CHEMOSENSORY AND VITICULTURAL STUDIES OF HYBRID AND NON-*VINIFERA* GRAPE SPECIES AND RESULTING WINES

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Native American grape species and hybrid varieties have several desirable properties compare to *Vitis vinifera*, but the effect of canopy treatment on them and the aromas inherent to most *Vitis* species are not well characterized.

Two canopy treatments were applied to two hybrid varities growing at commercial vineyards in the Finger Lakes region of New York State, including Marechal Foch and Coort noir. The experiments were conducted for two growing seasons.

For Marechal Foch, yields and clusters were reduced while berry weight was increased by shoot thinning. Shoot thinning reduced crop load and increased soluble solids in 2008. Shoot thinning increased berry anthocyanins, but no corresponding increase was observed in wine anthocyanins. Delaying harvest resulted in increases of soluble solids, berry and wine anthocyanins. Both treatments resulted in decreased six-carbon alcohols in finished wines. The total concentration of tannin in Foch fruit was comparable to that of some vinifera. However, the extractability of tannins during winemaking was very low compared to most *vinifera*. Sensory panelists reported that later harvest 2008 wines were more “fruity” than their early harvest counterparts for both treatments and that shoot thinning did not affect fruitiness.

For Corot noir, yield was reduced by cluster thinning (CL) but not shoot thinning (ST) in 2008. CL increased Brix in both of years. The treatments had variable impacts on wine anthocyanin, berry skin tannin, berry seed tannin, and wine tannin depending on year. Wine tannin and tannin extractability were both very low in comparison to *vinifera*. Panelists reported ST+CL wines were more "fruity" than the control in both years.

The key odorants in wine produced from the American grape species, *V. riparia* and *V. cinerea* were determined. Non-*vinifera* wines had higher concentrations of odorants with vegetative and earthy aromas: eugenol, *cis*-3-hexenol, 1, 8- cineole, isobutylmethoxypyrazine (IBMP) and isopropylmethoxypyrazine (IPMP). Concentrations of IBMP and IPMP were well above sensory threshold in both non-*vinifera* wines and some grape accessions. We expect that this knowledge will facilitate the selection of interspecific hybrids by grape breeders, or could be used to identify targets for viticultural or enological studies on interspecific hybrids.

BIOGRAPHICAL SKETCH

The author was born in Ningbo of Zhejiang Province, a coastal city of eastern China. She received her B.S and M.S. degree in food science from Southwest Agricultural University (now as Southwest University) in 2002 and 2005, respectively. She always feels fortunate to be a food science major.

In 2005, she married Guoping Feng in Shanghai, China. They met when they were both undergraduates in the food science program of the same university in 1998. She was enrolled in the food science Ph.D. program by Cornell Univerisy in 2007.

She is interested in product development and will work for International Food Network, as a food scientist in Ithaca, New York.

To mom, dad, sister and husband

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CHAPTER 1

INTRODUCTION

WINE SPECIES

Vitis vinifera wine species

Vitis vinifera is a breed of grapes native to Europe. It is also called the European grapevine, and is the major grape species used to produce wine (1). There are at least 1500 different varieties being used for commercial wine making, including well-known red wine grapes like Cabernet Sauvignon, Cabernet Franc, Pinot noir, Merlot and white wine grapes such as Riesling, Chardonnay, Gewurztraminer, Sauvignon blanc. *Vitis vinifera* is an ancient species, emerging between 130 and 200 million years ago (1). Many studies have been done in the area of *Vitis vinifera* with topics ranging from understanding effects of viticultural practices, aroma chemistry, clonal variation, color chemistry, and new variety development.

However, *Vitis vinifera* production has challenges.The greatest weakness of *Vitis vinifera* is its susceptibility to an aphid called *Phylloxera vastatrix*, originally native to eastern North America. In the late 19th century the phylloxera epidemic destroyed most of the vineyards for wine grapes in Europe, most notably in France (1). To save *Vitis vinifera*, the preferred method is grafting *Vitis vinifera* scions onto the roots of a resistant *Vitis aestivalis* or other American native species.

In addition, the *Vitis vinifera* vines generally are less cold hardy than the American grape species used to produce interspecific hybrids. Therefore, unlike the winter-hardy native grapes, traditional European wine grapes can't thrive in the regions with harsh winters and deep freezes, and are more susceptible to winter and cold injury. Also, *Vitis vinifera* have little or no inherent resistance to several diseases native to New York State. These include fungal diseases such as powdery mildew, black rot, and downy mildew. Consequentially, production of *Vitis vinifera* in

the Finger Lakes wine region in New York State is challenging, especially for most of varieties which need long growing season length.

Non-*vinifera* wine species

Non-*vinifera* grape species (North American grape) are widely grown in the eastern United States and Canada. These native varieties grow well because of their resistance to disease and insects and their adaptation to the regional climate. There are totally 30 species of *Vitis* including *vitis vinifera* in North American, with another 30 species in the Old World, mostly in East Asia (2). Six native species that had been growing in North America long before European settlers arrived, including *Vitis rotundifolia* (muscadine), *Vitis aestivalis* (summer grape), *Vitis riparia* (frost grape), *Vitis labrusca* (fox grape), *Vitis mustangensis* (Mustang grape), and *Vitis rupestris* (sand grape). Among which, four main species are used for wine production—*Vitis aestivalis, Vitis labrusca, Vitis riparia, and Vitis rotundifolia*. However, *Vitis vinifera* accounts for over 99 percent of the world's wines, due to the perception that it is difficult to make fine wines from the grapes of non-*vinifera* species.

However, over the last hundred years some interest has been given to these wild American species. While these grapes are not as widely cultivated or commercialized as *vinifera* varieties, they do show potential for making enjoyable wines and deserve to be recognized. Several varieties produced from non-*vinifera* species have achieved consumer acceptance. The Scuppernong grapes of *Vitis rotundifolia*, grown in southern states, are used for wine production (Horvat and Senter, 1984) (3).Norton and Cynthiana, predominantly *Vitis aestivalis* (Michx) (Reisch et al. 1993) (4) are premium native wine grapes, and are grown widely in the Midwest (Tarara et al. 1991; Kaps and Odneal 2001) (5, 6). In the northern states, the Clinton, a *Vitis riparia* variety, is gaining acceptance. In general, grapes with non-*vinifera* parentage are thought to be inferior for winemaking, but there is breeding improved cultivars for quality wine

production.. The successfully bred varieties should be disease resistant and well adapted to the climate of eastern United States.

Vitis riparia

Vitis riparia is commonly known as river bank grape since it is found along the banks of streams, in ravines, on the islands of rivers, and in wet places. It is the most widely distributed native American grape species, which is found north to New Brunswick, Quebec, Manitoba and Montana, south to Tennessee, northern Texas, Colorado, and Utah (7). The vine of *Vitis riparia* is vigorous to very vigorous. Clusters are small to medium, generally compact with short peduncle and many berries. Berries are also small to medium with black color (8). Because of high acid and average sugar content of berry, sugar and water are added to the must for winemaking. Unlike *Vitis labrusca*, *riparia* has no foxy or wild taste. Berry usually has 2 to 4 small seeds and the flesh is neither pulpy nor solid (9). *Vitis riparia* blooms early but ripens late. Its resultant fruit, if harvested late, would be the best for wine production.

Vitis riparia is very cold hardy, which can withstand temperatures to - 60 ° F. It is also moderately drought resistant so that it can survive in Southern State. *Riparia* is more resistant to the excessive lime than other species. Like other native species, it is very resistant to phylloxera and diseases such as powdery mildew, but it is susceptible to the leaf-hopper. Due to these characters, *Vitis riparia* has been widely used as grafted rootstock in hybrid grape breeding.

Vitis rupestris

As a native American species, *Vitis rupestris* is survived in southern Missouri to Kentucky, western Tennessee, Arkansas, Oklahoma, eastern and central Texas to the Rio Grande, westward into New Mexico.native to the Southern. In the nature it can be found on dry, sandy and rocky river beds. Therefore, it is also called Rocky Grape and Mountain Grape. *Vitis rupestris* is a

small many branched shrub with around 6-8 feet height. Clusters are small with 12 to 24 berries. Berries are black or purple-black with currants size. The skin of berries is thin and pulp is pleasant and berries have small seeds. Berries tasting varies from sweet to sour, without foxy aroma. *Vitis rupestris* blossoms and ripens early from late June to August.

Vitis rupestris is drought resistant, and therefore it can tolerate hot, dry southern exposures. It is widely used in hybrid breeding program because it is also phylloxera-resistant.

Vitis cinerea

Vitis cinerea is commonly named as Winter Grape, which is native to southern and central Illinois; it is hard to find this species in the northern portion of the state. It survived in moist woodlands, areas adjacent to woodland paths, partially shaded riverbanks, thickets, fence rows, and powerline clearances in wooded areas. The vine of *cinerea* is very vigorous and it is up to 40 feet long with long tendrils and elongated slender gray or ashy or whitish tomentose young tip growths. *Vitis cinerea* is distinct from most other wild species due to the cobwebby hairs on its branchlets, petioles, and leaf undersides. *Vitis cinerea* has big clusters with many small black berries that are mildly unpleasant to eat. The blooming begins in the late spring and lasts about 2 weeks. The berry is about 3/8" long, juicy, and black and tasted sweet-tart when mature and edible. Each seed is 3-5 mm in length.

FRENCH AMERICAN HYBRID VARIETIES

French American hybrid grape is any cross between a French species (*Vitis vinifera*) and an American species (*Vitis riparia, Vitis rupestrics, Vitis cinerea*, etc.). Hybrids came into the wine world when the fatal phylloxera devastated European varieties in the late 19th century (1). Making pest-resistant hybrids at that time was best solution to save their vineyard. Hybrids occur naturally, but also is performed in a targeted fashion by breeders/geneticists. Therefore, French

hybrids are generally disease resistant, easy growing and also produce more fruit than *Vitis vinifera*. However, winemakers observe that French American grapes are not easy to make high quality wines because of "hybrid aroma". Even nowadays, French American wines are considered by Europeans as lower quality. Some wine regions in Europe forbid production of hybrid wines.

Marechal Foch

Marechal Foch (Kuhlmann 188.2) is a 'classic', widely-planted hybrid in Finger Lakes, which is an interspecific hybrid red wine grape variety, named after a French general. It is a cross between Goldriesling (cross of Riesling and Courtiller Musque) and a *Vitis riparia* - *Vitis rupestris* (101-14Mgt) (10). The parent and progeny relationship of Marechal Foch was shown in Fig.1.1. Marechal Foch was originated in the Alsace of France and bred by Eugene Kuhlmann (10). In 1946, Marechal Foch was introduced to Canadian vineyards with other French hybrids by Adhemar de Chaunac of Brights Vineyards at Niagara Falls of Canada and then imported to the eastern United States in 1951 by Philip Wagner (1). Nowadays, it is widely planted in both the eastern wine regions of the United States and Canada. In New York wine region, the acreage of Marechal Foch increased from 79 acres in 1990 to 144 acres in 2006 while in Finger Lakes region, the acreage increased from 48 to 87 acres (11, 12). Marechal Foch ripens very early (early to mid-September in the Finger Lakes region) and is cold-hardy (winter hardy to -25 °C to -30 °C). Like many varieties with North American parentage, Marechal Foch is resistant to fungal diseases, being moderately susceptible to black rot and powdery mildew, slightly susceptible to dawny mildew, botrytis bunch rot and crown gall (13).

However, Marechal Foch vines tend to over-crop (14), having more crop on shoots grown from base buds than normal vines (15). Also, the shoots tend to have a larger number of flower

clusters than those of *Vitis vinifera* and *Vitis labrusca* varieties. In New York State wine regions, there has been increased planting of French-American hybrids. But due to over-cropping, fruit composition and wine quality were affected, and vine vigor decreased in the following years. Balanced pruning would not provide adequate crop control for most French-American hybrids due to the facts that they tend to produce heavily fruited primary and secondary shoots and that fruiting shoots can arise from latent buds on cordons and from basal nodes that are not counted during balanced pruning (16).

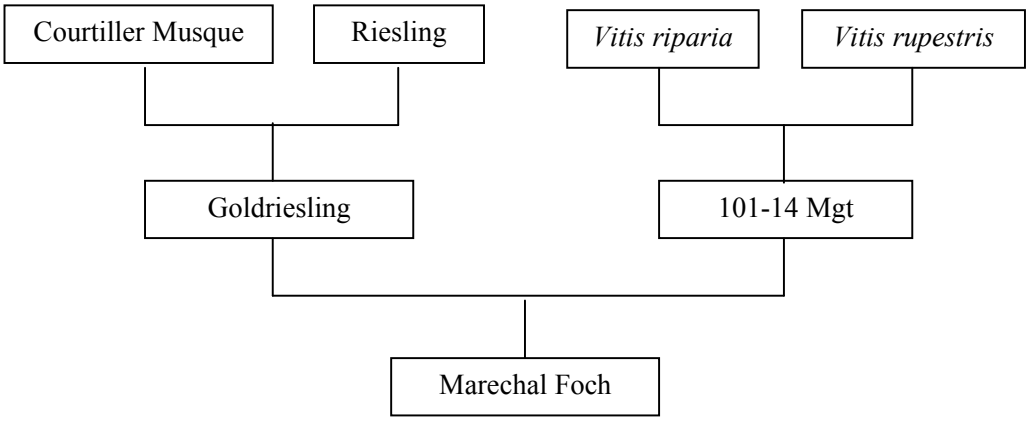


Fig. 1.1 Parent/progeny relationship of Marechal Foch

Corot noir

Corot noir is a complex interspecific red hybrid winegrape, which is a cross between Seyve Villard 18-307 x 'Steuben'. The pregiee was shown in Fig.1.2. The cross was bred in 1970, transplanted to a seedling vineyard in 1975, tested for wine characteristics in 1978, and released by Cornell University on July 7, 2006 (17). Corot noir ripens mid to late-season, is appropriate for either blending or the production of varietal wines, and is moderately winter hardy (-10°F) with moderate resistance to fungal diseases (17). According to the data from the breeding program of New York State Agricultural Experiment Station, it is hardier than some interspecific

hybrids, but not as hardy as *riparia*-derived varieties such as Maréchal Foch and Frontenac. The estimated temperature of 50% primary bud kill (LTF 50) for Corot noir was -15.1° F. Observations made at Geneva NY from 1996 – 2005 indicated that the vine size of Corot noir was smaller than GR7 (average pruning weight of 1.4 kg/vine compared to 2.1 for GR7), and observations made during roughly the same period at three locations in Indiana also suggest a small vine size (For example, average pruning weight of 0.6 kg/vine from 2000-2005 compared to 1.0 kg/vine for Marechal Foch at Vincennes, IN) (17). However, Corot noir growers in the Finger Lakes region of NY anecdotally describe the vine as highly vigorous with low cluster light exposure and high fruit yield, and in our own experience Corot noir is more vigorous than most if not all French-American hybrids grown in the region (http://www.nysaes.cornell.edu/hort/faculty/reisch/cultivars.html), although data to support this claim is lacking. According to Reisch et al, Corot noir has a dark red color and favorable cherry/berry fruit aromas without hybrid aromas. They noted that it may be used for varietal wine production or for blending.

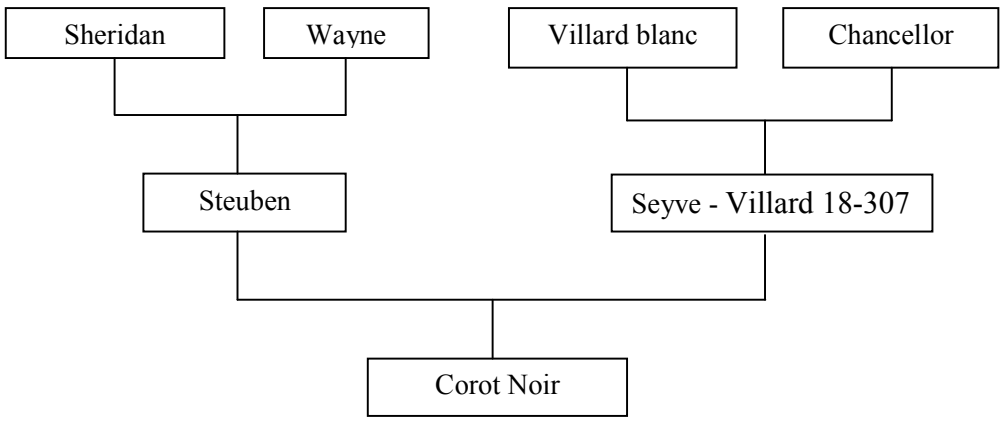


Fig.1.2 Parent/progeny relationship of Corot noir

CANOPY MANIPULATION PRACTICES IMPROVE WINE QUALITY

In viticulture, the canopy of a grapevine refers to the collective arrangement of the vine's shoots, leaves and fruit. Well-managed canopy will maximize the exposure of the grapes to sunlight and allow proper airflow, thus promote fruit ripening, prevent fungal disease and also make it easier to get access to the vines for pruning and picking. Ideal canopy is characterized by medium diameter shoots with moderate-length internodes and few lateral shoots and trained to an appropriate system.

Table 1.1 Canopy Manipulation Practices

Practices	When	Benefits	Negatives
Cluster Thinning	Flower cluster thinning; Post set thinning; Pre- or post-verasion thinning	control cropload; accelerate grape maturity and improve quality	reduce yield
Hedging	post-bloom; fully canopy; veraison	improve berry quality; facilitate the movement of manpower and equipment between vineyard rows; reduce vine vigor	stimulates growth of lateral shoots from the nodes below the cut position
Basal Leaf Removal	early (pea-sized) late (prior to veraison)	increased monoterpenes and norisoporenoids; reduced methoxypyrazines; reduced TA, pH and K; reduced bunch rot; better spray penetration	bird damage; slightly lower soluble solids; vegetative or cooked flavors
Shoot Thinning	Trunk suckering:1-3 shoot length; Condon: 8-12 shoot length	control cropload; reduce canopy density; improve berry quality	reduce yield
Trellising	depend on trellis system	Train and support the vine; improve a dense canopy	depend on trellis system

Many studies have shown that canopy manipulation practices improve grape and wine quality. The practices can improve light interception and light exposure of leaves and clusters, thus increase soluble solids and reduce titratable acidity (TA) and pH. There are many practices which are summarized in the Table 1.1.

Shoot thinning as a canopy practice

Pool et al. proposed shoot thinning may be an effective method to control over-cropping (18). In addition, shoot thinning is relatively cost-effective for crop control. Shoot thinning may achieve high wine quality. Reynolds (1986) repotted that reducing shoot density led to increased cane ripening, cluster exposure, berry weight and improved fruit composition of Seyval Blanc (19). Reynolds (1989) found that shoot thinning to 24 shoots per meter of row for Riesling provided some improvement in yield components and ºBrix. Reynolds (1994) repotted that aged wine from the lowest shoot densities have the most fruity flavor and the least green flavor and perceived acidity for Riesling (20). Too much shoot thinning would cause shady canopy because the remaining shoots would grow vigorously with many lateral shoots and larger leaves, which increase leaf area per canopy. However, using 20 shoots/m of row is based on an interpolation of other reports. Smart (1988) indicated that 15 shoots/m of row was probably ideal for 'Gewurztraminer' (21) from New Zealand. Kiefer and Crusius (1984) recommended 21 to 29 shoots/m for 'Riesling', 14 to 22 shoots/m for 'Muller-Thurgau' and 14 to 17 shoots/m for 'Silvaner' from Germany (22). Nikov (1987) recommended 22 to 37 shoots/m for 'Merlot' from France (23). Reynolds (1988) reported a density of 25 shoots/m may be good for moderately vigorous 'Riesling' in British Columbia (24), 4 shoots/30cm of row for Seyval Blanc (19). It is believed that properly shoot thinning could improve the quality of Marechal Foch wines by increasing fruity and floral characters, decreasing herbaceousness, and improving color and tannin intensity.

