THESIS

WHITE DRUPELET DISORDER OF RED RASPBERRY
RUBUS IDEAUS L.

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY PAMELA A. HOSMAN ENTITLED WHITE DRUPELET DISORDER OF RED RASPBERRY RUBUS IDEAUS L. BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

WHITE DRUPELET DISORDER OF RED RASPBERRY

White Drupelet Disorder (WDD) is a condition where individual drupelets of red raspberry fruit lack red pigmentation, but are white and fully turgid. This has been associated with high temperature and high UV-B radiation.

Five cultivars were evaluated for susceptibility to WDD using UV-B radiation and high temperature (42°C) in a growth chamber. Results of statistical analyses of differences in susceptibility to WDD were mixed but because of possible interactions with date of testing, were determined to be equally susceptible to WDD.

Cellulose fibers were used for coating fruit as a potential protectant against WDD. Dry coatings of the fruit were not significantly different from uncoated treatment. However, cellulose fibers applied wet resulted in significantly greater WDD than the control and dry application of fibers.

Pigment extracts of red and white fruit were evaluated for responses in color and absorbance level at 535 nm with variations in pH. Extracts from drupelets exhibiting WDD failed to develop red pigment with pH change, thus indicating the absence of the colorless forms of anthocyanins in the fruit.

Glucose and sucrose of both red and white drupelets of red raspberry cv. Heritage from field and laboratory samples were analyzed by gas chromatography for soluble sugars. No differences in glucose levels were observed in the red or white drupelets from either field or laboratory samples. However, red drupelets from both field and laboratory exhibited twice as much sucrose as white drupelets.

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TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Red raspberries are a high value fresh market crop. However, the occurrence of a physiological whitening of some drupelets known as the white drupelet disorder (WDD), in both the red and gold fruited cultivars, decreases their value by as much as 30-40% due to cullage. WDD has been observed in regions with high light intensity, and excessively high temperatures and UV-B. Fruit produced in geographical elevations which typically have intense UV-B radiation, are particularly susceptible. Even though the culls exhibiting WDD can be used in juices and preserves; the prevention of WDD is important in maintaining the high value of fresh fruit.

WDD is prevalent in Colorado especially on the Western Slope. Researchers in California, New Mexico, Australia (Renquist et al., 1987), and Oregon (personal communication, Helen Kahn, Oregon raspberry grower, 1991) have also reported the disorder. Understanding the causes of WDD may lead to a means of preventing it and thus retain better crop value (Renquist et al., 1987, 1989).

Renquist et al. (1987, 1989) reported that WDD is a physiological/environmental disorder caused by an interaction between UV-B radiation and excessively high temperatures in drupelets on the sun exposed side of the fruit. Temperatures recorded from probes inserted in drupelets on the sun exposed side of the fruit were 7.3°C higher than the ambient air temperatures which ranged from 31.8 to 37.4°C. WDD usually occurs in August when daytime temperatures may exceed 33°C. The affected drupelets are turgid and visually identical to normal fruit but are white due to the lack of anthocyanin development. Pathogenic diseases are not evident since there is no browning, necrosis or desiccation of the fruit.

Research by Renquist et al. (1987) has demonstrated that the use of shade fabrics can significantly reduce WDD. However, the cost of material and increased labor limits its potential application. The objective of this research was to further characterize WDD and determine if a means of prevention might be possible. Specifically, eperiments were designed to:

1) determine the relative susceptibility of five red raspberry cultivars to WDD,

2) further describe the pigmentation of WDD drupelets in comparison to normal fruit,

3) evaluate the use of an edible cellulose fiber application for prevention of WDD,

4) determine if relative sugar levels are associated with the lack of anthocyanin formation in WDD, and

5) determine if cellular pH differs between white and red drupelets and if so, is this related to the lack of red pigment.

CHAPTER 2

LITERATURE REVIEW

White Drupelet Disorder

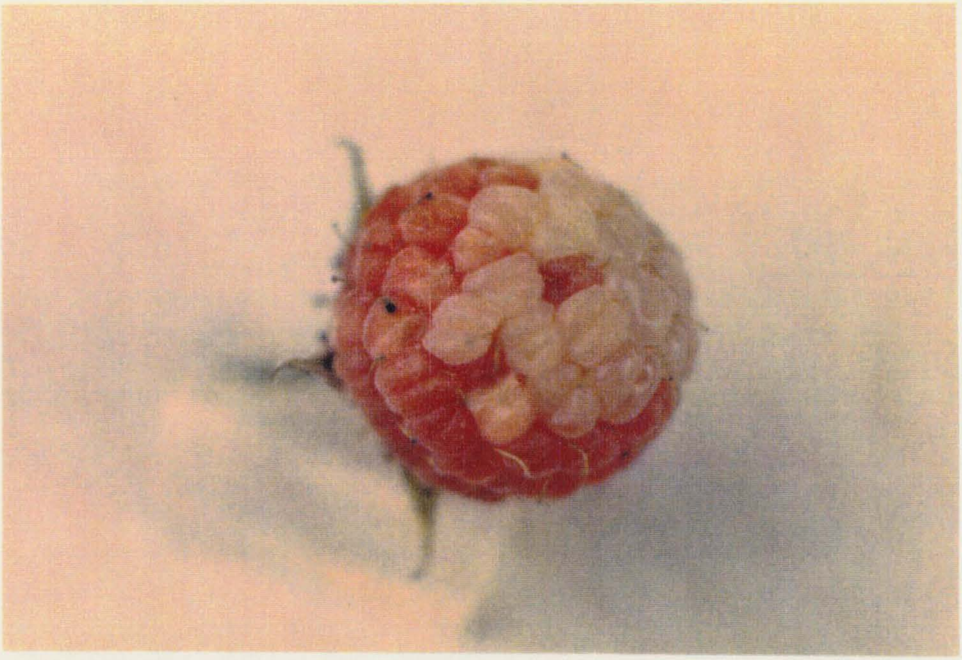
White Drupelet Disorder (WDD) in Rubus ideaus L. raspberries, is characterized by fully developed drupelets that are visually healthy except for the lack of anthocyanin pigment, Figure 1. WDD drupelets are of comparable size and turgidity to the pigmented ones on the same fruit but, remain white at maturity. Insects or pathogens are not associated with the condition.

WDD occurs primarily when ambient air temperatures are above 33°C and solar radiation is intense. Fruit temperatures conducive to WDD has been reported to be 7.3°C higher than ambient air temperatures which ranged from of 31.8 to 37.4°C, and usually involved one or more drupelets per fruit. Observations reported by Renquist et al. (1987) noted that in some years more than 30 to 40% of the fruit produced on the Western Slope of Colorado may be affected by the WDD in mid-August.

Raspberry Fruit

An understanding of fruit development is important in characterizing WDD. The raspberry fruit is an aggregate of individual fruits termed drupelets. Many ovaries develop from a single flower and adhere to a common receptacle. The drupelets are held together by unicellular linear trichome epidermal hairs. They are abundant on the base and sides of each drupelet and are so enmeshed that the drupelets

A.



B.

Figure 1: A. White drupelet disorder present in 'Heritage' raspberries. B. Fruit exhibiting grasshopper damage in 'Heritage' raspberries and normal fruit.

cannot be separated without tearing the "skin". The interior portion of the drupelet is composed of a thin wall of parenchymatous cells which radiate from the pyrene in the center to a region of larger oval cells underlying the epidermis (Jennings, 1988). The epidermis is one to three cells thick. Cultivar variations, due to structural differences, are partially due to cell diameter, tissue compactness and overall cell size. Anthocyanins develop within the cells near the epidermis, where they most intercept sunlight (Salisbury and Ross, 1985).

Raspberries ripen within 30 to 36 days after pollination. Even though each drupelet is an individual fruit, the aggregate of drupelets usually ripen at the same time. The ripening occurs in three, 10-12 day, stages. Stage one includes rapid growth associated with cell division. Stage two growth is slower as the embryo develops and the endocarp hardens. Rapid growth due to cell enlargement occurs during the third stage of ripening (Jennings, 1988). When ripening occurs, ethylene levels increase and respiration decreases as fruit color begins to develop. Maximum ethylene levels are reached when the fruit is fully ripe. Respiration, as measured by CO_2 produced per gram of fruit, decreases as fruit continues to ripen (Jennings, 1988).

