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Abstract approved:

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Raspberries have been popular since the time of the ancient Greeks. Red raspberries are cultivated throughout the world, particularly in the Pacific Northwest region of the United States and Canada. The *raspberry bushy dwarf virus* (RBDV) causes significant reduction in yield and crumbly fruit in raspberries, blackberries and raspberry-blackberry hybrids. There is no effective treatment once a plant is infected and the only effective prevention is using resistant varieties. Resistance is difficult to attain using traditional cultivation techniques. Through transgenic modification, five lines of resistant red raspberries have been developed from the 'Meeker' cultivar.

To investigate trueness to type, flavor characteristics for raspberries grown in Oregon and Washington during 2004 and 2005 were compared. Aroma volatiles important to the raspberry aroma profile were quantified, as well as the sugar and

organic acid profiles, to compare the five transgenic ‘Meeker’ lines with the wild type ‘Meeker’, ‘Chilliwack’, ‘Tulameen’, ‘Willamette’ and ‘Yellow Meeker’.

Thirty aroma compounds ((Z)-3-hexenol, 4-isopropylbenzyl alcohol, 6-methyl-5-hepten-2-ol, 2-nonanol, hexanal, (E)-2-hexenal, (Z)-3-hexenyl acetate, ethyl hexanoate, methyl nonanoate, 2-heptanone, 2-nonanone, raspberry ketone, zingerone, δ-octalactone, δ-decalactone, para-cymene, geraniol, α-ionone, β-ionone, limonene, linalool, myrcene, nerol, α-phellandrene, α-pinene, sabinene, α-terpinene, γ-terpinene, α-terpineol, terpinen-4-ol and terpinolene) were selected and quantified using stir bar sorptive extraction (SBSE) paired with gas chromatography-mass spectrometry (GC-MS) in all raspberry samples. The chiral stereoisomers of some volatile compounds in these raspberries were also studied using a cyclosilB GC column.

Different raspberry cultivars varied widely in titratable acidity, sugar and acid concentration, and volatile composition, particularly α-ionone, linalool and (Z)-3-hexenol. Chiral compositions were also quite different among the cultivars studied.

For the wild type ‘Meeker’ and RBDV-resistant varieties, flavor variations were observed from sites to sites and year to year. However, overall volatile profile had much less variations compared with sugar and organic acid profiles. None of the analyses separated the transgenic ‘Meeker’ raspberries from the wild type ‘Meeker’, and result was consistent for both of the locations and years studied. The results suggested that the transgenic RBDV-resistant ‘Meeker’ varieties were not different from the wild type ‘Meeker’ raspberry.

Flavor Composition of Transgenic Raspberry Bushy Dwarf Virus-Resistant 'Meeker' Raspberries

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sarah Marie-Mahler Malowicki, Author

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CONTRIBUTION OF AUTHORS

Dr. Robert Martin developed the transgenic raspberry lines investigated in this study. And harvested or obtained and delivered the samples to Oregon State University for analysis. He was further involved in assisting in the understanding of the *Raspberry bushy dwarf virus* and the development of all of the chapters.

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This thesis is dedicated to:

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INTRODUCTION

Red Raspberries

Raspberries, commonly grouped with blackberries and blackberry-raspberry hybrids as 'brambles' and 'caneberries', grow naturally in all temperate regions of the northern hemisphere and are grown commercially in the United States, Europe, Chile, New Zealand, and Australia (*1-3*). Cultivation of raspberries began by the 4th century A.D. This cultivation was begun by the Romans and spread throughout the Roman empire (*1*). During the late 19th and early 20th centuries numerous new cultivars were developed by gardeners and nurseries including 'Pyne's Royal' and 'Lloyd George' (*1*). 'Lloyd George' is still an important cultivar today particularly in England and is important in the United States as a parent cultivar of 'Willamette' and 'Meeker' cultivars among numerous others (*4*).

Domesticated raspberries are easily distinguished from wild raspberries. Wild raspberries produce numerous, thin, short canes and short thin laterals and small flowers, while cultivated raspberries produce fewer canes that grow taller (*1*). Fruit production in wild raspberries is also markedly different than in cultivated

plants. Cultivated fruits are generally 2 to 3 times larger than wild clones, while wild fruit is often quite soft and crumbles easily due to poor druplet cohesion, although fruit size can vary with location as some investigators have found wild forms with large fruit and superior flavor (*1*).

Two main types of cultivated raspberries are grown widely, primocane fruiting raspberries and floricane fruiting raspberries (*5;6*). Primocane fruiting raspberries, also known as ever-bearing raspberries, are less common. They produce fruit on the top half of first year canes in the fall of the year the canes first grew, followed by fruiting on the bottom half of the canes the second year in late spring or early summer (*3;5;7*). Floricane fruiting raspberries produce fruit on second year canes which grew the previous summer. Following summer fruiting both types of canes die and new canes grow each year while second year canes produce fruit (*3;5;7*).

Raspberries are a good source of many nutrients and neutraceuticals. Neutraceuticals in plants are primarily phytochemicals such as flavonoids, anthocyanins and other phenolic compounds (*8*). Raspberries are a good source of carbohydrates, dietary fiber, vitamins and minerals, including vitamins A and C, calcium and iron (*2;3*). Antioxidant vitamin C and carotenoids are also found in raspberries (*2;3*). These vitamins have been shown to delay the onset of degenerative diseases associated with aging through counteracting oxidative processes linked with these diseases (*2;9*).

Ellagic acid is one of the important neutraceuticals in raspberries. Ellagic acid is a naturally occurring phenolic compound that can exist in several forms including ellagitannins, ellagic acid glycosides and free ellagic acid (*10*). Ellagic acid has been found to have anticarcinogenic effects through inhibition of mutagens, suppression of ultimate carcinogen activity and suppression of the metabolic activation of carcinogens (*2;10;11*). As a glycoside it may protect fruits from microbial infections (*2*). Anthocyanins, responsible for the red and blue color present in berries, have been shown to have significant antioxidant activity similar to that of other flavonoids (*12;13*). These flavonoids can inhibit low-density lipoprotein oxidation and have been shown to have vasoprotective and antiinflamatory activity (*8*). The overall antioxidant activity of red raspberries has been found to be comparable to that of blackberries, but is lower than that of black raspberries (*8;14*). The antioxidant properties can vary based on cultivar, maturity, processing and other environmental factors (*11;14;15*).

Red Raspberry Production in the Pacific Northwest

Red raspberries are the most widely grown type of bramble (*3*). The Pacific Northwest, made up of Oregon and Washington states, is the largest production area for red raspberries in the United States (*16;17*) as the area has largely ideal growing conditions. This production has increased dramatically within the last fifteen years due to the implementation of machine harvesting and the use of higher yielding cultivars (*18*). 'Meeker' raspberry is currently the most widely planted cultivar in

the Pacific Northwest, replacing ‘Willamette’ which was the leading cultivar until the early 1980s (*18*).

‘Meeker’ Red Raspberry

‘Meeker’ is currently the most widely grown cultivar for commercial production in the Pacific Northwest accounting for 80% of the red raspberry acreage in Washington state, which is the largest producer (*19*). This cultivar is known for its good flavor, superior yield, resistance to root rot, a long harvest season, good fruit color and firmness, machine harvestable characteristics, and vigorous growth (*5;19;20*). This cultivar has become popular partially due to the suitability for both fresh and processed markets (*19*).

‘Meeker’ was developed and released from Washington State University in 1967 by C.D. Schwartze (*21*). This cultivar was developed from a cross of ‘Willamette’ and ‘Cuthbert’ cultivars (*4*).

Cultivar is an important consideration in the selection of raspberries for commercial production, as production and harvest characteristics vary considerably between cultivars and are important concerns for producers. However, flavor characteristics also vary considerably by cultivar due to genetic variations (*22*), and can sometimes be overlooked in the quest for higher yield and easier harvesting. A favorable aroma profile is a key element for successful cultivars.

Aroma Volatile Generation

Aroma profiles are highly specific and vary by compound and quantity in different cultivars and fruits (*23*). Volatiles are generated through biosynthesis pathways and from various parent compounds, while the pathways are often similar in various fruits the final concentration of the volatiles may vary. Important aroma compounds are generally separated into 11 important groups: hydroxyl compounds, aldehydes, ketones, acids, esters, nitrogen compounds, sulfur compounds, oxygen heterocycles, nitrogen heterocycles, sulfur heterocycles and other compounds (*24*). Of these, hydroxyl compounds, aldehydes, ketones, and esters are generally thought to be important in fruit. Aroma compound precursors are generally from three primary chemical classes: fatty acids, amino acids and carbohydrates.

Volatile aroma compound concentrations are affected by numerous factors including cultivar, fruit maturity, growing environment and post-harvest storage conditions (*22;23;25-27*). The development, storage, degradation and variation of aroma volatiles have been studied in numerous fruits and vegetables including: onions and garlic (*28*), olive oil (*29*), bananas (*30*), strawberries (*31;32*), grapes (*33;34*), blackberries (*26;35*) and raspberries (*23;25;36*).

Fatty Acids

Fatty acids are metabolized into volatile compounds through the β-oxidation and lipoxygenase (LOX) pathways (*37*). The β-oxidation pathway is responsible for the aroma compounds derived from fatty acids in intact fruits (37). The LOX

pathway is responsible for secondary aromas, due to the disruption of plant tissues, although aroma generation from fatty acids is not restricted to either pathway (*37*).

β-oxidation Pathway. The β-oxidation pathway has been well documented in animals and microorganisms, and is believed to function in fruits and vegetables as well. Studies concerning the presence of the pathway in plants have evaluated substrates and products rather than directly evaluating enzymatic function (*37*).

The β-oxidation pathway produces various esters and alcohols in fruits and vegetables (*37-39*). These molecules are formed through enzymatic degradation of fatty acids involving the enzymes acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacetyl-CoA dehydrogenase and acetyl-CoA acetyltransferase (or thiolase) (*37*). First the fatty acid is changed to an acyl-CoA derivative, following this step acyl-CoA dehydrogenase forms a double bond at C2 (*37*). Enoyl-CoA hydratase then hydrolyzes the C2 double bond, followed by another dehydrogenase reaction catalyzed by 3-hydroxyacyl-CoA dehydrogenase, which produces a ketone at C3 (*37*). These steps are followed by the enzymatic action of acetyl-CoA acetyltransferase, which transfers an acetyl group to a free coenzyme A to form an acetyl-CoA (*37*). These steps are continued with the additional activity of enoyl-CoA isomerase activity to transfer the fatty acid double bonds and finally 3-hydroxyacyl-CoA epimerase, which could be responsible for the enantiomeric forms of esters found in fruits and vegetables (*37*).

As a final step in volatile aroma formation alcohol acyltransferase can form esters from the acyl-CoA molecules produced in the fatty acid degradation although the mechanism for ester formation is still unclear (*37;38*). The final products in various fruits will differ depending on the final acyl-CoA molecule composition (*38*).

The β-oxidation pathway is also believed to be part of the pathway through which lactones are formed (*37*). Lactone biosynthesis uses oxo-acids and hydroxyacids as precursors. These precursors are then further metabolized, through reduction, hydration and elongation, although the pathways for this metabolism are currently unknown (*37*).

Lipoxygenase Pathway. The LOX pathway is generally believed to form various C_6 and C_9 volatiles. These molecules are formed through enzymatic oxidative degradation involving acylhydrolases, lipoxygenase, hydroperoxide lyase, and depending upon the end product, isomerase, alcohol dehydrogenase and alcohol acyltransferase (*29;37*). Acylhydrolases are responsible for freeing the polyunsaturated fatty acids from the triacylglycerols, phospholipids or glycolipids. The LOX enzyme changes a (1Z,4Z)-pentadiene structure to a conjugated pentadiene structure by shifting a cis-double bond to a conjugated trans-double bond (*29;37*). This enzymatic process is followed by the action of hydroperoxide lyase (HPL), which cleaves these fatty acid hydroperoxides to aldehydes and oxoacids, these enzymes can be categorized into three types: 9-HPL, 13-HPL and nonspecific HPL depending on where the incorporated hydroperoxide is located prior to enzymatic

action (*29;32;37*). By controlling the concentration of hydroperoxides available to the specific HPL enzyme various fruit types control the final compound concentration and composition (*37*). Following these steps, various combinations of enzymes can alter the molecules, including isomerase, alcohol dehydrogenase and alcohol acyltransferase (*29;37*).

This pathway is generally seen as a secondary pathway, where products, particularly (E)-2-hexenal and (Z)-3-hexenal, are formed primarily due to tissue damage to the fruit (*37;40*). However, it is believed that this pathway can also be active in mature fruit. In immature fruit emission of LOX pathway volatiles is impossible due to the sub-cellular enzymatic locations, however as the fruit matures, the cellular membranes become more permeable to various substrates (*37*). In apples, the intracellular oxygen content is believed to regulate this pathway, explaining the reduced aroma volatile production in apples stored in low-oxygen atmospheres (*37*).

This pathway can form alcohols, aldehydes, acids and esters from linoleic and linolenic acids to produce 'green' odor aromas (*29;32;37*). Some common examples of the odor aroma compounds produced from this pathway include hexanal, heptanal, hexanol, (Z)-3-hexenol, (E)-2-hexenol, (Z)-3-nonenol, (E)-2-nonenol, and various hexyl esters (*29;32;37;40;41*). The relative volatile concentrations vary based on which types of LOX enzymes are present (*42*).

Amino Acids

In fruits, amino acid derived aroma compounds are generally produced directly from the amino acid, rather than from amino acid derivatives that are further derived to form aroma compounds after cell disruption, as is an important pathway for aroma volatiles in vegetables (*37*). Free amino acid content can vary during ripening; these variations could contribute to the varying aroma profile associated with different fruit and different fruit maturity (*31;37*).

Metabolism of amino acids, in strawberries, toward aroma products seems to occur through two consecutive steps involving deamination and decarboxylation (*31*). The first step of deamination is accomplished by transferring the amino group from the amino acid to another compound and was inferred with the detection of glutamic acid being produced from 2-oxoglutarate (*31;37*). This deamination step is followed by decarboxylation accomplished by an enzyme complex similar to pyruvate dehydrogenase or 2-oxoglutarate dehydrogenase which oxidizes the 2-oxoacid and requires various cofactors (*37*). Another possibility requires an enzyme similar to pyruvate decarboxylase producing the non-oxidative decarboxylation of 2-oxoacids (*31;37*). This decarboxylation step could also utilize α-ketoacid dehydrogenase/decarboxylase to form branched chain aldehydes or branched chain acylCoAs with a final product of branched chain esters following a alcohol dehydrogenase and/or a alcohol acyltransferase step (*30*). This complex of steps provides a substrate for alcohol dehydrogenase, aldehydes oxidase, or alcohol acyltransferase (*31;37*).