Cluster thinning as a canopy practice

Cluster thinning is commonly used as a corrective viticultural measure to accelerate maturity, and improve fruit composition and wine quality. Cluster thinning is often used for *Vitis vinifera*

vine, especially for red cultivars to advance fruit ripening. Ough reported that cluster thinning increased wine quality of Caberner Sauvignon (25). Naor found that cluster thinning with high shoot density showed higher sensory parameters and total score of Sauvignon blanc (26). Leonardo found that cluster thinning increased the content of anthocyanin and improved phenolic composition of Syrah (27). Cluster thinning also can be used for white cultivars. Reynolds reported that cluster thinning reduced the yield and clusters per vine but increased Brix, pH and potential volatile terpenes of Chardonnay Musque (28). Reynolds reported that cluster thinning improved vine size, cane periderm formation and berry weight, thus improve berry and wine quality of Riesling in the Okanagan Valley of British Columbia of Canada. Arfelli reported that cluster thinned berries of Trebbiano in northern Italy had high sugar content because the treatment advanced accumulation of sugar during fruit ripening (29).

Cluster thinning also affects hybrid grape and wine composition. Cluster-thinned Vidal blanc had higher cluster weight and soluble solids/acid ratio (30, 31). The effect also was found in Chambourcin (32), De Chaunac (33, 34), Seyval blanc (35), and Chancellor (35).

Harvest date

Changes in physical and chemical parameters during grape ripening have been studied (36, 37). Some of these factors to some extent contribute to the sensory quality of berry and wine. Several studies have considered the impact of grape maturity on the volatile composition of *V. vinifera* grapes or resulting wines, but the effects of harvest date on hybrid grapes,excepting *V. labruscana* (38), are not reported. In *vinifera*, the free and bound concentrations of the major monoterpenes (linalool, geraniol, and nerol) are reported to increase with maturity (39, 40), although they may decrease in overripe fruit (41, 42) Accumulation of bound C13-norisoprenoids (e.g. precursors of TDN) is also reported to increase with maturation (43). The C_6

compounds (e.g. trans-2-hexenal, cis-3-hexenol) are derived by enzymatic oxidation of unsaturated fatty acids following disruption of the cell walls (44), and their concentration following crushing is generally reported to decrease with increasing grape maturity (45, 46), particularly the C_6 aldehydes (47). The production of many fermentation derived aroma compounds produced de novo by yeast are also influenced by grape maturity. For example, the concentration of branched chain fatty acid ethyl esters (ethyl 2-methylpropanoate, ethyl 3-methylpropanoate) in Pinot noir wines are reported to decrease with increasing grape maturity (48). Anecdotally, harvest date of Marechal Foch are reported by growers to significantly impact the aroma qualities of the resulting wines, but no data exists to evaluate these claims.

Based on the above information, we could hypothesize that different harvest date would change the quality of Marechal Foch. Finding an optimal harvest date for Marechal Foch in the Finger Lakes region may be beneficial for grape growers.

GRAPE AND WINE AROMA

Vitis vinifera and some American grape varieties aroma

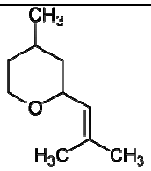
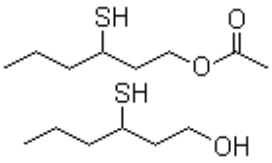
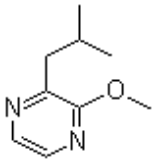
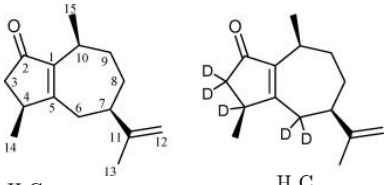
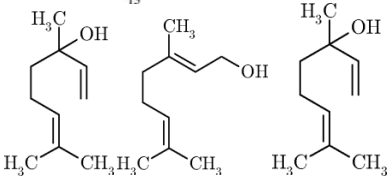
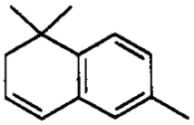
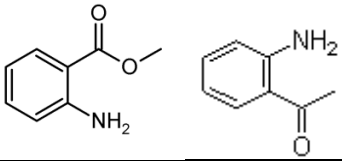
There are numerous reports about the aroma of *Vitis vinifera* and some American varieties. Impact odorants contribute to varietal aromas of selected wines, which are summarized in the Table 1.2.

Hybrid aroma

Although there are numerous reports about the aroma of *Vitis vinifera* and some American varieties, there are only a few reports about aromas of French American hybrid, and little indication of what compounds may be impact odorants. Studies have shown that various viticultural practices (59-63), winemaking (64-67) and phenolic composition (68) influenced the

wine quality of French American hybrids. But wine aroma profile was not examined in any detail while exploratory sensory and descriptive analysis has been performed on Missouri Seyval Blanc

Table 1.2 Impact Odorants of *Vitis vinifera* varieties

variety	aroma compounds	Structure
Gewurztraminer	*cis*-rose-oxide (49)	
Sauvignon blanc	3-mercaptohexyl acetate 3-mercapto-1-hexanol (50)	
Cabernet Sauvignon Cabernet Franc	2-methoxy-3-isobutyl pyrazine (51, 52)	
Shiraz	Rotundone (53, 54)	
Muscat	Terpenols (55)	
Riesling	1,1,6-trimethyl-1,2-dihydronaphthalene, or TDN (56)	
Concord	Methyl anthranilate o-aminoacetophenone (57, 58)	

wine (69), Vidal Blanc wine (70). The aroma of Seyval Blanc and Vidal Blanc wine has been compared to that of Riesling (71). But, there is no report for profiling aroma volatiles in red French American hybrid wines so far.

ANALYTICAL METHODS FOR EXTRACTION OF WINE AROMA COMPOUNDS

Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is a traditional method, but it is time-consuming, tedious and requires large amount of pure solvent. It requires an evaporation step to remove excess solvent through which some low boiling-point compounds will be lost, and can also have problems with emulsion formation and contaminants from solvent impurities (72).

Solid phase extraction (SPE)

In solid-phase extraction (SPE), solvent volume is greatly reduced. It is easy to automate and has high recovery for polar compounds. However, solvent elution is still required to finish extraction (72).

Stir bar sorptive extraction (SBSE)

Stir bar sorptive extraction (SBSE) is a novel extraction technique, which has higher volumn of polymeric coating compared to SPME. But it only has one coating face, poly (dimethylsiloxane) (PDMS). Therefore, the recovery for polar compounds is not good (73).

Static headspace method

Static headspace method has low sensitivity for trace volatiles while the results can be interfered with by water and ethanol by dynamic headspace method.

Solid phase micro extraction (SPME)

SPME was introduced by Pawliszyn in the early 1990s as a relatively novel sampling and sample-preparation technique. It is an inexpensive, simple, fast and effective technique which

does not require solvent and integrates sampling, isolation and enrichment into one single step. Since its introduction, SPME has been widely adopted for the sampling and analysis of aroma volatiles in a variety of different samples. Numerous articles about SPME applications for volatiles in wines have been reported. It can be applied for analyzing complex aromatic chemicals such as terpenes, sulfur compounds or single compounds like diacetyl, 2,4,6-trichloranisole, methyl isothiocyanate, methoxypyrazines (74-96). Therefore, SPME may be a useful tool to identify wine aroma profile.

IDENTIFICATION AND QUANTIFICATION OF AROMA COMPOUNDS

Identifying key aroma compounds: Gas chromatography - olfactometry (GCO)

GC-O is a technique that based on sensory evaluation of the eluting from the chromatographic column. GC-O use human nose as a detector in place of a more conventional detector, such as a flame ionization detector (FID), or a mass spectrometer (MS) to characterize odor-active compounds of samples by collecting its odor chromatogram. It is well known that human nose is often more sensitive than instrumental detector. Odorants which have very low concentration but high sensory impact can be detected by GC-O. Usually, the olfactometric port is connected in parallel to conventional detectors, such as GCO-FID of GCO-MS (Fig. 1.3). The flow of the eluting is split into two detectors simultaneously, thus both signals can be compared. GCO-MS is particularly used for identification of odor-active analytes. However, the retention time of the analytes might differ among the two detectors because they work under different conditions. The mass spectrometer works under vacuum condition while the olfactometric detector requires atmospheric pressure condition.

Aroma compounds are characterized by their odor descriptor and kovats retention index (RI). GC-O is widely used in different research areas. In wine research, GC-O has been used to profile

the aroma compounds from *Vitis vinifera* (97-105), French American hybrid and native American varieties (106, 107) (Table 1.4). The GC–O method has been developed into three general applicable approaches as follows.

Aroma extract dilution analysis (AEDA) and charm analysis

Both AEDA and Charm Analysis are simple sensory techniques, which are based on sensory evaluations of stepwise aroma extract dilutions until no odour is perceived, Fig. 4 (108, 109). The values are either in Flavor Dilution (FD) for AEDA or Charm for Charm analysis. FD and Charm value are calculated according to the following equations.

$FD = d^n$

where d is the dilution factor and n the number of dilution necessary for the odors to be no longer perceived.

$dv = F^{n-1}di$

$Charm = \int_{peak} dv$

Where dv is the dilution value, F is the dilution factor and n is the number of coincident odour responses detected at a single retention index, di.

The higher FD and Charm value are, the more potent the odor component. Due to numerous dilution steps, both of two methods are time consuming. Also, more panellists are required for a repeatable result for these two methods because of human olfactory sensitivity vary. AEDA and Charm Analysis are often used due to their simplicity.

Detection frequency analysis (DFA)

DFA use a panel of 8 to 12 judges to detect an eluting odor at any given retention time (110). In contrast to the AEDA and Charm Analysis, DFA utilize multiple replicates using the same panelist and do not require any dilution step. Therefore, this method is not based on individual

detection thresholds, and the aim is to detect all odorant compounds present in the given sample. DFA reflects the relative importance of the odor component. The method measures the odorant compounds' intensity, which is expressed as NIF (nasal impact frequency). Due to the enough panelists, specific anosmia of panelists is minimized.

Time intensity methods – odor specific magnitude estimation (OSME)

OSME method is developed by McDaniel et al. Miranda-Lopez and Sanchez in1990 (111), which is a quantitative bioassay method used to measure the intensity of an odor using magnitude estimation technique. A panel of 8 to 12 judges evaluates the intensity of the compound odor using a time-intensity device, thus giving an odor peak. At the same time, verbal descriptors of the odor are recorded.

Contrary to AEDA and Charm Analysis, this method is not based on odor detection thresholds. McDaniel et al. and Miranda-Lopez identified significant odor active peaks in wine using Osme method (111). Miranda-Lopez also successfully used GC-O (Osme) to compare odor profiles of Pinot noir wines from grapes harvested at different maturities (112). Osme method was reported be sensitive, effective and reproducible.

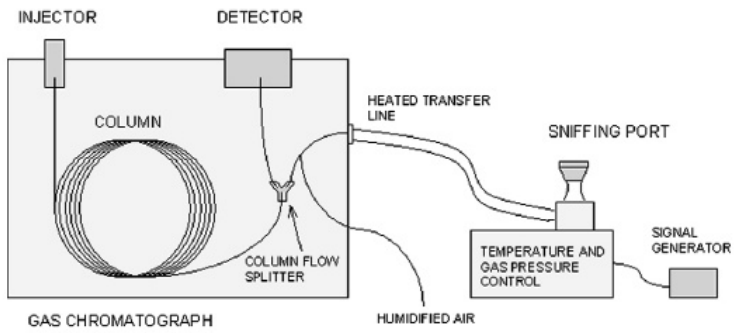


Fig. 1.3 GC- olfactometry

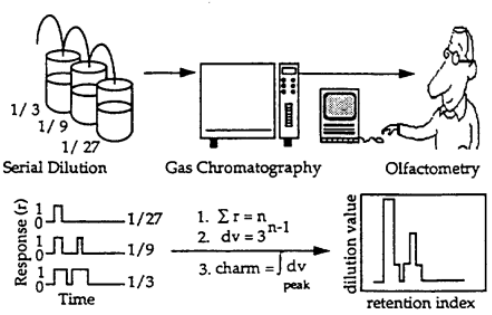


Fig. 1.4 Schematic procedure for GC-O using CharmAnalysis

Table 1.3 GC-O in wine aroma analysis

	Variety	Year published	Region	Potent Volatile Compounds
Red	Cabernet Sauvignon (113)	2007	Brazil	furaneol 2-methoxy-3-isobutylpyrazine
	Merlot (114)	2006	California Australia	3-methyl-1-butanol, 3-hydroxy-2-butanone, octanal, ethyl hexanoate, β-damascenone, etc.
	Pinot Noir (115)	2005	Oregon, USA	2-phenylethanol, 3-methyl-1-butanol, ethyl 2-methylpropanoate, ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate, and benzaldehyde
	Spanish Wines (116)	2004	Spain	1-nonen-3-one, 2-acetylpyrazines, etc.
	Kalecik Karasi (117)	2003	Turkey	isoamyl alcohol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, 2-phenyl ethanol and octanoic acid
	Aged Red Wines from Rioja (118)	2001	Spain	methyl benzoate, 4-ethylguaiacol, (E)-whiskey lactone, 4-ethylphenol, β-damascenone, fusel alcohols, isovaleric and hexanoic acids, eugenol, Furaneol, phenylacetic acid, (E)-2-hexenal, etc.
White	Sweet Fiano (119)	2006	Italy	terpenes, β-damascenone, lactones, aldehydes and ketones
	Madeira (120)	2005	Portugal	sotolon, phenylacetaldehyde
	Passito Wines (121)	2005	Italy	3-methylbutanoic acid, dimethyloctendiol, hexanal, linalool, 1-terpinen-4-ol, 3-mercapto-1-hexanol, 4-

			ethylguaiacol, 2-methoxy-4-vinylphenol
Gual, Verdello, Marmajuelo, Listan and Malvasia (122)	2003	Canary Islands, Spain	3-mercaptohexyl acetate, 3-methylbutyl acetate, â-damascenone, ethyl octanoate
Scheurebe (123)	1997	Germany	4-mercapto-4-methylpentan-2-one, etc.
Gewurztraminer (123)	1997	Germany	*cis*-rose oxide, etc

Quantification methods

According to many reports, major compounds of high concentration in wine were quantified by GC-FID (124, 125). Trace compounds were extracted by solid phase extraction method and quantified by GC-MS, using external or internal calibration (126). Unlike external or internal calibration method, standard addition method can resolve the matrix effect problem which happens when sample has impurities (127).

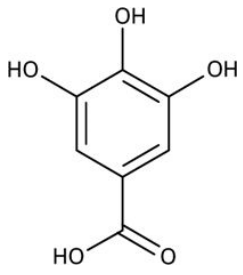
Therefore, standard addition method combined with SPE and GC-MS may be a good choice for quantification of trace volatile compounds in wine samples.

TANNIN

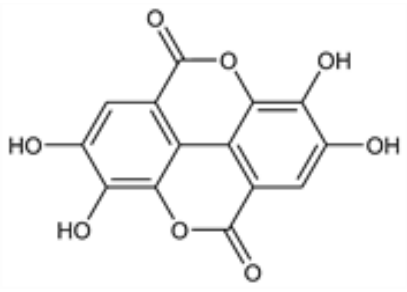
Tannins, a class of polyphenols, are a group of compounds found in plants. These polyphenols are mostly soluble in water. There are two major types of tannins, including hydrolyzable tannins and condensed tannins (proanthocyanidins), whose structures were show in Fig.1.5. Hydrolyzable tannin is tannin acid, which is able to be broken down by interaction with water. It was often found in the bark and wood of oaks and used commercially in tanning leather. Condensed tannins are a class of flavanols, which are the polymers of 2 to 50 (or more) flavonoid units that are joined by carbon-carbon bonds. Condensed tannins are less susceptible to being cleaved by acid hydrolysis. They are often found in tea, pomegranates, grape seeds and grape skins.

As a compound with astringent mouth feel, tannin plays an important role in the sensory characteristics of wine and is closely related to red wine quality. Tannin also can interact with anthocyannin in wines to form polymeric pigments during winemaking and aging, which are responsible for stabilizing the color of older red wines (128). Many previous studies focused on assessment of the concentration of tannin of *Vitis vinifera* (129, 130), and development of simple, robust and selective assays for quantification of grape and wine tannin, such as methyl cellulose precipitable tannin assay (MCP) and protein based tannin precipitation (131). There are a few reports about tannin concentration of hybrid grape and wine (132, 133). But the extractability of tannin in making hybrid wines has not been reported. Therefore, it is worthwhile to investigate the extraction ratio of tannin or alternative methods to improve extraction in wine making since hybrid wines tend to be low in astringency due to low tannin content.

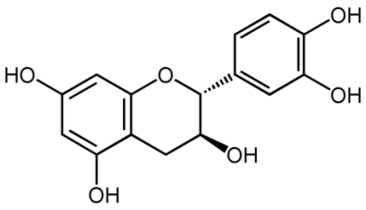
Seed, skin and wine tannin concentration were measured using a protein precipitation assay built by Harbertson et al (134) because it is a relative simple and robust method, which can be used as a routine method for tannin in winery. It is believed that to study the tannin of hybrid grape and wine and its extractability may assist winemakers to achieve better sensory properties and thus quality of finished wines.



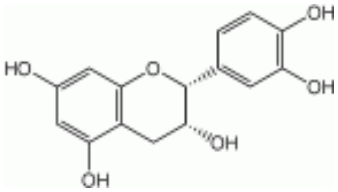
Gallic acid



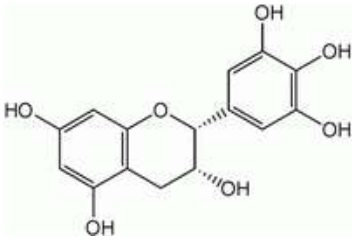
Ellagic acids



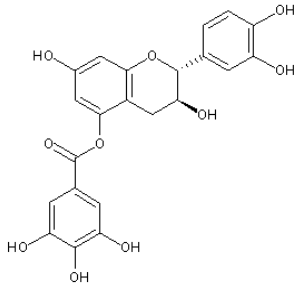
Catechin (C)



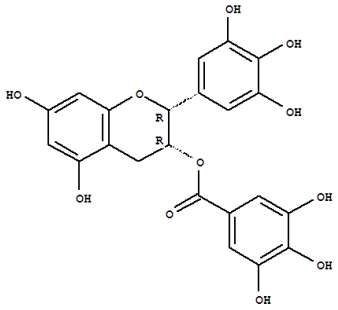
Epicatechin (EC)



Epigallocatechin (EGC)



Epicatechin gallate (ECG)



Epigallocatechin gallate (EGCG)

Fig. 1.5 The structure of hydrolyzable tannins and condensed tannins

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CHAPTER 2

IMPACT OF SHOOT THINNING AND HARVEST DATE ON YIELD COMPONENTS, FRUIT COMPOSITION, AND WINE QUALITY OF MARECHAL FOCH

ABSTRACT

Marechal Foch grapevines were subjected to shoot thinning (~15 shoots per meter of row and no shoot thinning) in combination with two harvest dates (early harvest and late harvest) in a factorialized treatment arrangement for two years (2007 and 2008). With shoot thinning, yields were reduced by 3.1 to 7.2 kg per vine and clusters were reduced by up to 59 clusters per vine, while berry weight increased by 0.03 to 0.09 g. Shoot thinning reduced crop load by 4.3 to 7.8 kg yield per kg pruning weight, and increased soluble solids in 2008 by 0.7 to 1.2 Brix. Shoot thinning increased berry anthocyanins by 1.25 to 2.24 mg/g fresh skin weight malvidin-3-glucoside, but no corresponding increase was observed in wine anthocyanins. Delaying harvest resulted in increases of soluble solids (0.5 to 2.3 Brix) and berry anthocyanins (0.32 to 1.48 mg/g) and significantly higher anthocyanins in finished wines. Both late harvest and shoot-thinning treatments resulted in decreased six-carbon alcohols (3 to 33%) in finished wines. The total concentration of tannin in Foch fruit was comparable to that of some vinifera (0.75 to 1.05 mg/berry catechin equivalents). However, the extractability of tannins during winemaking was very low compared to most vinifera (2 to 4%), in part likely due to the low skin tannin concentration. Using a two-alternative forced choice test, panelists reported that later harvest 2008 wines were more "fruity" than their early harvest counterparts for both treatments and that shoot thinning did not affect fruitiness.