Causes of White Drupelet Disorder

'Heritage' is currently the cultivar of choice in Colorado. In a yield trial it yielded twice the amount of fruit as 'Amity'. However it was twice as susceptible to WDD. Primocane cultivars such as 'Heritage' and 'Amity' are more suited to Colorado's variable weather patterns versus biennial cane cultivars which have poor winter survival of buds unless labor intensive protection is followed. During a yield study of 'Amity', fruit showed significantly less damage due to WDD than 'Heritage' (Renquist and Hughes, 1987). An indication of genetic variation means it may be possible to identify or develop cultivars which are less vulnerable to solar injury. Black raspberries, which have a different pigment makeup, have not shown symptoms of solar injury (Renquist et al., 1987).

Renquist et al. (1989) characterized the laboratory effects of high temperature and UV radiation, alone and in combination, by disrupting red pigment development, Figure 2. Fruit of Rubus ideaus cv. Heritage were exposed to UV-B for seven hours in a GE Precision Scientific 805 growth chamber that was pre-heated and held at 42°C. Fruit were then transferred to another growth chamber, where color was allowed to develop at 20°C with high humidity. Fruit that was harvested in the green stage resulted in an average of 0.2 white drupelets per fruit after exposure to UV-B and was thus determined to be resistant. Raspberries harvested at the white stage developed an average of 8.6 white drupelets while fruit at the pink stage developed 25.8 white drupelets. The pink stage was determined to be the most susceptible, but at this stage the color was reported to be arrested after UV-B exposure. The white stage was thus chosen for further testing under UV-B. Renquist et al. (1989) reported that the white stage, which ripens in two to three days, subjected to high temperature without UV radiation within the range of fruit surface temperatures observed in the field (≥43°C) had no effect. Also, UV radiation for ≥9 hr had no effect up to 17 hr at 37°C. Injury approximating that observed in the field (Renquist et al., 1989) only occurred with a combination of 42°C or higher and 4 to 7 hr of UV exposure.

Sunscald

Sunscald is a term applied to plant tissue damage due to heat and/or UV radiation injury. Photodynamic sunscald is an apparent blockage of pigment synthesis in which chemical lesions are included

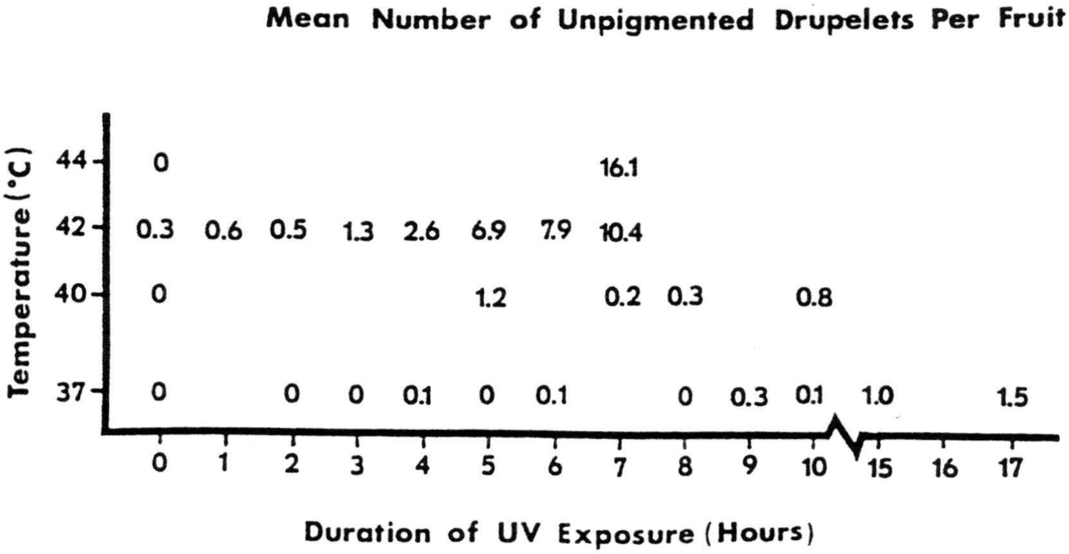


Figure 2: Effects of high temperature and UV radiation in disrupting pigment development. Values are the average of one to four means at each temperature-UV combination (Renquist et al., 1989).

(Barber and Sharpe, 1971; Teramura, 1983). It may occur following UV stress and have similarities to photobleaching, the loss of chlorophyll following UV stress (Smillie and Hetherington, 1983). Most sunscald injury is identified with discoloration as silvering, yellowing, browning or blackening, and desiccation (Barber and Sharpe, 1971).

Barber and Sharpe (1971) defined three patterns of sunscald injury:

1) Discoloration as expressed by silvering, yellowing, browning or blackening which may occur instantaneously in green apples or take several days to occur in white vegetable marrow such as squash. Damage varies from superficial blemishes, affecting a single layer of epidermal cells, to complete fruit destruction.

2) Droplet formation on the surface is the first sign of sunscald injury in light and dark green vegetable marrows. Browning (bright yellowing or bleaching followed by desiccation) occurs following subsequent exposure to the sun.

3) Water soaked blister formation is the first symptom of sunscald in tomato (*Lycopersicon esculentum* Mill.) and peppers (*Capsicum annum*). Green varieties undergo rapid bleaching followed by desiccation.

Although WDD in raspberries is similar to sunscald in that it is induced by solar radiation and temperature, the symptoms are not the same. Unlike the silvering, yellowing or browning that occurs in green apples or squash, the drupelets affected in raspberries remain consistently white. Raspberry fruit does not form water soaked blisters or show bleaching followed by desiccation. WDD is unique in that the fully formed drupelets that were affected never develop appropriate color, red or gold, on the cultivars tested (Renquist et al., 1987). The pigment blockage could possibly be a type of photodynamic sunscald (Barber and Sharpe, 1971; Teramura, 1983) caused by absorption of visible energy by photosensitive pigmented cells which result in temperature induced chemical lesions. Metabolic processes are known to be impaired by temperatures ranging from 37°C to 65°C (Barber and Sharpe, 1971).

Protection against WDD

Shade cloth was found to be effective in reducing WDD in 'Heritage' red raspberries. Renquist et al. (1987) reported a 41% incidence of WDD in the field without shade protection in mid-August of 1986 when temperatures were greater than 33°C. Black Sarlon fabric, 60% shading, resulted in the greatest reduction of the disorder as compared to other tested fabrics (Renquist et al., 1987). The mean percent of WDD incidence was 8% for late (August 14) application of shading as compared to 41% for controls. Black Sarlon fabric, 30% shade, showed an incidence of 12 and 15% with early and late application respectively, while white DuPont Reemay fabric, 25% shade, resulted in only 16% WDD.

Recorded surface temperature of exposed fruit was 4°C to 1°C lower than the control with the use of black Sarlon, (60% shade), and white DuPont Reemay (25% shade) fabrics, respectively. Thus, fruit temperature was implicated as a factor in WDD incidence.

Fans were used in the field to cool raspberry fruit without shade fabric to determine if cooling alone would reduce the incidence of WDD. A 1% reduction of fruit surface temperature occurred with the use of the fans, resulting in only 23% WDD. While fans cooled fruit and reduced the incidence of WDD, the use of shade fabrics showed the greatest reduction.

The cost effectiveness of either shade fabric or fans would likely be prohibitive, although not determined. It is also likely that the fabrics would reduce photosynthesis which may in turn result in an overall yield reduction.

Ultraviolet-B Radiation

Visible light (380-775 nm), and ultraviolet light (UV-A, 320-380 nm; UV-B, 280-320 nm; and UV-C, 320-180 nm), except for UV-C which does not reach the earth's surface, interact with living systems in different ways. UV-B has been shown to be damaging in human and plant living systems. Some specific effects of UV-B on living systems are noted, Figure 3 (Caldwell et al., 1986).

Becwar et al. (1982) reported that stratospheric ozone was predicted to decrease from 5 to 15% per hundred years at the current (1982) estimated chlorofluoromethane release rates. However, more recent measurements of the ozone depletion in the earth's stratosphere (beneficial ozone) and UV-B radiation, indicates UV-B radiation (280-320 nm) reaching the earth has dramatically increased. As the ozone layer presently appears to be dropping at the rate of 1 to 5% per decade in the latitudes of the United States, UV-B radiation will increase as barriers thin (Appenzeller, 1991).

The United Nations Environment Program, together with the World Meteorological Organization, have made measurements which show the ozone shield is eroding over temperate latitudes in the summer. Crops and people are therefore exposed to greater dosages of ultraviolet light just when they are most vulnerable (Appenzeller, 1991). NASA researchers reported that the ozone hole of the Antarctic hit a record depth in 1991 (Appenzeller, 1991). UV-B flux varies according to season, tropospheric ozone, latitude, and altitude where the distance to the earth's surface is shorter (Caldwell, 1981). Current and potential increases in UV radiation at the earth's surface raises the spectra of ever greater problems with WDD.