Amino acid degradation to aroma compounds produces alcohols, carbonyls, acids and esters that can be aliphatic, branched or aromatic (*30;37*). The final composition is under genetic control as the substrate type and compostition have been shown to vary according to cultivar and thus develop into different products and product distributions in various cultivars (*30*).

A second pathway for aromatic amino acids may exist. This pathway could account for the formation of cinnamic acid as an intermediary for numerous compounds with phenolic and spicy aroma odor notes (*37*). This pathway involves a combination of the following enzymes phenylalanine ammonia lyase, cinnamic acid hydroxylase, and phenolase (*37*). Phenylalanine ammonia lyase is responsible for the removal of the amino group to form a double bond forming cinnamic acid (*37*). Following this step cinnamic acid can be used to form benzoic acid derivatives following the removal of an acetate or cinnamic acid hydrolase can form p-coumaric acid (*37*).

This pathway is also believed to be responsible for the formation of the raspberry ketone, the key flavor compound in raspberries (*37*). Coumaric acid, formed by cinnamic acid hydrolase from cinnamic acid, can then be further hydrolyzed by phenolase to form phenolic acids and phenol esters or can incorporate a free coenzyme A to eventually form the raspberry ketone (*37*).

Carbohydrates

As sugars are the end-product of photosynthesis, carbohydrates are naturally the original source of all aroma compounds in plants. However, those aroma

compounds derived directly from carbohydrate metabolism are more limited. The major pathway responsible for aroma compound generation from carbohydrates is the isoprenoid pathway.

Isoprenoid Pathway. The isoprenoid pathway is responsible for terpene formation. The steps leading to the formation of isopentenyl diphosphate (IPP) are assumed to be the same as those involved in the formation of other isoprenoid compounds (*37*). The initial process is the formation of mevalonic acid (MVA) from three molecules of acetate, although the steps are not clear for this process, it is known to involve acetyl-CoA acetyltransferase, hydroxymethylglutaryl-CoA synthetase and hydroxymethylglutaryl-CoA reductase (*37*). MVA is then phosphorylated by mevalonate kinase to mevalonic acid 5-phosphate (MVAP), followed by a second phosphorylation catalyzed by 5-phosphomevalonate kinase forming mevalonic acid diphosphate (MVAPP) (*37*). The final step in the formation of IPP is the decarboxylation and dehydration of MVAPP by 5-diphophomevalonate decarboxylase (*37*).

Prenyltransferases, responsible for the formation of monoterpenes, require one molecule to be in the dimethylallyl diphosphate (DMAPP) form, which is produced by isopentenyl diphosphate isomerase (*37*). Prenyltransferases, such as geranyl diphosphate synthase, then catalyze the condensation of two isoprenoid diphosphates, such as IPP and DMAPP to geranyl diphosphate (GPP) (*37*). Geranyl diphosphate is believed to be the precursor for monoterpenes, and the formation of these terpenes requires several enzymatic reactions including hydrolysis, cyclations

and oxidoreductions (*37*). The most important of these reactions is often cyclation of GPP, although oxygenation is often necessary to produce volatile monoterpenes (*37*).

Other Factors Affecting Flavor

Numerous factors affect flavor of small fruits. These factors include cultivar, growing conditions, fruit maturity, storage and processing. These factors can also affect anthocyanin, polyphenolic and antioxidant concentrations and properties (*15*). The aroma volatile, antioxidant capacity and flavonol variations between some red raspberry cultivars have been studied several times, particularly for important aroma impact compounds (*11;14;23;25;36*). Varying proportions of chiral compounds can be caused by cultivar and growing condition variations, these differences can also cause flavor variations in small fruits (*43-47*).

Cultivar. Cultivar variations in flavor and phenolics have been studied by several groups, however the findings vary. Minimal variation in raspberry ketone, α-ionone and β-ionone concentrations between 'Camenzind,' 'Chilcotin,' 'Glen Clova,' 'Glen Moy,' 'Glen Prosen,' 'Meeker,' 'Rutrago,' 'Skeena,' 'Veten' and 'Zenith' cultivars has been reported although the raspberry ketone concentration varied from 1.09 ppm to 4.20 ppm (*25*), but in a different study a statistically significant difference (p-value=0.01), for the raspberry ketone, β-ionone, and no values for α-ionone for the cultivars 'Chilliwack,' 'Comox,' 'Malahat,' 'Meeker,' 'Tulameen' and 'Willamette' with raspberry ketone concentration varying from 0.01 mg/ml – 0.05 mg/ml (*23*). These papers used different methods to isolate the aroma compounds and compared different cultivars and the overall reported value for the

raspberry ketone in the studies differs by an order of magnitude, causing difficulties in determining the actual concentration of the raspberry ketone.

The volatile developments between 'Lloyd George' and 'Rose de Côte d'Or' have been compared, this study indicated not only that terpene hydrocarbons, esters, geraniol, and dihydro- β-ionone increased with maturity, but that the concentration of these volatiles varied in each stage between cultivars (*36*).

Volatile concentration differences between 'Newburgh' and 'Novost Kuzmina' cultivars were reported by Forney (*22*). 'Novost Kuzmina' reported significantly more volatiles than 'Newburgh' with more alcohols and carbonyl compounds while 'Newburgh' contained more terpenoid compounds (*22*).

Phenolic variations between cultivars 'Heritage,' 'Kiwigold,' 'Goldie' and 'Anne' are demonstrated in the study by Liu et al (*48*), although no statistical analysis of the data is presented. Large variations in the concentrations of quercetin and kaempferol, flavonoid phenolic compounds, have been seen in ripe 'Golden,' 'Heritage,' 'Veten,' 'Norna,' 'Malling Seedling,' 'Malling Promise,' Skeena,' 'Chilcotin,' 'Willamette' and 'Meeker' raspberries (*11*).

Growing Conditions. Growing conditions can affect the flavor and aroma volatile concentration in small fruits (*1;26*). The aroma volatile compositions of small fruits of the same cultivar grown in different locations are generally similar, although the aroma impacts and concentrations of these compounds can vary between locations (*26;49*).

Flavor variations between areas are generally related to the quantity and intensity of sunlight available (*1*). Areas with warm dry summers tend to produce more aromatic fruit with higher sugar and lower acid contents (*1*).

Based on growing conditions the aroma impact of cultivars can vary significantly. Wang et al (*26*) described 'Chickasaw' blackberry variation between samples grown in Oregon and samples grown in Arkansas. The mean high and low temperatures, humidity, and precipitation were lower in Oregon during the ripening season of June and July producing fruit with 'cut grass,' 'green,' 'fruity,' 'citrus,' and 'watermelon' aromas versus 'cinnamon,' 'piney,' 'floral,' 'sweet,' and 'caramel' aromas for Arkansas, showing significant flavor variation due to growing conditions (*26*). Significant year-to-year differences (p-value ≤ 0.05) have also been shown in raspberries grown on the same plot for raspberry ketone, alcohol, aldehyde, ester, ketone, and terpene concentrations studied by Moore et al (*23*). These variations were also witnessed between plots for raspberries in this study, although the reported differences were generally smaller than the differences reported for year-to-year variations (*23*).

Ripening conditions have been shown to be very important in volatile terpene concentrations in various grape cultivars and these variations could be similar to those in raspberries. The volatile terpene concentrations are related to the bound forms, which are found in higher concentrations prior to ripening (*33*). The total terpene content rises during ripening while the bound form varies with temperature

(*33*). Riesling grapes have shown that norisoprenoid content varies in relation to °Brix during berry development (*33*).

Fruit Maturity. Fruit development in raspberries occurs in three stages, each lasting 10-12 days, for a total of 30-36 days of growth and ripening, following pollination: a period of rapid cell division and growth, a period of embryo development and endocarp hardening and finally a period of cell enlargement (*1*). The final stage of cell enlargement is where berry ripening occurs.

Sugar concentrations, generally measured by soluble solids or °Brix, maintain a constant level during early growth and development (*34*). These levels then increase drastically during ripening, this increase continues through the overripe stage (*15*).

Acid concentrations increase early in fruit development, but decrease as the fruit ripens (*1*). In grapes acid development, increases during the first few weeks of berry formation then decreases, until finely reaching a steady stage several weeks later (*34*). This trend could be similar to that seen in red raspberries. The sugar:acid ratio provides an accurate maturity index, which indicates ripeness in blackberries although the expected ratio varies with cultivar (*15*).

Flavor compounds generally develop during the ripening stage in raspberries with concentrations of camphene, β-myrcene, limonene, α- phellandrene, α-pinene, α-ionone, β-ionone, methyl acetate, ethyl acetate, 2-methyl-1-butanol and *cis*-3-hexenol increasing, while *cis*-β-ocimene and *trans*-β-ocimene, compounds associated with green leaves, decrease in concentration (*22*).

Guichard (*36*) reported dramatic increases in terpene hydrocarbons during ripening and more regular increases in esters, although specific compounds in different cultivars responded differently in the overripe phase. A clear difference was shown between maturity stages for green-pink stage, pink stage and ripe/overripe stages, illustrating that the flavor changes occurring as fruit reaches the overripe stage are not as evident as those that occur before reaching the ripe stage (*36*).

Storage and Processing. Because only a very small portion of raspberries are sold fresh, less than 2% of Washington production (*19*), the effects of processing and storage are an important consideration.

Following harvest, strawberry, and possibly all small fruit, volatile concentrations continue to increase, but under-ripe fruit never reaches a full-ripe concentration level (*22*). Although increases occur in nearly all volatile classes, the increases are not equal in all volatiles; ethyl esters increased in strawberries stored at 1°C, while methyl esters did not, but at higher temperatures, 15°C, the methyl esters increased while ethyl esters did not (*22*).

Individually Quick Frozen (IQF) berries are an important part of the raspberry market. These berries are separated and frozen at -35°C in a blast freezer prior to packaging (*35*), the flavor changes due to this processing method have not been reported.

Chirality. Chirality is common in organic molecules. A chiral center exists when four different groups are attached to a single carbon. These centers can have

significant results in sensory studies. Chemically produced compounds will generally have an equal proportion of all isomers possible, however due to sterospecific enzymes, compounds produced in plants will often favor one isomer over others (*43*).

Different enantiomeric compounds often have different sensory thresholds and descriptors (*43-47*). Stereochemistry strongly influences the sensory thresholds, which can vary by factors of 1000 (*44*). These character and intensity variations make the isomeric ratio important to the overall sensory perception for small fruits.

RBDV and Resistance

Raspberry bushy dwarf virus (RBDV), genus *Idaeovirus,* occurs naturally worldwide and can infect raspberries, loganberries, boysenberries, and blackberry-raspberry hybrids (*4;50-52*). The virus was found to be widespread throughout England and Wales (*51*). It was present in 67% of primocane cultivars and 34% of floricane cultivars tested in the Pacific Northwest (*53*), although later tests detected RBDV in 31 of 46 fields tested in Washington state with higher than 50% infection (*18*). It has been found in 25 of 75 cultivars tested in British Columbia (*54*) and in 31.6% of field samples from the Czech Republic (*52*).

Characteristics

The virus is transmitted through pollen and seeds from infected plants and can spread rapidly through fields (*4*). The virus is often associated with reduced

yield, reduced cane vigor, crumbly fruit, and interveinal chlorosis, leaf discoloration, and in combination with other viruses, such as the *Black raspberry necrosis virus*, can cause dwarfing and shoot proliferation (*4;50*). The only effective method of control has been the use of resistant cultivars. Heat treatment combined with meristem tip culture can be used to eliminate RBDV from an infected plant, but there are no methods for eliminating the virus from plants in the field (*55;56*).

The intensity of these effects varies by cultivar (*57*) and to a lesser extent by virus strain (*58*). In cultivars that exhibit leaf symptoms, disease onset generally begins with interveinal chlorosis or an irregular, oak leaf, pattern of discoloration on the leaves (*57*). Reduced cane vigor and production are often associated with RBDV infection and crumbling fruit is sometimes absent. Once a field is infected, the virus can spread rapidly throughout the entire field, increasing from less than 3% to greater than 80% in two years (*18*). Following initial infection of highly susceptible cultivars, a field generally will be completely infected and require plant removal in five years if one wishes to produce fruit for the fresh or individually quick frozen (IQF) markets. Replanting generally includes: soil fumigation, replacing irrigation and trellising and being out of production for 1 – 1.5 years (*59*).

Several different strains of the virus have been described and have important management implications. There are three recognized strains of RBDV: S-isolates, the largest and most studied group; RB isolates, those capable of overcoming resistance conferred by the *Bu* gene; and B isolates, those isolated from black raspberries and shown to be serologically distinct from the S and RB types (*58*).

Europeans are primarily concerned with S- and RB-isolates as very few black raspberries are grown in Europe (*52*). The resistance breaking strain has not yet been detected in North America, but S and B strains are common (*18;52*).

Resistance

Several cultivars of red raspberry have natural resistance to RBDV, including 'Willamette,' however these cultivars have less desirable growth and harvest characteristics for commercial production (*4*). Resistance is believed to be linked to the *Bu* resistance gene, although it is likely to be controlled by complimentary dominant genes (*4;18*). These complimentary genes have produced mixed results in attempts to produce resistant plants through conventional breeding techniques (*4*). Results included resistance in cultivars where the parent cultivars were susceptible and susceptibility in cultivars where the parent cultivars were resistant (*4*).

Difficulties in producing resistance through conventional breeding methods have led to attempts to engineer resistance through directed mutagenesis of the movement protein gene in the virus and transformation of the mutated sequence into 'Meeker' raspberry, preventing the cell-to-cell movement of the virus within the raspberry plant (*18;59*). Of the 249 transgenic lines that resulted from these efforts, five resistant lines have been selected as having similar production characteristics to the wild-type 'Meeker', testing these lines for trueness-to-type regarding flavor characteristics was one of the goals of this research (*18*).

