was not used. Furthermore, in the later stages of dormancy, ABA does not correlate with dormancy in many cases, and high concentrations of exogenous ABA can not inhibit bud break. Powell (1987) proposed instead that a promoting agent may become dominant at the later stages of dormancy and override the effect of ABA. Thus ABA may or may not play a role in bud dormancy and dormancy release.

The general objective of this thesis research was to determine if H_2CN_2 can break dormancy of floral and vegetative buds of blueberries. If H_2CN_2 is successful in promoting bud break of blueberries, then it is possible that blueberries could be grown in tropical climates. Blueberries require chilling temperatures similar to peaches (Eck, 1988). Their adaptability ranges from the high-chill areas for the Northern highbush type, to the low-chill areas for Southern highbush and Rabbiteyes. There are also evergreen types indigenous to central Florida. Recognizing this wide range of adaptability there is a great possibility that blueberries can be grown in tropical climates; naturally or in combination with dormancy breaking agents. Therefore, we conducted several experiments to investigate blueberry dormancy. Specifically, we tested the effect of concentration and timing of H_2CN_2 application on bud break of blueberries. In addition, hot water and bud scale removal were also tested for their

effect on dormancy release. Finally, we investigated the effect of removing floral buds on dormancy of vegetative buds.

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Chapter Two: Hydrogen Cyanamide Concentration and Chilling Exposure Affect 'Tifblue' Rabbiteye Blueberry Bud Break

Abstract

'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade) have poor or delayed bud break in warm growing regions because they do not receive enough chilling to satisfy their requirement. Treatments applied at sub-lethal dosages can overcome endodormancy in buds of some temperate deciduous plants. Hydrogen cyanamide is one of the most effective dormancy breaking chemical that can break bud endodormancy. We tested the effect of hydrogen cyanamide (H_2CN_2) concentration on floral and vegetative bud break of 'Tifblue' cut shoots and whole plants at different chilling exposures.

Hydrogen cyanamide was effective in promoting floral bud break of 'Tifblue' whole plants between 300 to 500 hours of chilling, but was not effective in cut shoots. Hydrogen cyanamide at 125 mM and above was phytotoxic to floral buds of cut shoots. In whole plants however, H_2CN_2 was phytotoxic to floral buds at 300 chilling hours and below. After 500 chilling hours, floral buds became more tolerant to H_2CN_2.

Time to first floral bud break of two-year-old plants were delayed by H_2CN_2 at 750 chilling hours. Chilling requirement of two-year-old 'Tifblue' blueberry floral buds was 500 hours. Chilling fasten floral bud break of 'Tifblue' in both whole plants and cut shoots.

Hydrogen cyanamide at 125 and 250 mM induced optimum vegetative bud break of 'Tifblue' whole plants and cut shoots respectively. Hydrogen cyanamide phytotoxicity to vegetative buds was apparent only at 500 mM in both cut shoots and whole plants. Time to first vegetative bud break of whole plants was fasten by H_2CN_2 (125 mM and below) at 100 chilling hours. Chilling treatment in controls (0 mM H_2CN_2) did not fasten time to first vegetative bud break of whole plants, but interaction between chilling and H_2CN_2 fasten vegetative bud break.

Introduction

Temperate woody plants generally require exposure to chilling temperatures before bud break (Samish, 1954; Vegis, 1964). Lack of chilling in warm regions reduced and delayed bud break in temperate deciduous fruits (Darrow, 1942; George et. al, 1988; and Shulman et. al, 1986). This problem can be overcome by planting cultivars with low chilling requirement or using dormancy breaking agents such as hydrogen cyanamide (H_2CN_2). Hydrogen cyanamide is one of the most successful dormancy breaking agents in woody plants (Nee, 1986, and Shulman *et. al*, 1983 and

1986). Hydrogen cyanamide effectively broke bud dormancy of apples, grapes, and peaches (Shulman et. al, 1986); 'Redhaven' peach (Seiler et. al, 1991); red raspberry (Iona, 1983) and red-osier dogwood (Nee, 1986). However, it use has been limited because its effectiveness depends on time of application, and concentration (Nee, 1986). Applying H_2CN_2 at excessive concentrations for the stage of bud development kills the buds.

Winter dormancy, rest, deep dormancy or innate dormancy was recently defined as endodormancy (Lang, 1987). Endodormancy is regulated by physiological factors inside the affected structure itself. Internally inhibited buds normally require prolonged exposure to chilling temperatures before they can resume growth.

The effectiveness of H_2CN_2 in breaking endodormancy of blueberries has not been reported. If H_2CN_2 can break bud dormancy in blueberries, then chill requiring cultivars such as 'Tifblue' blueberry could be grown in warmer regions.

Our hypothesis was that endodormancy in 'Tifblue' floral and vegetative buds can be overcome by applying H_2CN_2 at certain times (chilling exposures) and concentrations.

Materials and Methods

Plant material. Dormant shoots 15-25 cm long were cut from field-grown 'Tifblue' bushes at the Southern Piedmont Experimental Station, Blackstone, Virginia on four dates. They were harvested on Oct. 18, Nov. 8, and Nov. 29, 1991, and Jan. 13, 1992; after they had received 0, 62, 190, and 605 chilling hours, respectively. Harvested shoots were kept moist in polyethylene bags during transport to the laboratory at Virginia Tech, Blacksburg. Shoots collected at each harvest date were divided into three groups. The first group was used immediately. The other two groups of shoots were stored moist at 6 ± 1°C for 168 and 504 hours, respectively. Afterwards, the leaves (that were still on the plants) were removed and the shoot base was recut. The shoots were left to stand in 2 cm deep distilled water at room temperature before treating with H_2CN_2. Two-year-old 'Tifblue' plants were supplied by the Finch Blueberry Nursery, Bailey, North Carolina. Before shipping to Blacksburg, VA; plants were detopped, roots washed and treated with Terra-sorb to retain moisture. The bare rooted blueberries were planted the first week of September, 1991 in 18.5 X 19.5 cm containers filled with 1:1 peat moss and perlite mixture. A slow release fertilizer compound 15:15:15 ($N:P_2O_5:K_2O$) was applied at the rate of 2 g/container every two months. Plants were watered daily, as required, for 9 months. Temperature in the greenhouse was at 18/25°C (day/night), with natural lighting. After floral buds

were fully developed, the plants were moved into a dark cold room at 6 ± 1°C (on March 6, 1992).

Hydrogen cyanamide. Commercial grade H_2CN_2 at 50 % A.I. (w/v) was diluted to the required concentrations with distilled water. For experiments using cut shoots, H_2CN_2 concentrations of 0, 50, 125, 250, and 500 mM were used. For shoots of whole plants, H_2CN_2 concentrations were 0, 50, 75, 125, and 500 mM.

