ALTERING GRAPEVINE CROP-LOAD AND CANOPY ARCHITECTURE THROUGH CULTURAL AND GENETIC METHODS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

North Dakota's extremely short growing season leads to premature harvest of unripe, high acid grapes with atypical fruit chemistry. To combat the short growing season, grape growers utilize labor intensive grapevine canopy management practices to expose grape clusters to sunshine for enhanced ripening potential. This research examined leaf removal and shoot-thinning, two widely utilized canopy management practices, for their effect on fruit chemistry of two regionally important, cold-hardy grapevines, ‘Frontenac’ and ‘Marquette’. These management techniques were effective at altering fruit chemistry as measured by sugar; however, they are costly to employ commercially due to yield reduction and labor requirements. Furthermore, they rarely impacted the targeted fruit component, acidity of grape must. Ultimately, we sought to mitigate labor expenses associated with on-farm canopy management practices by breeding cold-hardy grapevines with reduced single leaf area. To accomplish this goal, we utilized a novel mutant grapevine with highly dissected, technically compound leaves, ‘Chasselas Cioutat’. After crossing ‘Chasselas Cioutat’ with native, North Dakota isolated, wild riverbank grapes (*V. riparia*) and two interspecific breeding parents (‘Frontenac gris’ and ‘E.S. 5-8-17’), we observed developmental transitions within leaf shape of young seedling grapevines. Newly emerged leaves became progressively more lobed between main veins as seedlings exited juvenility. However, the targeted compound leaf form was not observed in outcrossed progeny, with one rare exception. For these reasons, grapevines were maintained under greenhouse conditions and advanced to the next generation. Within the first generation of inbreeding, segregation was observed for the distinct compound leaf trait. On-going work to isolate quantitative trait loci is merging genetic maps from multiple sequencing technologies with image analysis to create the first high-resolution understanding of genetic control of compound leaf shape in grapevines. Overall, these efforts have provided the foundation for future breeding aimed at incorporating functional leaf shapes (compound leaf morphology) in the vineyard. Future work in North Dakota grapevine management may need to focus on techniques that promote winter survival and early ripening. Future breeding efforts should continue to focus on the use of novel germplasm material to overcome biotic stresses, improve fruit quality, and increase environmental resistance.

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LIST OF ABBREVIATIONS

AGDD	Accumulated growing degree days
BpH	Harvest ratio: Brix × pH2
B:TA	Harvest ratio: Brix: Titratable acidity ratio
CN	Cluster number
CV	Coefficient of variation
DTA	Differential thermal analysis
FZLR	Fruit-zone leaf removal
FSLR	Fruit set leaf removal
GPA	Generalized Procrustes analysis
GY	Growth-yield relationship
LTE_{50}	Lethal temperature exotherm
OIV 601	Length of vein N_1
OIV 602	Length of vein N_2
OIV 603	Length of vein N_3
OIV 604	Length of vein N_4
OIV 605	Length from petiole sinus to upper lateral leaf sinus
OIV 606	Length from petiole sinus to lower lateral leaf sinus
OIV 607	Angle between N_1 and N_2
OIV 608	Angle between N_2 and N_3
OIV 609	Angle between N_3 and N_4
OIV 618aR	Angle of petiole sinus
OIV 618R	Length of petiole sinus
PC	Principal component
PCA	Principal component analysis

pH	Potential of hydrogen
PM	Dormant pruning mass
RI	Ravaz index
SBM	Single berry mass
SCM	Single cluster mass
SSC	Soluble solid content
TA	Titratable acidity, expressed as tartaric acid equivalents
TBLR	Trace bloom leaf removal
VRLR	Veraison leaf removal
YLD	Single vine yield

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CHAPTER 1. LITERATURE REVIEW