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FLAVOR COMPOSITION OF RASPBERRY CULTIVARS GROWN IN THE PACIFIC NORTHWEST

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by

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Abstract

Four raspberry cultivars ('Chilliwack', 'Tulameen', 'Willamette' and 'Yellow Meeker') grown in Washington during 2005 were compared to 'Meeker' red raspberries commercially grown in different locations in Washington state during 2005 for components important to flavor, including: °brix, titratable acidity, sugar (fructose, glucose and sucrose) concentrations, organic acid (citric and malic acid) concentrations, volatile aroma compound concentration and chiral compound ratios. Sugar analysis (measured by HPLC) found sucrose levels to be low in 'Meeker' raspberries, but these levels were considerably higher for the other cultivars studied. Thirty volatile aroma compounds ((Z)-3-hexenol, 4-isopropylbenzyl alcohol, 6-

methyl-5-hepten-2-ol, 2-nonanol, hexanal, (E)-2-hexenal, (Z)-3-hexenyl acetate, ethyl hexanoate, methyl nonanoate, 2-heptanone, 2-nonanone, raspberry ketone, zingerone, δ-octalactone, δ-decalactone, para-cymene, geraniol, α-ionone, β-ionone, limonene, linalool, myrcene, nerol, α-phellandrene, α-pinene, sabinene, α-terpinene, γ-terpinene, α-terpineol, terpinen-4-ol and terpinolene) were quantified using stir bar sorptive extraction (SBSE) paired with gas chromatography-mass spectrometry (GC-MS). α-Ionone, β-ionone, geraniol, linalool, (Z)-3-hexenol and raspberry ketone are particularly useful in separating raspberry cultivars. These compounds showed all of the cultivars to vary considerably from 'Meeker', particularly for α-ionone, (Z)-3-hexenol and linalool. Several chiral compounds were separated using a CyclosilB column to analyze the isomeric ratios of several aroma compounds. Variation from the 'Meeker' cultivar for the chiral ratios was seen for 6-methyl-5-hepten-2-ol and linalool for all cultivars studied.

(**Key words: raspberry, volatile, aroma, SBSE, quantification**)

Introduction

Raspberries including red raspberry and black raspberry are the most widely grown small fruits(*1;2*). The Pacific Northwest, Oregon and Washington in the USA, and southwestern British Columbia in Canada, are the primary regions in North America for raspberry production. Ninety-seven percent of the raspberries grown in Oregon and Washington are the red variety, and the majority of them are processed into a variety of food products. Many different cultivars of red raspberry

are grown in this region, with ‘Meeker’, ‘Willamette’ and ‘Tulameen’ being the most popular.

Aroma compounds in raspberry have been studied extensively. Many compounds including raspberry ketone, α-ionone, β-ionone, linalool, (Z)-3-hexenol, geraniol, nerol, α-terpineol, furaneol, hexanal, β-ocimene, 1-octanol, β-pinene, β-damascenone, ethyl 2-methylpropanoate, (E)-2-hexenal, heptanal and benzaldehyde, have been identified to contribute raspberry aroma (*3-7*). Among them, α-ionone, β-ionone, geraniol, nerol, linalool, and raspberry ketone could be particularly important to red raspberry aroma (*7-10*).

Fruit flavor is influenced by numerous factors including cultivar, climate, soil, ripeness and many other variables (*1;11-14*). Differences among cultivars variations are common, although the significance of these variations depends on the cultivars and flavor compounds under discussion (*10;12;15*). Significant variations in raspberry ketone and β-ionone concentrations have been seen between ‘Chilliwack,’ ‘Comox,’ ‘Malahat,’ ‘Meeker,’ ‘Tulameen,’ and ‘Willamette’ red raspberries (*12*). Overall volatile concentration differences have also been reported with ‘Novost Kuzmina’ having significantly higher concentrations of alcohols and carbonyl compounds while ‘Newburgh’ contained more terpenoids (*11*). Volatile aroma compounds are secondary metabolites generated from carbohydrates, amino acids and fatty acids (*16*). Variations in precursors and enzyme activities in different cultivars will result in the variation of final concentrations of volatile aroma compounds in the fruit (*14*).

Flavor compositions in small fruits are dramatically influenced by the degree of ripening (*11*). Aroma volatiles generally increase during the ripening process, but different volatiles increase to different degrees (*15*). Some of these compounds reach their peak concentration during the ripe stage and, then maintain these levels into the overripe stage (β-ionone and most esters), while other compounds continue to increase into the overripe stage (α-ionone and terpene hydrocarbons), and yet others increase at the early ripening stage and then decrease in concentration as ripening progresses (hexanol and dihydro-β-ionone) (*15*). Although these changes vary with cultivar, the general trend of increasing, steady and decreasing remains the same (*15*). Free volatiles can also be converted to the bound glycoside form and thus be unavailable for sensory contribution (*17*).

Different enantiomeric compounds often have different sensory thresholds, which can vary by factors of 1000 (*18*). In some cases, different enantiomeric compounds may even have different aroma descriptors (*18-22*). Cultivar variation and production practices could influence the enantiomeric ratios of aroma compounds, although one isomer is generally predominant, because some of the enzymes involved in the aroma formation are generally stereospecific (*22-24*).

Flavor extraction and quantification is historically a long and laborious process. Liquid-liquid extraction has been used widely for aroma compound identification and quantification, and the recovery is highly dependent upon the specific compounds and the solvent used for extraction. Static headspace analysis is only useful for compounds with high volatility (*7;11;15*). Solid-phase micro-

extraction (SPME) allows for simple sample preparation paired with minimal extraction time to establish a volatile spectrum, however, saturation and competitive adsorption are the major concerns for quantification. Stir Bar Sorptive Extration (SBSE) is a new technique for volatile extraction, it has a higher sensitivity than SPME and minimum competition and saturation effects due to the increased volume of absorbent phase (*25;26*). The high sensitivity and flexibility of SBSE makes it an effective and time saving method for extracting trace volatile compounds from complex matrices (*26*). SBSE extraction coupled with gas chromatography-mass spectrometry (GC-MS) has provn to be a valuable technique to quantify volatile aroma compounds in foods and beverages(*27*). The objective of this study was to develop and use a SBSE-GC-MS technique to quantify aroma-active compounds in raspberries grown in Pacific Northwest.

MATERIALS AND METHODS

Chemicals

Hexanal, (E)-2-hexenal, (Z)-3,7-dimethyl-2,6-octadien-1-ol (nerol), 5-isopropyl-2-methylcyclohexa-1,3-diene (α-phellandrene), 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (α-ionone), 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (β-ionone), 4-methyl-isopropyl-benzene (*para-c*ymene), ethyl hexanoate, 5-decanolide (δ-decalactone), γ- nonalactone, and 2,6-dimethyl-2,7-octadien-6-ol (linalool) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). (Z)-3-Hexenol and (Z)-3-hexenyl acetate were obtained from Bedoukian

Research (Danbury, CT). (-)-4-Isopropyl-1-methyl-1-cyclohexen-4-ol (terpinen-4-ol) was obtained from TCI Japan (Tokyo, Japan). 4-(*p*-Hydroxyphenyl)-2-butanone (raspberry ketone) was obtained from Acros Organics (Geel, Belgium). Methyl nonanoate was obtained from Eastman Blue (Rochester, NY). 5-Octanolide (δ-octalactone) was obtained from Alfa Aesar (Ward Hill, MA). 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one (cis-jasmone) was purchased from Pfalz & Bauer (Waterbury, CT). 1-Isopropyl-4-methyl-1,4-cyclohexadiene (γ-terpinene), (E)-3,7-dimethyl-2,6-octadiene-1-ol (geraniol), 3-cyclohexene-1-methanol (α-terpinene), 1-methyl-4-(1-methylethylidene-1-)-cyclohexene (terpinolene), 2-nonanol, 4-(4-hydroxy-3-methyoxyphenyl)-2-butanone (zingerone), 1-isopropyl-4-methylenebicyclo [3.1.0]hexane (sabinene), 6-methyl-5-hepten-2-ol and 4-isopropyl-benzyl-alcohol, 2-heptanone, 2-nonanone, 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene), 1-methyl-4-isopropenylcyclohex-1-ene (limonene), 2-(4-methylcyclohex-3-enyl)propan-2-ol (α-terpineol) and 7-methyl-3-methylene-1,6-octadiene (myrcene) were obtained from K&K Laboratories (Jamaica, NY).

Raspberry Samples

'Chilliwack,' 'Tulameen,' 'Willamette,' and 'Yellow Meeker' raspberries were hand harvested from research plots in Lynden, Washington in July 2005, when the fruits were fully ripe. The fruits were chilled and transported to the laboratory where they were individually quick frozen (IQF) and stored at -37°F until analyses were performed. 'Meeker' red raspberries were also obtained from commercial

producers in 2005, the raspberries were stored at -37°F until analyses were performed.

°Brix and Titratable Acidity

°Brix was analyzed at room temperature using a PAL-1 pocket refractometer (Atago U.S.A., Inc., Bellevue, WA). Titratable acidity was measured by mixing 7 ml of juice sample with 50 ml boiled water and titrating with 0.1 N NaOH to an end point of pH 8.1.

Sugar and Organic Acid Analysis

One hundred grams of red raspberries were thawed at room temperature for 3 hrs. The berries were blended with 50 g of boiling distilled water at high speed in a blender for 30 seconds. This mixture was placed in a boiling water bath for 5 min to deactivate enzymatic activity. The berry mixture was then centrifuged at 2000 rpm for 20 minutes and the supernatant was collected for further analysis.

. *Sugar Analysis.* The juice was diluted in a 1:2 ratio with acetonitrile to precipitate the pectin. One and a half milliliters of the supernatant was collected for sugar analysis. Twenty microliters of sample was injected onto a Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) system equipped with a Restek ultra amino column (3 μm, 200 x 4.6mm, Bellefonte, PA) and a refractive index (RI) detector. The mobile phase was 81% (v/v) acetonitrile-water solution with a flow rate of 1.2 mL/min. The column was maintained at 30°C. Each sample was run in triplicate.

Organic Acid Analysis. The juice was diluted in a 1:2 ratio with 0.005 M sulfuric acid, and 6 mL of the sample was passed through a C_{18} Sep-Pak Cartridges (200 mg, Waters Corporation Milford, MA) that had been conditioned with 10 mL methanol, water and dried with air prior to use. The first 4 ml of filtrate was discarded and the last 2 ml was collected for analysis.

Ten microliters of sample was injected onto a Shimadzu HPLC system equipped with a Bio-Rad Aminex ion exclusion column (HPX-87H, 300 x 7.8 mm, Richmond, CA) maintained at 30°C. A mobile phase of 0.005 M sulfuric acid was programmed at a constant flow rate of 0.4 mL/min. A UV-Vis spectrophotometric detector at wavelength of 210 nm was used for detection. Each sample was run in triplicate.

Extraction of Volatile Compounds

One hundred fifty grams of red raspberries were thawed for 3 hours. The berries were blended with 1% $CaCl_2$ and 10% NaCl in a commercial blender for 30 seconds. The calcium chloride was added to inhibit enzyme activity and the sodium chloride was added to increase sensitivity (*28*). The mixture was centrifuged at 2000 rpm for 20 minutes and the supernatant collected.

A Stir Bar Sorptive Extraction (SBSE) stir bar (1 cm long, 0.32 mm O.D. 0.5 mm film thickness) with a polydimethylsiloxane (PDMS) phase was used for the extraction of volatile compounds. The stir bar was cleaned with 80% acetonitrile in methanol overnight, allowed to air dry for 1 hour, then conditioned for 45 minutes at 300°C with 50 mL/min nitrogen flow. Ten grams of juice samples were weighed

into 20 mL clear glass vials (I-Chem, New Castle, DE) with polytetrafluorethylene septum caps and 10 µL internal standard mixture in methanol was added. The juice was extracted at room temperature for 1 hr at 1500 rpm. All samples were analyzed in triplicate.

Gas Chromatography-Mass Spectrometry (MS)

The analysis of volatile compounds was carried out by using an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose TDU autosampler with a CIS-4 cooled injection system (Gerstel USA, Baltimore, MD). The analytes were thermally desorbed at the TDU in splitless mode, ramping from 35ºC to 300ºC at a rate of 700ºC/min, and held at the final temperature for 3 min. The CIS-4 was cooled to -80°C with liquid nitrogen during the sample injection, then heated at 10°C/second to 250°C for 3 min. Solvent vent mode was used during the injection with a split vent purge flow of 50 mL/min beginning at 3 min. The helium column flow was 2.0 ml/min. Separation was achieved using a ZB-FFAP column (30m x 0.32mm I.D., 0.5 µm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40°C for 2 min, then ramped to 180°C at a rate of 6°C/min, then increased to 240°C at a rate of 4°C/min and held at the final temperature for 20 min. Standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 35 to 350 amu. System software control and data management/analysis were performed through Enhanced ChemStation Software

(Agilent Techonologies, Inc.). Compounds were identified through mass spectra comparison and retention index comparison with the pure standards.

Chiral Analysis

The samples were extracted using the same procedure as described previously, however internal standards were not added. Separation was achieved using a CyclosilB column (30 m. 0.25 mm I.D., 0.25 micron film thickness, Agilent). The oven temperature was programmed at 40ºC for 2 min, then increased to 90°C at a rate 6°C/min, then to 135°C at a rate of 1°C/min, increased to 220°C at a rate of 10°C/min and hold at the final temperature for 5 min. Isomeric ratios were determined using the relative total mass ion abundance for each compound peak.

Compound Quantification

An internal standard solution was prepared in methanol containing 784 ppm and 395 ppm of γ-nonalactone and cis-jasmone respectively. An aliquot (5μL) of the internal standard mixture was then added to 10 ml of water to yield a final concentration of 392 and 197 ppb respectively. Pure standards of all compounds quantified were divided into four groups for sample preparation and analysis. Standard stock solutions of 2000 ppm of each compound (except zingerone and raspberry ketone) were prepared in methanol. The stock solution was then used to create calibration curve solutions in water with concentrations of 1000 ppb, 750 ppb, 500 ppb, 100 ppb, 50 ppb, 5 ppb and 1 ppb with 5 μL of internal standard solution. MS-quantification was carried out using specific mass ions to avoid interference between co-eluted compounds. The ions used for these identifications were listed in

Table 2.1. Each ion was chosen for relative abundance, with qualifier ions used for further identification. Quantification curves were built by plotting selected ion abundance ratio of target compounds with their respective internal standards against the concentration ratio. The concentrations of target compounds in the samples were calculated based on the individual calibration curves (Table 2.1).

Statistics

A 95% 'Meeker' confidence interval, constructed using the average for each of the 'Meeker' values, then adding and subtracting 2 standard deviations, was used as the variation range for 'Meeker' from different sites.