Hydrogen cyanamide solutions were stored at 7°C, and kept on ice during application, since H_2CN_2 looses activity at warmer temperatures.

Hydrogen cyanamide was applied with a paint brush so that a thin film of solution was transferred onto each floral and vegetative bud. For whole plants, H_2CN_2 was applied together with a sticking agent (T.20). Distilled water at 0°C was used as control.

Experiment 1. Cut shoots from field grown-plants were used. A two-factor experiment, with five levels of H_2CN_2 (0, 50, 125, 250, and 500 mM); and four levels of chilling exposures (0, 62, 190, and 605 hours) as estimated under field conditions (refer to Data collection).

Dormant cut shoots of each chilling exposure were treated with H_2CN_2 and placed in a test tube containing 2 cm deep distilled water. Shoots were forced to break bud under florescent light at 26°C. The level of distilled water in each test tube was checked daily and refilled as required. Each week the base of each shoot was cut to remove plugged xylem vessels.

Experiment 2. Whole plants grown in the greenhouse were used. A two-factor experiment was conducted with five levels of H_2CN_2 (0, 50, 75, 125, and 500 mM); and five levels of chilling exposure (0, 100, 300, 500, and 750 chilling hours). Four shoots with floral and vegetative buds, from 4 different plants, were used for each treatment (i.e. 4 replications with 1 shoot per replication).

On March 6, 16 plants were moved from the greenhouse into the cold room at 6 ± 1°C. Four plants were left in the greenhouse as controls. Following 100, 300, 500 and 750 hours of chilling, 4 plants were moved out of the cold room at each time interval. Leaves were removed, and H_2CN_2 concentrations of 0, 50, 75, 125, and 500 mM was applied to floral and vegetative buds. Non-chilled plants (in the greenhouse) were treated with the same concentrations of H_2CN_2 as the chilled plants. Shoots with buds to be treated were selected randomly within each replicate (plant) for all chilling levels (including 0 chilling). Each H_2CN_2 concentration was applied to each plant. All floral and vegetative buds on each shoot were treated with H_2CN_2.

Floral and vegetative buds of treated plants were forced to break in the greenhouse, under natural lighting at 18/26°C (day/night) during spring season 1992.

Data collection. Number of days to first bud break, percent bud break, and the percent dead buds were recorded for floral and vegetative buds of each treatment. Percent bud break and percent dead buds were calculated. Observations were made for four weeks, following treatment application.

Floral bud break was defined as the protrusion of the first corolla. Vegetative bud break was defined as the opening of the first leaf.

Chilling hours accumulated in the field (experiment 1) were estimated from the daily minimum and maximum temperatures recorded at the Southern Piedmont Experimental Station. Hours accumulated between 0 and 7.2°C were considered as effective chilling (Darrow, 1942). Estimation was done by calculating hours accumulated between 0°C and 7.2°C. A linear response of daily temperature change from a minimum to a maximum temperature against time was assumed.

Area (0°C to 7.2°C) divided by total area (minimum to maximum temperatures), is equal to the number of chilling hours divided by 24 hours. Estimated chilling hours for shoots taken from the field on Oct. 18, Nov. 8, Nov. 29, of 1991, and Jan. 13, 1992; were 0, 62, 190, and 605 hours, respectively.

Statistical analysis. Polynomial regression was carried out on data collected at all levels of chilling exposures and H_2CN_2 concentrations. Data was transformed using the formula $\arcsin\sqrt{P}$.

Results

Floral bud break. Hydrogen cyanamide was effective in releasing floral buds only in whole plants. We observed in cut shoots, H_2CN_2 treatment did not increase floral bud break at any level of chilling (Table 2.1). In whole plants however, there

was an interaction between H_2CN_2 and chilling exposure on floral bud break. At 300 to 500 hours of chilling, 50 to 125 mM H_2CN_2 increased floral bud break (Table 2.2). Hydrogen cyanamide was not effective in increasing floral bud break of whole plants at other chilling exposures (Table 2.2).

Chilling treatment increased floral bud break significantly in both cut shoots and whole plants. Floral bud break increased linearly as chilling exposure increased, following treatment with 0 to 125 mM H_2CN_2 (Table 2.1 and 2.2). About 60 percent floral buds broke in whole plants after 500 hours of chilling (at 0 mM H_2CN_2) (Table 2.2). While on cut shoots, floral bud break for controls started after 190 hours of chilling (Table 2.1).

Hydrogen cyanamide was phytotoxic to floral buds of cut shoots and whole plants at all level of chilling (Table 2.3 and 2.4). In cut shoots, H_2CN_2 at 125 mM and higher was phytotoxic to floral buds at all except at 0 hrs. chilling (Table 2.3). On whole plants, 50 mM and above was phytotoxic to floral buds that were chilled at 300 hours and below (Table 2.4). Hydrogen cyanamide at $\leq$ 75 mM was not phytotoxic to floral buds as chilling increased to 500 and 750 hours (Table 2.4).

In addition to phytotoxic effects on floral buds, H_2CN_2 also delayed floral bud break in whole plants when applied above 125 mM following 750 hours of chilling (Table 2.5). Chilling treatment hastened the time to first floral bud break as chilling increased (Table 2.5).

Vegetative bud break. Hydrogen cyanamide effectively increased vegetative bud break. In cut shoots, H_2CN_2 increased vegetative bud break after 0, 190, and 605 hours of chilling (Table 2.6). Hydrogen cyanamide at 125, 250, and 500 mM increased vegetative bud break of cut shoots, as chilling increased to 190 hours (Table 2.6). Optimum vegetative bud break for cut shoots occurred at 250 mM H_2CN_2 (Table 2.6). For whole plants, H_2CN_2 increased vegetative bud break when artificially chilled at 100 to 500 hours (Table 2.7). For optimum vegetative bud break of whole plants, 125 mM H_2CN_2 was required (Table 2.7).

Chilling treatment did not overcome vegetative bud endodormancy. There was no significant relationship between vegetative bud break and chilling hours in controls of both cut shoots and whole plants (Table 2.6 and 2.7). Vegetative bud break was affected by the interaction of H_2CN_2 and chilling in cut shoots. Hydrogen cyanamide and chilling interaction on vegetative bud break was not significant in whole plants (Table 2.7).

Hydrogen cyanamide was less phytotoxic to vegetative buds compared to floral buds. High percentages of dead vegetative buds occurred only after applying 500 mM H_2CN_2 (Table 2.8 and 2.9).