Key words: canopy treatment, anthocyanin, tannin, aroma compounds

INTRODUCTION

Marechal Foch (Kuhlmann 188.2) is an interspecific hybrid red winegrape variety produced from a cross of Goldriesling (cross of Riesling and Courtiller Musque) and 101-14 Millardet et de Grasset (cross of Vitis riparia and Vitis rupestris) (1). Marechal Foch is widely planted in the eastern wine regions of the United States and Canada, partly because of its early ripening (early to mid-September in the Finger Lakes region) and its cold hardiness (winter hardy to -26°C to-29°C) (http://viticulture.hort.iastate.edu/info/pdf/cultivars08.pdf). Foch vines tend to be overcropped because of the fruitfulness of noncount shoots (2), resulting in negative impacts on fruit composition and wine qualities and reduced vigor in the following year. Balanced pruning does not provide adequate crop control for most French-American hybrids given the production of heavily fruited primary and secondary shoots and fruiting shoots can arise from latent buds on cordons and from basal nodes that are not counted during balanced pruning (2).

In the Finger Lakes region of New York, growers of Marechal Foch anecdotally report "beet" or "radish" aromas in the grapes in some years. As viticultural management of Foch in the region is generally not intensive given the low price value of the cultivar, we were interested in investigating low-cost viticultural practices that could improve the aroma of Foch wine. We hypothesized that increased exposure of clusters and reduced crop load as a result of shoot thinning, as well as increased ripening time, would reduce the negative aroma characteristics reported in locally produced Foch.

To our knowledge there are no reports on the impact of canopy management practices (including shoot thinning) on Marechal Foch. Shoot thinning is a common, and well researched, viticultural practice for Vitis vinifera (3), hybrids (Vitis sp.), and Vitis labruscana. It is generally reported to be an effective and inexpensive method for reducing yields and increasing canopy

openness in hybrids prone to overcropping (4), often leading to increased canopy photosynthesis, berry temperature, bud fruitfulness (5), and vine hardiness (6). Fruit composition is often improved (2). Similarly, several studies have considered the impact of grape maturity on the volatile composition of V. vinifera grapes or resulting wines (7, 8), but the effects of harvest date on hybrid grapes, excepting V. labruscana (9), are not reported. Several studies on V. vinifera have also considered the impact of canopy management practices or harvest maturity on polyphenols responsible for astringency (condensed tannins) and color (anthocyanins) (10, 11), but by comparison, there are relatively few studies on the impact of canopy management on phenolic species in red hybrid winegrapes (12). In particular, to our knowledge, quantitative measurements of tannins or tannin extractability by tannin precipitation assays such as the Adams-Harbertson assay have not been previously reported for French-American hybrids.

Quantitative gas chromatography-olfactometry (CHARM GC-O) was used recently to identify 56 aroma compounds with flavor dilution values >1 in Marechal Foch wine (13). The majority of odorants detected by GC-O in Marechal Foch wine was similar to those previously reported in V. vinifera wines (14), although a few compounds with “vegetal” and “musty” aromas unique to Marechal Foch are not yet conclusively identified. While not all compounds detectable by GC-O are necessarily critical to the aroma of the resulting wine (15), the GC-O data set does provide a useful starting point for understanding how growing practices influence wine flavor chemistry.The objective of this study was to assess the effects of two inexpensive viticultural practices, shoot thinning and harvest date, on the yield, wine and fruit composition, and wine sensory qualities of Marechal Foch in the Finger Lakes region of New York State.

MATERIALS AND METHODS

Vineyard site and experimental design. This study was conducted in 2007 and 2008 with 32-year-old Marechal Foch vines at a commercial winery on the west side of Seneca Lake in Penn Yan, New York. The vines were grafted onto 3309C. Soil in the block was a well-drained Lima silt loam (USDA-NRCS soil maps). Vines were spaced at 2.1 m x 2.4 m (vine x row) in north-south oriented rows and trained to the Umbrella-Kniffen system. Drip irrigation was installed throughout the vineyard. Standard pest control practices for the region were used.

The experimental design consisted of two canopy treatments (no shoot thinning and shoot thinning) combined with two harvest dates (early and late) in a randomized complete block design with four replications. Treatments were designated as no shoot thinning, early harvest (CE); no shoot thinning, late harvest (CL); shoot thinning, early harvest (SE); and shoot thinning, late harvest (SL). Each experimental unit consisted of five panels of vines, with two panels randomly selected at the beginning of the experiment for data collection. For the shoot-thinning treatment, approximately 15 primary shoots were retained per meter and all secondary, tertiary, and noncount shoots were removed. Shoot-thinning treatments were applied when shoots reached ~51 to 127 mm in length in May. The harvest dates were based roughly on the beginning and end of the Foch harvest in the Finger Lakes region for each season. “Early” harvests occurred on 11 Sept 2007 and 10 Sept 2008 and “late” harvests occurred on 18 Sept 2007 and 23 Sept 2008.

Yield components. Vines were individually harvested by hand on 11 Sept (early harvest) and 18 Sept (late harvest) in 2007, and 10 Sept (early harvest) and 23 Sept (late harvest) in 2008. Yield per vine was quantified using a hanging scale (Salter Weigh-Tronix, Fairmont, MN) and cluster number per vine was counted. Cluster weights were calculated by dividing yield by

cluster number on a per vine basis. A random sample of 15 to 20 clusters per panel was collected at harvest and stored at -20°C until analysis. Subsamples of 100 berries were weighed to determine mean berry weight. Berry number per cluster was calculated by dividing cluster weight by berry weight. Total shoots, base shoots, primary shoots, and secondary shoots were counted prior to pruning. Pruning weights were collected in early January in 2008 and 2009. Crop load was calculated by dividing yield by pruning weight on a per vine basis.

Canopy characterization. Enhanced point quadrat analysis (EPQA) (16) was used to characterize canopy light environment at approximately veraison in both years. A sharpened thin metal rod was inserted into the canopy at regular 10-cm intervals, and sequential contacts of leaves, clusters, and canopy gaps from one side to the other were recorded. Photon flux measurement was performed according to a previously described method (Meyers and Vanden Heuvel 2008). Canopy parameters were analyzed by EPQA and CEM Tools, version 1.6 (Cornell University, Ithaca, NY). Parameters included occlusion layer number (OLN), the number of shade-producing contacts (leaves and clusters per insertion); cluster exposure flux availability (CEFA), the percentage, expressed as a decimal of above-canopy photon flux that reaches clusters; and leaf exposure flux availability (LEFA), the percentage, expressed as a decimal of above-canopy photon flux that reaches leaves.

Berry and wine composition. A-100 berry sample was collected randomly in duplicate from each sample that was kept frozen at -40°C until analysis. The frozen berries were thawed at room temperature before collection. The berries

were juiced by a blender and the slurry was pressed through cheesecloth. Brix was measured using an Abbé temperature-compensated refractometer (ATAGO, Bellevue, WA). Berry

and wine pH were measured using an Orion 3-Star pH meter (Thermo-Fisher Scientific, Waltham, MA), and titratable acidity (TA) was determined on a 10 mL sample by Digital Buret autotitration (BrandTech Scientific, Essex, CT) using 0.1 M NaOH to an endpoint of pH 8.2. Wine alcohol concentration was measured by ebulliometer (DuJardin-Salleron, Arcueil Cedex, France). Wine-free SO_2 was measured by FIAstar 5000 analyzer (FOSS, Eden Prairie, MN). Berry and wine anthocyanins and tannins were determined by the Adams-Harbertson protein precipitation assay, using 20 berries (17).

Winemaking. Wines were made in duplicate after replicates for each treatment had been combined in the field. Fruit was destemmed, crushed, and treated with 50 mg/L sulfur dioxide. Diammonium phosphate (DAP) (Presque Isle Wine Cellars, North East, PA) was added to a concentration of 1 g/kg, Fermaid K (Lallemand, Rexdale, ON, Canada) to 0.1 g/L, and Goferm (Lallemand) to 0.15 g/L. Skin fermentation was done in jacketed 114-L fermentors. Cap management was performed twice per day by manual punchdowns. The must was brought to 20°C and inoculated with EC1118 (Lallemand) to 0.26 g/L. The temperature profile of the fermentations was controlled by a connected computer. During the first three days of fermentation, the must was warmed slowly from 20°C to a maximum between 30 and 35°C. Temperature limits were set at 20°C and 30°C for the remainder of the alcoholic fermentation. Fermentation was complete when residual sugar was measured as less than 0.5% using Clinitest tablets (Bayer, Etobicoke, ON, Canada). Wines were pressed, topped, and inoculated with Alpha (Lallemand) to start malolactic fermentation (MLF). Upon completion of MLF, sulfur dioxide was added to maintain 40 mg/L free sulfur dioxide. Wines were cold stabilized at 2°C. Titratable acidity was adjusted to 6.5 g/L by addition of tartaric acid or potassium carbonate after cold stabilization. The wines were screened for faults by an expert

panel prior to bottling. Bottling and screwcapping were performed manually. Quantification of wine aroma compounds. Analysis of aroma compounds was adopted from previously reported methods (Lopez et al. 2002). Solid phase extraction (SPE) of a 50 mL wine sample containing 0.25 mg/L 2-octanol (Sig-ma-Aldrich, St. Louis, MO) (quantification internal standard) was performed on a LiChrolut EN column (Merck, Darmstadt, Germany) preconditioned with 4 mL dichloromethane (DCM), 4 mL methanol, and 4 mL 12% ethanol (all Fisher Scientific, Pittsburgh, PA). Following sample loading, the SPE column was dried under nitrogen (2 mL/min) for 15 min, and analytes were eluted by 1.3 mL DCM containing 1 mg/L 2-ethyl hexanoate (Sigma-Aldrich) as a quality-control internal standard. After extraction, compounds were quantified either by GC-FID (for higher concentration analytes) or by GC-MS. For GC-FID quantification, standard curves were generated for analytes in model wine with respect to the 2-octanol internal standard over the range observed in wine. Calibration curves were not prepared for the GC-MS semiquantification, but previous work (14) demonstrated >90% recovery and good linearity for most the analytes in wine under study in our current work. The commercial source for each analyte, their method of quantification, and the calibration ranges used are shown (Table 1). Identification of compounds in samples was performed by comparison of linear retention indices and mass spectra to those of authentic standards.

GC-FID analyses were performed in duplicate on a CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a split/splitless injector and a CP-Wax 58 FFAP fused capillary column (30 m x 0.32 mm i.d. x 1.2 μm). Wine samples (3 μL) were injected into the column in splitless mode, with a purge time of 0.75 min. High-purity helium was used as carrier gas with flow rate of 3 mL/min. The injector temperature was 250°C and the FID detector temperature was 300°C. The oven temperature was held at 55°C for 5 min,

then increased to 163°C at 3°C/min, then increased to 250°C at 10°C/min, then held at 250°C for 15min. Galaxie Workstation ver. 1.9.3.2 (Varian) was used for data acquisition and analysis.

Table 2.1 Chemical standards and quantification methods

Compounds	Retention Index (CP-WAX)	Odorant description	Commercial source	Purity (%)	Quanti-fication method	Quanti-fication ion (*m/z*)	Calibration Range (mg/l)
Isobutanol	1105	solvent	SAFC Supply Solution	99	FID		2-240
Isoamyl acetate	1135	banana	Aldrich	98	FID		0.05-5
1-Butanol	1161	fruit	Acros Organics	99	FID		2-220
Isoamyl alcohol	1234	chocolate	SAFC Supply Solution	98.5	FID		5-250
Ethyl hexanoate	1251	apple	Acros Organics	99	FID		0.05-3
Hexyl acetate	1273	fruit	Aldrich	99	FID		0.020-1
Ethyl lactate	1352	fruit	Acros Organics	95	FID		2.5-250
cis-3-Hexenol	1356	grass	SAFC Supply Solution	98	GC-MS	67	
1-Hexanol	1368	green	Fluka (Sigma–Aldrich)	99	FID		0.250-25
trans-2-Hexenol	1406	grass	SAFC Supply Solution	95	GC-MS	57	
Butyric acid	1593	sweat	Aldrich	99+	FID		0.2-6
α-Terpineol	1680	flower	Acros Organics	97+	GC-MS	59	
Isovaleric acid	1694	cheese	Aldrich	99	FID		0.1-10
Diethyl succinate	1704	fruit	Aldrich	99+	FID		0.23-23
Methionol	1743	potato	Aldrich	98	FID		0.1-3
Citronellol	1769	flower	Aldrich	95	GC-MS	71	
β-Phenethyl acetate	1835	rose	Acros Organics	98+	FID		0.023-1.2
β-Damascenone	1844	cooked apple	SAFC Supply Solution	1.1-1.3wt	GC-MS	69	
Ethyl dihydrocinnamate	1857	flower	Aldrich	98	GC-MS	91	
Hexanoic acid	1871	sweat	Aldrich	99.5	FID		0.09-9
Guaiacol	1883	smoke	Aldrich	98	GC-MS	109	
Benzyl alcohol	1905	sweet	Acros Organics	99+	FID		0.02-1
β-Phenyl ethanol	1938	honey	Aldrich	99+	FID		3.5-70
γ-Nonalactone	2035	coconut	K & K Laboratories, Inc.	98	GC-MS	85	
Octanoic acid	2084	sweat	Aldrich	98	FID		0.09-9
Ethyl cinnamate	2131	flower	Aldrich	99	GC-MS	131	
Eugenol	2191	bandaid	Aldrich	99	GC-MS	164	
4-Vinylguaiacol	2225	clove	SAFC Supply Solution	98	GC-MS	135	

GC-MS analyses were performed using a CP-3800 gas chromatograph coupled to a Saturn 2000 ion trap mass spectrometer (Varian). Chromatographic separation was achieved on a CP-Wax column (50 m x 0.25 mm x 0.2 μm) (Varian). High-purity helium was used as a carrier gas with flow rate of 1 mL/min. The injector was operated at 250°C and the detector at 300°C. The temperature program for the column oven was 40°C for 6 min, then 140°C to 170°C at a rate of

10°C/min, then 170°C to 250°C at a rate of 5°C/min, and finally 250°C, held for 20 min. Saturn GC-MS version 6.3 software (Varian) was used for data acquisition and analysis.

Sensory tests. The 2007 wines were evaluated and compared in February 2009 for all four treatments by triangle test. Wines were aged in bottle for approximately one year prior to evaluation. Clear, tulip-shaped 220-mL wineglasses coded with 3-digit numbers were used to serve wines in a sensory room illuminated with fluorescent lighting. Wine samples (25 mL) were poured into glasses and evaluated at room temperature. Panelists were separated from each other. All panelists expectorated wine samples and rinsed their mouths with water between tests. Water and plain bread were provided as cleansers (18). Each test was carried out by 12 panelists with wine evaluation experience. Two sessions were conducted in the morning and afternoon comparing wines from the four different treatments. In session one, comparisons were made between SE versus CE and SL versus CL. In session two, comparisons were made between SE versus SL and CE versus CL. Each comparison was duplicated. The 2008 wines were evaluated and compared in October 2009 for all four treatments by two-alternative forced choice (2-AFC) test. Wines were aged in bottle for approximately 6 months. The use of the 2-AFC test was based on the sensory test result of 2007 wines. Fourteen panelists with wine evaluation experience were selected for the test. A pair of coded samples for comparison was presented to panelists, who were asked to select the sample with the stronger fruitiness (19, 20). Wines from each treatment were compared to one another. Each comparison was duplicated. One wine sample was randomly selected for sensory evaluation from duplicate wines.

Statistical analysis. Mixed-model ANOVA was performed using JMP software (ver. 8.0; SAS Institute, Cary, NC). Probabilities for the triangle test were calculated by Excel (version 2007; Microsoft, Redmond, WA) using the formula: p = 1- BINOMDIST (r-1, n, 1/3, TRUE),

where r is successes out of n trials and n is the number of trials. The 2-AFC test statistical analysis was performed by an established method (19).

RESULTS

Yield components. In 2007, shoot thinning reduced yield per vine, cluster number per vine, and berry number, but increased berry weight (Table 2.2). Yield reductions ranged from 3.1 to 4.7 kg/vine, primarily as a function of cluster number, which was reduced by up to 26 clusters per vine. Cluster weight and crop load were not affected by shoot thinning treatment. In 2008, yield per vine was reduced to a greater degree than in 2007 (with reductions ranging from 6.4 to 7.2 kg/vine) due to large decreases in cluster number per vine with shoot thinning (up to 59 clusters per vine). Shoot thinning reduced crop load, but increased cluster weight and berry weight (Table 2.2).

Table 2.2 Impact of shoot thinning and harvest date on yield compositions of Marechal Foch, 2007-2008. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

Treatment	Yield /vine (kg)	Clusters /vine	Cluster wt. (kg)	Berries /cluster	Berry wt. (g)	Crop load (kg yield/kg pruning weight)
			2007			
Control, early (CE)	14.7	91	0.17	162	1.03	21.9
ST, early (SE)	10.0	65	0.17	150	1.12	19.7
Control, late (CL)	14.5	89	0.16	160	0.99	24.9
ST, late (SL)	11.4	69	0.16	156	1.02	24.2
P value for shoot thinning	0.001	0.002	0.642	0.002	0.0009	0.347
P value for harvest date	0.523	0.814	0.477	0.112	0.0003	0.356
P value for shoot thinning x harvest date	0.556	0.858	0.765	0.019	0.0080	0.491
			2008			
Control, early (CE)	23.2	154	0.15	146	0.99	23.2
ST, early (SE)	15.9	95	0.17	154	1.08	18.9
Control, late (CL)	21.5	145	0.14	146	1.00	24.4
ST, late (SL)	15.1	91	0.17	153	1.09	16.6
P value for shoot thinning	<0.001	<0.001	0.043	0.016	0.003	0.011
P value for harvest date	0.347	0.369	0.560	0.723	0.619	0.821
P value for shoot thinning x harvest date	0.752	0.693	0.602	0.689	0.866	0.382

Harvest date only reduced berry weight in 2007 and had no impact on yield components in 2008. In 2007, there were significant interactions between shoot thinning and harvest date for berries per cluster and berry weight (Table 2.2). The SE treatment showed a significant decrease in berries per cluster compared to the CE treatment. The SE treatment increased berry weight to a greater extent than the CE treatment, but there was no significant difference between CL and SL.

Vine canopy. Shoot-thinning treatments increased CEFA from 0.16 to 0.21 in 2007 and 0.12 to 0.19 in 2008. LEFA increased by 0.35 to 0.40 in 2008 by shoot-thinning treatments. Shoot thinning did not affect OLN in both years. Harvest date had no effect on CEFA, LEFA, and OLN.

Berry composition. In 2007, shoot thinning had no effect on berry pH and Brix, but increased TA (Table 2.3). Berry anthocyanin concentration, as malvidin-3-glucoside equivalents, increased as a result of shoot thinning as did berry skin tannin (catechin equivalents) (Table 2.4). In 2008, shoot thinning increased Brix but had no effect on pH and TA (Table 2.3). Berry anthocyanin and skin tannin were also increased by shoot-thinning treatment (Table 2.4). In 2007, berry pH, Brix, and TA were increased by the CL and SL treaments (Table 2.3). Berry anthocyanin was increased by CL and SL (Table 2.4). In 2008, CL and SL treatments increased pH and Brix. Harvest date had no effect on berry TA (Table 2.3). Berry anthocyanin increased, while berry seed tannin was decreased by CL and SL treatments (Table 2.4).