Background of Cold-hardy Hybrid Grapevines

Viticulture in cool and cold-climates is typified by the production of high acid containing wine and grapes, *Vitis* spp. The two major acids found in wine grapes are tartaric and malic acid, with a third, citric acid, being present to a minor extent. In a survey of Vitis conducted by Kliewer (1967) only *Vitis vinifera* L., *Vitis labrusca* L., *Vitis rotundifolia* Michx., and a *Vitis* spp. accession from Afghanistan were reported to contain predominantly tartaric acid, with all other species having a higher percentage of total titratable acidity due to malic acid content. The main acid component in *Vitis riparia* Michx. derived cold-hardy hybrid grapes grown for wine production in the Upper Midwest and Great Plains States is malic acid (Rolfes, 2014; Vos, 2014). Malic acid is ranked as the most perceived sour tasting acid at both the same pH level and titratable acidity (TA) concentration (Amerine et al., 1965). The grape berry typically undergoes a three stage, double-sigmoidal growth curve during which malic acid concentration is at its highest during the final days of stage II, lag phase to veraison (Volschenk et al., 2006). Ratios of malic:tartaric acid and sugar:organic acid are most dictated by the climate during stage III, post veraison berry ripening. Cool climatic conditions with shorter ripening seasons result in slower rates of respiration of L-malic acid, this contributes to higher acidity. The acidity of cold-hardy grapes may be compounded in colder than usual years, and it may also be impacted by substandard management of vines.

The sour and “green” flavor of malic acid may be reduced post-harvest through the use of lactic acid bacteria, such as *Oenococcus oeni* (Garvie) Dicks et al. (Kunkee et al., 1964). Through the process of malolactic fermentation (MLF), malic acid is consumed and lactic acid is formed as a byproduct; however, diacetyl, butane-2,3-dione, is an additional organic compound resulting as a byproduct of MLF that may impart a buttery, cheese-like, undesirable flavor, which at high concentrations may be viewed as a wine flaw (Davis et al., 1985). When tartaric acid exceeds acceptable standards, it may be reduced through cold stabilization, forcing tartrate crystals to form, falling out of the wine solutions. Cold stabilization, yeast strain selection, and MLF methods have been used to successfully reduce titratable acidity of wines with exceptional acidity, such as ‘Frontenac’, ‘La Crescent’, and ‘King of the North’; however, due to either extreme stress of the wine environments, or the high native concentration of malic acid, treatments did not completely remove the acid components as was anticipated (Olson, 2016).

Within the states of Iowa, Minnesota, Montana, Nebraska North Dakota, and South Dakota, acres with bearing grapes increased over 500% in the ten-year period from 2002 to 2012 (USDA-NASS, 2017). Examining North Dakota, the final frontier for wine, reveals even greater expansion of the grape industry in the last U.S. state to produce a commercial wine; there has been a 2000% increase in acres bearing and non-bearing from 1997 to 2012. With expanding plantings of grapes in the Upper Midwest and Great Plains states, there is a growing necessity for information geared towards producer’s needs.

Commonly grown cold-hardy red grapes include ‘Marquette’ [48% of surveyed production in North Dakota and South Dakota commercial vineyards according to Tuck and Gartner (2014)], ‘Frontenac’ (28%), ‘Valiant’ (7%), ‘King of the North’ (6%), and ‘Sabrevois’ (5%). The most widely grown white grapes include ‘Brianna’ (38%), ‘La Crescent’ (31%), ‘Frontenac gris’ (19%), and ‘Prairie Star’ (6%).

Of the red grapes evaluated for North Dakota at the NDSU Absaraka Horticulture Research Station, only three were deemed climatically adapted, ‘King of the North’, ‘Valiant’, and an unreleased selection ‘ES 12-6-18’ (Hatterman-Valenti et al., 2014). With reduced growing degree day (GDD) accumulation in brief growing seasons, sometimes the majority of cultivars failed to achieve suitable soluble solid content (SSC); yet, all were suggested to ripen when GDD accumulation exceeded 1200, based on a minimum of 10°C and a maximum of 30°C. Though the highest yielding in most years, ‘King of the North’ also produced fruit with the highest TA values, followed by ‘La Crescent’ and ‘Frontenac’.

Near Absaraka, ND, Olson (2016) found ‘Frontenac’ pH to range from 3.02 to 3.30 and TA to range from 11.25 to 17.78 by year and training system. SSC was typically high, consistently measured above 25 Brix. However, in the still establishing vineyard, yield was not economically substantial, with all training systems failing to exceed three kg/vine and the lowest yielding treatment within year, only slightly above 1 Mg/ha. Lower titratable acidities were reported for ‘Frontenac’s gris colored bud sport mutation, ‘Frontenac gris’, grown in nearby Buffalo, ND (Aipperspach, 2013).