In addition to stimulating more buds to break, H_2CN_2 also hastened bud break. At 100 hours of chilling, H_2CN_2 at 500 mM hastened vegetative bud break of whole plants (Table 2.10). An interaction between H_2CN_2 at 75 to 500 mM, and chilling, hastened first vegetative bud break of whole plants (Table 2.10).

Discussion

Hydrogen cyanamide promoted 'Tifblue' rabbiteye blueberry floral bud break only in whole plants. This occurred at between 300 to 500 hours of chilling and H_2CN_2 concentrations at 125 mM and below (Table 2.2). The specificity of H_2CN_2 on 'Tifblue' floral bud break confirmed the findings of Nee (1986).

Floral bud break increased as chilling exposure increased. Chilling requirement in whole plants was about 500 hours. The 500 hours chilling requirement for 'Tifblue' blueberry agreed with the chilling requirement found by Darnell and Davies (1990) and Spiers (1976). The 190 hours chilling requirement we found in floral buds of cut shoots, appeared to be misleading. The method we used to estimate chilling hours in the field might have not been accurate. Chilling temperatures between 0 to 7.2°C only was considered effective (Darrow, 1942). It is possible that temperatures above 7.2°C can fulfill chilling requirements in blueberries too. If so, we may have underestimated chilling in the field. A chilling model for blueberries, has yet to be developed.

Hydrogen cyanamide was more effective in breaking vegetative buds than floral buds. The effectiveness of H_2CN_2 in increasing vegetative bud break occurred at a wider range of chilling exposures than floral bud break. Hydrogen cyanamide increased vegetative bud break in both cut shoots and whole plants, whereas floral bud break was increased in whole plants only. The effectiveness of H_2CN_2 in increasing

vegetative bud break of 'Tifblue' was in agreement to those found in peaches (Seiler, 1991), and 'Radiant' crabapple and red- osier dogwood (Nee, 1986).

Floral and vegetative bud break responded differently to chilling exposure. Chilling induced a positive linear response of floral bud break in both cut shoot and whole plants. However, vegetative buds of whole plants and cut shoots of controls did not respond to chilling. We therefore suggest that vegetative bud dormancy (endodormancy) of 'Tifblue' is not entirely controlled by a chilling factor, as in floral buds; but, may be controlled by other factors such as inhibitor(s) or the lack of growth promoter(s). We showed that H_2CN_2 can over come this problem of vegetative bud dormancy in both whole plants and cut shoots of 'Tifblue' blueberry. Hydrogen cyanamide at 125 and 250 mM was most effective in breaking vegetative bud dormancy of whole plants and cut shoots respectively.

Since 'Tifblue' rabbiteye blueberry floral and vegetative buds responded differently to chilling exposure, it is possible that floral and vegetative buds of other plant species will respond differently to chilling exposure too. Therefore, we suggest that, precaution should be taken when estimating chilling requirements of plant species, based on vegetative bud break alone.

In conclusion, we showed that H_2CN_2 was effective in promoting floral bud break of 'Tifblue' whole plants, and is effective only between 300 to 500 hours of chilling. Hydrogen cyanamide on the other hand, was effective in increasing vegetative bud break of both cut shoots and whole plants at a wider range of chilling exposure.

Hydrogen cyanamide was highly phytotoxic to floral buds than vegetative buds. Floral buds of 'Tifblue' exhibited a positive response of bud break after exposed to chilling temperatures. Vegetative buds on the other hand did not exhibit any relationship towards bud break after chilling exposure.

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Table 2.1. Cut shoot percent **floral bud break** after H_2CN_2 treatment. Percent floral bud break of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 4 different dormancy stages.

H_2CN_2	Chilling hours					
(mM)	0	62	190	605	*l*	*Q*
0	0	0	10	14	***	ns
50	0	0	12	17	***	ns
125	0	0	0	18	***	ns
250	0	0	0	9	*	ns
500	0	0	0	0	-	-
l	-	-	*	***		
Q	-	-	ns	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, *** is significant at $P < 0.05$ and $P < 0.001$ respectively. Data are mean values of n = 5 shoots. - no relationship.

Table 2.2. Whole plant percent **floral bud break** after H_2CN_2 treatment. Percent floral bud break of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	21	42	5	58	81	*	ns
50	28	31	19	75	89	*	ns
75	8	0	35	83	81	***	ns
125	17	0	13	99	50	*	ns
500	0	0	0	0	6	ns	ns
l	ns	*	ns	***	**		
Q	ns	*	ns	*	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, **, *** is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. Data are mean values of n = 4 shoots.

Table 2.3. Cut shoot percent **floral buds dead** after H_2CN_2 treatment. Percent floral buds dead of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 4 different dormancy stages.

H_2CN_2	Chilling hours					
(mM)	0	62	190	605	*l*	*Q*
0	0	0	0	0	-	-
50	0	0	0	0	-	-
125	0	20	65	42	*	**
250	89	89	79	63	ns	ns
500	92	99	86	92	ns	ns
l	***	***	***	***		
Q	*	*	***	**		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, *** is significant at $P < 0.05$ and $P < 0.001$ respectively. Data are mean values of n = 5 shoots. - no relationship.

Table 2.4. Whole plant percent **floral buds dead** after H_2CN_2 treatment. Percent floral buds dead of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	0	0	13	0	0	ns	ns
50	25	34	81	0	0	ns	ns
75	83	31	65	0	0	**	ns
125	67	88	88	0	75	ns	ns
500	99	98	99	53	94	ns	ns
l	**	***	**	***	***		
Q	**	**	*	ns	*		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, **, *** is significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. Data are mean values of n = 4 shoots.

Table 2.5. Whole plant **time (days)** to first floral bud break after H_2CN_2 treatment. Time to first floral bud break of 'Tifblue' blueberry was measured after treatment with a range of concentrations of H_2CN_2 followed by forcing to break bud. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	22	25	10	13	12	*	ns
50	28	15	20	15	12	*	ns
75	32	-	24	13	12	***	ns
125	33	-	20	12	13	***	**
500	-	-	-	-	17	ns	ns
l	ns	ns	ns	ns	***		
Q	ns	ns	ns	ns	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, **, *** is significant at $P< 0.05$, $P< 0.01$ and $P< 0.001$ respectively. Data are mean values of n = 4 shoots. - floral buds did not break by the end of the experiment.

Table 2.6. Cut shoot percent **vegetative bud break** after H_2CN_2 treatment. Percent vegetative bud break of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 4 different dormancy stages.