Wine composition. In 2007, shoot thinning increased wine pH (Table 2.3) and wine tannin (Table 2.4). In 2008, thinning slightly increased wine pH, alcohol, and TA (Table 2.3). The impact of late harvest was more pronounced in both years. In 2007, wine pH and alcohol were increased by CL and SL treatments while wine TA decreased (Table 2.3). Wine anthocyanin was increased by CL and SL treatments (Table 4). In 2008, wine pH and alcohol were increased by

Table 2.3 Impact of shoot thinning and harvest date on berry and wine composition of Marechal Foch, 2007-2008. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

	Berry			Wine		
Treatment	**pH**	**Brix**	**Titratable acidity (TA) (g/L)**	**pH**	**Alcohol (% v/v)**	**Titratable acidity (TA) (g/L)**
			2007			
Control, early (CE)	3.62	22.7	8.67	3.57	11.28	6.70
ST, early (SE)	3.66	22.9	9.36	3.60	11.50	6.55
Control, late (CL)	3.69	23.2	9.32	3.64	12.35	6.40
ST, late (SL)	3.70	24.3	9.50	3.72	12.80	6.20
P value for shoot thinning	0.276	0.107	0.0002	0.021	0.061	0.080
P value for harvest date	0.008	0.022	0.001	0.006	0.0008	0.012
P value for shoot thinning x harvest date	0.428	0.295	0.357	0.407	0.437	0.756
			2008			
Control, early (CE)	3.50	22.1	11.06	3.66	10.70	6.90
ST, early (SE)	3.55	23.3	11.04	3.72	11.00	6.60
Control, late (CL)	3.62	24.3	10.28	3.73	12.00	6.50
ST, late (SL)	3.68	25.1	11.01	3.77	12.10	6.45
P value for shoot thinning	0.020	0.005	0.094	0.0007	0.027	0.002
P value for harvest date	<0.0001	<0.001	0.057	0.0004	<0.0001	0.0004
P value for shoot thinning x harvest date	0.709	0.435	0.071	0.089	0.234	0.008

Table 2.4 Impact of shoot thinning and harvest date on berry and wine anthocyanin and tannin of Marechal Foch, 2007-2008. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

Treatment	**Berry anthocyanin (mg/g M-3-G[a] fresh skin weight)**	**Wine anthocyanin (mg/L M-3-G)**	**Berry skin tannin (mg/berry catechin)**	**Berry seed tannin (mg/berry catechin)**	**Wine tannin (mg/L catechin)**	**% tannin extraction**
		2007				
Control, early (CE)	5.78	478.5	0.21	0.64	48.55	3.53
ST, early (SE)	8.02	505.5	0.27	0.81	59.81	3.72
Control, late (CL)	6.73	644.0	0.19	0.66	45.06	3.15
ST, late (SL)	8.34	661.5	0.21	0.61	54.18	4.04
P value for shoot thinning	0.0002	0.413	0.030	0.472	0.024	
P value for harvest date	0.011	0.003	0.056	0.329	0.186	
P value for shoot thinning x harvest date	0.089	0.855	0.663	0.245	0.728	
		2008				
Control, early (CE)	4.64	753.0	0.18	0.81	39.55	2.38
ST, early (SE)	5.89	777.5	0.22	0.90	40.69	2.36
Control, late (CL)	5.45	861.5	0.19	0.70	29.28	1.98
ST, late (SL)	7.37	919.0	0.23	0.63	34.68	2.64
P value for shoot thinning	0.011	0.166	0.035	0.903	0.393	
P value for harvest date	0.032	0.007	0.305	0.026	0.076	
P value for shoot thinning x harvest date	0.396	0.533	1.000	0.292	0.567	

CL and SL treatments (Table 2.3). Wine anthocyanin increased (Table 2.4). The SE treatment decreased wine

Wine aroma chemistry. Based on previous GC-O work (13), 28 aroma compounds (six esters, five fusel alcohols, four fatty acids, three terpenoids, six shikimic acid derivatives, three C_6 alcohols, and one other compound) were selected for study (Table 2.5). Of these compounds, 17 were quantified against calibration curves based on authentic standards and 11 were semiquantified based on relative response with respect to the 2-octanol internal standard. As mentioned previously, not all odorants detected by GC-O in our earlier work have been confidently identified, including some compounds that are unique to Marechal Foch. Therefore, they are not included in the GC-MS analysis.

Because of the observed similarities in treatment effects to related compounds within a compound class and the high number of volatiles under investigation, we converted absolute changes to relative percent changes and pooled together related compounds. In 2007, the SE treatment increased esters and shikimic acid derivatives by 9% and 11, respectively, and decreased fatty acids, fusel alcohols, terpenoids, and C_6 alcohols by 6%, 5%, 12%, and 10%, respectively. The CL treatment increased esters by 7% and decreased fatty acids, fusel alcohols, terpenoids, shikimic acid derivatives, and C6 alcohols by 20%, 10%, 22%, 18%, and 18%, respectively. The SL treatment increased esters by 7% and decreased fatty acids, fusel alcohols, terpenoids, shikimic acid derivatives, and C6 alcohols by 27%, 16%, 42%, 1%, and 33%, respectively (Figure 2.1).

Table 2.5 Impact of shoot thinning and harvest date on wine aroma compounds of Marechal Foch, 2007-2008. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

Compounds	07CE	07SE	07CL	07SL	*P* value for shoot thinn	*P* value for harvest date	*P* value for thin x harvest	08CE	08SE	08CL	08SL	*P* value for shoot thin	*P* value for harvest date	*P* value for thin x harvest
Esters (mg/L)														
Ethyl lactate	146.0	144.1	140.6	131.6	0.556	0.176	0.374	86.24	81.91	73.30	70.25	0.026	<0.0001	0.664
Ethyl hexanoate	0.465	0.440	0.315	0.295	0.482	0.007	0.936	0.340	0.340	0.370	0.330	0.161	0.574	0.210
Hexyl acetate	0.130	0.130	0.130	0.130	1.000	1.000	1.000	0.140	0.120	0.120	0.120	0.0200	0.0060	0.0200
Isoamyl acetate	1.405	1.295	1.500	1.195	0.214	0.987	0.526	1.830	1.660	1.720	1.610	0.051	0.247	0.611
Ethyl succinate	2.715	5.145	3.135	4.260	0.500	0.119	0.479	0.840	0.880	0.990	1.070	0.086	0.0004	0.507
β-Phenethyl acetate	0.825	0.860	1.300	1.215	0.663	0.002	0.323	2.140	2.560	3.060	3.180	0.0003	<0.0001	0.016
Fusel alcohols (mg/L)														
Isobutanol	33.21	32.65	28.90	31.47	0.278	0.093	0.466	29.25	28.20	25.90	24.90	0.066	<0.0001	0.953
1-Butanol	2.925	3.045	3.450	3.275	0.812	0.025	0.2456	3.380	3.300	3.360	3.320	0.248	0.950	0.714
Methionol	2.240	1.755	1.630	1.375	0.283	0.173	0.720	1.210	1.090	1.210	0.960	0.031	0.417	0.399
Isoamyl alcohol	157.1	152.2	129.4	112.3	0.239	0.013	0.485	136.7	132.8	132.9	123.6	0.069	0.073	0.431
β-Phenyl ethanol	12.88	12.55	11.55	10.31	0.492	0.161	0.685	10.64	11.53	12.86	12.76	0.048	<0.0001	0.017
Terpenoids														
Citronellol	0.009	0.008	0.006	0.005	0.275	0.034	1.000	0.028	0.035	0.040	0.047	0.014	0.0004	0.868
α-Terpineol	0.016	0.016	0.014	0.012	0.047	0.0006	0.047	0	0	0	0			
β-Damascenone	0.019	0.014	0.015	0.008	0.057	0.092	0.836	0.070	0.090	0.070	0.090	<0.0001	0.800	0.613
Fatty Acids (mg/L)														
Caproic acid	2.435	2.320	1.490	1.240	0.386	0.006	0.737	2.020	1.820	1.770	1.610	0.155	0.077	0.864
Octanoic acid	1.530	1.385	0.955	0.825	0.306	0.008	0.952	1.030	0.910	0.790	0.690	0.002	<0.0001	0.7053
Isovaleric acid	1.345	1.280	1.460	1.325	0.094	0.155	0.486	1.630	1.710	1.420	1.320	0.877	<0.0001	0.014
Butyric acid	3.610	3.425	3.210	3.110	0.264	0.031	0.718	3.220	2.960	1.760	1.660	0.241	<0.0001	0.604
Shikimic Acid Derivatives														
Ethyl dihydrocinnamate	0.010	0.012	0.008	0.008	0.552	0.086	0.552	0	0	0	0			
Benzyl alcohol	0.380	0.335	0.335	0.345	0.441	0.441	0.250	0.430	0.440	0.350	0.230	0.0001	<0.0001	<0.0001
Ethyl cinnamate	0.046	0.035	0.032	0.027	0.066	0.025	0.424	0	0	0	0			
4-Vinylguaiacol	3.232	2.701	2.187	1.691	0.193	0.035	0.961	0.220	0.170	0.180	0.180	0.4494	0.6978	0.5096
Guaiacol	0.108	0.106	0.091	0.118	0.413	0.895	0.367	0.030	0.030	0.040	0.040	0.177	0.177	0.640
Eugenol	0.001	0.002	0.001	0.002	0.040	0.374	0.374	0.080	0.070	0.070	0.070	0.633	0.075	0.633
C_6 Alcohols														
cis-3-Hexenol	0.355	0.310	0.265	0.205	0.001	0.0001	0.320	1.020	0.870	1.010	0.790	<0.0001	0.028	0.085
trans-2-Hexenol	0.370	0.330	0.310	0.240	0.005	0.002	0.208	0.300	0.260	0.290	0.150	<0.0001	<0.0001	0.0003
1-Hexanol	5.000	4.645	4.330	3.935	<0.0001	<0.0001	0.3528	6.330	5.410	6.070	4.920	<0.0001	0.001	0.205
Other														
γ-Nonalactone	0.046	0.049	0.050	0.058	0.035	0.027	0.238	0	0	0	0			

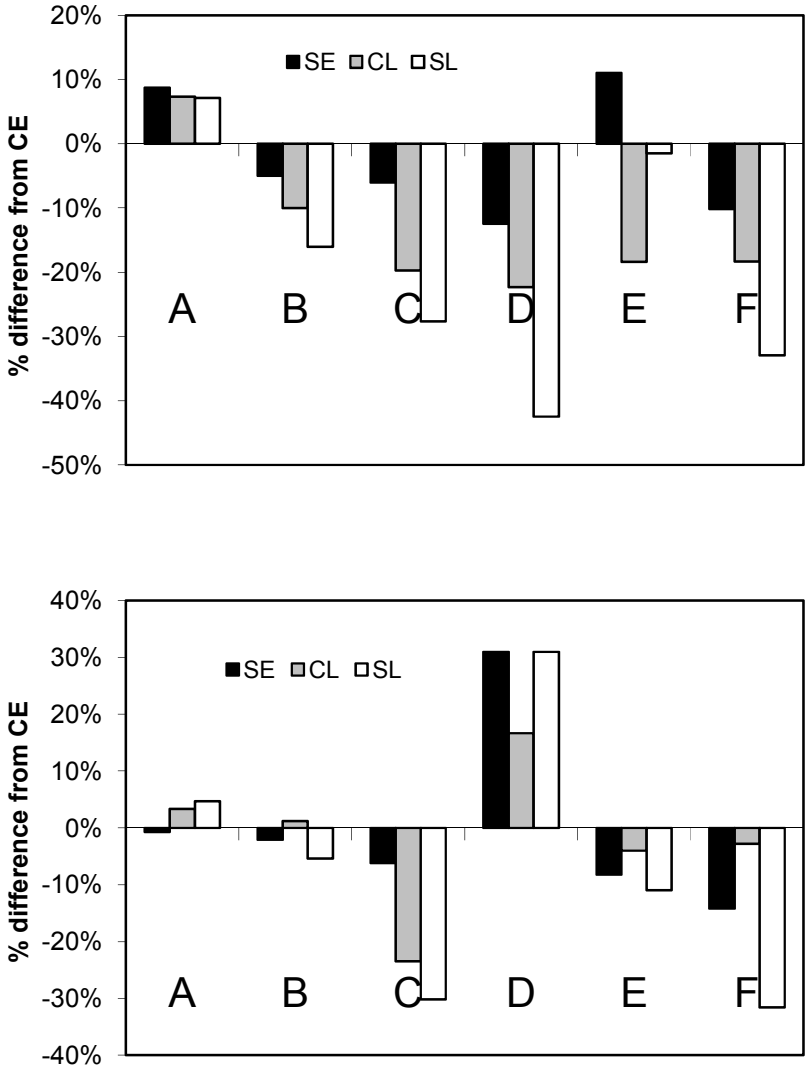

Fig. 2.1 Impact of shoot thinning and harvest date on wine aroma compounds of Marechal Foch, 2007 (top) and 2008 (bottom).
Y-axis: Average % change in compared to the CE treatment (normalized to 0%).
A: Esters; B: Fusel alcohols; C: Fatty acids; D: Terpenoids; E: Shikimic acid derivatives; F: C_6 alcohols.

In 2008, the SE treatment increased terpenoids by 31% and decreased esters, fusel alcohols, fatty acids, shikimic acid derivatives, and C_6 alcohols decreased by 1%, 2%, 6%, 8%, and 14%, respectively. The CL treatment increased esters, fusel alcohols, and terpenoids by 3%, 1%, and 17%, respectively, and decreased fatty acids, shikimic acid derivatives, and C_6 alcohols by 23%, 4%, and 3%, respectively. The SL treatment increased esters and terpenoids by 4% and 31%,

respectively, and decreased fusel alcohols, fatty acids, shikimic acid derivatives, and C_6 alcohols decreased by 5%, 30%, 11%, and 32%, respectively (Figure 2.1).

Sensory test. Panelists were able to distinguish between SL/CL and SE/SL at $p < 0.01$ for 2007 Foch wine (Table 2.6). Panelists were able detect differences in fruitiness between SE and SL, CE and CL ($p < 0.01$), and CE and SL ($p < 0.05$) for 2008 Foch wine (Table 2.7).

Table 2.6 Sensory results from triangle test of Marechal Foch, 2007. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

Treatment comparison	Correct responses out of 24	Probability of result by chance (%)
ST, early/Control, early (SE/CE)	5	0.941
ST, late/Control, late (SL/CL)	14	0.010
ST, early/ST, late (SE/SL)	14	0.010
Control, early/Control, late (CE/CL)	9	0.406

Table 2.7 Sensory results from 2-AFC test of 2008 Marechal Foch. Control = no shoot thinning, ST = shoot thinning (15 primary shoots per meter), early = early harvest (11 Sept. 2007, 10 Sept. 2008), late = late harvest (18 Sept. 2007, 23 Sept. 2008)

Treatment	Proportion 1"more fruity" than 2	d'	Variance of d'	Standard deviation of d'	*P* value
(1-SE/2-CE)	0.57	0.25	0.23	0.524	0.300
(1-CL/2-CE)	0.79	1.15	0.29	2.135	0.016
(1-SL/2-SE)	0.79	1.15	0.29	2.135	0.016
(1-SL/2-CL)	0.57	0.25	0.23	0.524	0.300
(1-SL/2-CE)	0.86	1.53	0.35	2.593	0.005
(1-SE/2-CL)	0.5	0	0.22	0.474	0.500

DISCUSSION

Effects of shoot thinning. The shoot densities in this study were 15 shoots per meter of row and no shoot thinning. Although vines were not very vigorous (2.5 to 3.2 OLN in 2007, 2.9 to 3.2 OLN in 2008) and were highly cropped in both years, shoot thinning improved CEFA in both years of the study. Higher LEFA of shoot-thinned vines in 2008 suggests a possible increase in canopy photosynthesis (21), which may be attributed to the improved Brix. Reducing the number

of shoots per vine also resulted in less clusters per vine; hence, lower yield and generally higher Brix (22).

Shoot thinning increased berry TA in 2007, but no effect was observed in 2008. The effect of shoot thinning on TA in previous reports is similarly inconsistent. The increase in anthocyanin in grapes but not in wines of shoot-thinned treatments may have been mediated by increased light exposure (23), although other reports have not observed an increase (11). It is not clear why the differences in berry anthocyanin concentration did not persist into the finished wines. Other differences in color composition or appearance may have occurred, such as changes in polymeric pigment or tristimulus values, but these were not measured in our study.

The shoot-thinning treatment resulted in higher berry skin tannin (10 to 30%) in both years, which is consistent with a previous report (11), although the increase was not apparent in wine tannin. Seed tannin was not affected by the shoot-thinning treatment, in contrast to other work (11). The Foch wines in our study had very low tannin (29 to 60 mg/L catechin equivalents), in concordance with anecdotal reports that wines produced from Marechal Foch and other French-American hybrids possess low astringency. By comparison, the mean tannin concentration in California, Oregon, and Washington State V. vinifera red wines is reportedly 544 mg/L, with less than 2% of wines reported to have <100 mg/L tannin (24). The standard Adams-Harbertson has a loss of accuracy for tannin concentrations <100 mg/L (25). However, even with a two-fold allowance for error, this work provides the first confirmation of low tannins in French-American hybrid wines by a protein-precipitation assay.

Skin tannin in our study ranged from 0.19 to 0.23 mg/berry and the total tannin concentration ranged from 0.82 to 1.12 mg/berry. Skin tannin concentration per berry in Foch is ~60% less

than values reported in Cabernet Sauvignon and Syrah (26), although the concentrations are more similar on a by-weight basis because of the smaller berry size of Marechal Foch. The seed tannin concentration per berry is similar to values reported in vinifera (26), where tannin extractability is calculated by dividing the tannin quantity in wines by the tannin in grapes and correcting for yield during pressing. We calculated that only 2 to 4% of tannin in Marechal Foch fruit of this study was extracted into wine during winemaking. For wine made from vinifera, extractability is reported to range from 4.9 to 61% (26), with the lowest extractability reported for Pinot noir. Both Foch and Pinot noir possess low levels of skin tannin, which is reported to be extracted more rapidly during fermentation than seed tannin. A low extractability of total tannin (9%) from Pinot noir during winemaking has been reported, with higher extractability (29% versus 6%) of skin tannin versus seed tannin (27). Using this reported extraction efficiency, we would expect a median concentration of 155 mg/L total wine tannin (91 mg/L from skin tannin and 64 mg/L from seed tannin) from Foch, or about a factor of 3 greater than what we observed. The very low tannin concentration of Foch wines compared to most V. vinifera wines appears to be due to both its lower skin tannin concentration and to lower tannin extractability (comparable to or less than Pinot noir). Factors that decrease tannin extractability from winegrapes during winemaking are poorly understood. Previous studies reported it is because of tannin binding to grape cell walls (28, 29). It is also hypothesized to be due to increased polysaccharide-tannin interactions during grape maturation. Further study will be necessary to determine if this is a general phenomenon for other hybrid winegrapes.

The aroma analysis did not identify any “beet” or “radish” aromas as reported by the local grape and wine industry in Foch. Shoot thinning impacted only a few aroma compounds in wines, and the impact of the treatment was often inconsistent across years or harvest dates. For example,

concentrations of some esters (ethyl lactate, hexyl acetate), a fusel alcohol (methionol), and fatty acids (hexanoic acid, octanoic acid) all decreased as a result of the shoot-thinning treatment in 2008, but this effect was not apparent in 2007. All compounds mentioned above are derived from fermentation. Although winemaking conditions were the same for all treatments, the initial soluble solids, pH, and composition of grape juice varied among treatments and between years, which may have affected formation of the compounds. For example, both total yeast assimilable nitrogen (YAN) concentration and the relative proportions of amino acids composition in juice are reported to modify concentration of esters, fusel alcohols, and fatty acids during fermentation (30). However, even in cases where the differences were significant,
the magnitude of the effect caused by shoot thinning was generally small (<20%).