Results and Discussion

Sugars and Organic Acids, °Brix and Titratable Acidity

All of the raspberry cultivars were grown in Washington state during 2005. The similar growing conditions makes a comparison between these cultivars useful for comparing the flavor variations between the cultivars.

Table 2.2 illustrates the results for sugar and organic acid concentrations, °brix and titratable acidity in various raspberry cultivars grown in Washington. Variation for °brix, titratable acidity, sugar and acid composition were observed in 'Meeker' fruits obtained from different sites. Sugars and organic acids are strongly affected by climate variations (*1;12;14*). Higher temperatures and lower humidity generally produce fruit with higher sugar and lower acid contents (1).

The major sugars in ‘Meeker’ raspberry were fructose and glucose and the ratio of fructose to glucose was around 1 to 1.1. ‘Meeker’ raspberry also had low concentration of sucrose. Significantly higher amounts of sucrose were found in other raspberry cultivars with sucrose being the major sugar in ‘Chilliwack’. ‘Yellow Meeker’ had much higher fructose, glucose and sucrose than ‘Meeker’. Sugar composition of several raspberries has been studied previously and a wide concentration range has been reported. Fructose concentrations were reported to vary between 1.83% and 2.92%, glucose varied from 1.13% to 2.63%, and sucrose varied from 0.12% to 1.51%(*29*). Although the cultivars and locations were different, the sugar composition for many samples were in agreement with previous study(*29*).

The major organic acid identified in these raspberries was citric acid. The average citric acid content in all ‘Meeker’ raspberries was 1.51% with a range from 1.33 to 1.72%. Very small amounts of malic acid were found in these raspberries. Compared with ‘Meeker,’ most other cultivars had higher acid concentrations. ‘Chilliwack’ had the highest amount of citric acid followed by ‘Willamette’. ‘Tulameen,’ had a citric acid content slightly greater than ‘Meeker’.

Citric acid concentrations are reported to vary between 1.06 and 2.48% with an average of 1.72% for red raspberries(29); ‘Chilliwack’ and ‘Willamette’ citric acid concentrations were above this range. Malic acid concentrations are reported to below 0.8% with an average of 0.4% for red raspberries(29), all raspberries studied

fell within this range. 'Yellow Meeker,' as the only golden raspberry studied, fell within the reported range for red raspberries for both acid and sugar concentrations.

The sugar to acid ratio is considered to be very important to the flavor perception of fruits. For most the cultivars, the high acids were balanced with high sucrose percentages, leading to a sugar/acid ratio that was very close to the 'Meeker' value. 'Willamette' is the one exception, the sugar/acid ratio was considerably lower than the average 'Meeker' value. Low sugar/acid ratio can lead to a "tart" sensory perception.

Volatile Quantification

Raspberry ketone, α-ionone, β-ionone, (Z)-3-hexenol, geraniol, linalool, and many other compounds have been identified to contribute raspberry aroma (*7;10;11;30;31*). Different proportions of these compounds give rise to different perceived raspberry odors from different cultivars, growing areas and growing conditions. Quantitative data on the concentration of these aroma-active compounds in raspberry is limited. In this study, a total of thirty-one compounds were quantified. These compounds were selected based on their previously reported importance to raspberry aroma as well as their representation to various chemical classes including alcohol, aldehyde and ketone, ester, and terpene and tepene alcohol. Calibration curves were constructed for the selected compounds using pure compounds and internal standards. Chromatographic conditions were selected to optimize resolution for the quantified compounds, quantifying and qualifing ions

were selected to eliminate interferance from coeluting compounds while providing good sensitivity. All of the volatile compounds were analyzed simultaneously by the SBSE-GC-MS technique. As shown in Table 2.1, good correlation coefficients (greater than 0.99) were achieved for most of the compounds, and the quantification limit for most of the compounds was at 1 μg/kg. The quantification of raspberry ketone and zingerone was also attempted, however, the PDMS phase has very low recoveries for these two compounds, thus, quantification of these two compounds by PDMS-SBSE technique was not reliable.

The concentrations of selected volatile compounds in 'Meeker' raspberry from different growing sites are listed in Table 2.3. Volatile concentration varied from site to site with standard deviation of 10-20% for most of the compounds investigated. Flavor variations between sites and years can be influenced by temperature, soil variation and the intensity of sunlight available during the growing and ripening seasons (*1*) (*14*). Year to year variations and location variations have been shown for the raspberry ketone, alcohols, aldehydes, esters, ketones and terpene concentrations in red raspberries (*12*). The year-to-year variations were more significant than the location variations (*12*). Areas with warmer and drier summers generally produce more aromatic fruit (*1*). As shown in Table 2.3, volatile concentration variations from different sites were much less than the variations observed for sugar and acids.

There were considerable differences between 'Meeker' red raspberry and other raspberry cultivars studied (Table 2.4). When a 95% confidence was applied,

'Chilliwack' appeared to be the cultivar that was the most different from 'Meeker', varying in concentrations in 22 of the 31 compounds studied. 'Tulameen' and 'Willamette' both varied from 'Meeker' in 18 of the 31 compounds studied. 'Yellow Meeker' was the most similar to 'Meeker,' varying in 14 of the 31 compounds.

Figure 2.1 illustrates the variations present between the cultivars sampled for aroma compounds that are considered to be particularly important in differentiating red raspberries: α-ionone, β-ionone, geraniol, linalool, (Z)-3-hexenol, and the raspberry ketone (*4;7;11*). All cultivars studied varied considerably from 'Meeker' for α-ionone, although 'Tulameen' and 'Willamette' were only just outside the range. 'Chilliwack' was the only cultivar to vary from 'Meeker' in β-ionone. 'Chilliwack' and 'Wilamette' varied for geraniol. All cultivars varied from 'Meeker' for linalool and (Z)-3-hexenol. These data illustrated the flavor profile variability that occurred within raspberries during development due to genetic variations. These variations may reflect different concentrations of enzymes and/or substrates necessary for the production of volatile compounds in the plant.

Volatiles are produced through various pathways from their precursors. These pathways are generally similar in plants, although naturally there are variations in the actual compounds produced and the concentrations of these compounds. Alcohols, aldehydes, and acids are generally formed from fatty acids through two major pathways (*16;32-34*). The β-oxidation pathway involves the enzymatic break-down of fatty acids to produce alcohols, aldehydes and ketones (*16;33*). This pathway is also believed to be responsible for lactone formation (*16*).

The lipoxygenase (LOX) pathway forms primarily 'green' odor notes through enzymatic oxidative degradation of fatty acids (*16;32*). The LOX pathway is found in mature fruit, where the cell membranes become more permeable or in injured fruit where the cell membranes have been damaged (*16;35*). The raspberry ketone is believed to be formed from the condensation of p-coumaryl-CoA and malonyl-CoA, followed by decarboxylation to p-hydroxyphenylbut-3-ene-2-one and reduction to raspberry ketone(*36*). Terpenes are formed primarily through the isoprenoid pathway, which metabolizes carbohydrates. Geranyl diphosphate, formed through the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by geranyl diphosphate synthase, is believed to be the precursor for most monoterpenes, which are formed through several enzymatic reactions including hydrolysis, cyclation and/or oxidoreductions depending on the final product (*16*). The most important of these steps is often cyclation, although oxygenation is often necessary for volatile formation (*16*).

Chiral Analysis

Natural flavor molecules are generally found with one enantiomer predominating, synthetic flavor molecules are generally found in racemic mixtures. Analysis of chiral compounds is a useful method for analyzing products produced with raspberries or raspberry flavor(*23*), particularly α-ionone, only the (R)(+)-enantiomer is found in fruit (*23*). It is of interest to know if the ratios of some chiral compounds differ between different cultivars.

Under experimental conditions, chiral isomers were separated for several compounds, while only one isomeric form was observed for α-ionone, β-ionone and the raspberry ketone. This supported a previous literature report that these compounds only have one isomer present in natural raspberry fruit (*23*).

The percent variations for the compounds found during the chiral analysis, 6-methyl-5-hepten-2-ol, δ-octalactone, δ-decalactone, linalool and terpinen-4-ol, are displayed in Table 2.5. Most of the compounds demonstrate a much higher percentage of one enantiomer over another, particularly 6-methyl-5-hepten-2-ol, and the lactones, with more than 90% of one isomer. Linalool is nearly a racemic mixture, with a slightly higher percentage of the later eluting isomer. Terpinen-4-ol is a 20/80 mixture favoring the later eluting isomer.

Little variation was seen between 'Meeker' samples for the isomeric compounds studied, however, there was considerably variation between 'Meeker' and the other cultivars. The largest variations between cultivars are seen for 6-methyl-5-hepten-2-ol and linalool, variations are seen for the other compounds compared, but these ratios are just outside the limit determined by the 'Meeker' raspberries. Linalool shows a more racemic mixture for non-'Meeker' cultivars, particularly 'Tulameen' and 'Willamette,' and 6-methyl-5-hepten-2-ol has a higher percentage of the later eluting isomer in the non-'Meeker' cultivars, particularly 'Willamette.'

Overall 'Chilliwack' red raspberries show the most variation from 'Meeker' for aroma compounds, 'Yellow Meeker' golden raspberries show the most variation

for sugar concentration and ‘Willamette’ red raspberries show the most variation for acid concentration and sugar/acid ratio.

Compound	Quantifier Ion	Qualifier Ion(s)	Quantification Range	Equation: $Conc = \frac{(Rc/Rs)}{M} * Cs$		Coefficient	Reference Internal Standard
				M	Cs		
(Z)-3-Hexenol	67	41	1 - 750	0.0007368	0.1969	0.9869	*cis* Jasmone
4-Isopropylbenzyl alcohol	135	150, 105	1 - 500	0.0001753	0.3922	0.9999	γ Nonalactone
6-Methyl-5-hepten-2-ol	95	69, 41	1 - 500	0.008595	0.1969	0.9998	*cis* Jasmone
2-Nonanol	45	69, 55	1 - 500	0.001183	0.3922	0.9925	γ Nonalactone
Hexanal	56	44	1 - 750	0.004221	0.1969	0.9788	*cis* Jasmone
(E)-2-Hexenal	41	55	1 - 750	0.001553	0.1969	0.9814	*cis* Jasmone
(Z)-3-Hexenyl-acetate	67	43, 82	1 - 500	0.05638	0.1969	0.9937	*cis* Jasmone
Ethyl hexanoate	88	99	1 - 1000	0.0009557	0.3922	0.9722	γ Nonalactone
Methyl nonanoate	74	87	1 - 750	0.001658	0.3922	0.9202	γ Nonalactone
2-Heptanone	43	58	1 - 750	0.01405	0.1969	0.9968	*cis* Jasmone
2-Nonanone	58	43	1 - 750	0.04101	0.1969	0.9790	*cis* Jasmone
Raspberry ketone	107	164	45 - 45000	0.000004828	0.3922	0.9869	γ Nonalactone
Zingerone	137	194, 45	10 - 5000	0.0004234	0.1969	0.9985	*cis* Jasmone
δ-Octalactone	99	71	1 - 1000	0.00006914	0.3922	0.9839	γ Nonalactone
δ-Decalactone	99	71	1 - 1000	0.0005589	0.3922	0.9973	γ Nonalactone
Para cymene	119	134	1 - 500	0.0005589	0.3922	0.9973	γ Nonalactone
Geraniol	69	41	1 - 750	0.002575	0.1969	0.9925	*cis* Jasmone
α-Ionone	121	93	1 - 500	0.002917	0.3922	0.9967	γ Nonalactone
β-Ionone	177	43	1 - 500	0.003951	0.3922	0.9994	γ Nonalactone
Limonene	68	93	1 - 500	0.04214	0.1969	0.9998	*cis* Jasmone
Linalool	71	93, 80	1 - 500	0.01521	0.1969	0.9938	*cis* Jasmone

Table 2.1. Calibration of volatile standard compounds by SBSE-GC-MS

Compound	Quantifier Ion	Qualifier Ion(s)	Quantification Range	Equation: $Conc = \frac{(Rc/Rs)}{M} * Cs$		Coefficient	Reference Internal Standard
				M	Cs		
Myrcene	41	93	1 - 750	0.02903	0.1969	0.9929	*cis* Jasmone
Nerol	69	41	1 - 750	0.01468	0.1969	0.9923	*cis* Jasmone
α-Phellandrene	93	77	1 - 750	0.02431	0.1969	0.9971	*cis* Jasmone
α-Pinene	93	77	1 - 500	0.04191	0.1969	0.9961	*cis* Jasmone
Sabinene	93	77, 79	1 - 500	0.06246	0.1969	0.9926	*cis* Jasmone
α-Terpinene	121	93	1 - 750	0.02974	0.1969	0.9963	*cis* Jasmone
γ-Terpinene	93	77	1 - 750	0.03418	0.1969	0.9913	*cis* Jasmone
α-Terpineol	59	93	1 - 750	0.006199	0.1969	0.9970	*cis* Jasmone
Terpinen-4-ol	71	93	1 - 750	0.01035	0.1969	0.9899	*cis* Jasmone
Terpinolene	121	93	1 - 750	0.04046	0.1969	0.9988	*cis* Jasmone

Rc: Compound Response
Rs: Internal Standard Response
M: Line Slope
Cs: Internal Standard Concentration

Table 2.1. Calibration of volatile standard compounds by SBSE-GC-MS (Continued)

	'Meeker' WA Site 1	'Meeker' WA Site 2	'Meeker' WA Site 3	'Meeker' WA Site 4	**'Meeker' Average**	**'Meeker' 95% Range**	'Chilliwack' Lynden, WA	'Tulameen' Lynden, WA	'Willamette' Lynden, WA	'Yellow Meeker' Lynden, WA
Percent Fructose	1.43	0.58	1.18	1.54	**1.18**	**0.39 - 1.97**	1.13	1.25	0.89	2.02*
Percent Glucose	1.23	0.44	0.96	1.35	**0.99**	**0.25 - 1.74**	0.75	1.01	0.67	1.75*
Percent Sucrose	0.17	ND	0.24	0.39	**0.20**	**0 - 0.49**	1.95*	0.84*	0.75*	0.83*
Total Percent Sugar	2.83	1.01	2.38	3.28	**2.38**	**0.59 - 4.17**	3.84	3.09	2.31	4.60*
Percent Citric Acid	1.45	1.52	1.72	1.33	**1.51**	**1.07 - 1.94**	2.63*	1.96*	2.53*	2.13*
Percent Malic Acid	0.03	ND	0.07	0.08	**0.04**	**0 - 0.11**	0.02	0.02	ND	0.07
Total Percent Acid	1.48	1.52	1.79	1.40	**1.55**	**1.09 - 2.00**	2.65*	1.98	2.53*	2.20*
Sugar/ Acid Ratio	1.91	0.67	1.33	2.34	**1.54**	**-**	1.45	1.57	0.91	2.09
°Brix	11.0	8.6	9.2	9.8	**9.6**	**7.6 - 11.6**	9.6	9.5	8.7	10.8
Titratable Acidity	1.28	1.22	1.47	1.14	**1.28**	**1.00 - 1.56**	2.23*	1.67*	2.10*	1.72*
Original pH	2.72	2.75	2.70	2.84	**2.75**	**2.63 - 2.88**	2.41*	2.48*	2.47*	2.55*
°Brix/TA Ratio	8.54	7.00	6.22	8.56	**7.51**	**-**	4.30	5.64	4.15	6.27