Gąstoł (2015) found ‘Frontenac’ to be the highest yielding red hybrid grape grown in Poland, while also having the greatest SSC. Acidity was still an obstacle; pH was lowest and titratable acidity was highest for ‘Frontenac’. The major acid component was malic acid, at over six times the concentration of tartaric acid. Similar reports indicate malic acid is the predominant acid component in ‘Frontenac’, as well as several other prominent cold-hardy grapes such as ‘La Crescent’ and ‘Edelweiss’ (Rolfes, 2014; Vos, 2014).

With 73% of vineyards being established since 2002, and 64% of those vineyards showing continued growth since establishment, regional vineyards within North and South Dakota are expanding and being challenged by management of diseases and vineyard labor (Tuck and Gartner, 2014). Management of vineyard labor is a critical component to maintaining vine health for grapes, a horticultural crop requiring hand planting, pruning, training, and harvest in a small scale vineyard setting. Within the region, viticultural practices currently utilized are all manual. Viticultural practices to improve fruit quality include canopy management such as leaf thinning, shoot thinning, and shoot positioning, as well as crop load management, nutrient management, and other cultural practices affecting vine physiology and ultimate wine quality (Smart, 1985).

The grapevine canopy can be altered to affect solar radiation, temperature, wind speed, humidity, and evaporation rates (Smart, 1985). Controlling shoot number through pruning severity and green shoot thinning, altering vigor through manipulation of crop load, enforced water stress, nutrient availability, rootstock selection, vineyard site selection, and training system are all methods employed to modify the grapevine canopy.

Grapevine Canopy Management

Fruit zone leaf removal (FZLR), leaf thinning the canopy specifically around grapevine clusters, has been shown to have many benefits for disease control and fruit quality (Sabbatini and Howell, 2010). Fungal diseases, specifically bunch rots, represent the most manually manageable disease. Disease incidence and severity may be reduced through manipulation of grapevine canopy microclimate architecture (English et al., 1989; Smart, 1985). ‘Pinot noir’ vines had a reduced level of TA, regardless of severity of defoliation of basal leaves during trace bloom (Sabbatini and Howell, 2010). Harvest rot incidence and severity were both increasingly reduced through the use of early leaf removal of 4 and 6 leaves in ‘Vignoles’, though the treatments also resulted in significantly lower yield per vine. In ‘Zinfandel’ and ‘Chenin’, leaf removal was found to significantly reduce the incidence and severity of Botrytis bunch rot (Botrytis cinerea Pers.) (English et al., 1989). Leaf removal increased the wind speed within the canopy, with most notable microclimate differences being found in the early morning or late afternoon; this increase in wind speed in conjunction with evaporative potential within the grapevine microclimate was suggested as an important environmental factor influencing the development of Botrytis infections.

Napa County, CA grown, quadrilaterally-cordon-trained ‘Sauvignon blanc’ vines receiving leaf removal treatments were lower in titratable acidity and malic acid content, and higher in SSC compared to control vines (Bledsoe et al., 1988). Leaf removal treatments also resulted in higher proportions of photon fluence rate (PFR) within the fruiting zone of the canopy, and PFR was found to negatively correlate with both malate and pH. Throughout the study, leaf removal severity, not timing, had the greatest impact on final fruit composition. In contrast with other reports, yield and yield components were not altered by any of the leaf removal treatments.

‘Cabernet Franc’ grapes grown in the cool viticulture climate of Benton Harbor, MI had lower TA in both 2011 and 2012 based on leaf removal treatments consisting of removal of six basal leaves at 50% berry color change (Frioni et al., 2017). Only TA was affected by treatments, pH and SSC were not in either year. Berry anthocyanin:Brix ratio was highest in 2011 for the treatment combining cluster thinning and leaf removal, while phenolic concentration was lowest for the control treatment. Berry weight, cluster weight, and number of berries per cluster were not affected by leaf removal treatments. Cumulatively, cooler summer conditions in 2011 were cited as the cause for observable treatment differences compared to a warmer 2012 growing season. They concluded that viticultural practices are more important and more useful during cooler years.