H_2CN_2	Chilling hours					
(mM)	0	62	190	605	*l*	*Q*
0	0	0	0	3	ns	ns
50	0	11	10	7	ns	ns
125	0	37	65	23	ns	**
250	20	19	79	46	ns	**
500	2	17	55	37	*	*
l	ns	ns	**	***		
Q	*	ns	***	**		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, *** is significant at $P< 0.05$ and $P< 0.001$ respectively. Data are mean values of n = 5 shoots.

Table 2.7. Whole plant percent **vegetative bud break** after H_2CN_2 treatment. Percent vegetative bud break of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	18	6	21	48	29	ns	ns
50	25	38	40	55	23	ns	ns
75	42	55	63	59	34	ns	ns
125	48	72	56	77	53	ns	ns
500	34	45	41	54	42	ns	ns
l	ns	ns	ns	ns	ns		
Q	ns	**	*	*	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, ** is significant at $P< 0.05$ and $P< 0.01$ respectively. Data are mean values of n = 4 shoots.

Table 2.8. Cut shoot percent **vegetative buds dead** after H_2CN_2 treatment. Percent vegetative buds dead of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 4 different dormancy stages.

H_2CN_2	Chilling hours					
(mM)	0	62	190	605	*l*	*Q*
0	0	0	0	0	-	-
50	0	0	0	0	-	-
125	0	0	0	0	-	-
250	0	0	0	0	-	-
500	0	60	0	28	ns	ns
l	-	***	-	***		
Q	-	*	-	*		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, *** is significant at $P< 0.05$ and $P< 0.001$ respectively. Data are mean values of n = 5 shoots. - no relationship.

Table 2.9. Whole plant percent **vegetative buds dead** after H_2CN_2 treatment. Percent vegetative buds dead of 'Tifblue' blueberry was measured 4 weeks after treatment with a range of concentrations of H_2CN_2. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	0	0	0	0	0	-	-
50	0	7	0	0	0	ns	ns
75	24	0	0	0	0	ns	ns
125	0	0	0	0	7	ns	ns
500	61	35	59	39	54	ns	*
l	*	**	***	***	ns		
Q	ns	ns	*	**	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, **, *** is significant at $P< 0.05$, $P< 0.01$ and $P< 0.001$ respectively. Data are mean values of n = 4 shoots. - no relationship.

Table 2.10. Whole plant **time (days)** to first vegetative bud break after H_2CN_2 treatment. Time to first vegetative bud break of 'Tifblue' blueberry was measured after treatment with a range of concentrations of H_2CN_2 followed by forcing to break bud. The treated buds were at 5 different dormancy stages.

H_2CN_2	Chilling hours						
(mM)	0	100	300	500	750	*l*	*Q*
0	19	20	15	16	16	ns	ns
50	16	16	9	14	16	ns	ns
75	19	18	14	15	13	***	ns
125	18	15	12	13	14	**	***
500	21	23	17	15	15	*	ns
l	ns	*	ns	ns	ns		
Q	ns	ns	ns	ns	ns		

l and *Q* is the linear and quadratic relationship of the polynomial regression model. *, **, *** is significant at $P< 0.05$, $P< 0.01$ and $P< 0.001$ respectively. Data are mean values of n = 4 shoots.

Chapter Three: Heat and Bud Scale Removal Influence Floral and Vegetative Bud Break of 'Tifblue' Rabbiteye Blueberry Excised Shoots.

Abstract

The Effect of hot water treatment and bud scale removal on floral and vegetative bud break of 'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade) shoots was investigated. Blueberry shoots were excised from field grown plants after they had received 0, 62, 190, and 605 chilling hours at the Southern Piedmont Experimental Station, Blackstone, Virginia. Some shoots were chilled artificially for 190, 694, 773, and 1109 hours before being treated with hot water (47°C) for 0, 5, 15, 30, 60, 90, 120, 180, and 240 minutes. In a separate experiment, shoots harvested from the field were treated by removing floral and vegetative bud scales. Percentage, and time taken to break floral and vegetative buds was recorded after 4 weeks of forcing under florescent light at 26°C.

Hot water (47°C) was effective in promoting floral bud break of 'Tifblue' blueberry cutting only at 190 chilling hours at 30 minutes exposure. In contrast hot water was more effective in increasing vegetative than floral bud break. The effectiveness of heat treatment on vegetative bud break was dependent on duration of

heat exposure, and varied with chilling hours. Hot water (47°C) immersion for 1 hour was the best heat treatment, since, vegetative bud endodormancy can be overcome at most levels of chilling exposures. Time to first floral and vegetative bud break was not hasten by hot water treatment.

Bud scale removal was not effective in releasing floral bud endodormancy of 'Tifblue' cut shoots. In vegetative buds however, bud scale removal increased bud break, but was nonsignificantly better than control. Removing bud scales was also not effective in reducing to time to vegetative bud break.

Introduction

Dormancy breaking agents have successfully promoted bud break of some endodormant deciduous fruit trees (Erez, 1987; Erez et. al, 1971), and H_2CN_2 is one of the most effective dormancy breaking agents (Fuchigami and Nee, 1987). Floral and vegetative bud endodormancy of many deciduous fruit trees (George et. al, 1988; Shulman et. al, 1986; and Seiler et. al, 1991) and vegetative buds of woody plants (Nee, 1986) can be overcome by H_2CN_2. However, commercial use of H_2CN_2 is limited, because its effectiveness depends on specific timing and concentration of application (Nee, 1986).

The mechanism by which dormancy breaking agents overcome dormancy in plants is not known. Stress treatments applied at sub-lethal dosages can overcome

dormancy (Erez and Lavee, 1974; Levitt, 1980). Fuchigami and Nee (1987) hypothesized that H_2CN_2 imposes sub-lethal stress by binding with the free thiol groups of reduced glutathione (GSH) and protein-bound thiol (PSH). Nee (1986) showed that the sub-lethal stress effects of hot water and freezing treatment correlated with ethylene production. How the binding of cyanamide with thiol groups, and the release of ethylene relate to one another is not understood. Ethylene however is thought not to be directly related in promoting bud break because ethephon application was less effective than H_2CN_2 in releasing bud break (Nee, 1986).

The effect of ABA in controlling bud dormancy has been controversial (Powell, 1987; Salisbury and Ross, 1992). ABA accumulated in buds during endodormancy (Emerson and Powell, 1978; Powell and Maybee, 1984; Rodriquez and Sanchez, 1986). Although ABA content was higher in bud scales than primordia, changes in ABA levels during chilling and bud break occurs only in primordia (Emerson and Powell, 1978). However removing bud scale hastens bud break. Emerson and Powell (1978) suggested that if there is any interaction between chilling and ABA, it is of secondary importance for bud break of grapes.