ND: not detected with detection limit of 0.01% for sugars and 0.01% for acids
*: different from Meeker raspberry 95% confidence interval

Table 2.2. Sugar and Organic Acid Compositions measured by HPLC, °Brix, Titratable Acidity and original pH for raspberries grown in Washington during 2005

Compound	Meeker' Site 1	Meeker' Site 2	Meeker' Site 3	Meeker' Site 4	Range
(Z)-3-Hexenol	312±6.9	256±17.3	263±14.7	279±3.3	228 - 327
4-Isopropylbenzyl alcohol	45±2.0	59±0.3	40±1.8	65±3.5	32 - 74
6-methyl-5-hepten-2-ol	70±0.7	60±2.4	62±2.7	71±0.4	55 - 76
2-Nonanol	7±0.5	9±0.1	4±0.1	9±0.3	2 - 12
Hexanal	56±8.8	37±4.2	40±2.6	54±3.8	27 - 66
(E)-2-Hexenal	315±17.3	278±18.5	331±18.8	311±3.4	260 - 357
(Z)-3-Hexenyl acetate	5±0.1	7±0.4	7±0.2	9±0.4	4 - 10
Ethyl hexanoate	6±0.8	12±0.5	10±0.8	10±0.6	5 - 13
Methyl nonanoate	ND	ND	1±0.2	1±0.1	0 - 1
2-Heptanone	71±9.3	94±9.0	81±2.6	80±0.9	61 - 102
2-Nonanone	20±1.1	25±1.4	15±0.4	29±0.8	11 - 34
Raspberry ketone	3797±737	3080±686	3770±1127	2500±1194	1297 - 5277
Zingerone	897±113	1107±127	815±153	925±125	620 - 1250
δ-Octalactone	814±25.7	799±8.6	696±36.7	918±25.3	636 - 978
δ-Decalactone	783±9.3	799±2.1	710±20.9	876±10.8	666 - 917
Para-cymene	12±1.0	16±0.5	12±0.3	21±1.4	8 - 23
Geraniol	149±8.0	126±4.9	118±5.0	156±6.5	102 - 172
α-Ionone	32±1.8	30±0.3	41±1.2	47±2.0	23 - 52
β-Ionone	67±3.8	71±1.3	71±2.4	89±2.5	56 - 93
Limonene	1±0.2	1±0.0	1±0.1	2±0.1	1 - 2
Linalool	39±0.7	37±1.3	34±1.0	42±0.3	31 - 44
Myrcene	5±3.1	3±0.2	4±0.8	5±0.7	1 - 8
Nerol	29±1.4	28±1.4	21±1.1	33±1.5	19 - 37
α-Phellandrene	57±18.4	69±3.2	52±2.7	87±3.5	26 - 100

Table 2.3. Volatile compound concentrations (ppb) in ‘Meeker’ red raspberry grown at different sites in Washington during 2005

Compound	Meeker' Site 1	Meeker' Site 2	Meeker' Site 3	Meeker' Site 4	Range
α-Pinene	27±5.0	17±1.4	14±0.4	21±0.6	11 - 27
Sabinene	20±1.8	25±1.2	17±1.3	29±1.4	13 - 32
α-Terpinene	14±4.9	23±0.9	18±0.9	33±0.3	4 - 39
γ-Terpinene	4±2.8	9±0.3	7±0.3	16±0.5	0 - 18
α-Terpineol	43±0.6	48±1.6	39±1.8	54±1.4	35 - 58
Terpinen-4-ol	154±4.0	160±7.6	111±4.9	175±3.6	100 - 201
Terpinolene	2±0.4	2±0.1	2±0.2	4±0.1	1 - 4

Table 2.3. Volatile compound concentrations (ppb) in 'Meeker' red raspberry grown at different sites in Washington during 2005 (Continued)

Compound	'Chilliwack'	'Tulameen '	'Willamette'	'Yellow Meeker'
(Z)-3-Hexenol	167±11.1	86±12.5	162±27.7	177±9.7
4-Isopropylbenzyl alcohol	3±0.2	2±0.0	2±1.9	36±4.8
6-Methyl-5-hepten-2-ol	17±1.0	29±3.0	10±0.2	55±5.1
2-Nonanol	ND	ND	2±0.1	5±1.0
Hexanal	178±7.9	70±5.4	114±30.8	147±35.6
(E)-2-Hexenal	402±27.6	418±36.4	252±151.2	464±197.1
(Z)-3-Hexenyl acetate	10±0.4	1±0.2	15±4.7	7±0.7
Ethyl hexanoate	2±0.2	8±0.3	3±2.3	3±1.9
Methyl nonanoate	1±0.0	1±0.0	ND	ND
2-Heptanone	61±4.1	65±4.1	90±51.1	57±24.0
2-Nonanone	11±0.4	2±0.1	13±1.2	20±1.9
Raspberry ketone	3247±1470	2852±347	3500±999	2103±238
Zingerone	169±44.1	982±158.4	1058±51.7	267±64.5
δ-Octalactone	106±4.1	601±16.5	203±40.6	390±45.7
δ-Decalactone	168±3.2	510±23.9	266±23.9	467±34.9
Para-cymene	4±0.1	3±0.2	1±1.6	12±2.0
Geraniol	38±0.9	155±12.9	48±9.0	130±6.9
α-Ionone	81±3.2	20±0.5	54±3.8	63±5.5
β-Ionone	115±6.2	62±1.9	73±1.3	70±3.0
Limonene	1±0.0	2±0.3	1±0.4	1±0.7
Linalool	14±0.7	145±13.1	20±2.6	16±1.5
Myrcene	4±3.1	6±1.0	6±3.6	10±4.0
Nerol	5±0.2	23±1.7	4±0.8	19±1.4
α-Phellandrene	6±2.9	20±3.5	20±4.5	47±16.5

Table 2.4. Volatile concentration (ppb) in 'Chilliwack', 'Tulameen ', 'Willamette', and 'Yellow Meeker' cultivars grown in Washington in 2005

Compound	'Chilliwack'	'Tulameen '	'Willamette'	'Yellow Meeker'
α-Pinene	6±0.2	4±0.4	22±5.1	30±4.5
Sabinene	4±0.3	8±1.1	4±4.9	12±10.6
α-Terpinene	3±0.3	10±1.0	5±2.3	15±5.8
γ-Terpinene	3±0.1	2±0.2	4±1.7	13±8.2
α-Terpineol	8±0.3	124±11.5	17±1.8	20±1.6
Terpinen-4-ol	23±1.0	74±6.8	46±6.1	123±52.5
Terpinolene	1±0.0	3±0.2	1±0.5	3±0.3

Table 2.4. Volatile concentration (ppb) in 'Chilliwack', 'Tulameen ', 'Willamette', and 'Yellow Meeker' cultivars grown in Washington in 2005 (Continued)

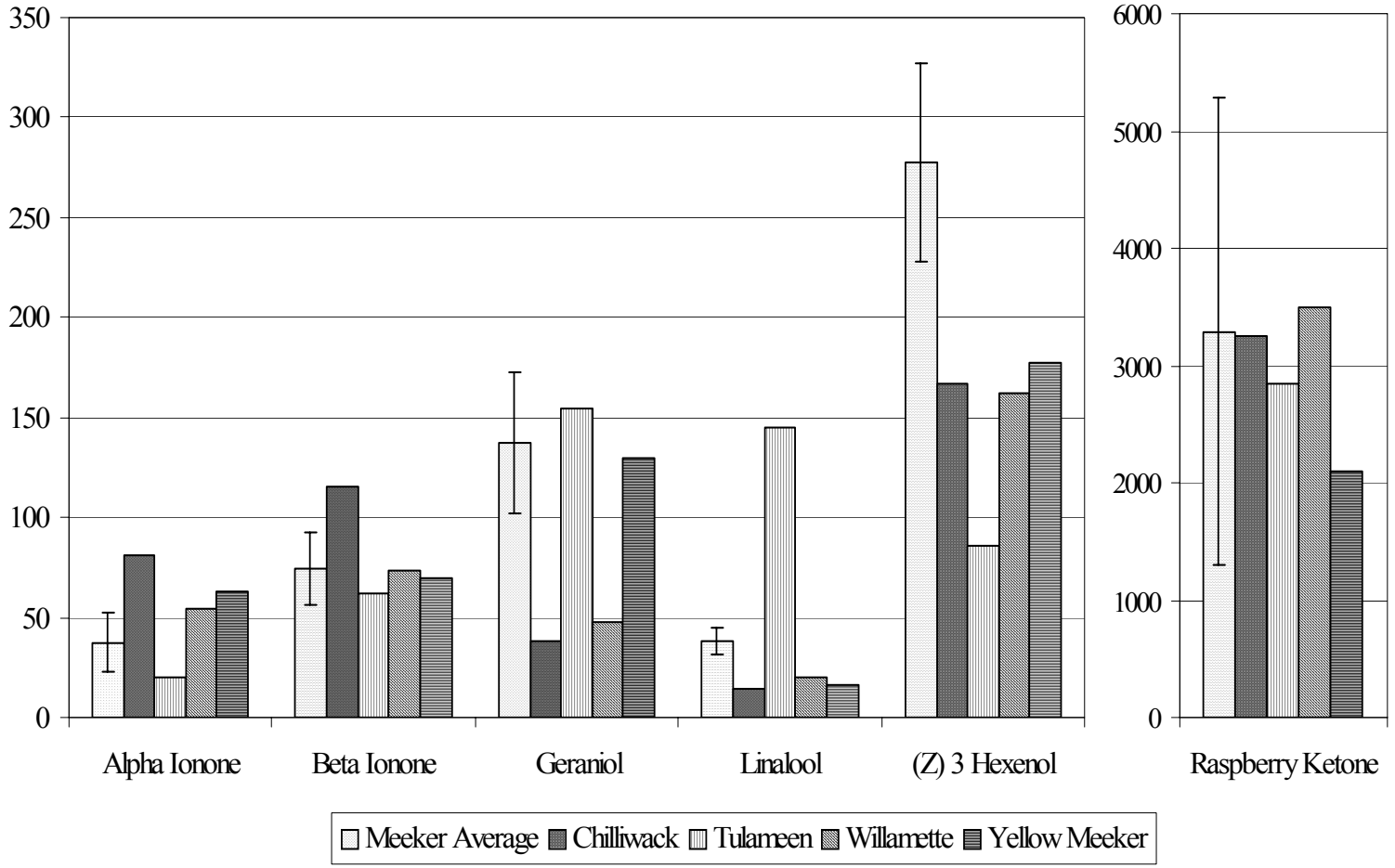

Figure 2.1. Comparison between cultivars for some important aroma compounds with error bars for 2 standard deviations for 'Meeker' average bar.

Location	6-Methyl-5-hepten-2-ol 1	6-Methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Wild Type 'Meeker' WA Site 1	94.7%	5.3%	98.1%	1.9%	96.8%	3.2%	41.4%	58.6%	20.7%	79.3%
Wild Type 'Meeker' WA Site 2	94.8%	5.2%	96.3%	3.7%	97.7%	2.3%	40.0%	60.0%	20.9%	79.1%
Wild Type 'Meeker' WA Site 3	95.5%	4.5%	96.5%	3.5%	97.8%	2.2%	42.1%	57.9%	20.8%	79.2%
Wild Type 'Meeker' WA Site 4	93.9%	6.1%	96.4%	3.6%	97.9%	2.1%	37.4%	62.6%	20.0%	80.0%
Wild Type 'Meeker' Average	**94.7%**	**5.3%**	**96.9%**	**3.1%**	**97.5%**	**2.5%**	**40.2%**	**59.8%**	**20.6%**	**79.4%**
Wild Type 'Meeker' 95% Range	**93.5 - 96.0%**	**4.0 - 6.5%**	**94.3 - 99.4%**	**0.6 - 5.7%**	**96.6 - 98.5%**	**1.5 - 3.4%**	**36.3 - 44.1%**	**55.9 - 63.7%**	**19.8 - 21.3%**	**78.7 - 80.2%**

Table 2.5 Chiral percentage comparison for raspberry cultivars grown in Washington during 2005

Location	6-Methyl-5-hepten-2-ol 1	6-Methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Chilliwack Lynden, WA	86.7%	13.3%	100%	0.0%	99.4%	0.6%	45.5%	54.5%	20.1%	79.9%
Tulameen Lynden, WA	86.1%	13.9%	100%	0.0%	100%	0.0%	48.8%	51.2%	17.6%	82.4%
Willamette Lynden, WA	77.4%	22.6%	100%	0.0%	99.7%	0.3%	51.2%	48.8%	19.6%	80.4%
Yellow Meeker Lynden, WA	96.3%	3.7%	100%	0.0%	99.1%	0.9%	45.7%	54.3%	19.6%	80.4%

Table 2.5 Chiral percentage comparison for raspberry cultivars grown in Washington during 2005 (Continued)

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FLAVOR QUALITY OF RASPBERRY BUSHY DWARF VIRUS-RESISTANT TRANSGENIC 'MEEKER' RED RASPBERRIES

Paper to be Submitted to…

by

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Abstract

Raspberry bushy dwarf virus (RBDV) causes significant reduction in yield and crumbly fruit in raspberries and raspberry-blackberry hybrids, there is no effective treatment once a plant is infected and the only effective prevention is the use of resistant cultivars. Genetic modifications were made to 'Meeker' red raspberries in order to impart resistance to RBDV while maintaining the desirable marketing characteristics of 'Meeker' including the sweetness, aroma profile and machine harvestability. These transgenic and wild-type 'Meeker' plants were grown in Oregon and Washington and harvested in 2004 and 2005 for fruit analysis. These raspberries were analyzed for °brix, titratable acidity, volatile aroma compound concentration and chiral compound ratios. Year-to-year and site-to-site variations were seen for °brix and titratable acidity, with Oregon raspberries having slightly

higher °brix and lower titratable acidity than Washington raspberries. Thirty volatile aroma compounds ((Z)-3-hexenol, 4-isopropylbenzyl alcohol, 6-methyl-5-hepten-2-ol, 2-nonanol, hexanal, (E)-2-hexenal, (Z)-3-hexenyl acetate, ethyl hexanoate, methyl nonanoate, 2-heptanone, 2-nonanone, raspberry ketone, zingerone, δ-octalactone, δ-decalactone, para cymene, geraniol, α-ionone, β-ionone, limonene, linalool, myrcene, nerol, α-phellandrene, α-pinene, sabinene, α-terpinene, γ-terpinene, α-terpineol, terpinen-4-ol and terpinolene) were quantified using stir bar sorptive extraction (SBSE) paired with gas chromatography-mass spectrometry (GC-MS). None of the variations between the transgenic lines and wild-type 'Meeker' seen within a site and year were repeated in the other site or year, indicating that the differences were due to environmental rather than genetic factors. Variations between harvest locations and season were larger than variation between wild-type 'Meeker' and the transgenic lines. Chiral analysis revealed very little variation between lines, locations or years for the compounds studied.