In North Dakota, Aippersbach (2013) found that 100% shade leaf removal reduced the TA of ‘Marquette’ substantially from 8.19 g/L down to 6.40 g/L when compared to vines with

0% shade leaf removal; however, there was no shade leaf removal treatment effect on 'Frontenac gris' vines within the same study. Leaf removal did reduce average dormant cane pruning weights of 'Marquette' and 'Frontenac gris' at the highest leaf removal intensity, but it only reduced yield in 'Frontenac gris'. The 2012 growing season resulted in higher SSC for 'Marquette' grapes, increasing from 25.95 to 27.07, though the growing season had no effect on pH. Timing of leaf removal treatments within the study varied by location and year from as early as 10 July to as late as 12 August. Olson (2016), found no significant differences for fruit quality measurements from leaf removal treatments to 'Frontenac' grapevines in Absaraka, ND. There were noted effects of training system on yield and cluster number, though not on point quadrat analysis of canopy structure.

There was no effect of cultural management practices for reduction of TA, tartaric acid, or malic acid in Adel, IA grown 'Marquette' grapes; however, pH was highest for the combined treatment of shoot thinning and leaf thinning and the combined treatment of shoot positioning and shoot thinning (Rolfes, 2014). As labor per vine increased, solar irradiance within the canopy increased in comparison to the control 'Marquette' vines, this was also observed in Madrid, IA with 'La Crescent' grapes. For 'La Crescent' grapes the tartaric:malic ratio was highest for the combined treatment of shoot thinning and leaf thinning, but with a value of 0.35, the ratio did not approach a commercially acceptable value for wine grapes of V. vinifera.

Combined across cultivars 'Frontenac', 'La Crescent', 'Marquette', and 'St.Croix', delaying harvest reduced TA, tartaric acid, and malic acid content (Vos, 2014). For both years of the study, late harvest 'Marquette' had significantly lower TA and higher SSC. Titratable acidity was as low as 6.2 g/L compared to 9.2 g/L for the early harvest in 2010. Similarly, in both years, the tartaric:malic acid ratio exceeded 1.0 for the late harvest ‘Marquette’ in conjunction with malic acid dropping to as low as 5.3 g/L in 2009.

In other cool-climate regions, such as Canada, basal leaf removal reduced TA of ‘Riesling’ fruit in three of five years, and it also resulted in an increased concentration of potentially-volatile terpenes in all years studied (Reynolds et al., 1996). Similarly, Reynolds et al. (2007) noted that basal leaf removal of ‘Chardonnay Musqué’ reduced wine TA compared to the control, non-thinned vines while having increased apple/pear and floral/perfume-like aromas in the 2002 vintage.

Repercussions of leaf removal include reduced fruit set, reduced cluster mass, and reduced fruit yield, all while requiring higher labor or machine input costs. Comparing defoliation of the first six leaves of ‘Barbera’ conducted at the pre-bloom stage with an untreated control, Poni and Bernizzoni (2010) noted reduced cluster compactness combined with a reduced berry number per cluster. Defoliation led to higher total phenols, anthocyanins, and SSC; likewise, within individual berries, the skin provided a greater proportion of the mass.

Berry skin thickness has been observed to be increased via leaf removal on numerous cultivars, potentially as a physiological response to the stress of defoliation and subsequent increased UV radiation (Verdenal et al., 2017). An increase in berry skin thickness is often, but not always correlated with higher levels of anthocyanin and phenolic substances. In cool-climate MI grown ‘Pinot noir’, no increase in anthocyanin content was observed, but phenolic content accumulation was higher for the higher rates of basal leaf defoliation (Acimovic et al., 2016). For the first year of the study SSC was highest and TA lowest for the highest rate of leaf removal; the same trends were not observed in the following season, moreover, rates of removal of eight and ten leaves resulted in uneconomic levels of yield below 5.3 t/ha.