The shoot-thinning treatment resulted in a consistent decrease in C_6 alcohols (1-hexanol, cis-3-hexenol, and trans-2-hexenol) in finished wines across both harvest dates and years of study. These C_6 alcohols possess herbaceous aromas and can be formed immediately following crushing of grape berries from lipid-precursors or by reduction of analogously formed C_6 aldehydes during fermentation (31). Several groups have reported that the total C_6 concentration of V. vinifera grapes (aldehydes + alcohols) decreases during grape ripening (31, 8), but to our knowledge the impact of canopy-management practices on resultant levels of C_6 compounds in wines has not been reported. Although the importance of the C_6 alcohols to Marechal Foch wines still needs to be demonstrated, the current work demonstrates that shoot thinning can be used to reduce these potentially negative compounds.

Effects of harvest date. Harvest date impacted basic fruit and wine chemistry as expected. Later harvest dates resulted in grapes with higher pH, higher Brix, and lower TA. The resulting

wines had higher ethanol concentration. Harvest date did not affect OLN, CEFA, or LEFA in either year.

Harvest date increased both berry and wine anthocyanins. The increase in berry anthocyanins was calculated as mg/g fresh skin weight and likely indicates continued accumula tion of anthocyanins during maturation. In 2007, the higher anthocyanin concentration of late harvest wines may also be partially due to berry dehydration (decrease of 0.075 g in average berry weight between early and late harvest). In 2008, the CL and SL berries contained lower seed tannin, but that did not translate into increased wine tannin, likely because of the low extractability of seed tannin.

Among the aroma compounds, the herbaceous C_6 alcohols showed the most consistent and greatest percent reduction as a result of the CL and SL treatments. Late harvest wines possessed lower 1-hexanol, cis-3-hexenol, and trans-2-hexenol than their early harvest counterparts. As mentioned previously, lower levels of C_6 aldehydes and alcohols are reportedly formed from more mature grapes following crushing (31, 8). Although the aldehydes are reduced to their corresponding alcohols during fermentation (29) (Joslin and Ough 1978), a recent report did not observe a clear correlation between C_6 compounds in wine and berry maturity (32). The shoot-thinning treatment also reduced C_6 alcohols, and no significant interaction term (harvest date x treatment) was observed with the exception of the 2008 trans-2-hexenol levels. Thus, in most cases, harvest date and shoot thinning appear to independently reduce C_6 alcohols. Potentially, growers could use a combination of later harvest and shoot thinning to reduce these herbaceous compounds in Foch, although future sensory studies are necessary to establish their sensory importance.

The other compounds measured in our study (esters, fusel alcohols, fatty acids, terpenoids, and shikimic acid derivatives) did not vary consistently among years between different harvest dates. One exception was the straight-chain fatty acids (octanoic and butanoic), which decreased in both years with both treatments. Production of straight-chain fatty acids by yeast during fermentation is linked to several factors, including the availability of unsaturated fatty acids, oxygen, and fermentation temperature (33). While the latter two factors are not expected to vary, the concentration of polyunsaturated fatty acids is reported to decrease with grape maturity (34), potentially resulting in greater mid-chain fatty acid production (35).

Sensory experiments. Results indicated that harvest date is generally more important than shoot-thinning treatment in affecting fruitiness. Thus, even though shoot thinning resulted in some changes to berry chemistry, that did not translate into differences in fruitiness. However, the panel also observed no difference in fruitiness between CL and SE treatments, indicating that shoot thinning may permit an earlier harvest to achieve similar levels of fruitiness.

CONCLUSION

Shoot-thinning treatments (15 shoots/m) on Marechal Foch grapevines resulted in improved canopy microclimate (CEFA, LEFA), decreased yield, and improvement in some chemical parameters (higher Brix and anthocyanins in berries and decreased concentrations of the herbaceous C_6 alcohols in resulting wines). However, the impact of shoot thinning was generally comparable to or less than the differences observed with late harvest. Similarly, sensory evaluations indicated that 2008 wines produced from CL and SL treatments were fruitier than their early harvest counterparts but that shoot-thinned treatments were not different than their nonthinned counterparts. Therefore, delayed harvest may have a larger impact on the flavor chemistry of Marechal Foch than shoot thinning. Finally, there was both low skin tannin

and low tannin extractability in Marechal Foch grapes and, consequentially, very low levels of tannin in the resulting wines. Increasing tannin extraction from Foch or other hybrids during winemaking may be an interesting direction for improving the chemosensory attributes of the resulting wines. Growers and winemakers should delay harvest on Foch to improve fruitiness and decrease herbaceousness of wines.

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CHAPTER 3

SHOOT THINNING AND CLUSTER THINNING IMPACT YIELD, FRUIT COMPOSITION, AND WINE QUALITY OF COROT NOIR

ABSTRACT

Cluster thinning (CL), shoot thinning (ST), and a combination of the two practices (ST+CL) were applied to 5-year-old vigorous Corot noir (hybrid sp.) wine grapes growing at a commercial vineyard in the Finger Lakes region of New York State. Yield was reduced by CL (by 3.6 kg/vine) but not ST in 2008, but by ST (by 1.0 kg/vine) and not CL in 2009, however high pruning weights (up to 4.5 kg/vine in 2008) and low cropload ratios (ranging from 2.3 – 7.1) indicated that vines in the study were undercropped in both years regardless of treatment. CL increased Brix by 1.3 in 2008, and by 0.4 in 2009, while ST increased Brix in 2009 by 0.45. The treatments had variable impacts on wine anthocyanin, berry skin tannin, berry seed tannin, and wine tannin depending on year. Wine tannin (40-60 mg/L) and tannin extractability (5-6%) were both very low in comparison to values typically observed in red wines produced from *vinifera*. Using a two-alternative forced choice test, panelists reported ST+CL wines were more "fruity" than the control and the ST only wines in both years of the study, and that the CL wine was more "fruity" than the control in 2008. An economic analysis indicated that in order for growers/wineries to maintain their economic welfare, bottle prices would have to increase by $0.02 - $0.41 depending on the practice and year to compensate for additional labor costs and lost yield.

INTRODUCTION

Corot noir is a complex interspecific red hybrid winegrape released by Cornell University in 2006 (1). It ripens mid- to late-season, is appropriate for either blending or the production of varietal wines, and is moderately winter hardy (-10°F) with moderate resistance to fungal diseases (1). Observations made at Geneva NY from 1996 – 2005 indicated that the vine size of Corot noir was smaller than GR7 (average pruning weight of 1.4 kg/vine compared to 2.1 for GR7), and observations made during roughly the same period at three locations in Indiana also suggest a small vine size (average pruning weight of 0.6 kg/vine from 2000-2005 compared to 1.0 kg/vine for Marechal Foch at Vincennes, IN) (Reisch et al. 2006). However, anecdotal observations by commercial growers in the Finger Lakes region of NY have characterized Corot noir as perhaps the most vigorous French-American hybrid grown in the region, exhibiting high fruit yield, vigorous canopy growth, and low cluster light exposure.

Both shoot thinning (ST) and cluster thinning (CL) are recommended viticultural practices for French-American hybrids due to their propensity to over-crop (2). ST has been shown to improve fruit quality of several French-American hybrids such as Seyval blanc (3), Aurore, Chancellor and Villard noir (4) and Marechal Foch (5). CL is commonly used as a corrective viticultural measure to allow the remaining fruit to reach a higher level of maturity by improving the leaf area to fruit ratio, and has been demonstrated to improve various quality metrics in Vidal Blanc (6, 7), Chambourcin (8), and De Chaunac (9, 10). While these studies indicate that canopy management practices can often result in improved fruit quality, in a commercial vineyard operation the additional costs associated with implementing CL and ST would need to be carefully considered along with the economic benefits of potential enhancements to fruit quality (11). Due to the recent release of Corot noir, there is little information available on best

management practices for this cultivar. The objective of this study was to investigate the impact of ST and CL treatments on yield components, fruit composition, and wine quality of Corot noir.

MATERIALS AND METHODS

Vineyard site and experimental design. Five-year-old own-rooted Corot noir vines located at a commercial winery on the west side of Cayuga Lake in Romulus, NY were subjected to canopy and cropload management treatments in 2008 and 2009. Soil in the block was a Darien-Danley Cazenovia silt loam (USDA-NRCS soil maps). Vines were spaced at 8 x 9 feet (vine x row) in north-south oriented rows and trained to a high wire cordon system (12) with three vines per panel. Because the northern end of the vineyard was lower in vigor than the southern end, mulch was applied in 2009 to the two replications of the experiment located in the northern end of the planting. Drip irrigation was installed throughout the vineyard. Standard pest control practices for the region were used.

The experimental design consisted of two ST treatments (no ST and ST) combined with two CL treatments (no CL and CL) in a randomized complete block design with four replications. Each experimental unit consisted of thirteen contiguous panels of vines, with two panels in each plot randomly selected at the beginning of the experiment for data collection. For the ST treatment, 15 primary shoots were retained per meter, and all secondary, tertiary, and non-count shoots were removed. ST was conducted when shoots reached approximately 2-5 inches in length in May. No ST was applied in the control plots. For the CL treatment, the distal cluster was removed from each shoot which had more than two clusters. CL was conducted when berries reached approximately pea-size, and no CL was applied in the control plots.

Yield components. Vines were individually harvested by hand on 21 October 2008, and 16 October 2009 just prior to commercial harvest. Yield per vine was quantified using a hanging

scale (Salter Weigh-Tronix, Fairmont, MN), and cluster number per vine was counted. Cluster weights were calculated by dividing yield by cluster number on a per vine basis. A random sample of 20 clusters per data panel was collected at harvest and stored at -40 °C until analysis. Subsamples of 100 berries were weighed to determine mean berry weight. Berry number per cluster was calculated by dividing cluster weight by berry weight.

In January 2010, vines were pruned according to grower specifications and pruning weights were collected on a per vine basis in each data panel. Cropload for 2009 was calculated by dividing yield by pruning weight on a per vine basis. In January 2009, the grower pruned all vines in the planting prior to data collection; as a result, pruning weights were estimated on a per panel basis by raking and weighing the vine prunings which were lying on the vineyard floor between the posts defining the data collection panels. Cropload for 2008 was calculated by dividing yield by pruning weight on a per panel basis.

Canopy Characterization. Enhanced point quadrat analysis (EPQA) (13) was used to characterize canopy light environment at approximately veraison in both years of the study. A sharpened thin metal rod was inserted into the canopy at 10cm intervals and sequential contacts of leaves, clusters and canopy gaps from one side to the other were recorded. Photon flux measurement was performed according to a previously described method (13) (Meyers and Vanden Heuvel 2008) using a Decagon ceptometer (Decagon, Pullman, WA). Canopy parameters were analyzed by EPQA and CEM Tools (version 1.6) (available from Jim Meyers, jmm533@cornell.edu).

Berry and wine composition. A 100-berry sample was collected randomly in duplicate from each sample that was kept frozen as described above. The frozen berries were thawed at room temperature before analysis. The berries were juiced by a blender and the slurry was pressed

through cheese cloth. Degrees Brix was measured using an Abbé temperature-compensated refractometer (ATAGO, Bellevue, WA). Berry and wine pH were measured using an Orion 3-Star pH meter (Thermo Fisher Scientific, Waltham, MA), and titratable acidity (TA) was determined on a 10 mL sample by autotitration (Digital Buret™, BrandTech Scientific, Essex, CT) using 0.1 M NaOH to an endpoint of pH=8.2. Wine alcohol content was measured by ebulliometer (DuJardin-Salleron, France). Wine lactate, acetate, glucose, and fructose were quantified by FT-IR (WineScan FT120 Basic, FOSS, Denmark) to confirm that fermentations went to dryness and spoilage did not occur (data not shown). Wine free SO_2 was measured by the Ripper method (data not shown). Berry and wine anthocyanins and tannins were determined by the Adams-Harbertson assay (14). Tannin extractability was calculated by dividing the tannin quantity in wines by the tannin in grapes and correcting for yield during pressing.

Winemaking. Wines were made in duplicate after combining field replicates for each treatment. Fruit was destemmed, crushed, and treated with 50 mg L^{-1} sulfur dioxide added as potassium metabisulfite. Diammonium phosphate (DAP) (Presque Isle Wine Cellars, PA) was added at a rate of 1 g kg^{-1}, Fermaid K (Lallemand, Rexdale, ON) at 0.1 g L^{-1} and Goferm (Lallemand, Rexdale, ON) at 0.15 g L^{-1}. Skin fermentation was performed in temperature controlled 114-liter stainless steel fermenters. Cap management was performed twice per day by manual punchdowns. The must was brought to 20 ºC and inoculated with ICV-GRE (Lallemand, Rexdale, ON) to 1 g $gallon^{-1}$. The temperature profile of the fermentations was computer controlled. During the first three days of fermentation, the must was warmed slowly from 20 ºC to a maximum between 30 ºC to 35 ºC. For the remainder of the alcoholic fermentation, the temperature was kept between 20 ºC to 30 ºC. Fermentations were stopped when residual sugar was <0.5% by Clinitest tablets (Bayer, Etobicoke, ON). At the end of fermentation, wines were

pressed, racked into glass carboys, and inoculated with Alpha (Lallemand, Rexdale, ON) to start malolactic fermentation (MLF). Upon completion of MLF, potassium metabisulfite was added to maintain 40 mg L^{-1} free sulfur dioxide. Wines were cold stabilized at 2 ºC. Titratable acidity was adjusted to 6.5 g L^{-1} by addition of tartaric acid or potassium carbonate after cold stabilization. The wines were screened for faults by an expert panel prior to bottling. Bottling and screwcapping were performed manually.

Sensory test. Wines from 2008 and 2009 were evaluated after bottle aging and compared in November 2010 for all four treatments by 2-alternative-forced choice (2-AFC) test (15, 16). Sixteen panelists with wine evaluation experience were recruited for the test. A pair of coded samples for comparison was presented to panelists. The panelists were asked to select the sample with the stronger fruitiness (15, 16). Wines from each treatment were compared to one another. Each comparison was duplicated. One wine sample was randomly selected for sensory evaluation from duplicate wines. Pre-sensory testing was performed to ensure the replicates did not differ.

Statistical analysis. Mixed model ANOVAs was performed using JMP (version 8.0; SAS Institute, Cary, NC). The 2-AFC test statistical analysis was performed by the method of Bi et al (15).

RESULTS AND DISCUSSION

Yield components and vine growth. In 2008, ST and CL separately decreased yield through a decrease in cluster number per vine compared to the control. The yield was reduced by 1.445 kg per vine and 3.565 kg per vine by ST and CL, respectively (Table 3.1). However, individual cluster weight was increased 45 g by ST and 45 g by CL (Table 3.1). Cropload ratio decreased

2.04 by ST and 2.78 by CL (Table 3.1). The ST by CL interaction was significant for cluster number per vine, berry number per cluster, and berry weight.

Table 3.1 Impact of shoot thinning and cluster thinning on yield components of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning

Treatment	Yield /vine (kg)	Clusters /vine	Cluster wt. (kg)	Berries /cluster	Berry wt. (g)	Pruning wt. (kg)	Cropload (kg yield/kg pruning weight)
			2008				
Control	15.4	76.9	0.20	98.7	2.22	2.7	7.1
ST + CL	10.4	36.4	0.29	113.7	2.43	4.5	2.3
CL	10.6	47.0	0.23	99.6	2.27	3.0	4.7
ST	12.8	54.7	0.23	93.1	2.29	2.6	5.5
P value for shoot thinning	0.0777	<0.0001	0.0002	<0.0001	0.0005	0.0747	0.0075
P value for cluster thinning	<0.0001	<0.0001	0.0007	<0.0001	0.0011	0.2540	0.0004
P value for ST x CL	0.1453	0.0354	0.2582	0.0007	0.0053	0.1803	0.5781
			2009				
Control	6.3	55.8	0.11	55.5	2.04	1.1	5.1
ST + CL	4.6	29.7	0.15	59.9	2.55	1.4	3.7
CL	4.6	34.4	0.13	57.8	2.31	1.3	4.2
ST	4.3	32.9	0.13	51.6	2.47	1.2	4.0
P value for shoot thinning	0.0254	<0.0001	0.0328	0.6433	<0.0001	0.2687	0.0488
P value for cluster thinning	0.1348	<0.0001	0.0015	0.0053	<0.0001	0.0328	0.1613
P value for ST x CL	0.0214	0.0008	0.5179	0.0668	0.0007	0.9014	0.3545

In 2009, CL resulted in 12 fewer clusters per vine compared to the non-cluster thinned treatments but the increased average cluster weight (due to an increase in berry number) in the cluster thinned treatments resulted in CL having no impact on yield per vine, similar to results reported for Seyval blanc (3). ST significantly reduced yield per vine through a decrease in cluster number (Table 3.1). Cropload ratio was reduced 0.78 by ST, while CL surprisingly had no impact on cropload at harvest. Individual cluster weight was increased 20 g by ST and CL separately (Table 3.1). There was a significant interaction between ST and CL for clusters per vine, berries per cluster, and berry weight in 2008. The yield, cluster per vine, berries per cluster and berry weight were also affected by both ST and CL in 2009 (Table 3.1). The ST by CL interaction was significant for yield per vine, clusters per vine, and berry weight in 2009.

Pruning weights and yields in both years confirm the grower reports of high vigor in Corot noir. Pruning weights reported here for 2008 (ranging from 2.5 to 4.5 kg/vine) are considerably higher than those reported for other hybrids on non-divided systems in other cool-climate growing regions (17, 18, 19) as well as in NY (5). Concords in NY are generally reported to have pruning weights below approximately 2 kg/vine (20, 21), suggesting that Corot noir, in some instances, may produce a larger canopy than Concord. However, hybrids in Arkansas have been reported to have similar vigor to that reported here for Corot noir (22, 4). Vines in all treatments of this study had croploads that were below the generally accepted range for hybrids (17, 23, 19) likely resulting in increased vegetative growth of vines.

ST and CL separately reduced canopy density as reflected by the reduction in OLN by 1.225 and 0.825 respectively in 2008, and a similar decrease in CEL of 0.37 and 0.29, respectively. While there were fewer shade-producing contacts in the canopy as well as fewer shading layers between clusters and the nearest canopy boundary, the percentage of photon flux that reached the clusters (CEFA) and leaves (LEFA) did not differ among treatments, suggesting that if changes in canopy density impacted fruit growth and composition it was not through changes in cluster or leaf light environment. ST and CL had no impact on OLN, CEL, CEFA, or LEFA in 2009 (Table 3.2).