(**Key words: Raspberry, Meeker, RBDV-resistant, transgenic, volatile, SBSE, quantification**)

Introduction

Raspberries, commonly grouped with blackberries and blackberry-raspberry hybrids as 'brambles' and 'caneberries', grow naturally in all temperate regions of the northern hemisphere, and are grown commercially in the United States, Europe, Chile, New Zealand and Australia (*1-3*).

The Pacific Northwest, made up of Oregon and Washington states, produces the largest volume of red raspberries in the United States (*4;5*). The area has ideal growing conditions for red raspberries. The production of raspberry has increased dramatically over the last fifteen years due to the development of machine harvestable and high yield cultivars and increased demand (*6*). Since the early 1980s, 'Meeker' has begun to replace 'Willamette' and is now the leading red raspberry cultivar grown commercially in the Pacific Northwest, accounting for 80% of red raspberry acreage in Washington state (*6;7*). 'Meeker' is popular due to high yields, a long harvest season, resistance to root rot and machine harvest characteristics (*6-11*). 'Meeker' fruit has a desirable color, firm texture and good sensorial qualities, including aroma, sweetness and acidity.

With the change in cultivars and increased planting density, there was a rapid increase in the incidence of *Raspberry bushy dwarf virus* (RBDV). RBDV, genus *Idaeovirus*, occurs naturally worldwide and can infect most bramble species and cultivars (*12-15*). RBDV reduces cane vigor and causes leaf discoloration, known as interveinal chlorosis; in combination with other viruses, such as the *Black raspberry necrosis*, RBDV can cause dwarfing and shoot proliferation (*14*). RBDV affected plants often have crumbly fruit and reduced yields.

RBDV is transmitted through pollen and seeds from infected plants and can spread rapidly through fields (*6;14*). Following the initial infection, a field will generally be completely infected and require plant removal, fumigation and replanting with new plants within 5 years (*16*). Heat treatment combined with

meristem tip culture can be used to eliminate RBDV from an infected plant, but there are no methods for eliminating the virus from plants in the field (*17;18*).

Conventional breeding methods have difficulties when attempting to produce resistant, commercially viable plants (*14*). These difficulties in producing resistance through conventional breeding methods have led to attempts to engineer resistance through directed mutagenesis of the movement protein gene in the virus and transformation of the mutated sequence into 'Meeker' raspberry, preventing the cell to cell movement of the virus within the raspberry plant (*6;16*). Previous work has generated 249 transgenic lines, among which 51 lines have remained free from RBDV infection after 4 years exposure to high RBDV pressure in which all 202 wild-type 'Meeker' plants in the plot became infected (*6*). Of these infection-free lines, 5 have been selected as having similar production characteristics to the wild-type 'Meeker' cultivar (*6*). However, the flavor characteristics of the fruits from these transgenic raspberry plants have not been fully evaluated for trueness-to-type.

Flavors in raspberry are mainly formed during a brief ripening period and are influenced by numerous factors (*19-21*). These factors include internal genetic makeup, and external agronomical factors, such as climate and soil type, as well as the ripeness and handling of the fruit (*1;22-25*). Sugars and organic acids are strongly affected by climate variations. Volatile compounds are generated through numerous pathways and their final concentrations are affected by environmental factors to various degrees due to effects on precursors and enzymatic activity within the fruit (*25;26*).

Flavor variation due to genetic makeup of cultivars are also common, although the significance of these variations depends on the cultivars and flavor compounds under discussion (*10;23;27*). Variations in the volatile pathways in raspberry can often be related to enzyme concentration and activity (*27*). The objective of this study is to determine if the volatile composition of RBDV-resistant lines will vary from the wild-type 'Meeker' raspberry.

Materials and Methods

Chemicals

Hexanal, (E)-2-hexenal, nerol, α-phellandrene, α-ionone, β-ionone, *para-c*ymene, ethyl hexanoate, δ-decalactone, γ-nonalactone and linalool were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). 2-Heptanone, 2-nonanone, α-pinene, limonene, α-terpineol and myrcene were obtained from K&K Laboratories (Jamaica, NY). γ- terpinene, geraniol, α -terpinene, terpinolene, and 2-nonanol were obtained from Lab Stock Flavor. (Z)-3-Hexenol and (Z)-3-hexenyl acetate were obtained from Bedoukian Research (Danbury, CT). terpinen-4-ol was obtained from TCI Japan. Raspberry ketone was obtained from Acros Organics. Methyl nonanoate was obtained from Eastman Blue (Rochester, NY). δ-Octalactone was obtained from Alfa Aesar. 4-(4-Hydroxy-3-methyoxyphenyl)-2-butanone (zingerone), 1-isopropyl-4-methylenebicyclo [3.1.0]hexane (sabinene), 6-methyl-5-hepten-2-ol and 4-isopropylbenzyl alcohol were obtained from laboratory stores. 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (cis-jasmone) was purchased from Pfalz & Bauer.

Red Raspberry Samples

Ripe wild type and transgenic 'Meeker' raspberries were harvested from Lynden, Washington in July 2004 and 2005 and Aurora, OR in June 2004 and 2005, when the fruits were fully ripe. The fruits from the transgenic lines and the wild-type at each location were harvested at the same time. The fruits were chilled and transported to the laboratory where they were quickly individually quick frozen (IQF) and stored at -37°F until analyses were performed.

°Brix and Titratable Acidity

One hundred grams of red raspberries were thawed at room temperature for 3 hrs. The juice collected from the thawing process was used to analyze °Brix using Pocket Pal-1 Pocket Refractometer from ATAGO (Bellevue, WA). The berries were blended with 50 g of boiling distilled water at high speed in a blender for 30 seconds. This mixture was then placed in a boiling water bath for 5 min to deactivate enzymatic activity. The berry mixture was then centrifuged at 2000 rpm for 20 minutes and the supernatant was collected for further analysis. Seven (7) milliliters of the juice collected above was combined with 50 mL of CO_2 free water, it was then titrated with 0.1N NaOH solution to an end point of 8.1 pH. The results were reported as percent (%) of citric acid.

Extraction of Volatile Compounds

One hundred fifty grams of red raspberries were thawed for 3 hours. The berries were blended with 1% $CaCl_2$ and 10% NaCl in a commercial blender for 30 seconds. The calcium chloride was added to inhibit enzyme activity and the sodium

chloride was added to increase sensitivity (*28*). The mixture was centrifuged at 2000 rpm for 20 minutes and the supernatant collected.

A Stir Bar Sorptive Extraction (SBSE) stir bar (1-cm long, 0.32-mm O.D. 0.5mm film thickness) with a polydimethylsiloxane (PDMS) phase was used for the extraction of volatile compounds. The stir bar was cleaned with 80% acetonitrile in methanol overnight, allowed to air dry for 1 hour, then conditioned for 45 minutes at 300°C with 50 mL/min nitrogen flow. Ten grams of juice samples were weighed into 20-mL clear glass vials (I-Chem, New Castle, DE) with polytetrafluorethylene septum caps and 10 µL internal standard mixture in methanol was added. The juice was extracted at room temperature for 1 hr at 1500 rpm. All samples were analyzed in triplicate.

Gas Chromatography-Mass Spectrometry (MS)

The analysis of volatile compounds was carried out by using an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose TDU autosampler with a CIS-4 cooled injection system (Gerstel USA, Baltimore, MD). The analytes were thermally desorbed at the TDU in splitless mode, ramping from 35ºC to 300ºC at a rate of 700ºC/min, and held at the final temperature for 3 min. The CIS-4 was cooled to -80°C with liquid nitrogen during the sample injection, then heated at 10°C/second to 250°C for 3 min. Solvent vent mode was used during the injection with a split vent purge flow of 50 mL/min beginning at 3 min. The helium column flow was 2.0ml/min. Separation was achieved using a ZB-FFAP column (30

m x 0.3 mm I.D., 0.5 μm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40°C for 2 min, then to 180°C at a rate of 6°C/min, then to 240°C at a rate of 4°C/min and held at the final temperature for 20 min. Standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 35 to 350 amu. System software control and data management/analysis were performed through Enhanced ChemStation Software (Agilent Techonologies, Inc.).

Chiral Analysis

The samples were extracted using the same procedure as described previously, however internal standards were not added. Separation was achieved using a CyclosilB column (30 m. 0.25 mm I.D., 0.25 micron film thickness, Agilent). The oven temperature was programmed at 40ºC for 2 min, increased to 90°C at a rate 6°C/min, then to 135°C at a rate of 1°C/min, to 220°C at a rate of 10°C/min and hold at the final temperature for 5 min. Isomeric ratios were determined using the relative total mass ion abundance for each compound peak.

Compound Identification and Quantification

The volatiles were quantified used the method described previous (*29*). An internal standard solution was prepared in methanol containing 784 ppm and 395 ppm of γ-nonalactone and cis-jasmone respectively. An aliquot (5μL) of this internal standard mixture was then added to 10 ml of water to yield a final concentration of 392 and 197 ppb respectively. Pure standards of stock solutions of 2000 ppm of each compound (except zingerone and raspberry ketone) were prepared in methanol.

The stock solution was then used to create calibration curve solutions in water with concentrations of 1000 ppb, 750 ppb, 500 ppb, 100 ppb, 50 ppb, 5 ppb and 1 ppb with 5 μL of internal standard solution. Zingerone and raspberry ketone stock solutions were prepared with 45,000 ppm and 90,000 ppm respectively. These stock solutions were used, with 5 μL of internal standard solution, to create calibration curve solutions in water with concentrations of 23 ppm, 17 ppm, 11 ppm, 2 ppm, 1 ppm, 0.1 ppm, and 0.02 ppm for zingerone and 45 ppm, 35 ppm, 24 ppm, 5 ppm, 2.5 ppm, 0.25 ppm, and 0.05 ppm for raspberry ketone. MS-quantification was carried out using specific mass ions to avoid interference between co-eluted compounds (*29*). Quantification curves were built by plotting selected ion abundance ratio of target compounds with their respective internal standards against the concentration ratio. The concentrations of volatile compounds in the samples were calculated based on the individual calibration curves.

Results and Discussion

Wild-type and transgenic 'Meeker' raspberries grown in Lynden, Washington and Aurora, Oregon were studied for two years. The average high temperatures in Lynden, Washington in June and July (20˚C and 23˚C, respectively) are slightly cooler than those in Aurora, Oregon (23˚C and 27˚C, respectively), while the temperatures at night are similar (9˚C and 11˚C) in Washington and (9˚C and 12˚C) in Oregon. The average rainfall for June and July in Washington (66 mm and 50.8 mm, respectively) is considerably higher than in Oregon (44.4 mm and 18.5 mm,

respectively). These differences allow for the comparison of transgenic lines and wild-type under different agronomic conditions.

°Brix and Titratable Acidity

The ˚brix, titratable acidity and ˚brix/TA ratio for the wild-type and transgenic 'Meeker' raspberries grown in Washington and Oregon during 2004 and 2005 are presented in Table 3.1. Both year to year variations and site to site variations were observed. For both Washington and Oregon sites, fruits from 2004 had higher Brix and lower acidity than fruits from 2005 although the degree of difference was dependent on the sites and year. For year 2004, all the raspberries grown in Washington had slightly lower °brix and higher titratable acidity than the raspberries grown in Oregon. This trend was also observed in year 2005 and with a much greater magnitude. On average, raspberries grown in Oregon in 2005 had 30% higher brix and 20% lower titratable acidity than those grown in Washington. This difference correlated well with the climate difference that Oregon had a higher average temperature than Washington. Climate variations are known to affect the flavor of fruits during the growing and ripening seasons, warmer and drier weather generally produce fruit with higher sugar and lower acid contents (*1*).

Sugar and acid contents are also affected by fruit maturity. Sugar concentration typically increases drastically during fruit ripening, and continues through the overripe stage (*21;30*). Acid concentration typically increases early in fruit development, but decreases as the fruit ripens (*1*). The high brix number always corresponded with a low titratable acidity value, this trend was consistent for

all the raspberry cultivars studied. Sugar, acid, and ˚brix/TA ratio are often used as maturity indicator (*30*). The ˚brix/TA ratio is particularly important for flavor perception.

During both 2004 and 2005 growing seasons, the °brix for all transgenic lines were not different from wild-type within a site and growing season. The °brix values for all lines grown in Washington in 2005 were within the range reported previously (*29*). The titratable acid values for all transgenic lines were similar to the wild-type for that location and growing season.

Volatile Quantification

A total of thirty volatile compounds were compared for all transgenic lines and the wild-type in Oregon and Washington for 2004 and 2005. These compounds were selected based on their previously reported importance to raspberry aroma (*11;22;31-33*) as well as their representation to various chemical classes including alcohol, aldehyde and ketone, ester, and terpene and tepene alcohol. The concentrations (in ppb) these volatiles were listed in Table 3.2-3.5.