Excessive leaf removal may also result in insufficient leaf area to ripen a crop leading to the opposite, undesirable effects on fruit quality including lower SSC, higher TA, and more acidic pH overall (Osrečak et al., 2015; Zoecklein et al., 1992). Leaf removal led to lower total acidity in ‘Pinot gris’ and ‘Riesling’ when it occurred during the early stages of flowering, BBCH 63, with no observed effect on total yield per plant (Molitor et al., 2011). Bunch rot severity was also reduced for ‘Auxerrois’, ‘Riesling’, and ‘Sauvignon blanc’ especially when leaf removal was conducted during the flowering and pre-veraison fruit development.

Annual variation of climatic conditions including temperature and light play a role in interannual variability of treatment effects to achieve measurable changes in anthocyanin, phenolics, and technological fruit maturity (Downey et al., 2004; Feng et al., 2015; Spayd et al., 2002; Tommaso et al., 2017). Typically, regardless of season, North Dakota grape growers delay harvest until as late in the season as possible, maximizing the crops ripening capacity. Thus, to further enhance ripening of regionally produced grapes, investigations into cultural practices are warranted.

Reducing the crop load can, but does not necessarily, result in improved fruit chemistry; cluster thinning of ‘Chambourcin’ failed to produce an effect on TA, though it did result in higher SSC (Dami et al., 2006). Additionally, low crop levels of ‘Seyval blanc’ resulted in higher SSC, but in one of the two years observed, low crop levels also resulted in greater malic acid content (Hummell and Ferree, 1998). Hummel and Ferree also observed reduction in TA and tartaric acid with reduced shading of clusters.

Leaf removal, shoot positioning, and shoot thinning were employed to successfully reduce the malic acid content of interspecific hybrid, ‘Norton’ grapevines (Jogaiah et al., 2013). Likewise, grown in Summerland, BC, ‘Okanagan Riesling’ on a Geneva double curtain had their

TA reduced through management practices such as leaf removal and shoot positioning (Reynolds et al., 1992).

The timing of shoot thinning can affect the treatments impact on TA and SSC of ripe grapes (Reynolds et al., 2005). In ‘Pinot noir’, a decreased effect on phenol content was observed as shoot thinning treatments were delayed, while an opposite trend was observed for TA potentially due to cluster exposure timing. Shoot thinning to a level of 15 shoots per meter reduced yield of ‘Marechal Foch’ without affecting TA of the crop, though it effectively reduced herbaceous compounds in wine (Sun et al., 2011). Likewise, shoot thinning reduced yield of Corot noir; however, it also resulted in reduced TA and higher SSC (Sun et al., 2012). For ‘Corot noir’, treatments such as shoot thinning and cluster thinning resulted in higher levels of perceived fruitiness of wine, and warranted additional compensating increases in price per tonne or cost per bottle for economic sustainability of producers of grapes and wine.

Shoot thinning of three high yielding hybrid cultivars, ‘Aurore’, ‘Chancellor’, and ‘Villard noir’ in Arkansas only had a minor observed effect on SSC of ‘Chancellor’, but it did result in increased proportions of the crop being derived from count nodes and subsequently larger cluster masses for ‘Chancellor’ and ‘Villard noir’ (Morris et al., 2004). Yield per vine was reduced through shoot thinning of ‘Barbera’ (Bernizzoni et al., 2011). The early season shoot thinning conducted in Piacenza, Italy on potted vines at between 15 and 20 cm shoot length increased anthocyanin content and SSC while decreasing TA. Contrastingly, shoot thinning of ‘Sauvignon blanc’ in Israel had no stable effect on TA, though decreased crop load resulted in more fruity and herbaceous tasting and smelling wines (Naor et al., 2002). TA of ‘Riesling’ grown in Kelowna, B.C., CAN was linearly reduced with reductions in shoot density from 36 to 16 shoots per meter row in 1987, but in all other years there was no statistically significant reduction in TA (Reynolds et al., 1994). Within the same study, increased crop-thinning led to a linear reduction in TA in two of the four years studied, and a linear reduction with increasing crop level for SSC and pH was observed in the majority of years examined.

It is critical for growers in cool climates with limited GDD available during the growing season to maximize their vine's capacity to ripen a crop; however, cultivars respond variably across both years and treatments, so further investigations are necessary to define cultural management practices, especially for the underexplored viticultural regions of the Northern Great Plains.