There are two known mechanisms by which plants protect themselves from UV-B radiation. These are avoidance and damage repair. In

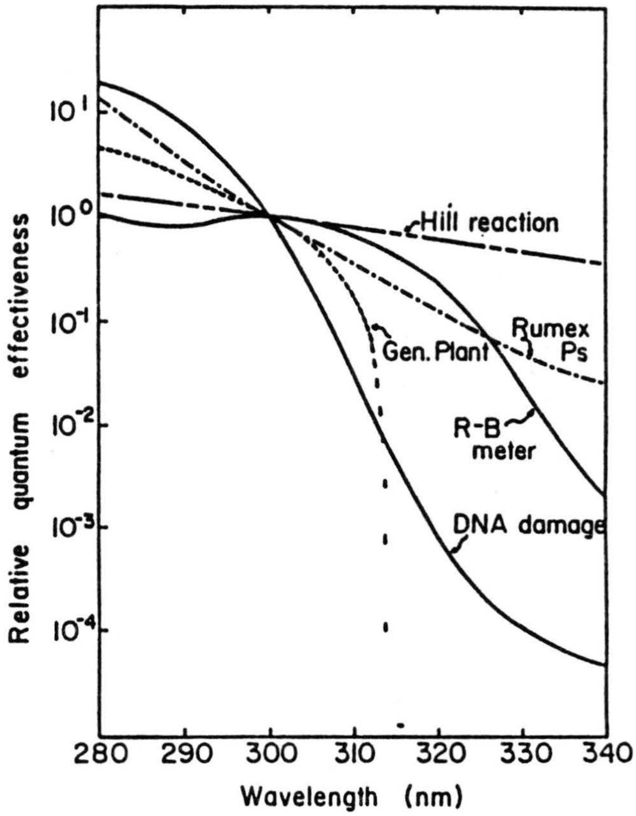


Figure 3: Ultraviolet action spectra (UV-B 280-320 nm, UV-A 320-400 nm, and UV-C 180-280 nm, not depicted) representing basic modes of plant tissue damage (Caldwell et al., 1986).

avoidance, foliage coverage of fruit or flowers or the leaf's positioning are protective mechanisms to decreased direct sunlight. Secondly, avoidance may take the form of UV attenuation through absorption by non-critical tissues such as the cuticle waxes and flavonoids present in many species. These absorb UV and transmit longer but less energetic wavelengths (Robberecht and Caldwell, 1978). In a study of 25 species, 43% to 80% of the UV attenuation was attributed to epidermal structures. UV-B absorbing pigments, such as flavonoids, resulted in 20 to 57% of the attenuation of UV-B radiation (Robberecht and Caldwell, 1986). They determined that flavonoids were induced after UV exposure in pigment cell extracts. These induced flavonoids were of the three groups, anthocyanins, flavonols, and flavones.

Genetic damage (DNA) can sometimes be reversed by corrective measures in the cell. Sutherland (1981) reported that pyrimidine dimers of cyclobutane can be removed through visible light-dependent enzymatic reactions. First, a photoreactivating enzyme or photolyase binds to the dimer site in the dark reaction. Second, light energy, with maximum effectiveness at about 350-450 nm, splits the dimer and restores the DNA to its original state. It was reported that this process may not work if the DNA damage is too severe and rendered irreversible. Murphy (1983) noted that changes in both DNA and RNA can cause secondary effects such as a decrease in membrane permeability, and ionic imbalance.

Some deleterious effects of UV radiation on organisms are reversible by concomitant or subsequent exposure to UV-A (315-400 nm) or visible radiation (Becwar et al., 1982). Teramura et al. (1980) found that UV-B radiation adversely affected net photosynthesis at low (less than ambient field levels) photosynthetically active radiation (PAR) levels but had little effect at PAR levels that saturated photosynthesis in the field (Teramura, 1983). Becwar et al. (1982) reported that deletion of UV-B radiation or a 52% enhancement of effective UV-B radiation at midday did not affect dry weight in pea, potato, radish, and wheat although wheat plant height was more sensitive than other parameters measured. Kasperbauer and Loomis (1965) showed that flowering was reduced when filters were used that prevented UV penetration. Once the filters were removed flowering increased. Specific plant sensitivity to UV-B has not been determined in all species or cultivars.

The effective solar UV-B radiation level in the San Luis Valley, a 3000 m site in Colorado, was 61 [mW • m^{-2}] (Becwar et al., 1982). In addition, Becwar et al. (1982) reported the Beltsville, MD. effective solar UV-B radiation level near sea level was 41 [mW • m^{-2}]. Both readings were taken at solar noon in the middle of August. These measurements are only at midday and cannot be used directly to estimate daily or cumulative effective UV-B radiation enhancement. The Colorado site had a 49% biologically weighted UV-B increase over the sea level intensity at Beltsville, MD.

Photosynthesis has been shown to be partially inhibited when intact leaves of many species were exposed to UV-B (Iwanzik and Tevini, 1982; Sisson, 1981; Sisson and Caldwell, 1976, 1977; Termura et al., 1980; and Tevini and Iwanzik, 1983). UV-B damage to the photosynthetic process is cumulative. Normal doses of UV-B did not cause significant chlorophyll concentration reduction, but decreased chlorophyll did occur in plant leaves exposed to particularly enhanced doses of UV-B (Brandle et al., 1977; Sisson and Caldwell, 1976; and Termura et al., 1980).

UV-B photoinhibition may be related to the inhibition of electron transport associated with photosystem II (Brandle et al., 1977; Yamashita and Butler, 1986). Research by Okada et al. (1976) supported the direct inhibition of primary photochemistry at the photosystem II reaction center and inactivation of photosystem I.

Water stress increases the sensitivity of plants to UV-B radiation. Although UV-B decreased vegetative growth in soybeans, UV-B stress plus water stress showed a greater decrease in vegetative growth (Teramura, 1986).

Cellulose Fibers

Cellulose forms the framework of all vegetable matter, and the main constituent of all woods. It is the basic material of cellulose products available for industry and bakery use. Industrially, cellulose has been diversely used commercially for the production of textiles, cigarette filter-tips, and plastics. It is cost effective, environmentally safe and economical in terms of environmental fuel use. Cellulose fibers morphologically may be hydrophilic or hydrophobic. Cellulose products used in foods such as methyl ethyl cellulose are only soluble in cold water; however, sodium carboxymethyl cellulose is soluble in both hot and cold water. This property depends on the solution added to the fiber as well as the structure of the cellulose. Resistance to microbiological and light degradation are attributes produced from cellulose with the addition of various chemicals such as nitrates, ethers, and acetates (Reveley, 1985).

Epidermal sunburn of apples induces corky tissue formation, resulting in a major profit loss of as much as 10% of the produce in Western Colorado. A field spray application of cellulose fiber was used by Matthew Rogoyski in reducing fruit sunburn of 'Golden Delicious' apples at Rogers Mesa Research Center in Hotchkiss, CO. during the 1990-91 growing season. Cellulose fibers were used with the intent of mimicking the pubescent hairs of desert plants in reflecting light and promoting a cooler microclimate in the apple experiment (personal communication, Matthew Rogoyski, Rogers Mesa Research Center, 1991). Thus, at least experimentally, cellulose has shown promising results in reducing sunburn of apples, though cellulose fibers do wash off apples 'smooth 'skin after several rains as the binding agent is a pectin (personal communication, Matthew Rogoyski, Rogers Mesa Research Center, 1991).

Chemistry of Anthocyanins

Anthocyanins are pigments which impart colors commonly found in red, purple, and blue flowers. They are also present in various other plant parts, such as certain fruits, stems, leaves, and even roots. These intensely colored pigments are water-soluble and often more than one is present in a particular fruit or organ. Anthocyanins are present as glycosides, usually containing one or two glucose or galactose units attached to the hydroxyl group in the central ring or to that of the 3-position in the ring. When the sugars are removed, the remaining parts of the molecules, which are still colored, are called 'anthocyanidins' (Salisbury and Ross, 1985).

Anthocyanidins are usually named after the particular plant from which they were first obtained. The most common anthocyanidin is 'cyanidin', which was isolated from the blue cornflower, Centaurea cyanus. 'Pelargonidin' was named after a bright red geranium of the genus Pelargonium. 'Delphinidin' obtained its name from the genus Delphinium (blue larkspur). These anthocyanidins differ only in the number of hydroxyl groups attached to the B ring of the basic flavonoid structure. Other important anthocyanidins include the reddish 'peonidin' (present in peonies), the purple petunidin (in petunias), and the mauve-colored purplish pigment 'malvidin' first found in a member of the Malvaceae, the mallow family. The chemical structure of raspberry pigments are illustrated, Figure 4.