There were very few differences in volatile concentrations between the transgenic lines and the wild-type 'Meeker'. Most of the transgenic lines and the wild-type 'Meeker' grown in Lynden, Washington during 2004 had very similar volatile composition (Table 3.2). However, transgenic 2174 BO appeared to have higher hexanal and (E)-2-hexenal than the wild-type 'Meeker' and the rest of transgenic lines. Hexanal and (E)-2-hexenal are generated from enzymatic oxidative

degradation of fatty acids (*26;34*) by lipoxygenase (LOX) and are responsible for the 'green' odor notes, and their concentrations typically related with fruit maturity.

Transgenic 2174 BO also had lower concentrations of most terpenoids than the wild-type and the other transgenic lines. Terpenoids are formed primarily through the isoprenoid pathway, starting from the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by geranyl diphosphate synthase to form geranyl diphosphate. Further hydrolysis, cyclation and/or oxidoreductions of geranyl diphosphate leads to the formation of various of terpernoids (*26*). However, this variation was not repeated for this variety grown in Oregon during 2004 or in Washington or Oregon during 2005, indicating that this variation is likely not due to genetic variance. Similarly, the volatile composition for all the transgenic lines and the wild type was very similar in Oregon during 2004.

In 2005, some of the volatile compounds had wider range of variations in Washington. Transgenic 2172 AG appeared to have lower (E)-2-hexenal, while transgenic 2174 BS had higher hexanal and (E)-2-hexenal, however, these variations were not seen in the Oregon grown plants for the same year, much less the 2004 raspberries. None of the variations were consistent from site to site or year to year. The results suggested that none of the transgenic lines were different from the wild-type 'Meeker'.

Much larger variations were observed between sites and harvest seasons than between transgenic lines and wild-type 'Meeker'. The raspberries grown in Washington in 2004 had similar hexanal and (E)-2-hexenal concentration to the

berries grown in Oregon, while they had much lower hexanal and (E)-2-hexenal in 2005. Volatile aroma compounds can increase, maintain steady, or decrease during the ripening process due to different pathways involved in volatile formation.(*27*). Hexanal and (E)-2 hexenal can be generated from β-oxidation or lipoxygenase-catalyzed oxidative degradation of fatty acids (*26;34-36*), and their concentrations in fruit typically decrease with maturity.

Raspberries grown in Oregon in 2005 also had lower α- and β-ionone than in Washington, which coincided with the higher concentrations of hexanal and (E)-2-hexenal. α- And β-ionones are generated from the degradation of carotenoids and their concentrations typically increase with maturity. The higher aldehydes and lower ionone concentrations suggest that the raspberries grown in Oregon in 2005 were less mature than the raspberries grown in Washington.

Raspberries grown in Oregon appeared to have higher concentrations of δ-octalactone, δ-decalactone, geraniol, and linalool. This trend was observed in both 2004 and 2005, indicating that these compounds are likely to be linked to agronomic differences between the locations, particularly the quantity and intensity of sunlight during the growing season (*1;25*).

The concentrations of many aroma compounds such as esters and terpenoids generally increase in fruits with maturity and the growing conditions, but the concentration at each stage and rate of change can vary between cultivars (*27*).

Varying concentrations and activity of enzymes between cultivars is partially responsible for volatile variation; variations can also be due to growing conditions.

Variations in growing conditions between years and soil variations have also been shown to have considerable influence on flavor volatile formation (*25*). Year to year variations and location variations have been shown in several raspberry cultivars for the raspberry ketone, alcohols, aldehydes, esters, ketones and terpene concentrations in red raspberries (*23*).

Chiral Analysis

Different isomers can have significantly different sensory thresholds and descriptors. Isomeric descriptors, distributions, thresholds and variations have been studied in various natural products, however possible variations due to growing conditions and locations have not been studied (*37-41*). Chemically produced compounds will generally have an equal, or racemic, mixture of all possible isomers, however due to stereospecific enzymes, compounds produced in plants will often favor one isomer over others (*41*).

The percent ratios for the compounds found during the chiral analysis, 6-methyl-5-hepten-2-ol, δ octalactone, δ decalactone, linalool and terpinen-4-ol, are shown in tables 3.6 and 3.7 for 2004 and 2005, respectively. Most of the compounds demonstrate a much higher percentage of one isomer over the other, particularly 6-methyl-5-hepten-2-ol, and the lactones, with more than 90% of one isomer. Linalool

is nearly a racemic mixture, with a slightly higher percentage of the later eluting isomer. Terpinen-4-ol is an 80/20 mixture favoring the later eluting isomer.

Little variation is seen for the isomeric compounds for either year studied (Tables 3.6 and 3.7). There is little difference between the years, states or varieties for isomeric ratios.

Table 3.1. °Brix, Titratable acidity, original pH and °Brix/TA ratio for wild type and transgenic raspberries grown in Oregon and Washington during 2004 and 2005

Location	°Brix	Titratable Acidity	Original pH	°Brix/TA Ratio
Wild Type 'Meeker' Lynden, WA 2004	12.9	1.13	3.10	11.36
Transgenic 2171 BJ Lynden, WA 2004	12.1	1.11	3.20	10.89
Transgenic 2172 AG Lynden, WA 2004	13.6	1.17	3.08	11.61
Transgenic 2174 BO Lynden, WA 2004	13.6	1.22	3.16	11.16
Transgenic 2174 BS Lynden, WA 2004	13.0	1.16	3.18	11.19
Wild Type 'Meeker' Aurora, OR 2004	13.7	1.24	3.03	11.01
Transgenic 2171 BJ Aurora, OR 2004	14.9	0.96	3.01	15.45
Transgenic 2172 AG Aurora, OR 2004	13.9	0.95	3.04	14.63
Transgenic 2172 BJ Aurora, OR 2004	15.2	1.09	3.02	13.91
Transgenic 2174 BO Aurora, OR 2004	14.6	1.11	3.05	13.16
Transgenic 2174 BS Aurora, OR 2004	15.9	1.14	3.02	13.99
Wild Type 'Meeker' Lynden, WA 2005	10.8	1.63	2.58	6.63
Transgenic 2171 BJ Lynden, WA 2005	10.5	1.52	2.57	6.93
Transgenic 2172 AG Lynden, WA 2005	10.4	1.50	2.65	6.88
Transgenic 2172 BJ Lynden, WA 2005	10.4	1.43	2.67	7.26
Transgenic 2174 BO Lynden, WA 2005	11.0	1.59	2.57	6.90
Transgenic 2174 BS Lynden, WA 2005	10.7	1.55	2.62	6.85
Wild Type 'Meeker' Aurora, OR 2005	13.4	1.24	2.82	10.78
Transgenic 2171 BJ Aurora, OR 2005	13.7	1.32	2.86	10.32
Transgenic 2172 AG Aurora, OR 2005	13.4	1.03	2.90	12.98
Transgenic 2172 BJ Aurora, OR 2005	13.7	1.33	2.82	10.27
Transgenic 2174 BO Aurora, OR 2005	13.2	1.28	2.82	10.29
Transgenic 2174 BS Aurora, OR 2005	13.4	1.35	2.85	9.85
2005 Washington Wild Type Range	7.6 – 11.6	1.00 – 1.56	2.63 – 2.88	-

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
(Z)-3-Hexenol	145±7.7	137±2.3	188±8.9	177±5.6	161±5.1	158±7.4	278	228-327
4-Isopropylbenzyl alcohol	23±0.8	17±0.7	24±4.1	29±1.6	48±1.3	24±0.2	52	32-74
6-Methyl-5-hepten-2-ol	79±4.9	85±2.5	71±9.2	78±3.1	102±7.2	84±3.2	66	55-76
2-Nonanol	3±0.1	2±0.0	2±0.5	2±0.1	13±0.2	3±0.1	7	2-12
Hexanal	182±14.7	234±14.6	123±30.9	155±9.5	408±118.2	178±9.9	47	27-66
(E)-2-Hexenal	422±18.2	421±48.0	221±114.9	394±14.7	600±16.1	374±17.5	309	260-357
(Z)-3-Hexenyl acetate	5±0.2	4±0.0	10±1.8	10±0.4	4±0.2	6±0.3	7	4-10
Ethyl hexanoate	5±0.6	3±0.1	2±0.9	3±0.2	3±1.8	7±0.3	10	5-13
Methyl nonanoate	1±0.1	1±0.1	1±0.4	2±0.2	1±0.4	1±0.0	1	0-1
2-Heptanone	85±1.9	79±3.0	51±20.5	83±4.1	67±17.0	123±9.6	82	61-102
2-Nonanone	26±0.6	24±0.2	17±2.3	28±1.0	17±0.6	28±0.7	22	11-34
Raspberry ketone	1395±942	1559±639	2065±614	2679±1161	1179±151	1851±198	3287	1297-5277
Zingerone	59±17.2	48±9.3	146±10.1	121±34.1	51±3.2	77±8.6	936	620-1250
δ-Octalactone	484±19.5	415±15.4	270±41.6	312±10.1	303±24.4	526±13.3	807	636-978
δ-Decalactone	540±14.7	469±6.7	401±26.9	441±1.0	326±7.9	552±6.9	792	666-917
Para cymene	14±0.5	10±0.7	6±4.0	14±1.0	4±0.6	12±0.6	15	8-23
Geraniol	121±4.2	123±3.2	113±4.1	149±5.9	95±8.3	129±3.1	137	102-172
α-Ionone	80±1.9	63±1.4	63±4.7	84±4.3	62±3.4	71±0.5	38	23-52
β-Ionone	93±0.7	75±1.1	72±2.2	83±4.6	71±0.8	87±0.5	75	56-93
Limonene	2±0.1	1±0.5	1±0.7	2±0.1	1±0.4	1±0.3	1	1-2

Table 3.2 Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Lynden, Washington during 2004 with the 2005 Washington ‘Meeker’ average and range from table 2.3

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
Linalool	8±0.4	6±0.1	8±0.5	8±0.2	7±0.3	7±0.1	38	31-44
Myrcene	5±4.8	10±4.4	6±0.9	7±3.2	3±5.9	7±6.0	4	1-8
Nerol	15±0.6	12±0.2	14±0.9	17±0.6	6±0.4	17±0.3	28	19-37
α-Phellandrene	28±25.7	34±4.4	30±4.7	30±26.9	11±3.8	53±3.3	66	26-100
α-Pinene	29±0.2	20±0.5	25±5.0	29±1.8	10±1.8	25±0.6	20	11-27
Sabinene	16±1.5	12±1.0	12±6.0	17±1.4	6±0.4	17±1.3	23	13-32
α-Terpinene	4±3.9	8±0.5	8±4.1	17±1.3	4±1.5	12±0.8	22	4-39
γ-Terpinene	11±0.9	10±0.1	8±5.7	21±1.3	5±0.4	14±0.2	9	0-18
α-Terpineol	12±0.7	9±0.1	13±1.2	11±0.4	6±0.3	11±0.3	46	35-58
Terpinen-4-ol	96±4.2	81±1.1	113±5.5	124±4.0	47±2.5	104±2.6	150	100-201
Terpinolene	2±0.2	2±0.1	1±0.9	3±0.2	1±0.5	2±0.1	3	1-4

Table 3.2 Aroma Compound Concentrations (ppb) for wild type and transgenic 'Meeker' red raspberries grown in Lynden, Washington during 2004 with the 2005 Washington 'Meeker' average and range from table 2.3 (Continued)

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG 4	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
(Z)-3-Hexenol	190±1.5	136±4.0	152±3.6	203±9.6	117±5.6	131±5.8	278	228-327
4-Isopropylbenzyl alcohol	30±0.2	24±0.9	34±1.0	11±0.8	39±0.2	25±1.0	52	32-74
6-Methyl-5-hepten-2-ol	92±0.2	82±4.0	83±2.1	87±3.4	66±4.1	77±4.1	66	55-76
2-Nonanol	4±0.2	6±0.2	5±0.1	2±0.1	3±0.3	9±0.1	7	2-12
Hexanal	90±3.7	155±21.1	105±5.3	137±10.0	89±4.9	146±10.2	47	27-66
(E)-2-Hexenal	289±7.9	358±16.9	240±1.2	473±29.8	272±14.4	277±7.4	309	260-357
(Z)-3-Hexenyl acetate	3±0.2	2±0.1	4±0.1	6±0.1	2±0.2	3±0.1	7	4-10
Ethyl hexanoate	5±0.2	14±0.7	10±0.4	6±0.5	5±0.4	25±0.4	10	5-13
Methyl nonanoate	2±0.1	ND	ND	1±0.2	1±0.1	ND	1	0-1
2-Heptanone	63±2.0	64±3.8	53±0.6	74±5.0	40±3.7	94±3.9	82	61-102
2-Nonanone	22±0.9	11±0.6	18±0.3	15±0.5	10±0.4	20±0.8	22	11-34
Raspberry ketone	1922±153	1908±75	1410±345	2102±301	3308±564	1811±1914	3287	1297-5277
Zingerone	109±7.2	219±15.5	92±6.9	127±12.2	173±10.8	291±62.5	936	620-1250
δ-Octalactone	546±10.8	785±36.1	576±22.4	361±7.8	781±5.4	1049±13.5	807	636-978
δ-Decalactone	602±3.5	706±6.3	561±4.2	396±10.7	668±10.4	841±19.5	792	666-917
Para cymene	12±0.2	13±0.9	14±0.6	8±0.6	9±0.6	18±0.3	15	8-23

Table 3.3 Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Aurora, Oregon during 2004 with the 2005 Washington ‘Meeker’ average and range from table 2.3

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG 4	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
Geraniol	167±7.4	149±2.8	133±0.3	188±1.9	127±7.3	161±7.8	137	102-172
α-Ionone	63±1.3	57±4.2	77±2.1	60±4.3	62±1.5	75±0.8	38	23-52
β-Ionone	76±1.9	75±4.0	74±1.5	63±3.3	79±1.3	88±1.5	75	56-93
Limonene	2±0.1	1±0.1	1±0.0	1±0.1	1±0.1	1±0.1	1	1-2
Linalool	12±0.4	18±0.4	13±0.0	11±0.3	12±1.2	16±0.8	38	31-44
Myrcene	6±0.3	6±3.0	3±0.6	5±4.3	3±0.1	6±1.8	4	1-8
Nerol	20±0.5	17±0.3	14±0.0	13±0.1	13±0.7	19±1.0	28	19-37
α-Phellandrene	42±6.9	30±1.7	23±3.0	27±1.3	21±2.1	43±2.6	66	26-100
α-Pinene	25±1.3	16±1.5	15±0.8	17±1.3	13±2.1	20±0.9	20	11-27
Sabinene	15±0.7	9±0.0	8±0.4	9±0.7	7±0.3	16±0.2	23	13-32
α-Terpinene	9±0.5	9±0.9	7±0.1	4±0.0	3±0.1	10±4.0	22	4-39
γ-Terpinene	8±0.4	7±0.8	6±0.2	3±0.1	1±0.0	13±0.5	9	0-18
α-Terpineol	16±0.2	18±0.3	16±0.2	12±0.1	16±0.5	18±0.9	46	35-58
Terpinen-4-ol	104±2.6	82±1.8	66±0.2	70±1.0	51±2.1	105±5.8	150	100-201
Terpinolene	1±0.1	2±0.1	1±0.0	1±0.0	ND	3±0.1	3	1-4