Foliar Morphological Variability

Leaves, the photosynthetically active site of gas exchange, vary in size and shape within Kingdom Plantae and across environmental gradients (Chitwood et al., 2012a; Givnish and Vermeij, 1976; Givnish, 1987; Nicotra et al., 2011). Leaf shape, integrated with vascular patterns, is developmentally regulated by hormones, such as auxin (Chitwood et al., 2012c; Dengler and Kang, 2001). Foliar morphology can be used to identify amongst genus and species. Within genus Vitis, grapevines, there is a dedicated field of study, ampelography, which seeks to characterize species, hybrids, and cultivars using their morphological traits (Chitwood et al, 2014; Galet, 1979). Leaf shape may vary greatly within differing environments and stages of development (Bodor et al., 2014, 2016a, 2016b; Chitwood et al., 2015, 2016). Leaves, especially compound leaves, exhibit phenotypic plasticity. Evaluation of cinquefoil, (Potentilla spp.), revealed similar variation amongst whole leaves and leaflets (Klingenberg et al., 2012). Tropical lianas exhibit larger leaves in sunny conditions and contrastingly smaller leaves when developing in shady environments (Givnish and Vermeij, 1976). While leaf shape and size may be modulated along a growing axis and within environments, leaf shape is a genetically heritable trait (Chitwood et al., 2013, 2014).

Work towards biological efficiency in maize, (Zea mays L.), investigating leaf area in relation to yield in three sets of diallel crosses revealed that hybrid combinations varied in yield efficiency per unit of leaf area (Rutger et al., 1971). Within grapes, there are varying reports on the necessary amount of leaf area to sufficiently ripen a season’s crop ranging from about 7 cm^2 to 15 cm^2 leaf area per gram of fruit (Amberg and Shaulis, 1966; Kliewer and Weaver, 1971; May et al., 1969). These reports differ by location and cultivar grown; however, it may be possible to integrate breeding work to select for genotypes that most efficiently ripen a crop with restricted leaf area.

Grapevine canopies are a three-dimensional construct. At times the canopy may be linearly compressed along vineyard rows such as in a vertically shoot positioned canopy. Shading of grapevine canopies can have negative effects on the health and quality of fruit. Through increasing sunlight penetration and airflow, it is possible to reduce the incidence of fungal diseases common to the Eastern United States, such as rots (*Aspergillus* sp., *Alternaria* sp. *Rhizopus* sp., *Penicillium* sp., and others), powdery mildew [*Uncinula necator* (Schwein.) Burrill], and Botrytis bunch rot (Scholberg et al., 2008; Smart, 1989). Grapevines are often managed in commercial operations either through machine or manual defoliation, shoot thinning, and other techniques to encourage an open canopy, especially around the fruit zone. Reducing single leaf area, by breeding for either a smaller leaf or a dissected leaf, may effectively lead to a more open canopy (Cousins and Prins, 2008).

Aberrant Leaf Shapes in Crop Plants

Investigations into abnormal leaf types such as variegated and yellow spot of common bean (*Phaseolus vulgaris* L.) reveal these foliar traits are often controlled by at most only several recessive genes (Smith, 1934; Coyne 1969; Parker, 1933; Zaumeyer, 1942). A single recessive gene for unifoliate leaves has also been shown (Yarnell, 1965). Rabakoarihanta (1979) found continuous, quantitative variation within distorted common bean leaf types. The results of F2 and backcross lines indicated distortion is inherited with a relatively high heritability based on regression of the F1 and mid-parent.

A mutant gene for non-lobed leaves (nl) has been described as incompletely dominant in watermelon, [*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai] (Mohr, 1953; Rhodes and Dane, 1999). Other mutant leaf types analyzed in watermelon, *Citrullus* spp., include seedling leaf variegation (slv) controlled by a single recessive gene, yellow spotted leaves and fruit controlled by a dominant gene (Sp), yellow leaf (Yl) a trait with incomplete dominance leading to yellow leaves, and tendrilless (tl) resulting in modified, gradually more triangular leaf forms (Poole, 1944; Provvidenti, 1994; Rhodes et al., 1999; Rhodes and Dane, 1999; Warid and Abd-El-Hafez, 1976; Zhang et al., 1996).