The color of anthocyanins depends first on the substituent groups present on the B ring. When methyl groups are present, as in peonidin, they cause a reddening effect. The anthocyanins are often associated

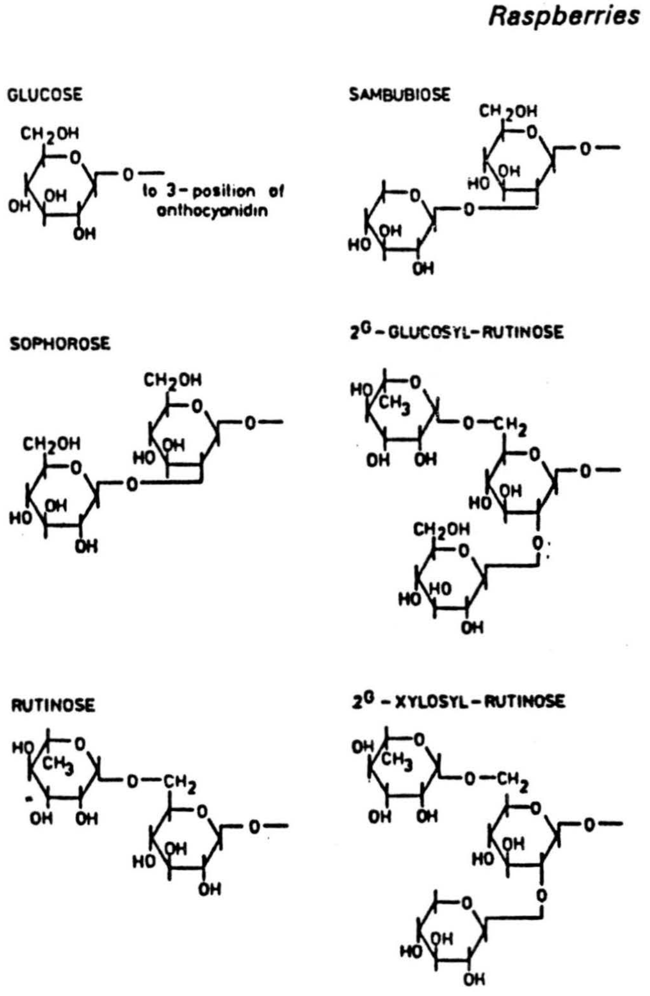


Figure 4: Chemical structure of pigments in raspberry (Jennings, 1988).

with flavones or flavonols which cause them to become more blue. When associating with each other in high concentrations, this can cause a reddening or a bluing effect, depending on the anthocyanin and the pH of the vacuoles in which they accumulate (Hoshino et al., 1981). Most anthocyanins are reddish in acid solution but become purple and blue as the pH is raised. The pH values of epidermal cells containing delphinidin increase from 5.5 to 6.6 during aging, and the color changes from reddish purple to purplish blue (Asen et al., 1975).

Environmental conditions including temperature, nutrition, and light affect anthocyanin formation. Light, especially blue wavelengths, promotes formation of flavonoids (Wong, 1976), and hence anthocyanin. A nutritional deficiency of nitrogen, phosphorus, or sulfur leads to accumulations of anthocyanins in some plant species (Salisbury and Ross, 1985). Low temperatures also increase anthocyanin formation in most flowers, and definitely contribute to the coloration of autumn leaves.

Most plants form anthocyanin pigments and other flavonoids in specialized cells of one or more of their organs. This process is frequently promoted by light, especially the blue wavelengths (Salisbury and Ross, 1985). An example is the earlier development of the red color in apple fruits on the south as compared to those on the north side of a tree.

The action spectra for anthocyanin production has been shown to be, in general, at maximum response in the red, far-red, and blue regions. Green (approximately 550 nm) has almost no effect (Mancinelli, 1983), while ultraviolet spectra has not been reported.

Enzyme pathways of flavonoid biosynthesis were studied for relative activity with light treatment (Hahlbrock and Griseback, 1975). Many flavonoid-synthesizing enzymes increased activity after light treatment however, no one enzyme or specific light influence on a particular enzyme for flavonoid synthesis, has been identified.

Raspberry Anthocyanins

Raspberry anthocyanins have been identified (Spanos and Wrolstad, 1987) and mean values reported as cyanidin-3-glucoside, 12.2%; cyanidin-3-sophoroside, 74.2%; cyanidin-3-glucorutinoside, 8.6%; cyanidin-3-rutinoside, 1.7%; and pelargonidin-3-sophoroside, 2.9%. Using different cultivars, Goiffon et al. (1991) could not detect any pelargonidin-3-glucoside, while pelargonidin-3-rutinoside was found in only trace amounts. These discrepancies were attributed to the difference in raspberry varieties tested.

Anthocyanin in Rubus fruit is determined by a series of genes. Some genes affect both the fruit and vegetative portions of plants, while other genes affect only the fruit or vegetative portion of the plants. The T gene is the most important because it affects all parts of the plant. The recessive t allele gives a very low concentration of anthocyanins and gives fruit a yellow color and non-pigmented spines in stems. In Russian wild raspberry, the i gene when homozygous, interacts with gene T and gives apricot-colored fruit and leaves (Keep, 1984). The dominant Y gene found in R. phoenicolasis causes a reduction in fruit pigment which results in yellow fruit but has no affect on pigments in vegetative tissues. The dominant effect of the Y gene is suppressed by the Ys gene which results in red fruits. Even though all of these genes limit the fruit pigment concentration, they do not prevent the development of any one anthocyanin in particular (Jennings and Carmichael, 1980).

pH

Anthocyanidins are unstable in aqueous solution, in the pH range of two to five. They rapidly undergo decomposition reactions and decolorize with the formation of colorless chromenols (Chang, 1981).

These chromenols are somewhat stable at room temperature and instantly regenerate the anthocyanin cation upon acidification. In contrast to the relative stability of chromenols of anthocyanins, chromenols derived from anthocyanidins are unstable and readily undergo ring fission to yield α-diketones. Such reactions have been reported as irreversible pH decomposition of anthocyanidin chromenols (Chang, 1981; Jurd, 1972). Raspberry fruit pH values range from 2.94 to 3.23 (Spanos and Wrolstad, 1987).

Sugars

Flavonoid production requires sugars as a source of the phosphoenolpyruvate and erythrose-4-P that provide carbon atoms needed for the B ring and acetate units in the A ring (Salisbury and Ross, 1985). These sugars, especially sucrose, can arise from photosynthesis in chlorophyll containing cells or degradation of starch. Plowman (1991) reported that sucrose in brambles ranged from 9.3 to 51.8% of the total sugars by weight. Other reported values were between 0 and 13% (Spanos and Wrolstad, 1987). However, the low values were attributed to the failure to inactivate invertase at an early stage during the preparation of the fruit for analysis. Inversion of sucrose has been observed to occur over the thawing time of strawberries and also as a result of the juicing process (Plowman et al., 1989). It was not determined whether reported sugar differences in raspberry cultivars were genuine and reflected the genetic origins of the cultivars examined. Furthermore, there could be possible regional variations reflecting day length and light intensity.

Plowman (1991) prevented inversion of sugars by treating fruit immediately after harvest. In this procedure, the fruit was homogenized, then heated in a boiling water bath for 5 min, centrifuged 15 min at $5^{\circ}C$, filtered through a $45\mu m$ cellulose nitrate fiter, and stored at -20°C. Spanos and Wrolstad (1987) used a different technique to prevent degredation of sugars. Fruit was quick frozen using liquid nitrogen, and then kept at ≈ -21°C until ready for testing.

With the use of high performance liquid chromatography (HPLC), the anthocyanidine profiles of most common red fruits, raspberry included, have been established (Goiffon et al., 1991). Eventually through HPLC programs, specific anthocyanin pigments may be used to identify differences among cultivars.

Stability of anthocyanins in aqueous environments is due to the glycosidic linkage at position 3 on the pyrilium ring with formation of a salt. In many anthocyanins a second sugar is combined with the phenolic hydroxyl group located at position 5. The sugars present in anthocyanins have been identified as monosaccharides, disaccharides, or trisaccharides (Chang, 1981).