Fruit and wine composition. In 2008, Brix was increased by approximately 1.3 with CL (Table 3.3), but was not affected significantly by ST. However, pH increased slightly and TA decreased (by approximately 0.78 g/L) as a result of ST, while CL had no impact on either parameter (Table 3.3). In 2009, berry pH and Brix were increased by ST and CL, however the increases were small (<0.5 Brix). TA decreased by <0.5 g/L as a result of ST (Table 3.3), but was unaffected by CL, which is a result that has been similarly reported in other hybrid CL

studies (17, 24). Decreases in TA during ripening can be due to both increases in potassium uptake and

Table 3.2 Canopy characterization of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning

Treatment	OLN	CEL	CEFA	LEFA
		2008		
Control	4.75	1.39	0.17	0.31
ST + CL	2.70	0.74	0.27	0.35
CL	4.37	1.24	0.23	0.33
ST	3.97	1.16	0.26	0.37
P value for shoot thinning	0.0026	0.0100	0.0758	0.1033
P value for cluster thinning	0.0318	0.0414	0.3542	0.9701
P value for ST x CL	0.2546	0.2538	0.4888	0.4794
		2009		
Control	5.02	1.79	0.06	0.23
ST + CL	5.89	2.10	0.07	0.22
CL	6.33	2.28	0.05	0.21
ST	5.79	2.02	0.11	0.23
P value for shoot thinning	0.8935	0.8040	0.1148	0.9869
P value for cluster thinning	0.1390	0.3016	0.2555	0.0856
P value for ST x CL	0.2274	0.5260	0.6563	0.5242

OLN = occlusion layer number, CEL = cluster exposure layer, CEFA = cluster exposure flux availability, LEFA = leaf exposure flux availability

increased respiration of malic acid. Malic acid respiration increases at higher berry temperatures, which can result from greater cluster exposure. However, since the treatments did not alter cluster light environment, greater potassium uptake may be a more plausible explanation for the decrease in TA observed with ST. Berry anthocyanin concentration, expressed as malvidin-3-glucoside equivalents, ranged from 950-1200 mg/kg berry weight across treatments and years. These concentrations are comparable to several of the more intensely pigmented *vinifera* cultivars such as Cabernet Sauvignon (25) (Table 3.3). Although no difference was observed in grape anthocyanin concentrations among treatments, a modest increase (20%) was observed in 2009 as a result of shoot thinning. The concentration of anthocyanins in wines ranged from 670-900 mg/L, or a mean of 67% of the concentration observed in grapes, with a maximum of 86% observed for the shoot thinned and cluster thinned treatment. Both of these values are about a

factor of 2 greater than typical anthocyanin concentrations (300-500 mg/L) and typical extraction efficiency (20-50%) for red wines (25) Anthocyanin extraction efficiency is well known to vary among cultivars and sites (26) although the factors that limit anthocyanin extractability are not well understood. The pH differential method employed is designed to quantify monomeric anthocyanins, and a possible explanation for our observed results is that the low tannin concentration of the Corot noir wines (described below) resulted in reduced polymeric pigment formation and consequentially less immediate loss of the anthocyanins. Interestingly, we also observed a significantly higher extraction ratio in 2009 than in 2008 (75% vs. 59%, $p<0.05$) although the reasons for this difference are not obvious. No significant difference in berry skin weight (data not shown) or berry weight were observed, so greater adsorption to solids seems unlikely.

Berry skin tannin and seed tannin concentrations ranged from 0.2-0.3 mg/g berry and 0.8-1.0 mg/g berry, respectively, which are comparable to some *V. vinifera* cultivars (27) Berry skin tannin was not affected by ST or CL, however berry seed tannin was reduced slightly by ST (Table 3.4). Neither skin nor seed tannin was affected by the CL treatment (Table 3.4). Berry skin tannin was decreased 0.070 mg/berry by CL, but was unaffected by ST; seed tannin was not affected by either practice. Wine tannin was decreased by both ST and CL (Table 3.4), but the concentrations of wine tannin (40-60 mg/L) were an order of magnitude below typical concentrations observed in red *V. vinifera* wines (28). The tannin extractability was very low (6%) (Table 3.4), compared to the wine made from *V. vinifera* species (4.9 to 61%) (27). This finding is consistent with a previous study which reported that the tannin extractability of hybrid cultivar Marechal Foch was 2-4% (5). The low tannin extractability is likely due in part to low

skin tannin concentrations in Corot noir (0.2-0.3 mg/berry) compared to *V. vinifera*, as skin tannin appears to be more rapidly and effectively extracted during fermentation (29).

Table 3.3 Impact of shoot thinning and cluster thinning on berry and wine composition of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning. Musts were chapatalized prior to fermentation to the same potential alcohol.

	Berry			Wine		
Treatment	**pH**	**Brix**	**Titratable acidity (TA) (g/L)**	**pH**	**Alcohol (% v/v)**	**Titratable acidity (TA) (g/L)**
			2008			
Control	3.60	15.0	8.6	3.51	9.7	5.8
ST + CL	3.75	16.7	7.5	3.68	10.0	5.8
CL	3.71	17.5	8.0	3.60	10.1	5.8
ST	3.66	16.6	7.6	3.58	9.6	5.7
P value for shoot thinning	0.0150	0.4924	0.0250	0.0007	0.1462	0.3739
P value for cluster thinning	0.2529	0.0289	0.2448	0.0003	0.0034	0.3739
P value for ST x CL	0.9930	0.0505	0.4867	1.0000	0.8512	0.3739
			2009			
Control	3.53	15.8	11.1	3.51	8.3	6.7
ST + CL	3.55	16.6	10.8	3.57	9.1	6.6
CL	3.58	16.5	11.0	3.56	9.0	6.6
ST	3.55	16.6	10.5	3.58	9.0	6.6
P value for shoot thinning	0.0381	0.0072	0.0149	0.0008	0.0347	0.1583
P value for cluster thinning	0.0002	0.0476	0.7788	0.0008	0.0232	0.1583
P value for ST x CL	0.0007	0.0649	0.2370	0.0075	0.0534	0.1583

Additionally, insoluble grape cell wall material can bind to tannins and limit its extractability (30). Potentially, the cell wall material of hybrid winegrapes may bind tannins more strongly than in *V. vinifera*, although this would need to be investigated.

ST and/or CL has been reported to improve fruit composition (particularly Brix) in the more vigorous hybrid cultivars, at least in some years (3, 6, 17). The results of this study were variable, but CL increased Brix in both years, while ST reduced TA and increased berry anthocyanin concentration in both years. However, in only one of two years was the improvement in berry anthocyanin concentration reflected in wine anthocyanin concentration.

Table 3.4 Impact of shoot thinning and cluster thinning on berry and wine anthocyanin and tannin of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning

Treatment	Berry anthocyanin (mg/kg M-3-G[a] fresh berry weight)	Wine anthocyanin (mg/L M-3-G)	Berry skin tannin (mg/berry catechin)	Berry seed tannin (mg/berry catechin)	Wine tannin (mg/L catechin)	% tannin extraction
		2008				
Control	1071	671	0.28	1.18	63.6	6.3
ST + CL	1260	705	0.24	0.98	49.9	6.5
CL	1098	700	0.27	1.06	44.0	4.9
ST	1260	686	0.25	0.87	48.2	6.4
P value for shoot thinning	0.2912	0.2928	0.3156	0.0158	0.2511	
P value for cluster thinning	0.9411	0.9575	0.6798	0.9201	0.0495	
P value for ST x CL	0.9394	0.3622	0.9174	0.1166	0.0524	
		2009				
Control	944	709	0.31	1.01	61.4	6.2
ST + CL	1045	897	0.19	0.84	42.1	6.1
CL	1100	735	0.22	1.08	53.6	6.6
ST	1147	874	0.24	1.07	52.1	6.6
P value for shoot thinning	0.6298	0.0014	0.0819	0.5904	0.0251	
P value for cluster thinning	0.7985	0.3095	0.0368	0.6436	0.0407	
P value for ST x CL	0.4705	0.9485	0.5004	0.3949	0.7229	

Wine sensory analysis. Panelists were able detect differences in fruitiness between ST+CL and the control from both vintages (Table 3.5). An additional difference in fruitiness was observed between ST+CL and ST only in both years, and between the CL and control in 2008 (Table 3.5). Surprisingly, results from the sensory analysis were reasonably consistent across both years, as the growing seasons differed greatly with respect to accumulated GDD and precipitation (data not shown).

Table 3.5 Sensory results from 2-AFC test of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning

Treatment	Proportion 1"more fruity" than 2	d'	Variance of d'	Standard deviation of d'	*P* value
		2008			
(1-ST+CL/2-Control)	0.75	0.95	0.23	0.482	0.024
(1-CL /2-Control)	0.75	0.95	0.23	0.482	0.024
(1-ST/2-Control)	0.18	0.00	0.12	0.346	0.500
(1-ST+CL/2-CL)	0.43	0.00	0.20	0.440	0.500
(1-ST/2-CL)	0.5	0.00	0.20	0.443	0.500
(1-ST+CL /2-ST)	0.93	2.16	0.48	0.694	0.001
		2009			
(1-ST+CL/2-Control)	0.75	0.95	0.23	0.482	0.024
(1-CL /2-Control)	0.31	0.00	0.16	0.411	0.500
(1-ST/2-Control)	0.31	0.00	0.16	0.411	0.500
(1-ST+CL/2-NSTCL)	0.43	0.00	0.19	0.440	0.500
(1-ST/2-CL)	0.5	0.00	0.20	0.443	0.500
(1-ST+CL /2-ST)	0.88	0.88	0.32	0.568	0.002

Cost of implementing practices. A simple accounting analysis of the costs of implementing CL and ST was performed in order to depict a realistic financial scenario for commercial Corot Noir growers regarding the potential costs and benefits of adopting these practices. Variable production costs of growing winegrapes in the Finger Lakes region of NY were estimated based on a published report of *V. vinifera* production (31). The two primary costs associated with implementing ST and/or CL are: (1) additional vineyard labor hours required to complete the thinning in consort with other ongoing field practices; and (2) potential opportunity cost if total vine yield is decreased as a result of either practice and thus there are fewer grapes to sell and/or make into wine. It is likely that the cost of labor estimates used in our analysis that were determined for *V. vinifera* (31) are lower than would be required for hybrids due to the generally larger canopy size of hybrids; therefore required prices to compensate for labor costs may be higher than reported here. If a grower has to spend money to implement cluster and/or ST, and then has fewer grapes to sell at the standard industry price, then the grower can expect to receive lower total revenue for their crop. Logically, if a grower expects to maintain the same level of

welfare (total revenue) following implementation of ST and/or CL, the price they charge for Corot Noir grapes would need to be above market price.

Table 3.6 Production cost and price analysis of Corot noir. Control = no shoot thinning and no cluster thinning, ST = shoot thinning (15 primary shoots per meter), CL = cluster thinning

Treatment	Additional production cost/ha	Additional production cost/tonne	Yield tonnes/ha	Expected revenue/ha[U]	Grower preferred price/tonne to maintain welfare[V]	Additional cost per bottle[W]
			2008			
Control	$0.00	$0.00	28.3	$16,138.92	$569.71	$0.00
ST+CL	$286.64[X]	$15.03	19.1	$10,862.74	$861.46	$0.41
CL	$153.21[Y]	$7.03	21.8	$12,414.56	$747.65	$0.25
ST	$133.44[Z]	$5.21	25.6	$14,587.10	$635.53	$0.09
			2009			
Control	$0.00	$0.00	11.7	$5,886.54	$502.58	$0.00
ST+CL	$286.64	$33.95	8.4	$4,243.78	$731.07	$0.32
CL	$153.21	$13.08	11.7	$5,886.54	$515.56	$0.02
ST	$133.44	$13.61	9.8	$4,928.26	$613.91	$0.15

[U]Expected revenue a commercial grape grower can expect to receive for Corot noir calculated by multiplying yield (tonnes) by the reported average industry price for Corot noir (2008=$569.71/tonne, 2009=$502.58/tonne)
[V] Price per tonne of Corot noir that a commercial grape grower would need to charge in order to compensate for the two main costs associated with implementing thinning practices: reduced grape yield and additional production costs.
[W] Additional cost per bottle produced after implementing thinning practices, if the commercial grower keeps the grapes to make wine instead of selling them at a market price. Assumes 491.4 L of wine (or 655.2 bottles) per tonne of grapes
[X] Additional production cost per hectare for shoot thinning and cluster thinning in *V. vinifera*, estimated from White (2008)
[Y] Additional production cost per hectare for cluster thinning in *V. vinifera*, from White (2008)
[Z] Additional production cost per hectare for shoot thinning in *V. vinifera*, from White (2008)

In 2008 and 2009 the average prices for Corot Noir grapes in the Finger Lakes were $569.71 and $502.58 per metric tonne, respectively (32, 33). As detailed in Table 6, the price per ton of Corot Noir grapes in 2008 that a grower would need to charge in order to maintain constant welfare would increase from a base market price of $569.71 per metric tonne for the control up to $861.46 per metric tonne for the shoot- and cluster-thinned vineyard (an increase of 51%). For the same parameters in 2009, the price per ton to maintain constant grower welfare would need to increase from $502.58 per metric tonne to$731.07 per metric tonne (an increase of 45%). The variability in these prices reflects the specific practices implemented (Table 3.6). The increase in price per 750 mL bottle required to compensate for the higher fruit costs plus additional vineyard labor ranged from $0.09-$0.41 in 2008, and from $0.02-0.32 in 2009 (Table 3.6). It is unclear

whether hybrid fruit growers who implement ST and/or CL would actually be able to re-capture their costs by charging substantially higher market prices, or whether grape buyers would be willing to pay such a premium without compelling enhancements to fruit quality or flavor. However, the sensory analysis results in this study indicate a consistent increase in fruitiness with CL (alone or combined with ST), a characteristic positively associated with consumer preference (34). Wine consumers may be willing to pay more for such wines, or may be more likely to purchase the wine again. Ultimately, a hybrid grape grower's decision of whether to implement thinning practices is best determined based on a rational analysis of the potential costs and benefits similar to the one presented here.

CONCLUSION

The Corot noir vines used in this study were extremely vigorous, with high pruning weights and low croploads. While the impact of ST on yield and yield components varied in the two years of this study, ST was consistent in reducing both cropload and TA, and increasing berry anthocyanin concentration, in both years. CL also demonstrated a variable impact on yield and yield components, but improved Brix in both years. Increasing retained node numbers during dormant pruning may have more successfully improved fruit composition by reducing overall vine vigor. Surprisingly, the impact of ST and CL on the wine sensory analysis was consistent: in both years of the study, the panelists reported that CL alone resulted in a more fruity wine when the vines were shoot-thinned, and that ST combined with CL increased the perception of fruitiness in wines when compared to the control. Poor tannin extraction from Corot noir was observed, which is consistent with previous work on Marechal Foch, and could potentially be the result of binding between cell wall material and tannins. If this occurs, it would compromise the effectiveness of exogenous tannin additions to hybrid red wine fermentations, as is sometimes

practiced commercially. Further investigation into the poor tannin extraction from hybrids is warranted. Lastly, implementing CL and/or ST would require a hybrid grape grower to charge considerably higher prices for their grapes in order to compensate for the lost yields and additional production costs. It is uncertain whether the quality or flavor enhancements to Corot noir fruit as a result of CL and/or ST would warrant such price increases under existing hybrid winegrape market conditions.

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CHAPTER 4

CHARACTERIZATION OF ODOR-ACTIVE COMPOUNDS IN NON-VINIFERA SPECIES GRAPE AND WINE

ABSTRACT

Native American grape species have several desirable properties for winegrape breeding, but the aromas inherent to most *Vitis* species other than *V. labrusca* and *V. rotundofolia* are not well characterized. We determined the key odorants in wine produced from the American grape species, *V. riparia* and *V. cinerea*, in comparison to wine produced from European winegrapes (*V. vinifera*). Volatile compounds were extracted by solid-phase-micro-extraction (SPME) and identified by quantitative gas chromatography olfactometry/mass spectrometry (GC-O/MS). Based on FD values, grape-derived compounds with fruity and floral aromas were at similar potency, but non-*vinifera* wines had higher concentrations of odorants with vegetative and earthy aromas: eugenol, *cis*-3-hexenol, 1, 8- cineole, isobutylmethoxypyrazine (IBMP) and isopropylmethoxypyrazine (IPMP). Elevated concentrations of these compounds in non-*vinifera* wines were confirmed by quantitative GC-MS. Concentrations of IBMP and IPMP were well above sensory threshold in both non-*vinifera* wines. In a follow-up study, IBMP and IPMP were surveyed in 31 accessions of *V riparia, rupestris,* and *cinerea*. Some accessions had concentrations of >350 ng/L IBMP or >30 ng/L IPMP, well above concentrations reported in previous studies of harvest-ripe *vinifera* grapes. We expect that this knowledge will facilitate the selection of interspecific hybrids by grape breeders that lack undesirable aroma characteristics, or could be used to identify targets for viticultural or enological studies on interspecific hybrids.

Keywords: non-*vinifera*, hybrid grape, SPME, methoxypyrazines, GC-O/MS, GCxGC-TOF-MS

INTRODUCTION

At least 60 species of grapes (*Vitis)* are reported worldwide (1). Of these species, *V. vinifera* (European wine grapes) account for the majority of world wine production, but *vinifera* can be challenging to grow due to their high susceptibility to diseases (e.g. powdery mildew) and poor cold hardiness. Native American species and interspecific hybrids of non-*vinifera* grape species and *vinifera* generally have better resistance to both abiotic and biotic stresses, and as a result are popular in continental and humid climates such as the midwestern and eastern North America (2). The flavor chemistry of some wild American species, notably those that demonstrate "foxy" aromas like *V. labrusca* and *V. rotundifolia,* are relatively well studied. Methyl anthranilate (MA) has long been known to be an impact odorant in Concord (*Vitis labruscana* Bailey cv. 'Concord') and several related *labrusca*-containing cultivars (3). Furaneol and 2-aminoacetephenone (2AAP) have also been implicated as critical to the perception of foxiness, especially since many "foxy-smelling" grapes have negligible MA concentrations (4). Furaneol and 2AAP are also suggested to be the characteristic odorants of Muscadine (*V. rotundifolia*) juice (5). In the wild, *labrusca* and *rotundifolia*, and related species are consumed primarily by small mammals, and the observed increase in 2AAP and MA in ripening fruit may serve as deterrent to birds (6).

By comparison, the aroma chemistries of the small-fruited American grape species used in grape breeding are poorly characterized. The major species used in breeding, *V. riparia* and *V. rupestris*, are perhaps best known for their importance to breeding phylloxera resistant rootstocks, but are also in the parentage of hybrid cultivars like Marechal Foch and Chambourcin. These grapes do not generally demonstrate foxy aromas. However, even without foxy aromas, these interspecific hybrids are generally believed to have inferior aroma qualities, and sensory studies in peer-reviewed journals have used terms like "green" and "vegetative" (7, 8). However,

odorants responsible for these negative characteristics are still not well defined, which serves as a hindrance for researchers, especially grape breeders interested in developing genetic markers for undesirable aroma characteristics in non-foxy cultivars.

The most potent volatiles in the interspecific hybrids Frontenac, Vidal blanc, and Seyval blanc have been determined by GC-O/MS (8, 9) although key odorants were not quantified in these studies to allow calculation of odor activity values, as has been reported in studies of *vinifera* wines. Several quantitative studies of volatiles in wines produced from interspecific hybrids have been reported, but these have targeted volatiles like linalool known to be important to *vinifera* (10). The volatile composition of *V. riparia* by GC-MS has been reported, but the focus of this earlier work was on profiling the quantitatively dominant volatiles rather than determining the most odor-active volatiles (11). Additionally, analyses were performed on grape juice rather than wine, and thus compounds derived from non-volatile precursors in grapes may have been overlooked.

In this work, we report on the key odorants in wines produced from *V. riparia* and *V. cinerea* species in comparison to a *V. vinifera* wine. As stated earlier, *V. riparia* is widely used by breeders due to its good cold hardiness properties, and is part of the parentage of several well known interspecific hybrids. *V. cinerea* has good disease resistance, although it is usually avoided in breeding winegrapes to poor flavor quality. We expect that this information will facilitate the selection of interspecific hybrids by grape breeders that lack undesirable aroma characteristics, and identify targets for viticultural studies.