Bassett (1981) described a single monogenetic dominant mutation for lanceolate leaf type in common bean fitting a 3:1 ratio in S1 progeny; however, due to the fact that F1 progeny did not fit an expected 1:1 normal to mutant segregation, rather a 2:1 segregation, some lethal action was suggested as possibly present, similar to observations in mung bean, [*Vigna radiata* (L.) R. Wilczek] (Singh and Saxena, 1959).In tomato (*Solanum lycopersicum* L.), mutant potato-like leaves arise from a single recessive gene, solanifolia (sf) (Rick and Harrison, 1959). Compared to normal leaf types, sf mutants exhibit entire, not serrated leaf margins, greater leaf area, and terminal leaflet area; the increased leaf area is possibly due to the higher number of total leaflets (Sekhar and Sawhney, 1990). Two additional mutant genes from *Solanum galapagense* S.C. Darwin & Peralta, Petroselinum (Pts) and galapagos dwarf (gdw) contribute to highly dissected observed in S. galapagense (Harevan et al., 1996; Kimura et al., 2008). Jannsen et al. (1998) suggest that lobes and leaflets arise from fundamentally the same differentiable cell source, and KNOX genes may dictate their expression within foliar morphology of *Solanum* spp.

A mutant leaf type, okra leaf shape in tetraploid upland cotton, (*Gossypium hirsutum* L.), is controlled by a single, incompletely dominant gene (Andres et al., 2013; Jones, 1982; Rahman et al., 2005). Cotton lines homozygous recessive for okra leaf shape have reduced single leaf area, variant leaf margins, and deep sinuses, while heterozygous lines form intermediate, sub-okra leaf types (Andres et al., 2013; Green, 1953). In diploid cotton, (*Gossypium arboreum* L.), Kaur et al. (2016) confirmed the monogenic control of laciniate leaf shape, an ortholog to the okra leaf shape observed in upland cotton.

A report in 1995 indicated okra leaf selections represented approximately 50% of plantings in Australia (Thompson, 1995). The leaf trait has economic implications due to reductions in rot, lint trash, evapotranspiration, and chemical requirements (Andres et al., 2013; Jones, 1982). Assessment of agronomic and physiological characteristics of okra leaf cotton give insight into the applicability of the trait in a breeding program. Comparison between two cotton isolines, one normal leaf and one okra leaf, revealed that okra leaf cotton more efficiently utilizes intercepted solar radiation (Gonias et al., 2011). Greater CO2-exchange rates and water use efficiency have been observed in okra leaf cotton (Pettigrew et al., 1993). No yield reduction of okra leaf type was observed compared to normal leaf cotton in Stoneville, MS (Wells and Meredith, 1986). Thomson et al. (1986) explored the effect of multiple novel leaf traits on the yield and quality of cotton lines, finding mutant genes had a minor impact on lint quality. Additionally, they noted that okra leaf genotypes yielded more than normal leaf genotypes under a low insecticide spray regime. Mapping of leaf traits can lead to increased tools for breeders to implement novel foliar phenotypes for the purposes of modulating chlorophyll content, CO2 exchange, canopy light interception, and other important physiological and morphological traits for a changing climate (Jiang et al., 2000; Saranga et al., 2001; Song et al., 2005).

In soybean [*Glycine max* (L.) Merr.] leaf shape (expressed as the ratio of leaf width/leaf length) and area have high broad sense heritability estimates, 80.9 and 82.4 percent, respectively (Suh et al., 2000). Leaf shape also has a high narrow sense heritability of 63.2 compared to leaf area at 43.4 percent. There was an observed tendency for progeny leaf shape to be lanceolate, rather than oval. Overall, the additive and total genetic variances observed for leaf shape indicate the trait may be utilized for its breeding value in future soybean breeding work.

Inheritance and Genetics of Foliar Characteristics with a Focus on *Vitis*

Variegation and albinism within seedling populations are other examples of extreme foliar phenotypes periodically observed within *Vitis* offspring that may hold aesthetic value (Marcotrigiano, 1997). Though albinos are incredibly rare, Reisch and Watson (1984) observed 16 percent of self-pollinated lines derived from ‘Ravat 51’ to exhibit the trait. Additionally, they proposed five lines, including ‘Riesling’, ‘Chardonnay’, and ‘Ravat 51’ to be heterozygous for variegation while noting the trait appeared to reduce plant survival in NY without being lethal.