High Temperature Stress

There are four possible temperature reactions in plants that lead to heat injury: (1) reversal of relative rates of reactions, due to differences in activation energies, (2) protein denaturation and aggregation, (3) hyperfluidity of membrane lipids, and (4) direct chemical decomposition (Levitt, 1980). These all point to membrane damage as the cause of primary, direct heat injury. With direct and indirect injury, the chloroplast membrane is most sensitive to high temperature, the mitochondrial membranes less affected, and the plasma membrane least sensitive. Salts and sugars have been reported as protective substances in high temperature stress situations (Levitt, 1980). For example, carbohydrate metabolism and growth is increased in tomatoes at 25°C, while at 13°C and 36°C the growth rate is only 50% of maximum (Weis and Berry, 1988). Sucrose levels increased at 30°C, while the export of carbon from leaves was inhibited.

The heat tolerance limit of leaves is related to the thermal sensitivity of the primary photochemical reaction in the thylakoid membrane. Tolerance limits are subject to acclimation with light exposure leading to an increase in tolerance to heat.

High temperature effects are often irreversible, as in the case of thermal sensitivity of the electron transport system in PS II. Heat may inactivate the oxygen evolving system, cause a disturbance of the pigment complexes, or block the photochemical reaction center. (Levitt, 1980).

CHAPTER 3

MATERIALS AND METHODS

Raspberry fruit from an established planting (7 yrs) located at the Colorado State University Cooperative Extension Arapahoe County demonstration plots, in Littleton, CO. were used. These cultivars had been grown as part of a cultivar trial in cooperation with the Arapahoe County Cooperative Extension. The Master Gardeners of Arapahoe County maintained the plants and used drip irrigation as needed for standard growing conditions. The soil was a sandy clay loam with a pH of 6.9 - 7.0, requiring no fertilization. Fruit of cv. Heritage from an established planting (6 yrs) located at the Colorado State University Horticulture Field Research Center at Fort Collins, CO. were used in the characterization of WDD and for the cellulose fiber applications in the laboratory. The Research Center located approximately 10 m northeast of the main university campus has heavy Nunn clay loam soil with a pH of 7.8 - 8.2. Drip irrigation and fertilization were used as needed for standard growth.

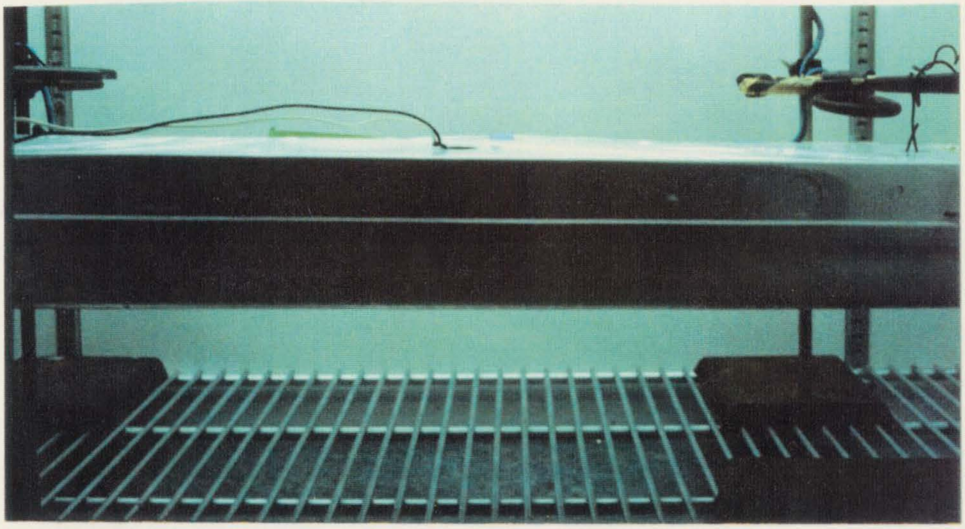
Fruit used in the experiments were randomly harvested within the canopy, resulting in samples with minimal prior exposure to solar radiation. The fruit selected were full sized, turgid, and without sign of disease or blemishes. The developmental growth stage selected for treatment was berries with no pink or yellow coloration. These were harvested approximately three days prior to complete ripening, a susceptible stage for induction of WDD (Renquist et al., 1989).

After harvesting, raspberries were placed in pint sized baskets and set on covered ice (1.2 cm thickness of newspaper) so that no part of the berry or pedicle was actually touching the ice. The raspberries were then transported directly from the field to the lab in a styrofoam cooler. Experiments were initiated within four hours of harvest. In all experiments, 50 ml S/P Tri-Angle plastic disposable beakers and cardboard, write-on, wax-impregnated caps were used. A 0.75 cm hole was punched in the center of each cap with a standard single hole hand punch. The pedicle of each fruit was immersed in deionized water through the cap hole. Caps were labeled with a code number that indicated the cultivar, column, and row position in the growth chamber. Each treatment category and number was recorded.

Two Percival 1E-45-B growth chambers were used based on previous work by Renquist et al. (1989), Figure 5. Chamber A was used to expose the raspberries to UV-B radiation and was held at 42°C. Chamber B was used to permit the raspberries to complete ripening and was held at 20°C. In Chamber A, a Westinghouse FS 20 Sunlamp was placed posteriorly, and a two bulb unit with one FS 20 Sunlamp and a standard fluorescent bulb was placed anteriorly in the chamber, Figure 5. Cellulose acetate, 1.5 mil, filters (permits passage of UV-B radiation only) were placed over the bulbs and replaced after three to four days use. Degradation of cellulose acetate was noted by Becwar et al. (1982) after three days use.

Light readings were taken in the growth chamber with a Lambda Li-185 radiometer. UV-B readings (1.49 Med/hr)were taken with a UV Biometer Model 501. One Med/hr = $5.83 * 10^{-6}$ W cm^{-2} ($5.83*10^{-2}$ W · m^2) of effective power for a MED of 21 mJ/cm^2 (210 J · m^{-2}) effective dose. The detector was calibrated for a clear sky, 30 degrees solar zenith angle, 2.7 mm ozone column thickness, at sea level and at a 25°C temperature of the phosphor. Lighting in Chamber B consisted of eight 36 inch 45 watt fluorescent lamps and four 25 watt incandescent bulbs. The light intensity at berry level was 70 μmol · m^{-2} · s^{-1} (PPF). This is considerably lower than a reading of 2000 μmol · m^{-2} · s^{-1} at solar noon in Colorado. After exposure to the UV-B in Chamber A, the cups with the raspberries were placed in plastic open top storage containers in Chamber B. To maintain humidity, three to five mls of water were placed

A.



B.

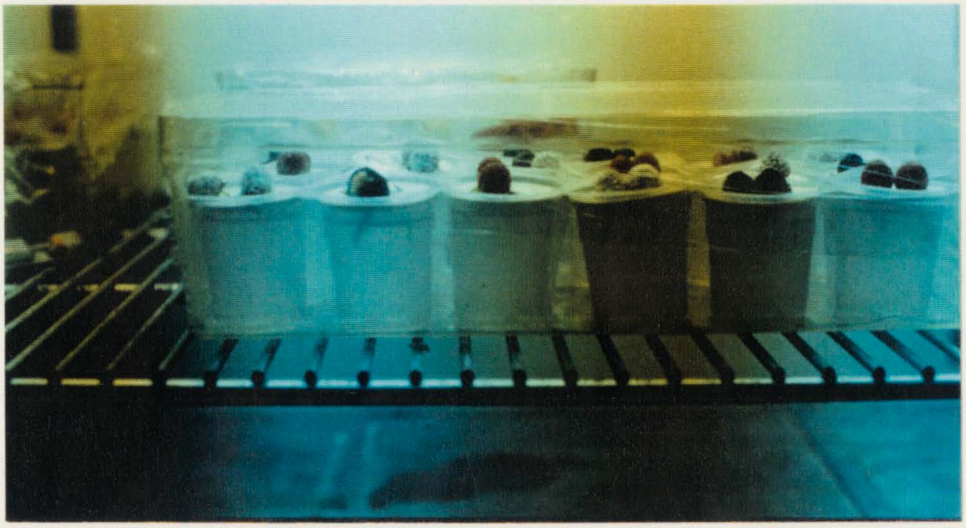


Figure 5. A. UV lamp set up in growth chamber A.
B. Fruit ripening in growth chamber B.

in the containers and a clear plastic wrap covered the top. The raspberries were held in this chamber two to three days until ripening was complete.