Our previous study (chapter 2) showed that H_2CN_2 is effective on floral bud break of 'Tifblue' blueberry Two-year-old plants at 500 hours chilling. Hydrogen cyanamide however was effective in promoting vegetative bud break on both cut shoots and whole plants at broader chilling regimes. The purpose of this study was to

determine the effect of hot water and bud scale removal on bud break of 'Tifblue' blueberry.

Materials and Methods

Plant material. Dormant shoots 15-25 cm long were excised from 'Tifblue' blueberry plants grown at the Southern Piedmont Experimental Station, Blackstone, Virginia. Shoots were harvested on Oct. 18, Nov. 8, and Nov. 29, 1991, and Jan. 13, 1992 after exposure to 0, 62, 190, and 605 chilling hours, respectively. Excised shoots were kept moist in polyethylene bags during transport to the laboratory at Virginia Tech, Blacksburg. Shoots were stored and prepared for experiments, as in chapter two.

Experiment 1. Excised shoots were treated with hot water after being exposed to 190, 694, 773, and 1109 hours of chilling. The amount of chilling was determined by combining chilling hours accumulated in the field to the artificial chilling. Cut shoots were chilled in the dark at 6-7°C, under moist conditions. Heat treatment was applied by immersing cut shoots in 47°C water for 0, 5, 15, 30, 60, 90, 120, 180, and 240 minutes. Five shoots were wrapped in plastic and immersed in hot water for each time interval. Untreated shoots (0 min.) served as the control. Treated shoots were placed in 2 cm of distilled water in test tubes and were forced to break bud under florescent light at 26°C. Distilled water level in test tubes were checked and adjusted daily. Every week the base of each shoot was recut to remove plugged xylem vessels.

Experiment 2. Scales of floral and vegetative buds of cut shoots were removed to test their effect on bud break. Shoots were harvested from the field on Oct. 18, Nov. 8, and Nov. 29, of 1991, and Jan. 13, of 1992, after being exposed to 0, 62, 190, and 605 chilling hours. At each harvest, bud scales of floral and vegetative buds were removed using forceps. Treatments included hot water (47°C for 1 hr) and H_2CN_2 (125 mM), and untreated buds were used as controls. Hot water treatment was applied as described in experiment 1. A thin film of H_2CN_2 solution was applied to floral and vegetative buds using a brush. Shoots were forced to break bud as described in experiment 1.

Data collection and analysis. Shoots were observed for four weeks after treatment application. Floral bud break was defined as the protrusion of the first corolla. Vegetative bud break was defined as the opening of the first leaf. Chilling hours accumulated in the field were estimated from the daily minimum and maximum temperatures recorded at the Southern Piedmont Experimental Station. Chilling hours accumulated in the field was estimated as described in chapter 2.

Treatments were completely randomized and replicated five times; one shoot was the experimental unit. Analysis of variance was performed, and mean separation was made by Duncan's Multiple Range Test.

Results and Discussion

Heat treatment was only effective in promoting floral bud break at 190 hours chilling at 30 minutes exposure time (Figure 3.1). Percent floral bud break was generally negatively related to time of hot water treatment (Figure 3.1). Increasing time of heat treatment increased floral buds killed. Compared to control, heat treatment did not enhance the time to break the first floral bud when combined with the chilling treatments (Appendix 1). Similarly, the time to break first vegetative bud was not enhanced by hot water treatment (Appendix 2).

In contrast, heat treatment increased vegetative bud break of cut shoots regardless of chilling hours (Figure 3.2). Time of heat exposure required to break vegetative bud endodormancy varied with chilling. Heat treatment for 60 to 120 minutes broke almost all vegetative buds at the 190-hour-chilled shoots (Figure 3.2). At 694 chilling hours, maximum bud break was induced by 180 minutes of heat exposure. However, at about 773 chilling hours, 5 to 90 minutes of heat exposure induced maximum bud break (Figure 3.2). Therefore the optimum hot water exposure time for increasing vegetative bud break varied with chilling exposure. The one hour exposure time selected for the second experiment appeared to be the optimal for vegetative bud break at all chilling exposures except 694 chilling hours (Figure 3.2). Our results confirm the findings of Nee (1986), where hot water was effective in promoting vegetative bud break of 'Radiant' crabapple.

Compared to control, vegetative bud scale removal increased vegetative bud break nonsignificantly after 190 hours of chilling (Figure 3.3). The increased in vegetative bud break might have been significant if we used a larger sample size. However, bud scale removal was as effective as heat treatment in increasing vegetative bud break at all chilling levels. Bud scale removal increased vegetative bud break less effectively than H_2CN_2 at 62 and 190 hours of chilling (Figure 3.3).

Floral bud scale removal did not significantly promote floral bud break at any dormancy stages. Therefore, we suggest that bud scale removal is not as effective in promoting floral bud break of 'Tifblue' as other treatments such as H_2CN_2 (chapter 2) and hot water (47°C for 1 hr).

Time to first floral and vegetative bud break was not enhanced by scale removal (Appendix 1 and 2). As discussed earlier, similar results were obtained following hot water treatment to break floral and vegetative buds.

In conclusion, we showed that heat treatment was effective in breaking vegetative; but on floral bud break, heat treatment was only effective at 190 chilling hours at 30 minutes of heat treatment. The effectiveness of heat treatment on vegetative bud break varied with chilling exposure, but depended on duration of heat exposure. Vegetative bud break increased slightly, but nonsignificantly, with scale removal.