MATERIALS AND METHODS

Reagents, samples, and standards. Ethyl hexanoate, 99%, ethyl octanoate, 99+%, ethyl laurate, 99+%, ethyl butyrate, 99%, octanoic acid, 99%, phenethyl acetate, 98+%, ethyl valerate,

99% were purchased from Acros Organics. Ethyl 2-methylbutyrate, 99%, eugenol 99%, β-citronellol, 95%, nerol, 97%, 1-octen-3-ol, 98%, ethyl trans-cinnamate, 99%, 2-Phenylethanol, 99+%, ethyl isobutyrate, 99%, butyric acid, 99+% were purchased from Aldrich. 1-hexanol, 99%, geraniol, 99%, (+) *cis*-rose-oxide, 99% were purchased from Fluka (Sigma–Aldrich). Isobutyl alcohol, 99%, (Z)-2-hexen-1-ol, 95%, δ-nonalactone, 98%, *cis*-3-hexenol, 98%, *p*-vinyl guaiacol, 98%, isoamyl alcohol, 98.5%, β-damascenone, 2-octanol, 97%, linalool, 97+%, γ-nonalactone, isoamyl acetate, α-terpineol, methionol, acetic acid, isovaleric acid, hexanoic acid, guaiacol, decanoic acid and ethyl caprate were purchased from SAFC Supply Solution (Sigma–Aldrich). A C7–C30 hydrocarbon mixture for determination of Kovats retention indices (RI) was obtained from Supelco (Bellefonte, PA, USA). Water was purified through a Milli-Q Water System (Millipore, Billerica, MA, USA). Absolute ethanol, 200 proof, was purchased from Pharmco-*AAPER* (*Shelbyville*, KY, USA). Dichloromethane, L-tartaric acid (99%) and sodium chloride (NaCl) were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Grape Sampling. *Vitis vinifera* (Cabernet franc and Lemberger) grapes were harvested in Sawmill Creek Vineyards on the east side of Seneca Lake in New York State on 10 October, 2009 and 5 October, 2010. *V. rupestris* (7 accessions), *V. riparia* (9 accessions) and *V. cinerea* (10 accessions) grapes were harvested from the USDA-ARS *Vitis* germplasm collection vineyard (Geneva, NY). The Brix of 2009 pooled samples was measured by refractometry (*vinifera*: 20 °; *riparia*: 21 °; *rupestris*: 17 °; *cinerea*: 21°). Samples in 2009 were used to produce wines for GC-O/MS studies, and the Brix of 2010 samples were measured by refractometry and used for quantification of MPs in individual accessions.

Winemaking. Accessions of the same species were combined, manually destemmed, and crushed. Musts were supplemented with 1 g/L diammonium hydrogen phosphate (Presque Isle

Wine Cellars, PA), 0.1 g/L Fermaid K (Lallemand, Rexdale, Ontario) and 0.15 g/L Goferm (Lallemand, Rexdale, Ontario) were added prior to inoculation with EC1118 (Lallemand, Montréal, Canada) at a rate of 0.26 g/L. Skin fermentations were performed in 4L glass fermenters fitted with airlocks. The fermentor was shaken 2 to 3 times per day to submerge the cap. Primary fermentation was determined to be complete when residual sugar was measured to be lower than 0.5% using Clinitest tablets (Bayer, Etobicoke, ON). Wine was pressed by hand with cheesecloth, and sulfur dioxide was added to maintain 40 mg/L free sulfur dioxide. Wines were cold stabilization at 2 °*C*, screened for faults by a trained panel, and bottled.

Volatile Extraction for GC-O. Three extraction techniques were initially compared, including solid-phase extraction (SPE) (12), Solid-phase microextraction (SPME) and liquid-liquid extraction (13). SPME was selected because it is an inexpensive, simple, fast and effective technique. In addition, SPME was often applied to gas chromatography-olfactometry dilution analysis (14). In this study, manual SPME was performed with a 50/30 μm fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Fibers were thermally conditioned for 1 h at 270 °C before their first use. Five mL of wine and 5mL water were added to a 20 mL SPME glass vial (Supelco, Bellefonte, PA, USA) containing 3g NaCl. The vial was tightly capped with a Teflon/silicone septum (Supelco, Bellefonte, PA, USA) and incubated at 40°*C* for 10 min. The SPME fiber was exposed to the sample for 50 min at 40°C and the vial was sonicated throughout the extraction.

Gas Chromatography-Olfactometry/Mass Spectrometry Analysis (GCO). Quantitative GC-O analyses were performed by on a CharmAnalysis system (Datu, Inc., Geneva, NY) equipped with either a DB-5 (30 x 0.25mm i.d., film thickness=0.25um) (Agilent) or a CP-wax 58 FFAP (25 m×0.25 mm i.d., film thickness=0.20um) (Varian) column. Following extraction,

the SPME fiber was inserted into the split/splitless injection port (held at 250°C) for 5 min. Dilutions were performed by adjusting the split to 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256. The oven temperature was held at 35°C for 3min, ramped at 6 °C/min to 250°C and then held for 5 min. The GC effluent was combined with a humidified air stream at 7 L/min before entering the sniff port. The sniffer was selected based on olfactory acuity according to a training procedure (*15*). Sniffing of all extract dilutions was repeated twice until no odor was detected. To determine retention indices for each column, the column outlet was manually switched to an FID detector, and a C7-C30 n-alkane standard run. Kovats retention indices were then calculated using standard approaches. The FD value was geometrically averaged from the data of two replicates using the equation $FD=2^{(a+b)/2}$.

Compound identification was performed by GC-MS using a HP6890 coupled to a HP model 5970 mass-selective detector (Agilent Technologies, Palo Alto, CA, USA) fitted with the same columns as used GC-O. Temperature of the injector was set at 250°C. Purge flow to split vent was 50mL/min for 2min and the helium carrier gas flow rate was 1.5mL/min. The oven temperature was held at 35°C for 3min and programmed at 6 °C/min to 250°C and held for 5 min isothermally. Mass spectra were acquired over the m/z range 33-250. The total ion chromatogram (TIC) acquired by GC-MS was used for peak area identification. Chemstation software version G1701EA E.02.00.493.33 was used for data acquisition. Compounds were tentatively identified by matching the retention index (RI) of the unknown compound with the RI of standard compounds as well as odor character and mass spectral data. Where possible, identification was confirmed by comparison against authentic standards.

Quantification of aroma compounds. Eugenol, 1,8-cineole and *cis*-3-hexenol were quantified by GC-TOF-MS, using solid phase extraction (8). The injector temperature was

250 °C. The oven temperature was held at 40°C for 3 min, then increased to 200 °C at 5 °C/min, then ramp to 240 °C at 10 °C/min, held for 15min. 3-Isobutyl-2-Methoxypyrazine (IBMP) and 3-Isopropyl-2-Methoxypyrazine (IPMP) were quantified by SPME-GCXGC-TOF-MS, using a previously described method (*16*). In brief, HS–SPME was performed by a LEAP CombiPAL Autosampler (Carrboro, NC) using a three-phase fiber (DVB/CAR/PDMS). The vial was incubated online at 650 rpm agitation rate under 80°C for 10 min before fiber insertion and equilibrium. After fiber insertion, the vial was agitated at 100 rpm for 30 min at 80°C. Quantification was performed by GCxGC–TOF–MS (Pegasus IV, Leco Corp, St. Joseph, MI) using two columns. The first column (30 m × 0.25 mm × 0.50 μm) was an RTX5 (Restek, Bellefonte, PA), and the second one (2.5 m × 0.10 mm × 0.10 μm) was a VF-WAXms (Varian, Palo Alto, CA). High-purity helium was used as a carrier gas with flow rate of 1 mL/min. The injector was held at 270°C. The temperature program for the column oven was at 40°C for 5 min, then ramp to 120°C at a rate of 5°C/min, then 120°C to 150°C at a rate of 2°C/min, and finally ramp to 250°C at 10°C/min, held for 15 min. The GCxGC modulation time and the MS transfer line temperature was set to 3 sec and 230°C, respectively. The TOF–MS was performed in EI mode with an ionization energy of 70 eV. The voltage of the electron multiplier was 1680 V. The data acquisition rate of the TOF–MS was set to 120 Hz in a mass range of m/z 20 to 400. The qualifier ions were m/z = 124, 151, 166 for IBMP and m/z = 126, 153, 168 for [^{2}H 2]-IBMP, respectively. The quantifier ions were m/z = 124 and 126, respectively. For IPMP, the qualifier ions were m/z=137, 124, and 152 and quantifier ion was m/z=137.

RESULTS AND DISCUSSION

Comparison of extraction techniques. We evaluated three extraction techniques, previously used for GC-O studies of wine volatiles: solid-phase extraction (SPE), solid-phase

microextraction (SPME) and liquid-liquid extraction (LLE). A few compounds were detectable in SPE and LLE extracts that were not detectable in SPME extracts: namely, the products of carbohydrate degradation, sotolon and furaneol (data not shown). These compounds were previously reported in SPE and LLE extracts (17, 18) that use medium polarity solvents and sorbents, but are frequently absent in GC-O studies that use SPME, stir-bar sorption extraction, or apolar LLE. Based on preliminary dilution analyses of wine made from non-*vinifera* species by three extraction techniques, no difference were detected except for furaneol. Therefore, SPME was thus selected due to its convenience.

As a caveat, all three of these techniques are not well suited for highly volatile compounds (19). In the case of SPE and LLE, the solvent generally co-elutes with early eluting compounds, and in SPME, highly volatile compounds are not efficiently extracted. The use of headspace analyses as a complementary technique has been recommended. In the case of wine, the majority of highly volatile compounds identified in headspace-GCO of alcoholic beverages are due to fermentation (20). Highly volatile grape derived compounds are expected to be lost to CO_2 entrainment during fermentation. However, in our studies, we cannot rule out the possibility that highly volatile odor-active compounds were overlooked.

Detection and identification of odor active compounds by quantitative GC-O. Forty odor-active aroma compounds were detected by GC-O using two different columns (nonpolar DB-5 and polar FFAP). The compound identities, flavor dilution (FD) values and means of identification are listed in Table 4.1. These compounds are sub-divided into three categories: fermentation-derived compounds, grape-derived compounds, and unknowns. 'Fermentation derived compounds' include ethyl esters, acetate esters, fatty acids, and fusel alcohols produced *de novo* via yeast metabolism from sugars and amino acids (21). Although important for the

Table 4.1 Odor-active compounds

No.	Volatile Compounds	RI		FD Value			Descriptor	Basis of Identification
		DB-5	CP-WAX	*Vitis vinifera*	*Vitis riparia*	*Vitis cinerea*		
	Grape-derived compounds							
1	β-damascenone	1385	1767	128	256	256	cooked apple	MS[a], RI[b]
2	ethyl cinnamate	1467	2141	32	16	32	floral	MS, RI
3	linalool	1098	1548	16	32	8	floral	MS, RI
4	β-ionone	1452	1890	16	16	32	sweet	MS, RIL
5	α-terpineol		1725	8	8	16	floral	MS, RI
6	3-isobutyl-2-methoxypyrazine	1180	1527	8	4	16	bell pepper	MS, RI
7	guaiacol	1092	1873	4	8	32	smoky	MS, RI
8	octen-3-ol	973	1404	4	8	2	mushroom	MS, RI
9	(+)-*cis*-rose oxide	1109		2	0	0	floral	MS, RI
10	3-isopropyl-2-methoxypyrazine		1424	1	64	64	earthy	MS, RI
11	eugenol	1357	2183	1	4	64	clove	MS, RI
12	citronellol	1313		1	4	1	floral	MS, RI
13	(Z)-linalool oxide	1065		1	2	1	floral	MS, RI
14	*cis*-3-hexenol	853	1390	0	2	16	green	MS, RI
15	1,8-cineole	1029	1192	0	2	4	minty	MS, RI
	Fermentation-derived compounds							
1	ethyl isobutyrate	750	947	128	128	128	apple	MS[a], RI[b]
2	isoamyl alcohol	726	1209	128	128	64	chocolate	MS, RI
3	ethyl hexanoate	998	1224	128	64	16	fruity	MS, RI
4	methyl furanthiol	862	1316	32	32	8	potato	RIL
5	ethyl 3-methylbutyrate	852	1058	32	16	16	fruity	MS, RI
6	phenyl ethanol	1113	1922	32	16	16	floral	MS, RI
7	ethyl phenylacetate	1242		32	16	16	floral	MS, RI
8	ethyl 2-methylbutyrate	846	1048	16	128	32	fruity	MS, RI
9	dimethyl trisulfide	965	1376	8	64	128	dirty	RIL
10	butyric acid	821		8	128	64	fruity	RI
11	isobutyl acetate		1013	8	32	32	fruity	MS, RI
12	ethyl acetate	608	907	8	8	16	solvent	MS, RI
13	isoamyl acetate	899	1118	8	4	2	banana	MS, RI
14	isovaleric acid		1671	4	32	128	potato	RI
15	ethyl butyrate	796	1031	32	64	64	fruity	MS, RI
16	isobutanol	654	1093	4	8	8	coca	MS, RI

17	diacetyl	636	960	4	2	8	butter	MS, RI
18	ethyl propionate	665	985	4	2	4	Fruity	MS, RI
19	ethyl octanoate	1228	1436	4	2	1	Floral	MS, RI
20	1-hexanol	874	1362	1	2	2	Green	MS, RI
21	(Z)-2-penten-1-ol	770		0	1	1	Rubber	MS, RIL
22	ethyl lactate		1345	0	0	2	Floral	MS, RI
				Unknown compounds				
1	unknown	1363		32	64	1	sweet	RIL[c]
2	unknown	668		32	64	16	dirty	RIL[c]
3	unknown	1049		1	4	1	Fruity	RIL[c]

[a](MS), compounds were identified by the MS spectra.
[b](RI), compounds were identified by comparing retention indices of standards.
[c](RIL), compounds were identified by comparing with retention indices from www.flavornet.org.

general perception of wine (22) in most cases these compounds do not contribute to varietal distinctiveness (23). 'Grape-derived compounds' include those primary odorants initially present in the grape as well as compounds likely to have been released during fermentation from non-odorous precursors.

Fermentation-derived compounds. The majority of compounds (24 of 40) detected and identified by GC-O/MS were likely derived solely from fermentation. Similar results have been observed in other GC-O/MS studies of wines. For example, 14 of the 26 most potent compounds (FD ≥16) in a Grenache rosé wine (18) were fermentation derived, and comparable results have been observed for Gewurztraminer (17). The most potent fermentation aroma compounds, ethyl isobutyrate, ethyl hexanoate, ethyl 3-methylbutyrate, ethyl 2-methylbutyrate, isoamyl alcohol, and phenylethyl acetate had FD values > 16 for all wines (Table 1, bottom). Again, these compounds have been reported to have high FD values not only in other *vinifera* and hybrid wines, but also in fermentations of model juice substrates (24) and in spirits (20).

The concentrations of fermentation derived compounds are well known to vary with initial sugar concentration, oxygen availability, must lipid composition, yeast assimilable nitrogen, and

fermentation temperature (25). However, we attempted to standardize fermentation conditions in our current work, and only modest differences in FD among fermentation derived compounds, generally less than a factor of 4, were observed in our study for nearly all compounds.

An exception to this generalization was observed for isovaleric acid, butyric acid, and ethyl butyrate, where FD values in *vinifera* were an order of magnitude less than the wild species wines. This observation was particularly odd because no difference was observed in ethyl 3-methylbutyrate, whose concentration should be linked to isovaleric acid. We performed a semi-quantitative analysis of these three compounds by SPE-GC-MS (data not shown) and observed only minor differences (< 12 %), even though larger semi-quantitative differences were apparent in SPME-GC-MS during compound identification. Thus, we suspect that the differences in FD values among wines for short chain fatty acids was an artifact of the SPME procedure. SPME is poor at extracting these semi-polar compounds, and thus susceptible to differences in matrix composition among wines (26).

In summary, fermentation-derived compounds are unlikely to explain differences among wines produced from different grape species.

Grape-derived compounds. Several classes of grape derived compounds are commonly reported in wines: methoxypyrazines (MPs), volatile thiols, volatile phenols, C13 norisoprenoids, and monoterpenes. Of these compound classes, only volatile thiols were not well represented in Table 1. 2-methyl-3-furanthiol was detected in all wines. Volatile thiols like 3-mercaptohexanol are readily oxidized, and it is possible that the small-scale winemaking or extraction conditions we used resulted in loss of some key aroma compounds.

Differences in fruity versus vegetative aromas are frequently the most important characteristic for distinguishing wines when evaluated by sensory descriptive analysis (27). Accordingly, the

grape derived compounds detected by GC-O were grouped according to their flavor class; Fruity/floral (cooked apple, sweet, floral); and Vegetative/earthy (green pepper, minty, green, earthy, smoky, mushroom), and the mean FD value for each group across wines is presented in Figure 1. We observed little difference in mean FD for compounds with a fruity/floral character, such as β-damascenone, ethyl cinnamate, linalool, β-ionone, α-terpineol, (+)-*cis*-rose oxide, citronellol and (Z)-linalool oxide (Fig.4.1). However, both *vitis riparia* and *vitis cinerea* had higher overall vegetative aromas based on mean FD values (Fig.1), Thus, the major differences between these non-*vinifera* and *vinifera* wines is likely better explained by the presence of higher concentrations of vegetative odorants in non-*vinifera* rather than the absence of fruity aroma compounds. Specifically, five aroma compounds had FD values greater than 2 dilution steps (> 4 fold) in non-*vinifera* wines as compared to *vinifera*: eugenol, isopropylmethoxypyrazine (IPMP), isobutylmethoxypyrazine (IBMP), *cis*-3-hexenol, and 1,8-cineole.

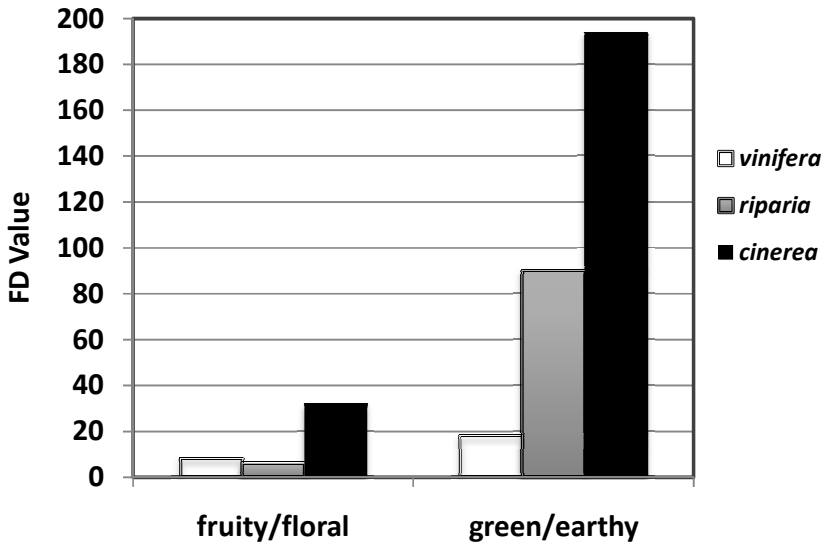

Figure 4.1 Comparison of cumulative flavor dilution values for grape-derived odorants detected by GC-O among wines produced from different *Vitis* species.

Quantification of vegetative aroma compounds from GC-O. We utilized SPE-GC-TOF-MS and SPME-GCxGC-TOF-MS to quantify the vegetative-smelling compounds identified as uniquely high in non-*vinifera* wines in the previous section, and quantitative data along with sensory thresholds and odor activity values are summarized in Table 4.2.

Table 4.2 The concentration of aroma compounds in wines

	Sensory Threshold	Typical concentrations from literature	*vinifera*	*riparia*	*cinerea*
eugenol (µg/L)	6	0 – 15 [30, 48] (unoaked wines) 20-116 [30, 48] (oaked wines)	5^{a}	18^{b}	105^{c}
1,8-cineole (µg/L)	1.1	<0.8 (white wines) ≈1.7 (red wines) [32]	n.d.	2.7^{a}	5.2^{b}
***cis*-3-hexenol (µg/L)**	400	40 – 240 [35]	71^{a}	200^{b}	3987^{c}
IBMP (ng/L)	10-15	5-20 ng/L in Bordeaux cultivars (Cab Sauvignon, Sauvignon blanc) [43]	15.5^{a}	56.6^{b}	57.4^{b}
IPMP (ng/L)	0.2-1.5	n.d-2 ng/L in Bordeaux cultivars [43]	n.d.	2.86^{a}	5.63^{b}

Different subscripts ($^{a, b, c}$) in the same column indicate significantly different concentrations.