Cultivar Evaluation

Raspberries were harvested and tested as previously described to induce white drupelet disorder. A completely random design of a total of 150 raspberries were placed within 5 to 7 cm of the UV-B lamps in Chamber A. Each replication had six fruit per cultivar and each were replicated 5 times. After 7 hrs under the UV-B lamps, they were transferred to Chamber B to permit complete ripening (Renquist et al., 1989). All raspberries were exposed to the same treatment of UV-B light. Cultivars were evaluated for presence or absence of WDD and then Chi-square was used to analyze the null hypothesis (there were no differences between cultivars) using 'yes' the berry had white drupelets and 'no' the berry did not have white drupelets. Secondly, a factorial analysis of variance using square root transformations to stabilize variance, and the AB Error Factor was used to conservatively analyze the number of drupelets exhibiting WDD per berry per cultivar, and susceptibility of WDD between cultivars (Kleinbaum et al., 1988). If differences were seen, Student-Neuman-Keul's test (a more conservative multiple range test) was to be used to determine which cultivar or cultivars were most susceptible to WDD.

Cellulose Fibers

Fine cellulose fibers in powder and liquid forms were evaluated for potential use as a means of reducing the incidence of WDD. The fibers obtained from the James River Corp. in Suttlebrook, N.J. were 40 microns in length (B W 300 FCC). 'Heritage' raspberries (180) were used in the three treatments which included; 1) a control with no cellulose fibers, 2) a coating of dry powdered fibers, 3) and cellulose in solution (0.01% Tween 80, 3% cellulose fibers,and 96.09% deionized water). All raspberries were placed under the UV-B lamps at 42°C and held for 7 hrs. Fruit temperatures in the growth chamber were recorded on treated (dry cellulose fiber coating) and non treated berries using an Omega remote sensing thermocouple thermometer (copper constant 36AWG Type T). After exposure to UV-B and 42°C, the fruit was then transferred to chamber B and held until ripening was complete. Cellulose fiber data were analyzed in the same procedures as mentioned in cultivar evaluation with Chi-square and factorial analysis of variance (with square root transformations using the conservative AB error factor) followed by Student-Neuman-Keul's test if differences were found (Box et al., 1978; Kleinbaum et al., 1988).

In all experiments, each individual white drupelet of all treated raspberries were counted and recorded as WDD. The drupelets appeared completely white with no indication of disease or environmental damage, and were fully turgid.

pH

Fruit from the field plots in Fort Collins were treated with UV-B in the laboratory as described previously. An approximate 1 gm sample of frozen whole red raspberry (1.042 gm) and of frozen whole white drupelets (1.035 gm) were used. Grinding was performed with a mortar and pestle. A 10 ml extracting solution of 95% ethanol and O.5N HCL in an 85:15 ratio separated the red pigment from drupelet tissue. The extraction liquid with red pigment was passed through a Buchner funnel, and Whatman #1 filter aided by vacuum suction. The extracted solution was transferred to a 25 ml beaker mixed with a stir bar on a Corning Hot Plate Stirrer (PC - 351). The pH was measured with a Corning 245 pH meter and the color and absorbance noted. Drops of 0.5N KOH were added to the solution and color noted with pH changes. Absorbance spectra set at 535 nm was correlated with increasing pH on a Spectronic 20. The light action spectra of 535 nm was determined by Beckman Model 35 Spectrophotometer and Recorder with red pigment of raspberry.

Sugars

Mature fruit of 'Heritage' were harvested (with and without WDD) and transported from the Colorado State University Horticulture Field Research Center as noted previously. The white drupelets were separated from the red drupelets on each fruit, and the red fruit were maintained whole. The samples were frozen using liquid nitrogen, put into clear zip lock bags and maintained at -25°C in a General Electric freezer (20.3 cu ft, model CB 20 DA).

Fruit was also selected in the white development stage and exposed to the laboratory procedure for induction of WDD. After ripening, the fruit that developed WDD and fruit that were totally red were selected, divided as above, and frozen using liquid nitrogen and maintained at -25°C. All fruit were then freeze-dried in a Virtis Freeze Dryer (Model# 1073 10-MRTR Gardener, New York) and returned for storage at -25°C until samples were prepared for gas chromotography (GC) tests four months later.

Sample Preparation: The white drupelets and red drupelets from field and laboratory treated fruit were selected. The fruit were ground separately in a mortar and pestle. Seeds were removed and the remaining material was passed through a 100 mesh sieve. The resulting powder was then labelled and returned to the -25°C freezer until trimethylsilyl (TMS) derivitization.

Using an internal standard method modified from Sweeley et al. (1963) to help identify soluble sugars, a standard mix for the GC was made. Glucose, raffinose, stachyose, sorbitol, malto-triose, sucrose, fructose, maltose, galactose, myoinositol, trehalose, and methyl-α-D-glucopyranoside (internal standard) were mixed and frozen in small aliquots. During derivitization, trimethylchlorosilane (TMCS), hexamethyldisilazane (HMDS, 98%), high purity pyridine and n-hexane were used. All reagents used were of reagent grade quality or better and were purchased from Sigma Chemical Co. Double distilled water was used in formulating the standard solutions.

A Hewlett-Packard (HP) 5840A gas chromatograph equipped with an electronic integrator and a dropping needle injection port was used in the analysis of TMS derivitized samples. All weight measurements were performed on a CAHN C-31 microbalance with a rated accuracy of 0.0012% and estimated precision in weighing of .5 μg.

<u>Preparation of Internal Standard:</u> The internal standard (IS) solution of 1 μg/μl was made by dissolving 5 mg of methyl-α-D-glucopyranoside in 5 mls of H_2O. A 25 μl aliquot of this solution was added to tubes and blown dry. The standard mixture consisted of the above mentioned sugars and were mixed together in 5 mls of H_2O. An aliquot of 50 μl was then placed in a screw-capped glass vial containing 25 μl of IS solution. The resulting mixture was then blown dry and ready for the trimethylsilylation procedure.

<u>Trimethylsilylation Procedure</u>: This method used a known quantity (near 1 mg) of powdered sample placed in each screw-capped vial already containing 25 μg of internal standard (IS). A 400 μl aliquot of pyridine, 80 μl of HMDS, and 40 μl of TMCS were then added in that order. The tubes were heated, caps tightly sealed, at 80°C for 20 minutes. Immediately following this heating period, the vials were

uncapped and blown dry at room temperature. A 0.5 ml aliquot of n-hexane was added to the resulting dried residue of each tube. After a few seconds, to allow the solvent to solubilize the derivitized sugars, the solvent was carefully pipetted (in order to exclude residue particles) and dispensed into a clean dry tube. Last, the n-hexane solution was blown dry and 200 μl of fresh n-hexane added completing the solution process. The solution was then ready for injection into the GC.

Quantitative Analysis: The amount of each analyte in the chromatogram was determined using the internal standard method. In order to account for non-linearities in the flame ionization detector (FID) response for a given sugar, the standard mixture consisting of the analyte sugars to be quantified was also subjected to the GC for analysis under identical conditions as the samples. The wt% (dwb) could then be calculated from the following expression:

$$wt\% = Aas/Aiss \times Aissm/Aasm \times Masm/Ms \times 100$$

Where Aas is the area of the analyte saccharide peak in the sample chromatogram, Aiss is the area of the internal standard peak in the sample chromatogram, Aissm is the area of the internal standard peak in the standard mixture chromatogram, Aasm is the area of the anaylte saccharide peak in the standard mixture chromatogram, Masm is the mass of the analyte saccharide in the standard mixture (μg), and Ms is the mass of the powdered sample (μg).

Glucose and sucrose values in M/g x 10^{-5} were noted in white and red drupelets for possible variation with analysis of variance on 12 sub-samples. Although sophorose is another sugar required in pigment formation, a standard sample was unavailable for testing.

CHAPTER 4

RESULTS AND DISCUSSION

Cultivar Evaluation

Induction of white drupelets by use of UV sunlamps in the growth chamber was confirmed as reported by Renquist et al. (1989). However, statistical differences in susceptibility to WDD of the five cultivars used in this study varied. Differences among cultivars was indicated using a Chi-square comparison of the five cultivars Heritage, Pathfinder, Trailblazer, 67-12, and Fall Gold, where fruit that had one or more white drupelets was counted as having WDD, Table 1. 'Heritage'showed the greatest number of fruit affected with WDD. However, the two-way Chi-square test assumes independence of individual berries. There was some evidence that this assumption was not valid in that there was apparent clustering of berries with WDD at a given date, which may have been a factor in this experiment. Cultivar susceptibility to WDD may be influenced by changes in environmental fluctuations of solar irradiation and temperature during and prior to fruit harvest. Further research with greater numbers of harvest dates and coordination of measurements of daily UV-B radiation and temperatures is recommended. It would clarify any differences among cultivars where temperature and solar irradiation show interactions.

Red raspberry cultivars also varied in the number of drupelets per berry that exhibited WDD. A two factor analysis of variance was used to compare the average number of white drupelets per berry by cultivar and date for an analysis in which dates were considered fixed. Significant differences among cultivars were indicated when an F test used the mean square error in the denominator, data not shown. However, when the dates were considered as random factors with an F test in which the

Table 1. Relative susceptibility to white drupelet disorder of five red raspberry cultivars.