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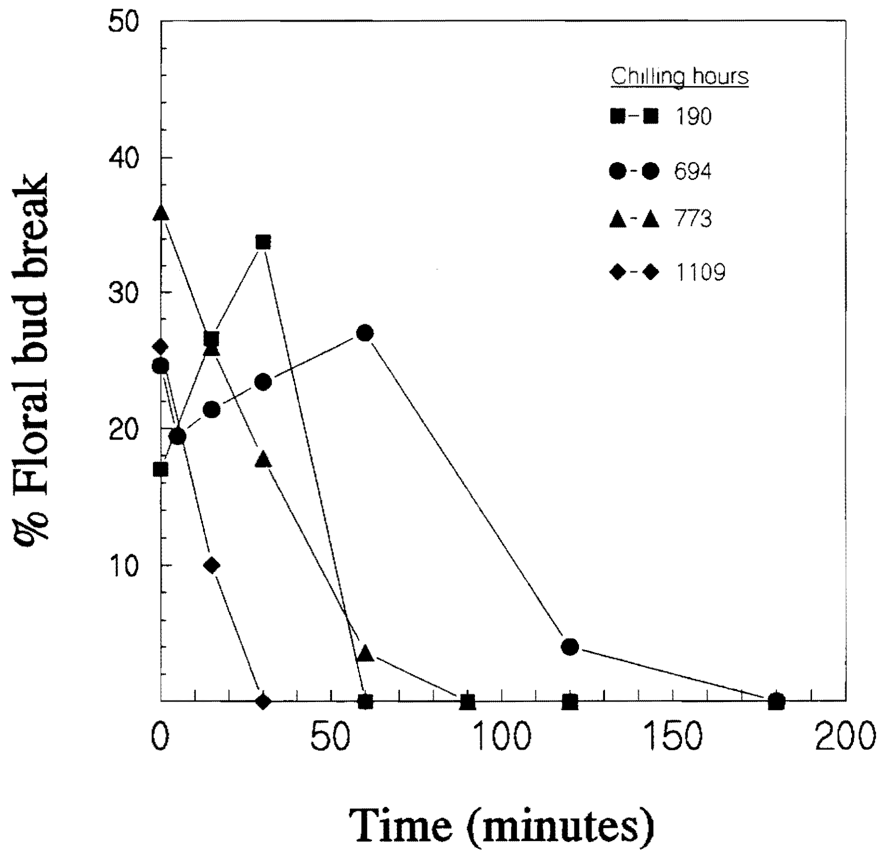

Figure 3.1. Effect of hot water (47°C) immersion time on percent floral bud break of 'Tifblue' shoots. Shoots were excised, and artificially chilled for 190, 694, 773, and 1109 hours prior to hot water treatment. Floral bud break was recorded after 4 weeks of forcing (n = 5 shoots).

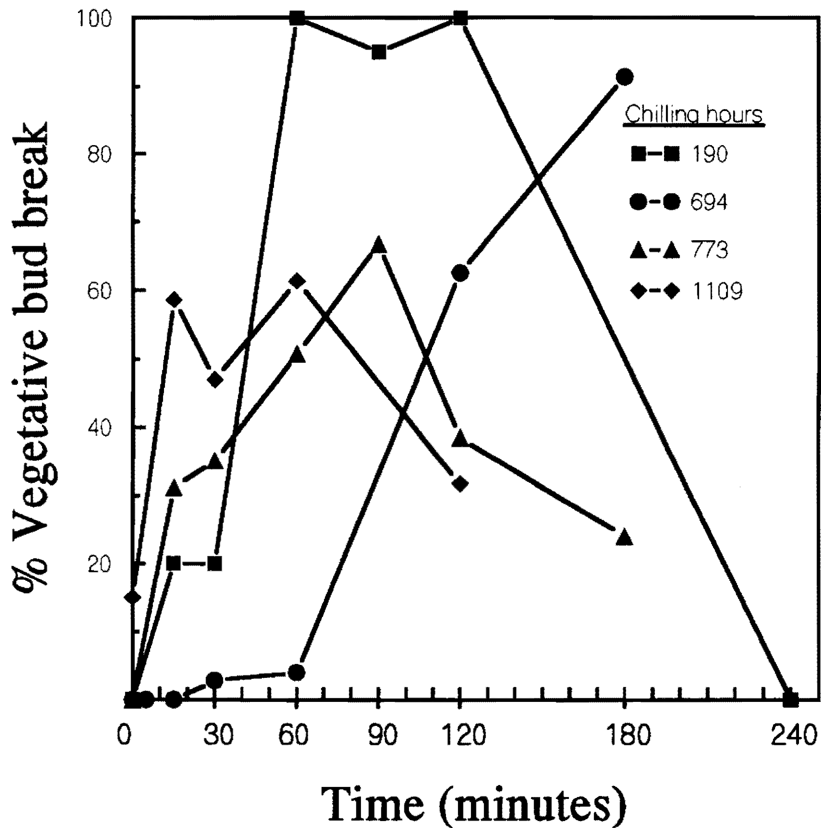

Figure 3.2. Effect of hot water (47°C) immersion time on percent vegetative bud break of 'Tifblue' shoots. Shoots were excised, and artificially chilled for 190, 694, 773, and 1109 hours prior to hot water treatment. Vegetative bud break was recorded after 4 weeks of forcing (n = 5 shoots).

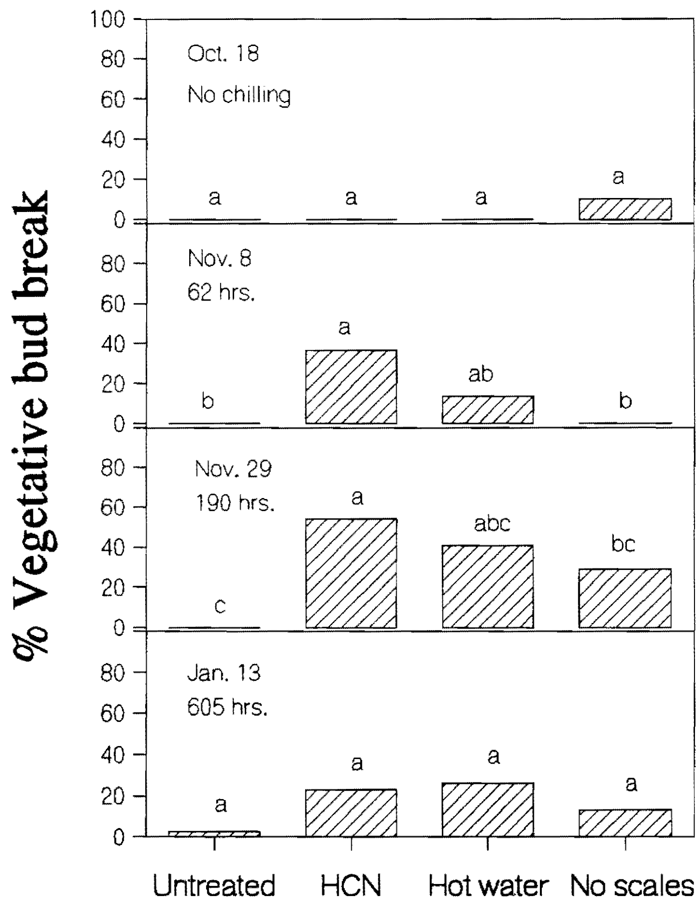

Figure 3.3. Effect of H_2CN_2 (125 mM), hot water (47°C for 1 hr), bud scale removal on percent vegetative bud break of 'Tifblue' shoots. Shoots were excised from field grown plants exposed to 0, 62, 190 and 605 hours of natural chilling. Vegetative bud break was recorded after 4 weeks of forcing (n = 5 shoots).