Table 3.3 Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Aurora, Oregon during 2004 with the 2005 Washington ‘Meeker’ average and range from table 2.3 (Continued)

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
(Z)-3-Hexenol	149±8.1	141±4.3	168±6.9	152±24.0	153±10.5	188±18.0	278	228-327
4-Isopropylbenzyl alcohol	45±0.6	43±2.0	32±1.8	57±0.8	37±0.6	34±3.3	52	32-74
6-Methyl-5-hepten-2-ol	63±5.3	54±1.6	66±1.3	56±3.2	57±2.6	69±6.7	66	55-76
2-Nonanol	3±0.1	5±0.1	3±0.3	10±0.3	3±0.0	4±0.1	7	2-12
Hexanal	132±3.0	120±5.1	126±15.6	133±15.2	101±8.2	213±13.6	47	27-66
(E)-2-Hexenal	416±21.8	349±3.8	285±14.7	365±15.4	309±14.5	538±45.0	309	260-357
(Z)-3-Hexenyl acetate	6±0.7	5±0.2	7±0.0	5±0.4	5±0.1	5±0.3	7	4-10
Ethyl hexanoate	6±0.4	8±0.3	7±0.5	8±0.3	8±0.4	6±0.2	10	5-13
Methyl nonanoate	1±0.3	1±0.4	ND	1±0.1	ND	ND	1	0-1
2-Heptanone	84±5.9	96±5.2	66±45.7	102±2.3	88±7.7	117±7.1	82	61-102
2-Nonanone	20±2.1	44±1.9	22±0.1	49±3.0	24±0.9	26±1.2	22	11-34
Raspberry ketone	2941±292	2613±1187	1957±1189	3070±471	1738±491	2350±1592	3287	1297-5277
Zingerone	220±12.7	187±47.9	291±98.9	350±29.1	177±27.1	236±102.0	936	620-1250
δ-Octalactone	375±14.0	438±22.3	327±19.9	493±12.3	418±3.6	395±40.8	807	636-978
δ-Decalactone	476±24.5	498±5.5	433±8.5	523±9.0	497±5.8	462±29.9	792	666-917
Para cymene	20±1.5	20±1.7	10±1.7	24±1.7	18±1.8	16±1.2	15	8-23

Table 3.4. Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Lynden, Washington during 2005 with the 2005 Washington ‘Meeker’ average and range from table 2.3

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
Geraniol	126±12.1	121±7.7	131±1.4	148±7.3	123±7.0	132±8.7	137	102-172
α-Ionone	89±5.2	82±1.5	53±1.3	75±3.4	75±0.1	80±1.6	38	23-52
β-Ionone	94±5.3	94±2.7	72±0.6	96±4.3	88±0.4	90±1.5	75	56-93
Limonene	2±0.2	2±0.1	1±0.1	2±0.2	2±0.2	2±0.2	1	1-2
Linalool	14±1.1	10±0.2	10±0.2	12±0.3	13±0.4	12±1.0	38	31-44
Myrcene	5±1.0	4±0.6	16±7.5	4±1.1	9±6.6	2±1.8	4	1-8
Nerol	21±2.7	20±1.5	21±1.3	29±1.7	20±1.2	20±1.5	28	19-37
α-Phellandrene	57±15.7	51±10.5	33±19.1	72±45.9	59±24.7	54±24.8	66	26-100
α-Pinene	25±1.8	28±0.8	30±3.4	39±2.2	34±3.1	29±0.9	20	11-27
Sabinene	22±2.8	24±2.1	15±7.4	37±0.9	23±1.3	23±2.3	23	13-32
α-Terpinene	23±1.8	22±0.4	10±4.9	40±10.1	20±0.8	21±2.1	22	4-39
γ-Terpinene	24±2.3	21±0.9	6±6.7	49±4.0	20±1.6	24±0.5	9	0-18
α-Terpineol	18±1.4	16±0.4	14±0.1	22±0.7	19±0.8	17±1.6	46	35-58
Terpinen-4-ol	135±14.2	145±6.8	139±2.4	214±8.9	138±6.6	158±12.0	150	100-201
Terpinolene	4±0.4	4±0.1	3±0.1	8±0.7	4±0.3	4±0.2	3	1-4

Table 3.4. Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Lynden, Washington during 2005 with the 2005 Washington ‘Meeker’ average and range from table 2.3 (Continued)

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
(Z)-3-Hexenol	249±18.4	204±13.0	218±2.8	189±8.2	210±25.1	192±1.9	278	228-327
4-Isopropylbenzyl alcohol	64±2.3	55±10.0	50±1.8	64±4.4	63±4.4	61±1.3	52	32-74
6-Methyl-5-hepten-2-ol	107±6.3	81±11.6	103±6.7	84±6.5	86±9.6	96±1.2	66	55-76
2-Nonanol	7±0.4	5±0.9	6±0.3	6±0.1	8±0.2	7±0.1	7	2-12
Hexanal	170±16.6	175±31.6	176±6.0	126±7.6	168±15.8	155±5.3	47	27-66
(E)-2-Hexenal	425±30.5	385±210.9	468±0.7	467±11.4	481±26.2	477±11.1	309	260-357
(Z)-3-Hexenyl acetate	11±0.7	4±0.7	5±0.4	6±0.2	6±0.5	7±0.3	7	4-10
Ethyl hexanoate	11±0.7	6±2.4	2±0.1	8±0.1	12±1.6	10±0.6	10	5-13
Methyl nonanoate	1±0.2	1±0.8	1±0.4	1±0.2	1±0.4	1±0.1	1	0-1
2-Heptanone	108±7.5	59±38.8	87±0.9	87±4.8	85±7.4	105±5.8	82	61-102
2-Nonanone	36±2.4	24±2.7	34±1.0	31±1.5	33±1.5	33±2.1	22	11-34
Raspberry ketone	2443±682	2196±532	1672±72	2582±319	1728±743	2746±476	3287	1297-5277
Zingerone	234±44.4	181±21.7	160±10.5	218±20.1	182±37.0	206±35.1	936	620-1250
δ-Octalactone	547±24.2	542±63.2	446±12.9	583±13.6	518±52.9	595±4.6	807	636-978
δ-Decalactone	625±21.8	740±275.1	516±4.8	627±10.1	591±24.1	622±6.0	792	666-917
Para cymene	24±2.8	13±10.7	7±7.2	23±1.0	22±1.3	23±2.0	15	8-23

Table 3.5 Aroma Compound Concentrations (ppb) for wild type and transgenic ‘Meeker’ red raspberries grown in Aurora, Oregon during 2005 with the 2005 Washington ‘Meeker’ average and range from table 2.3

Compound	Wild Type 'Meeker'	Transgenic 2171 BJ	Transgenic 2172 AG	Transgenic 2172 BJ	Transgenic 2174 BO	Transgenic 2174 BS	Average	Range
Geraniol	155±5.9	163±13.2	161±1.0	166±8.2	166±10.8	150±6.67	137	102-172
α-Ionone	53±2.6	63±23.1	56±1.2	54±1.6	52±2.0	59±1.2	38	23-52
β-Ionone	73±3.3	84±34.9	66±1.0	70±1.3	72±2.9	74±1.0	75	56-93
Limonene	2±0.4	1±0.5	2±0.2	2±0.1	2±0.2	2±0.4	1	1-2
Linalool	15±0.9	16±0.8	16±1.1	17±1.0	20±1.6	17±1.2	38	31-44
Myrcene	4±5.4	9±2.3	23±0.6	13±6.2	15±12.1	20±5.8	4	1-8
Nerol	27±1.8	21±3.6	25±0.2	25±0.9	27±1.3	25±0.6	28	19-37
α-Phellandrene	49±20.5	44±11.9	61±6.3	58±46.7	45±16.7	59±7.8	66	26-100
α-Pinene	33±3.5	31±5.3	26±0.5	28±1.5	28±3.7	27±1.9	20	11-27
Sabinene	30±4.3	14±0.0	21±2.8	25±1.0	23±1.9	23±3.4	23	13-32
α-Terpinene	25±5.2	13±3.8	15±1.9	20±2.1	17±2.0	19±2.4	22	4-39
γ-Terpinene	25±3.1	13±9.4	14±1.3	23±2.6	6±5.5	18±1.8	9	0-18
α-Terpineol	22±1.3	20±1.2	19±0.5	23±1.0	25±2.1	21±0.9	46	35-58
Terpinen-4-ol	172±7.4	128±36.8	137±1.9	162±9.3	161±10.7	153±6.9	150	100-201
Terpinolene	4±0.5	3±0.5	2±0.2	4±0.4	3±0.3	3±0.3	3	1-4

Table 3.5 Aroma Compound Concentrations (ppb) for wild type and transgenic 'Meeker' red raspberries grown in Aurora, Oregon during 2005 with the 2005 Washington 'Meeker' average and range from table 2.3 (Continued)

Compound	6-methyl-5-hepten-2-ol 1	6-methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Wild Type 'Meeker' Lynden, WA 2004	97.1%	2.9%	95.6%	4.4%	99.3%	0.7%	46.9%	53.1%	20.1%	79.9%
Transgenic 2171 BJ Lynden, WA 2004	97.2%	2.8%	100%	0.0%	99.0%	1.0%	46.9%	53.1%	20.7%	79.3%
Transgenic 2172 AG Lynden, WA 2004	96.2%	3.8%	100%	0.0%	99.2%	0.8%	45.8%	54.2%	20.4%	79.6%
Transgenic 2172 BJ Lynden, WA 2004	96.6%	3.4%	100%	0.0%	99.1%	0.9%	46.1%	53.9%	20.9%	79.1%
Transgenic 2174 BO Lynden, WA 2004	93.8%	6.2%	100%	0.0%	99.3%	0.7%	45.3%	54.7%	19.9%	80.1%
Transgenic 2174 BS Lynden, WA 2004	94.0%	6.0%	100%	0.0%	99.3%	0.7%	45.2%	54.8%	20.1%	79.9%
Wild Type 'Meeker' Aurora, OR 2004	95.1%	4.9%	96.1%	3.9%	99.2%	0.8%	44.7%	55.3%	20.8%	79.2%
Transgenic 2171 BJ Aurora, OR 2004	96.4%	3.6%	100%	0.0%	99.2%	0.8%	44.4%	55.6%	20.8%	79.2%
Transgenic 2172 AG Aurora, OR 2004	96.1%	3.9%	100%	0.0%	99.2%	0.8%	47.7%	52.3%	20.8%	79.2%

Table 3.6. Chiral percentages for wild type and transgenic ‘Meeker’ raspberries grown in Washington and Oregon during 2004

Compound	6-methyl-5-hepten-2-ol 1	6-methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Transgenic 2172 BJ Aurora, OR 2004	95.0%	5.0%	100%	0.0%	99.3%	0.7%	49.3%	50.7%	21.1%	78.9%
Transgenic 2174 BO Aurora, OR 2004	95.7%	4.3%	100%	0.0%	99.3%	0.7%	47.8%	52.2%	20.5%	79.5%
Transgenic 2174 BS Aurora, OR 2004	95.5%	4.5%	100%	0.0%	99.1%	0.9%	47.5%	52.5%	20.9%	79.1%

Table 3.6. Chiral percentages for wild type and transgenic 'Meeker' raspberries grown in Washington and Oregon during 2004 (Continued)

Compound	6-methyl-5-hepten-2-ol 1	6-methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Wild Type 'Meeker' Lynden, WA 2005	95.9%	4.1%	96.4%	3.6%	99.1%	0.9%	47.8%	52.2%	20.3%	79.7%
Transgenic 2171 BJ Lynden, WA 2005	95.8%	4.2%	100%	0.0%	99.0%	1.0%	48.3%	51.7%	21.0%	79.0%
Transgenic 2172 AG Lynden, WA 2005	94.6%	5.4%	100%	0.0%	99.1%	0.9%	42.4%	57.6%	21.0%	79.0%
Transgenic 2172 BJ Lynden, WA 2005	94.1%	5.9%	100%	0.0%	99.2%	0.8%	42.1%	57.9%	20.6%	79.4%
Transgenic 2174 BO Lynden, WA 2005	95.6%	4.4%	100%	0.0%	99.3%	0.7%	45.5%	54.5%	20.1%	79.9%
Transgenic 2174 BS Lynden, WA 2005	93.4%	6.6%	100%	0.0%	99.2%	0.8%	44.0%	56.0%	20.3%	79.7%
Wild Type 'Meeker' Aurora, OR 2005	96.7%	3.3%	96.1%	3.9%	99.2%	0.8%	45.0%	55.0%	21.3%	78.7%
Transgenic 2172 AG Aurora, OR 2005	93.9%	6.1%	100%	0.0%	99.2%	0.8%	41.5%	58.5%	21.5%	78.5%

Table 3.7. Chiral percentages for wild type and transgenic ‘Meeker’ raspberries grown in Washington and Oregon during 2005

Compound	6-methyl-5-hepten-2-ol 1	6-methyl-5-hepten-2-ol 2	δ Octalactone 1	δ Octalactone 2	δ Decalactone 1	δ Decalactone 2	Linalool 1	Linalool 2	Terpinen-4-ol 1	Terpinen-4-ol 2
Transgenic 2172 BJ Aurora, OR 2005	95.4%	4.6%	100%	0.0%	99.0%	1.0%	40.3%	59.7%	21.3%	78.7%
Transgenic 2174 BO Aurora, OR 2005	95.5%	4.5%	100%	0.0%	99.2%	0.8%	41.6%	58.4%	21.2%	78.8%
Transgenic 2174 BS Aurora, OR 2005	96.7%	3.3%	100%	0.0%	99.4%	0.6%	43.4%	56.6%	21.0%	79.0%

Table 3.7. Chiral percentages for wild type and transgenic 'Meeker' raspberries grown in Washington and Oregon during 2005 (Continued)

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