Cultivar	White Drupelets Yes	 No	Means
Heritage	20	10	0.67
Pathfinder	14	16	0.47
Trailblazer	11	19	0.37
Fall Gold	10	20	0.33
67-12	10	20	0.33

Total Chi-square = 9.77

Degrees of freedom = 4

Probability = 0.04*

* Significant differences among Chi-square values at $p < 0.05$.
This test assumes that individual berries are independent and does not analyze the effect of date variability.
Sample size = 30 fruit per cultivar.

denominator was date by cultivar interaction (AB error term) was used. Thus, the factorial analysis with square root transformations, indicated no significant differences among the five cultivars over the dates tested, Table 2. However, when the means are examined, 'Heritage' again had the greatest number of white drupelets per berry.

The low PPF may not have allowed for correction of UV-B damaging effects in the laboratory treatment. Further studies correlating laboratory results with field studies are indicated because of the possible influences of low PPF. Additionally, other cultivars should be included in comparison studies to determine if cultivar differences in susceptibility to WDD are evident.

Field observation of WDD was apparent in each cultivar at the Arapahoe County Research Field Center during three separate observations. It was particularly apparent during the last week of August, 1991, when 2 to 12 white drupelets per affected raspberry were observed. In previous research, Renquist and Hughes (1987) determined that cvs. Amity and Heritage showed significant differences in susceptibility to WDD. Since these differences might have been associated with natural leaf shading in 'Amity', further examination of these cultivars is indicated. If, in fact, it is not due to natural shading, laboratory evaluation of 'Amity' may aid in the development of a more precise cultivar evaluation scheme in the laboratory.

A total of 30 drupelets from red and WDD affected fruit were observed microscopically (Olympus dissecting microscope at 20x). The red raspberries had pink-red trichomes on and between each drupelet. White drupelets showed a variation in tricome coloration; most trichomes were white on white drupelets while other trichomes on the drupelet were white and pale pink at the juncture of the epidermis. Also, the epidermis of white drupelets had pale pink areas approximately the diameter of the trichomes; these pink pigmented areas were scattered in no immediately recognizable pattern. These pigmented areas may be

Table 2. Relative susceptibility to white drupelet disorder as indicated by average numbers of white drupelets per fruit among five raspberry cultivars.

Source	Means*	df	Mean Square	F Value	
(Factor A) Harvest Dates		4	0.28	0.17	NS
(Factor B) Cultivars		4	2.70	1.68	NS
Heritage	2.87**				
Pathfinder	1.77				
Fall Gold	1.10				
67-12	0.90				
Trailblazer	0.70				
AB		16	1.60	1.88	NS
Error		125	0.85		

NS: Not significant at $p < 0.05$.

* Data analyzed in square root transformations. Means transformed back for table.

** Though analysis does not show significant differences among cultivars, cv. Heritage has the greatest number of white drupelets. Sample size = 30 fruit per cultivar.

individual cells, and if so, they may be organelle specific. Some pink as well as white drupelets on otherwise fully ripened red fruit were observed in the field. This would suggest that pigment formation was stopped abruptly even though drupelet growth continued.

Cellulose Fiber Evaluation

The application of cellulose fibers did not reduce the incidence of WDD, Table 3. Factorial analysis for number of drupelets (WDD) per berry per cellulose fiber application using Student-Neuman-Keul's multiple range test to rank differences also showed no decrease, Table 4. In fact, the highest WDD incidence, significantly different at the 5% level of WDD, was observed when the treatment of cellulose fibers in solution remained wet during of UV-B and high temperature exposure. Water probably acted as a lens, magnifying UV-B intensity. There were no differences among the control treatments and dry applications of fiber on raspberry fruits. The analysis of variance indicated no differences associated with dates of picking raspberries, Table 4.

No differences between harvest dates were indicated in the analysis of variance, Table 4. Use of powdered cellulose fibers does not appear to be a viable protectant to WDD on red raspberry. There is the possibility that UV-B exposure in the laboratory was so intense as to cause WDD even though there could have been some minor benefit with the wet application of cellulose fibers allowed to dry prior to exposure. Field observation of sunscald in apples indicated some preliminary benefits of decreased sunscald through the use of cellulose fibers (personal communication, Matthew Rogoyski, Rogers Mesa Research Center, 1991). Therefore, field evaluation is suggested before one could eliminate the possibility of fiber as a potential protectant.

Mean temperatures of control fruit (no fiber application) in the growth chamber was 41.1°C while fruit with a dry cellulose application

Table 3. Cellulose fiber application effects on presence of white drupelet disorder in red raspberry cv. Heritage.

Observed values:	Chi-square Values	Means
Control (no fiber)	13.12	0.43
Dry Fiber application	2.96	0.55
Wet Fiber application	28.55	0.98

Total Chi-square = 44.63

Degrees of freedom = 2

Probability = 0.0000-*

* Highly significant differences among values at p <0.001.
Sample size = 60 fruit per cultivar.

Table 4. Relative susceptibility to white drupelet disorder of cv. Heritage with cellulose fiber application.

Source	Means**	df	Mean Square	F Value	
Factor A Harvest Dates		4	25.93	0.31	NS
Factor B Treatments		2	196.99	19.60	0.000*
Wet Fiber	18.83 A***				
Dry Fiber	4.10 B				
No Fiber	0.92 B				
AB		8	84.05	10.51	NS
Error		165	0.99		

NS: Not significant at $p < 0.05$.

* Highly significant at $p < 0.001$.

** Data analyzed in square root transformations. Means transformed back for table.

*** Mean separation using Student-Neuman-Keul's test for significance at $p < 0.05$. Means not followed by a common letter differ significantly. Sample size = 60 fruit per treatment.

recorded 39.8°C. The cellulose fiber application apparently lowered the fruit temperature 0.3°C due to a shading effect as compared to fruit without the coating. However, this was not enough to reduce the incidence of WDD.

Even though edible, the powders in any application leave a white residue that would be undesirable to the consumer. The powder could not be easily washed off without damaging the thin epidermis of the fruit, which would reduce shelf life and hence the fresh market value. Therefore, the potential of the cellulose fibers for use as a protectant is minimal.

pH

The pH and corresponding absorbance of an extract from red and white drupelets were noted in cv. Heritage, Table 5. At the time of initial extraction of the pigment from red fruit the pH was 2.13, pink-red color, and absorbance of 1.5 at 535 nm. As the pH was increased to 7.21, the solution became almost clear (0.5 absorbance). Particles were observed in the pigment solution and a yellow color was apparent. At higher pH the color turned darker to a pink-brown shade. This color change may have been associated with the particles in the pigment solution, and/or possible contamination of seed proteins from grinding the fruit for samples.

The white drupelet extract had a light pink color prior to pH adjustments. The pH level with the lightest color and least absorbance was between 3.0 and 4.0, Table 5. As pH increased, the color and absorbance turned darker and became a pink-brown in color. This solution did not turn red, indicating that colorless anthocyanin was not present or in amounts that would be observable. Since there was minimal pink color change from the extract of the white drupelets, it would

Table 5. Observed changes in color and absorbance levels with pH adjustments of extracts from red and white drupelets of cv. Heritage.

pH	Color changes	Absorbance level
	Red drupelets	
2.13	pink red	1.5
3.07		1.1
4.11	light pink	0.8
5.07		0.6
6.04	very lt. pink	0.56
7.21	almost clear	0.5
8.01	clear of pink/ lt.yellow color	**
8.04	light pink	0.525
9.31	pink brown	0.64
10.23	dark pink brown	0.75
	White drupelets	
2.03	light pink	0.345
3.03	lighter pink	0.36
4.01	lighter pink	0.37
4.98	pink brown	0.38
6.48	darker pink brown	0.48

** The rapid pH change prevented an absorbance reading until the pH had reached equilibrium at 8.04.

indicate that an anthocyanin influenced by pH was not present. Therefore, pH was not a factor in expression of red pigment in WDD.

Another contamination factor, in the white drupelets used for pH evaluation, was red pigments from adjoining red drupelets which were not completely removed prior to extraction. However, red pigment was observed microscopically on white drupelets, and would have been found in a small amount in pigment extraction regardless. Since red pigment was observed in white drupelets, it indicates that pigment formation was abruptly interrupted by the temperature/UV treatments. Identification of the red pigment in white drupelets plus any other anthocyanins, colored or not, may direct which interrupted path to explore further.

The absorbance spectrum of the red raspberry pigment extraction at pH 1.1 was 535 nm, Figure 6.