Chapter Four: Floral Bud Removal and Hydrogen Cyanamide Release Vegetative Bud Dormancy of 'Tifblue' Rabbiteye Blueberry Cuttings

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In temperate climates, buds of blueberries and other deciduous fruit crops become dormant during autumn and require exposure to chilling to overcome dormancy (Amling and Amling, 1980; Darnell and Davies, 1990; Darrow, 1942; Gilreath and Buchanan, 1981; Spiers, 1976; Spiers and Draper 1974). Winter

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dormancy is classified as endodormancy, since physiological factors within the are thought to bud impose dormancy. Paradormancy, such as apical dominance of lateral buds, is regulated by factors outside the bud. We discovered that blueberry vegetative buds exhibit paradormant characteristics during endodormancy. We also tested several other deciduous species for winter paradormancy.

Dormant 'Tifblue' blueberry shoots 15-25 cm long were taken from field plots in Blackstone, VA on 13 Jan., 1992 after they had received 605 chilling hours, between 0 to 7°C. The cut shoots were placed in distilled water, wrapped with plastic, and stored in the dark at 6-7°C for 850 hours. Shoots were then defoliated (no new leaves developed in cold storage), and treated by either: 1) removing the apical bud, 2) removing all floral buds, 3) hydrogen cyanamide (H_2CN_2), or 4) first removing all floral buds and then applying H_2CN_2, 5) control-untreated. Hydrogen cyanamide (125 mM) was applied with a paint brush to floral and vegetative buds until a thin film covered each bud. The buds of five shoots per treatment were forced to break bud under florescent light at 27°C for three weeks.

'Tifblue' vegetative buds have an estimated chilling requirement of 500 chilling hours (Darnell and Davies, 1990). In contrast we observed that vegetative buds did not break dormancy even after 850 chilling hours. However, removing of floral buds greatly increased vegetative bud break (Table 4.1). Removing apical buds alone did not affect vegetative bud break, while applying H_2CN_2 increased vegetative bud break. Floral bud removal plus H_2CN_2 application also increased vegetative bud break, but

was no better than removing floral buds (Table 4.1). Thus it appears that vegetative buds can be inhibited by ecodormant floral buds. The inhibitory effect of floral buds can only be partially overcome by H_2CN_2.

To determine whether other deciduous species exhibit paradormant characteristics in winter we collected shoots of flowering Dogwood (*Cornus florida*), Higan cherry (*Prunus subhirtella*), Oriental cherry (*Prunus serrulata*), Sand cherry (*Prunus x cistena*), Koreanspice Viburnum (*Viburnum carlesii*), Peach (*Prunus persica*), and Easter Red bud (*Cercis canadensis*) on 31 March, 1992. Floral buds were removed and vegetative buds forced to break under the conditions described for blueberries. Vegetative bud elongation was recorded by measuring bud length (cm) after two weeks of forcing.

Removing floral buds of *Cornus florida*, *P. subhirtella* and *C. canadensis* enhanced vegetative elongation (Figure 4.1). In *Prunus serulata*, *Prunus x cistena*, *V. carlesii*, and *P. persica* floral bud removal did not significantly increase vegetative bud elongation. Positional relationships of floral and vegetative buds influenced the bud break pattern (Figure 4.1). Vegetative buds were inhibited when floral buds were closer to the shoot apex than vegetative buds. Vegetative buds were not inhibited when floral buds were at the base or in close association with the vegetative buds.

The inability of vegetative buds to break even after receiving excessive chilling hours, could be explained by an inhibitory effect of floral buds. We observed that floral bud removal hastened vegetative bud break. This effect of organ removal in

blueberry was also reported when leaves were removed (Spiers and Draper, 1974). Supporting our observations, insufficient chilling affected floral buds differently compared to vegetative buds (Darnell and Davies, 1990; Spiers and Draper, 1974). This suggests that other factor(s), such as inhibitors are influencing dormancy release. Alternatively bud removal or leaf removal may elicit a bud breaking agent or growth promoter instead of removing an inhibitory agent. Therefore, vegetative buds of blueberries may be paradormant rather than endodormant as previously suggested (Spiers and Drapers, 1974). Since H_2CN_2 is able to break dormancy similar to removing floral buds, it may be doing so by influencing the same inhibitor/promoter as floral bud removal. We found that in species other than blueberry, some dormant vegetative buds were stimulated by floral bud removal and others were not. If a promoter or inhibitor was involved, the relative position of vegetative to floral bud may have been important.

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Table 4.1. Effect of floral bud removal and exogenous H_2CN_2 on vegetative bud break of 'Tifblue' Rabbiteye blueberry. Bud break was recorded after 3 weeks of forcing, following treatment application.

Treatment	Mean % Vegetative Bud Break
Control	0.0 c[z]
Apical bud removed	4.0 c
All flower buds removed	62.6 a
125 mM H_2CN_2	23.3 b
All flower buds removed + 125 mM H_2CN_2	53.3 a

[z]Mean separation within columns by Duncan's multiple range test ($P < 0.05$).

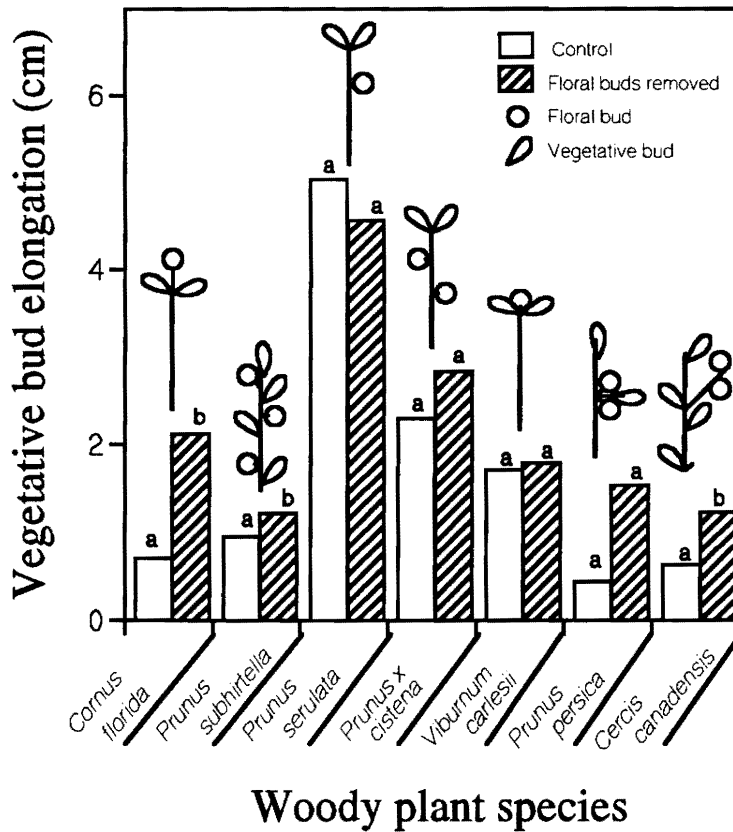

Figure 4.1. Effect of floral bud removal on vegetative bud elongation (cm) of selected woody plants. Shoots from selected woody plant species were incubated at 27°C under light after floral buds were removed. Vegetative bud growth was measured after 2 weeks. Drawings above the bar graph are morphological representations of the relative positions of floral and vegetative buds. Means compared by F-test ($P < 0.05$).