Eugenol is reported to have a 'clove' like aroma, and its concentrations were significantly higher in *Vitis riparia (*18 µg /L) and *Vitis cinerea* (105 µg /L) than in the vinifera wine (5 µg/L), and greater than the sensory threshold of eugenol in a 12% ethanol/water matrix (28, 29). Eugenol was also previously detected in the *riparia* containing hybrids, Frontenac and Marechal Foch, although exact quantification was not performed. While eugenol can be detected as a bound, glycosylated precursor in grapes, high concentrations are usually derived from oak (30). The mean concentration in Spanish red wines is reportedly 29 µg /L (range = 4-73 µg/L) (11). The upper end of this range is slightly below the concentration observed in our *V. cinerea* wine. To our knowledge, eugenol aromas are not generally considered a defect in wine, but their presence in unoaked wines may be undesirable.

1, 8-cineole, also known as eucalyptol, has been reported to contribute a "eucalyptus" aroma to wine (29), and has a threshold of 1.1 µg/L in red wine (31). The concentration of 1, 8-cineole in

both *Vitis riparia* (2.7 μg/L) and *Vitis cinerea* (5.2 μg/L) are above threshold, while it is undetectable in *Vitis vinifera* (Table 2), consistent with observed differences in FD values in Table 1. The presence of 1, 8-cineole at concentrations up to 20 μg/L has been reported in red wines, potentially due to exogenous contamination of grapes by eucalyptus tree emission, aka "eucalyptus taint" (32) , although this phenomenon seems unlikely in upstate New York. Endogenous formation of 1,8-cineole has been reported to occur pre-veraison before decreasing during maturation, but it is not possible to infer 1,8-cineole behavior in our study since only a single time point was sampled. Alternatively, Farina et al. suggested that other monoterpenes (terpineol, limonene) could serve as precursors of 1,8-cineole in Tannat (33). We did not attempt to quantify 1,8-cineole or these potential precursors in our current work.

Cis-3-hexenol ("leafy-grassy" aroma) one of several 6-carbon alcohols and aldehydes formed by enzymatic oxidation of lipids following mechanical damage to grapes, especially underripe grapes (34) or green tissue. While *cis*-3-hexenol is detectable immediately following crushing, it is also possible that some is formed during fermentation by reduction of *cis*-3-hexenal (34). Literature reports of concentrations of *cis*-3-hexenol in *vinifera* wines generally range from 50-250 μg/L (35, 36). While this is below the reported sensory threshold for *cis*-3-hexenol in 10% ethanol (400 μg/L) [21], it is suggested that peri-threshold concentrations could increase or modify perception of herbaceousness caused by methoxypyrazines (37). In our work, we found much greater *cis*-3-hexenol concentrations in *cinerea* wine (3987 μg/L) than in either the *riparia* wine (200 μg/L) the *vinifera* wine (71 μg/L) or in the aforementioned studies. While we did not perform sensory experiments, it seems very likely that *cis*-3-hexenol would have a noticeable impact on cinerea wine aroma at its concentration 10 fold over threshold. Potentially, the higher concentrations of *cis*-3-hexenol in *riparia* or *cinerea* wines could be due either to higher

concentrations of linolenic acid, the likely precursor of *cis*-3-hexenol; or due to higher activity of key enzymes associated with *cis*-3-hexenol formation (e.g. hydroperoxylyase, lipoxygenase). Two MPs, isobutylmethoxypyrazine (IBMP) and isopropylmethoxypyrazine (IPMP), were determined to have higher FD values by GC-O in one or both of the non-*vinifera* wines as compared to the *vinifera*, and these differences were confirmed by quantitative analysis. IBMP and IPMP are generally described as having "herbaceous" and "earthy" aromas and thresholds of 10 - 15 ng/L (38, 39) and 0.2-1.5 ng/L (40) in wine, respectively. While MPs are not observed in all grape cultivars (42), at harvest, the *vinifera* wine in this study contained 50% Cabernet franc, a cultivar known to have detectable IBMP, and the concentration of IBMP in the *vinifera* wine (15 ng/L) is within the range of values previously observed for Finger Lakes Cabernet franc (16). Similar to this previous study, IPMP was undetectable. By comparison, IBMP in *riparia* and *cinerea* wines was nearly 60 ng/L, higher than in wines from all other reports except for one Australian Cabernet Sauvignon. Similarly, IPMP is usually undetectable in wines (43), but was present in *riparia* (2.8 ng/L) and *cinerea* (5.3 ng/L) wines well above the reported sensory threshold.

Concentrations of MPs in *V. cinerea*, *rupestris*, and *riparia* accessions. While MPs can contribute to varietal character in some wines, they are considered undesirable at concentrations well in excess of threshold, especially in red wines (41). Since MP concentrations are reported to be well correlated between grapes and wines, reducing MPs early in the selection process would seem to be a logical target for grape breeders interested in eliminating selections with poor flavor potential. Since the 2009 wines used in the GC-O/MS studies were blends of multiple accessions from the USDA Grape Germplasm collection, it was not possible to determine if the MPs were uniformly high in all *riparia* and *cinerea* accessions. In 2010, we performed a survey of the 10

cinerea and 14 *riparia* accessions we used in wine production in 2009. We also included 7 accessions of *V. rupestris*, since this cultivar is widely used in grape breeding for cool- and humid climates. Accessions were sampled on the same day. Although a wide range in maturity, based on Brix, was observed, the mean value (20 Brix) is within the range commonly observed for vinifera in the Finger Lakes at harvest. The concentration of IBMP in some *cinerea* and *riparia* accessions was remarkably high (Table 3). We observed IBMP ranging from 13 to 353 ng/L in *cinerea* and from 79 to 310 ng/L in *riparia*. IBMP concentrations were less variable in *rupestris* accessions (14 to 29 ng/L), and more comparable to the range reported in Cabernet Sauvignon and related cultivars. The highest IBMP concentrations detected (>300 ng/L) in the non-*vinifera* accessions are well above any concentrations reported in *vinifera* at harvest, and are comparable to concentrations found in at the pre-veraison maximum (44), In *vinifera*, high IBMP at harvest can either arise from greater accumulation of IBMP pre-veraison or slower degradation post-veraison, but since only a single time point was sampled it is not clear if IBMP dynamics in *vinifera* are similar to non-*vinifera* species. No correlation was observed between Brix and IBMP ($p>0.05$), so differences in maturity seem unlikely to explain observed differences in IBMP. IPMP was not found in all non-*vinifera* accessions, ranging from undetectable to 31 ng/L in *Vitis cinerea*, from undetectable to 13 ng/L in *Vitis riparia* and from undetectable to 1 ng/L in *Vitis rupestris* (Table 4.3). In all accessions, IBMP > IPMP, as has been observed in *vinifera*. The concentrations of the two MPs were positively correlated, although the correlation was modest ($r=0.55$, $p<0.05$)

Although the final step of MP biosynthesis in grapes (methylation of a hydroxypyrazine intermediate) is reasonably well defined, earlier steps in biosynthesis are still not well characterized (45). Potentially, the higher concentrations of IBMP and IPMP in several of the

accessions in this study would make appropriate studies for either mapping studies or biochemical investigations.

Table 4.3 The Concentration of Methoxypyrazine in Grapes

Species	Accession	IBMP (ng/L)	IPMP(ng/L)	Total soluble solid (Brix)
cinerea	1	13±1	10±3	20.5
	2	110±9	7±2	20.3
	3	143±8	17±3	18.0
	4	17±1	1±0.4	15.0
	5	18±2	n.d.	20.1
	6	286±12	16±4	23.5
	7	251±10	31±5	20.6
	8	52±6	n.d.	15.3
	9	353±16	n.d.	20.4
	10	33±5	8±2	21.2
riparia	1	50±5	n.d.	17.9
	2	50±3	n.d.	23.9
	3	166±10	n.d.	24.8
	4	109±9	4±1	17.8
	5	79±8	n.d.	20.6
	6	310±13	13±3	25.7
	7	82±8	n.d.	19.5
	8	65±6	n.d.	23.4
	9	89±6	n.d.	22.3
	10	36±5	n.d.	20.4
	11	33±5	n.d.	18.4
	12	43±6	n.d.	19.2
	13	13±1	n.d.	20.1
	14	36±5	n.d.	22.3
rupestris	1	24±6	1±0.5	19.5
	2	29±6	1±0.5	20.1
	3	14±2	n.d.	17.8
	4	14±2	n.d.	19.6
	5	16±0	n.d.	18.2
	6	13±1	n.d.	19.8
	7	11±1	n.d.	18.4

n.d. means below limits of detection (25 pg/mL for IBHP, 1.2 pg/mL for IBMP)

Since neither MP was detected in previous GC-O/MS studies on *riparia*-containing hybrids (8, 9), and since MPs are readily detectable in the grape berries, it seems plausible that breeders have unknowningly selected against this trait when developing new winegrape cultivars. However, grape breeders interested in using *cinerea* or *riparia* material should benefit from having genetic markers for high MP concentrations, as this would allow them to select low MP offspring in the first year without needing to wait an extra year for grape production. A similar approach has been proposed for selecting other desirable traits in fruit, such as seedlessness in table grapes and powdery mildew in wine grapes (46, 47).

In summary, we have used GC-O/MS to characterize the aroma profile of wines produced from non-*vinifera* (*riparia* and *cinerea*) grape species without "foxy" characteristics in comparison to wine produced from European winegrapes *(vinifera)*. In agreement with previous studies, most compounds with high FD values were derived solely from fermentation (e.g. ethyl esters, acetate esters, fatty acids, and fusel alcohols) and did not differ among wines. Grape-derived aroma compounds with floral and fruity characteristics (e.g linalool and β-damascenone) also did not differ in FD value. However, based on cumulative FD values, non-*vinifera* wines had more aroma compounds with vegetative and earthy aromas, and this was confirmed by quantitative GC-MS studies. A survey of MP concentrations in *riparia* and *cinerea* from a germplasm collection indicated revealed that some accessions had >350 ng/L IBMP and 30 ng/L IPMP, well above concentrations reported in previous studies of ripe *vinifera* grapes. As a broad conclusion, grape breeders interested in developing *riparia* and *cinerea*- containing cultivars with acceptable aroma could expedite the selection process by focusing on eliminating crosses with high potential to produce MPs or other off-aromas. Currently, this requires at least 2 year old vines to have fruit available, but development of molecular markers could allow for marker

assisted selection immediately after crossing. Interestingly, the "foxy, grapey"-smelling methyl anthranilate (MA) and 2-aminoacetophenone (2AAP) were not detected by GC-O in *riparia* or *cinerea*. These compounds are readily detectable with high dilution values in *labrusca* and *rotundifolia* grapes using GC-O (5). While we did not attempt to quantify these compounds by GC-MS, their absence is not unexpected, as MA and 2AAP are bird deterrents. and *riparia* and *cinerea* are consumed by birds.

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CHAPTER 6

CONCLUSIONS AND FUTURE WORK

CONCLUSIONS

Shoot-thinning treatments improved canopy microclimate (CEFA, LEFA) and some berry and wine chemical parameters of Marechal Foch. However, the impact of shoot thinning was generally comparable to or less than the differences observed with late harvest. Delayed harvest may have a larger impact on the flavor chemistry of Marechal Foch than shoot thinning. Finally, there was both low skin tannin and low tannin extractability in Marechal Foch grapes and, consequentially, very low levels of tannin in the resulting wines. Growers and winemakers in cool climate wine regions should delay harvest on Foch to improve fruitiness and decrease herbaceousness of wines.

The Corot noir vines used in the study were extremely vigorous, with high pruning weights and low croploads. While the impact of ST on yield and yield components varied in two years. ST was consistent in reducing both cropload and TA, and increasing berry anthocyanin concentration. CL also demonstrated a variable impact on yield and yield components, but improved Brix in both years. Increasing retained node numbers during dormant pruning may have more successfully improved fruit composition by reducing overall vine vigor. The impact of ST and CL on the wine sensory analysis was consistent: in both years of the study, the panelists reported that ST combined with CL increased the perception of fruitiness in wines when compared to the control. Similar to Marechal Foch, poor tannin extraction from Corot noir was observed. Lastly, implementing CL and/or ST would require a hybrid grape grower to

charge considerably higher prices for their grapes in order to compensate for the lost yields and additional production costs. It is uncertain whether the quality or flavor enhancements to Corot noir fruit as a result of CL and/or ST would warrant such price increases under existing hybrid winegrape market conditions.

The aroma profile of wines produced from non-*vinifera* (*riparia* and *cinerea*) grape species was characterized by GC-O/MS in comparison to wine produced from European winegrapes *(vinifera)*. In agreement with previous studies, most compounds with high FD values were derived solely from fermentation (e.g. ethyl esters, acetate esters, fatty acids, and fusel alcohols) and did not differ among wines. Grape-derived aroma compounds with floral and fruity characteristics (e.g linalool and β-damascenone) also did not differ in FD value. However, based on cumulative FD values, non-*vinifera* wines had more aroma compounds with vegetative and earthy aromas, including eugenol, *cis*-3-hexenol, 1, 8- cineole, IBMP and IPMP. This was confirmed by quantitative GC-MS studies. A survey of MP concentrations in *riparia* and *cinerea* from a germplasm collection indicated revealed that some accessions had >350 ng/L IBMP and 30 ng/L IPMP, well above concentrations reported in previous studies of ripe *vinifera* grapes. As a broad conclusion, grape breeders interested in developing *riparia* and *cinerea*- containing cultivars with acceptable aroma could expedite the selection process by focusing on eliminating crosses with high potential to produce MPs or other off-aromas. Currently, this requires at least 2 year old vines to have fruit available, but development of molecular markers could allow for marker assisted selection immediately after crossing. Interestingly, the "foxy, grapey"-smelling methyl anthranilate (MA) and 2-aminoacetophenone (2AAP) were not detected by GC-O in *riparia* or *cinerea*. These compounds are readily detectable with high dilution values in *labrusca*

and *rotundifolia* grapes using GC-O. This was also confirmed by semi-quantitative GC-MS studies.

FUTURE WORK

Based on two hybrid varieties study, we found that the tannin extraction ratio of the hybrid wines was very low compared to that of vitis-vinifera wines. This is consistent with the phenominum that hybrid wines tend to be low in astringency. That could potentially be the result of binding between cell wall material and tannins. If this occurs, it would compromise the effectiveness of exogenous tannin additions to hybrid red wine fermentations, as is sometimes practiced commercially. Increasing tannin extraction from hybrids during winemaking may be an interesting direction for improving the chemosensory attributes of the resulting wines. Further investigation is necessary to confirm the poor tannin extraction from other hybrids. Also, more study need to do to increase the extraction ratio of tannin by alternative methods such as extended maceration.

APPENDIX

Odorants Found in the GC-Olfactometric Analysis of Marechal Foch and Cabernet Franc Wines

No.	Volatile compounds	RI		FD factor		Descriptor	Basis of identification
		DB-5	CP-WAX	Marechal Foch	Cabernet Franc		
1	β-damascenone	1383	1828	256±1	256±1	cooked apple	MS, A, RI
2	ethyl isobutyrate	746	952	128±2	152±2	fruity	MS, A, RI
3	isoamyl alcohol	722	1208	90±3	152±2	chocolate	MS, A, RI
4	ethyl 3-methylbutyrate	845	1060	76±2	152±2	fruity	MS, A, RIL
5	ethyl 2-methylbutyrate	821	1050	23±4	108±2	apple	MS, A, RIL
6	ethyl hexanoate	996	1235	128±2	107±2	apple	MS, A, RI
7	phenylethanol	1110	1903	54±6	54±4	rose	MS, A, RI
8	ethyl cinnamate	1467	2044	5±2	54±2	flower	MS, A, RI
9	β-ionone		1928	91±4	45±4	sweet, raspberry	MS, A, RIL
10	phenethyl acetate	1254	1846	76±2	19±7	rose	MS, A, RI
11	ethyl butyrate	794	1028	45±2	16±3	fruity	MS, A, RIL
12	linalool	1100	1548	27±2	16±1	flower	MS, A, RI
13	octen-3-ol	976	1404	7±2	16±3	mushroom	MS, A, RI
14	isobutanol	662	1095	6±2	16±1	solvent	MS, A, RI
15	γ-nonalactone		2017	1±1	16±1	coconut, wood	MS, A, RIL
16	diacetyl	632	961	23±11	13±4	cream	MS, A, RI
17	guaiacol	1089	1868	23±4	11±4	smoky	MS, A, RI
18	isoamyl acetate	871	1120	16±2	11±1	banana	MS, A, RI
19	butyric acid		1609	4±1	11±1	cheese	MS, A, RI
20	unknown	770	992	8±1	8±1	plastic	
21	ethyl valerate	898	1127	6±2	8±2	fruity	MS, A, RIL
22	*cis*-rose-oxide	1120	1365	4±1	8±2	rose	MS, A, RIL
23	isovaleric acid	862	1685	6±1	7±1	cheese	MS, A, RI
24	eugenol	1356	2135	13±2	6±2	bandaid	MS, A, RI
25	1-hexanol	851	1378	11±7	6±3	green	MS, A, RI
26	*cis*-3-hexenol	867	1388	11±1	4±1	grass	MS, A, RI
27	γ-decalactone		2051	8±2	4±1	peach, fat	MS, A, RIL
28	unknown		1303	4±1	4±1	mushroom	
29	vinyl guaiacol		2164	6±1	3± 1	smoky	MS, A, RI
30	citronellol	1228	1766	4±1	3±1	flower	MS, A, RI
31	geraniol	1315	1850	4±1	2±1	flower	MS, A, RI
32	ethyl acetate	650	905	4±1	2±1	fruity	MS[a], A[b], RI[c]
33	(E)-farnesol		2320	4±1	1±1	burnt	MS, RIL
34	isobutyl acetate	748	1007	2±1	1±1	fruity	MS, A, RIL[d]
35	acetic acid	634	1441	2±2	1±1	sour	MS, A, RI
36	furfural		1453	1±1	1±1	sweet, fruity	MS, A, RIL
37	δ-decalactone		2161	1±1	1±1	coconut	MS, A, RIL
38	2-nonenal	1155	1504	1±1	1±1	earthy	MS, A, RIL
39	ethyl decanoate		1641	1±1	1±1	coca	MS, RI
40	nerol	1246	1770	3±1	1±1	flower	MS, A, RI

41	ethyl benzoate		1654	1±1	1±1	flower	MS, A, RIL
42	α-terpineol		1700	1±1	1±1	fresh	MS, RI
43	hexanoic acid		1855	1±1	1±1	sweat	MS, A, RI
44	ethyl dihydrocinnamate	1345	1887	1±1	1±1	flower	MS, A, RIL
45	ethyl octanoate	1212	1395	1±1	1±1	fruity	MS, A, RI
46	ethyl dodecanoate		1858	1±1	1±1	fruity	MS, A, RI
47	octanoic acid	1200	2038	1±1	1±1	sweat, cheese	MS, A, RI
48	decanoic acid		2245	1±1	1±1	rancid, fat	MS, A, RI
49	isoeugenol		2314	1±1	1±1	flower	MS, A, RIL
50	unknown	1160		4±1	0	vegetative, potato	
51	unknown	1179		11±1	0	musty	
52	unknown	1216		8±1	0	green	

[a](MS), compounds were identified by the MS spectra. [b](A), compounds were identified by the aroma descriptors. [c](RI), compounds were identified by comparing retention indices of pure standards. [d](RIL), compounds were identified by comparing with retention indices from www.flavornet.org.

FD value is geometrically averaged from the data of two sniffers. Geometric standard deviation is calculated by

$$\sigma_g = \exp\left(\sqrt{\frac{\sum_{i=1}^{n}(\ln A_i - \ln \mu_g)^2}{n}}\right)$$