The results of this study suggest that close observation in the field and under the microscope would further aid in the characterization of WDD. This is particularly important since microscopic examination identified the presence of pigment in the epidermis and on some trichomes of white drupelets.

Sugars

Glucose and sucrose content of samples of both red and white drupelets from field and laboratory were derivitized and analyzed. There were no significant differences among red and white drupelets from field and growth chamber, Table 6. However, in field and growth chamber trials, the red fruit had significantly higher sucrose levels than the white drupelets, Table 7. Sucrose levels of red drupelets from the field and the growth chamber had no significant differences between them, nor did the white drupelets from the field and lab, Table 7. Sucrose can be broken down to form more glucose or have additions to

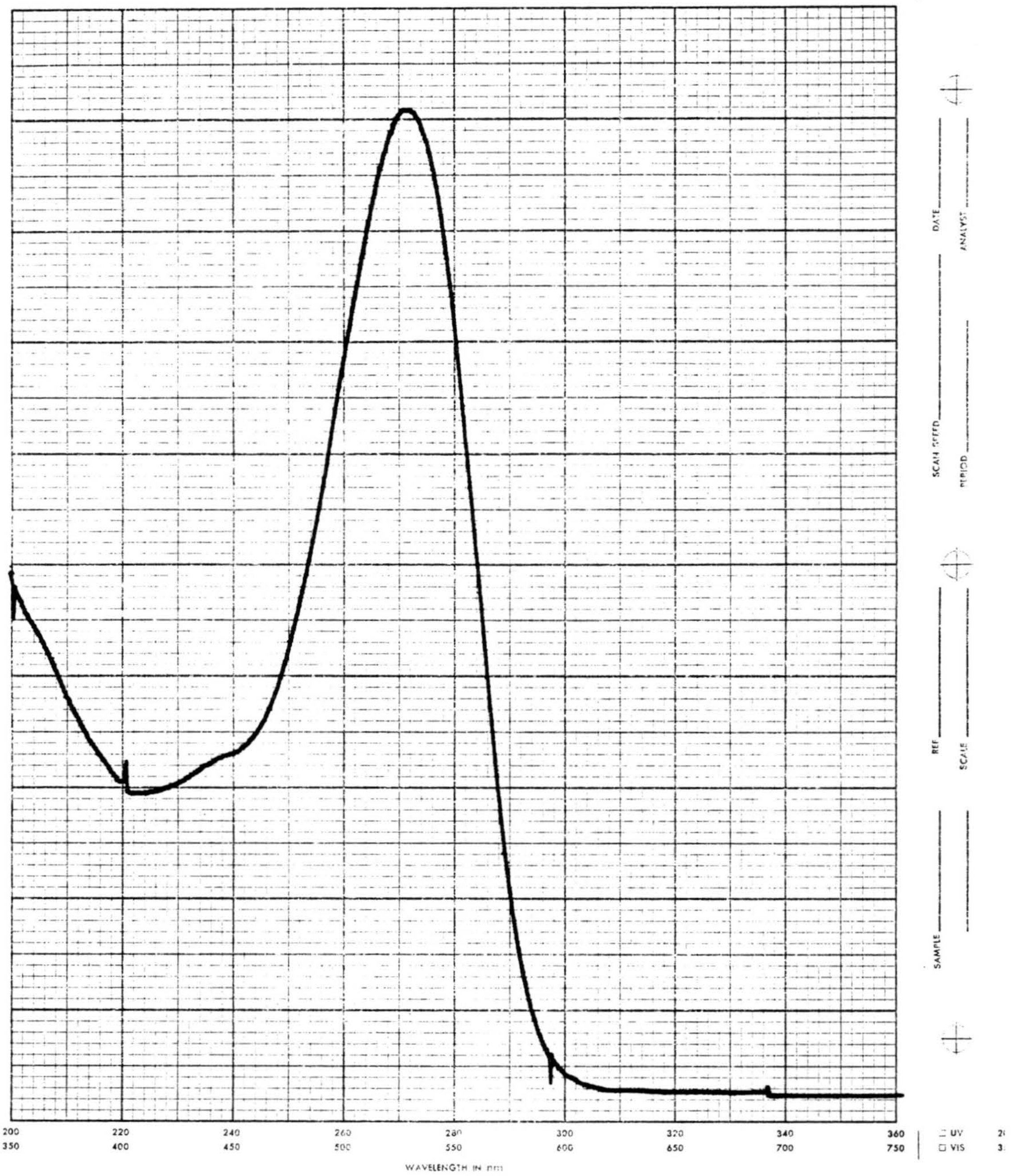


Figure 6. Active light spectra of anthocyanin pigments of 'Heritage' red raspberry fruit extract at 535 nm.

Table 6. Field and growth chamber effects on levels of glucose in red and white drupelets of red raspberry cv. Heritage.

Source	Means (M/μg)	df	Mean Square	F Value	
Glucose:					
Treatment		3	1469.67	2.82	0.10 NS
Red drupelets:					
Field	101.20				
Laboratory	104.70				
White drupelets:					
Field	138.45				
Laboratory	132.90				
Replication		3	122.40	0.24	0.87 NS
Error		9	520.47		

NS: Not significant at $p <0.05$. Sample size = 4.

Table 7. Field and growth chamber effects on levels of sucrose in red and white drupelets of red raspberry cv. Heritage.

Source	Means** (M/μg)	df	Mean Square	F Value	
Sucrose:					
Treatment		3	1111.197	41.46	0.0000*
Red drupelet:					
Field	33.60 A				
Laboratory	35.37 A				
White drupelet:					
Field	4.28 B				
Laboratory	7.15 B				
Replication		3	34.61	1.29	0.34 NS
Error		9	26.80		

* Very highly significant above $p < 0.001$.

** Mean separation using Student-Neuman-Keul's test for significance at $p < 0.05$. Means not followed by a common letter differ significantly. Sample size = 4.

become a larger sugar molecule. In anthocyanin formation, the colored pigments have glucose and other sugars (but not sucrose specifically) attached to the pigment. Glucose is only one precursor for pigment formation, thus finding amounts that are not significantly different in either field or growth chamber tests in both red or white drupelets of 'Heritage', indicates that glucose is not a factor associated with WDD.

Directly identifying anthocyanins and/or their precursors should indicate the differences in sugar molecule attachment and variations in anthocyanins between red and white drupelets. HPLC has indicated specific anthocyanins in red drupelets of several red raspberry cultivars, however, white drupelet anthocyanins have not been identified yet.

Enzyme presence and activity should be investigated in white drupelets. There could be inhibition of enzymes in the early stages of the flavonoid biosynthetic pathway that could be completely blocking flavonoid synthesis, or perhaps in the later stages of synthesis that result in a shift from anthocyanin synthesis to synthesis of non-colored flavonoids with a strong UV absorption but not in the visible region (personal communication, Alberto L. Mancinelli, Columbia University, 1992). Mancinelli (1992) suggested checking the enzyme activity of the phenylpropanoid pathway first. If no differences are indicated, the pathway of chalkone synthase and chalkone isomerase would next be logically investigated. If the activity of these enzymes are not different in the white and red drupelets of raspberry, critical enzymes could be determined by the differences in the flavonoids, accomplished through extraction, separation, and identification of the flavonoids.

CHAPTER 5

CONCLUSIONS

1. There appear to be no genetic differences in relative susceptibility to WDD among cultivars tested when date variability is taken into account in the analysis. However, further research is required since previous field observation of cvs. Amity and Heritage had indicated significant differences in relative susceptibility to WDD.

2. Laboratory evaluations of cellulose fibers indicate they provide no protection against WDD. In fact, a wet application increased relative susceptibility to WDD. Again, field evaluations are necessary to verify these results under natural conditions.

3. Microscopic observations of white drupelets of red raspberry exhibiting WDD indicated the presence of minute areas of pink-red coloration and pink-red trichomes. Observed with the unaided eye, these drupelets appeared entirely white.

4. Observations of pH effects on extracts from red and white drupelets verified that pH was not affecting the expression of the anthocyanin pigment.

5. The comparison of glucose and sucrose levels among red and white drupelets from field and laboratory fruit revealed that white drupelets from both field and laboratory treatments had reduced sucrose levels, but comparatively equal glucose levels. Thus, glucose was not determined to be a factor in the lack of pigment formation. However, the highly significant differences of sucrose between red and white drupelets in both field and growth chamber were evidenced, indicating sucrose formation may be associated with the lack of red pigment formation.

6. Though this is a preliminary test of subsamples, this appears to confirm that laboratory treatment with high UV and temperature duplicates the field induction of WDD.

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