Appendix 1

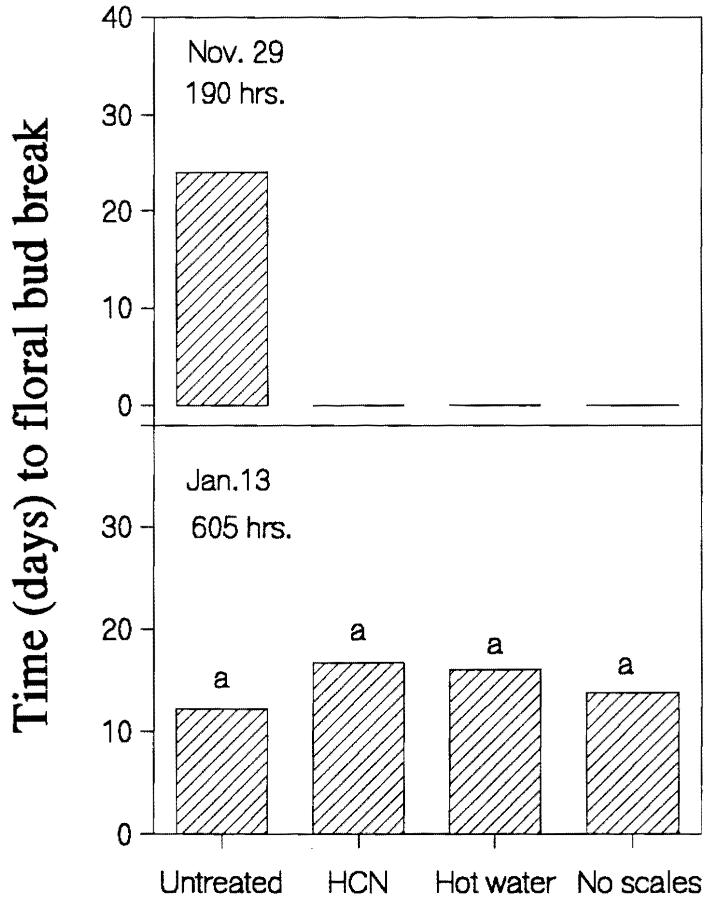

Figure 1. Effect of hot water (47°C for 1 hr) and bud scale removal on time to first floral bud break of 'Tifblue' rabbiteye blueberry excised shoots. Shoots were excised from field grown trees exposed at 190 and 605 hrs. of natural chilling. Untreated and H_2CN_2 (125 mM), were used as controls. Flower bud break was recorded after 4 weeks of forcing. Mean values of n = 5 shoots.

Appendix 2

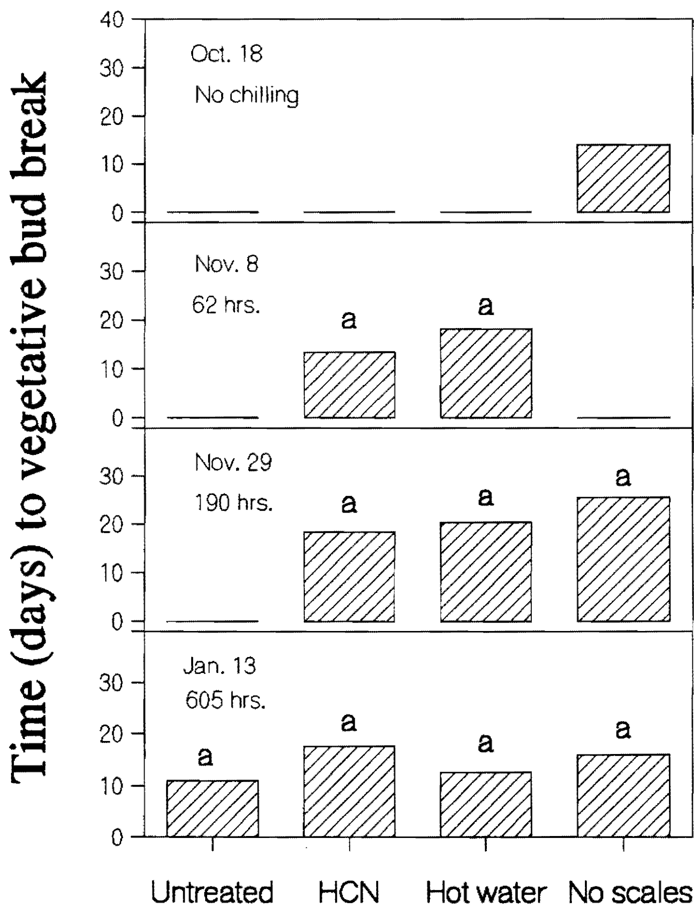

Figure 2. Effect of hot water (47°C for 1 hr) and bud scale removal on time (days) to first vegetative bud break of 'Tifblue' rabbiteye blueberry excised shoots. Shoots were excised from field grown trees exposed at 0, 62, 190 and 605 hrs. of natural chilling. Untreated and H_2CN_2 (125 mM), were used as controls. Vegetative bud break was recorded after 4 weeks of forcing. Mean values of n = 5 shoots.

Vita

Mohd. Ridzuan Mohd. Saad was born on 28 March, 1957, in Kodiang, Kedah, Malaysia. He graduated from University Pertanian Malaysia (UPM) in 1977 and held position as an assistant researcher for four years at the Malaysian Agricultural Research and Development Institute (MARDI). He returned to UPM to earn his Bachelor's degree in Agriculture in 1984, and continued his service at MARDI in rice production research, and later in the Technology Promotion Division. In December of 1992, he earned a Master's of Science in Horticulture specializing in bud dormancy, from Virginia Polytechnic Institute and State University, Blacksburg, VA.

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