Progeny of ‘Southern Home’, a rare, lobed leaf muscadine [*Muscadinia* spp. ($2n=2x=40$)] cultivar with *Vitis* spp. in its genetic background segregated for leaf shape, lobed vs. standard, indicating ‘Southern Home’ is not homozygous dominant for the trait (Mortensen et al., 1994; Sandefur et al., 2010). With no consistent maternal or paternal effect evident in the subpopulations, neither a 2:1 nor a 1:1 segregation ratio was fully confirmed (Sandefur et al., 2010).

Investigating *Vitis* leaf diversity, Chitwood et al. (2014) performed GWAS assessment of over 1,200 grape accessions leading to the identification of four significant marker associations with leaf shape metrics; one inner landmark PCs, one outer landmark PC, and circularity were found on chromosome 1 and one inner landmark PC was found on chromosome 6. In the proximity of both identified loci are previously identified regulators for leaf development. Overall heritability across the sampled genotypes was highest for outer landmark PC2, approaching 0.5. Further RNA-Seq analysis comparing ‘Chasselas Dore’ (DVIT 0689) and the mutant leaf shaped ‘Chasselas Ciotat’ (DVIT 0372) revealed 2,977 genes significantly down-regulated in ‘Chasselas Ciotat’ and an additional 2,370 genes significantly up-regulated when compared to its proposed clonal progenitor ‘Chasselas Dore’ (Myles et al., 2011; Chitwood et al., 2014).

In testing mode of inheritance for leaf shapes within 90 progeny of a ‘Muscat Hamburg’ × ‘Villard blanc’ cross, four foliar traits fit the expected Chi-sq values: size of leaf blade, number of lobes, shape of teeth, and shape of petiole sinus; however, both leaf hair inheritance hypotheses were rejected (Nikolić, 2015). Categorizing 130 seedlings of ‘Drenak crveni’ × ‘Afuz-ali’ according to O.I.V. descriptors based on six leaf traits, Bešlić et al. (2005) found segregation ratios in the F1 generation fit the X2 values for two traits, shape and upper leaf margin form.

Unique accessions and cultivars with novel traits, such as ‘Chasselas Ciotat’ with a highly dissected, nearly compound (NC) leaf offer the opportunity for combining foliar form and functionality. Breeding for practical aesthetics, such as the NC leaf trait, may enable commercial vineyards to reduce or eliminate certain chemical and cultural practices associated with ensuring fruit exposure and control of pests and pathogens (Cousins and Prins, 2008).

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CHAPTER 2. CROP ‘TIL YOU DROP: ACIDITY AND COLD-HARDINESS NOT CORRELATED WITH CROP-LOAD IN NORTH DAKOTA GROWN ‘FRONTENAC’ GRAPEVINES

Abstract

In North Dakota, high titratable acidity (TA) and low pH of grapes at harvest are two key limiting fruit components that minimize fruit and wine value. In 2017 and 2018, shoot-thinning treatments, a technique that reduces crop-load and canopy density, were applied to create a linear gradient of feasible yield levels on ‘Frontenac’ grapevines grown within three trellis-training systems, Geneva double-curtain (GDC), single-high wire curtain (HW), and mid-wire with vertical shoot positioning (VSP). The Pearson product-moment correlation coefficients among fruit composition and viticultural traits, including pruning mass, Ravaz-index, cluster number, and yield were examined within years. No meaningful correlations between the critical acid component, TA and viticultural traits were detected. Relationships between soluble solid content (SSC) and pruning mass, Ravaz-index, cluster number, and yield were identified; these correlations were further explored via regression model fitting techniques. For the 2017 and 2018 data sets, the best models incorporated trellis-training system and cluster number to predict SSC. Based on these models, farmers may anticipate an estimated 1 Brix reduction in SSC for every 29 to 33 clusters retained per vine. Across eight total cold-hardiness screening test dates during the 2017-2018 and 2018-2019 dormant seasons using differential thermal analysis techniques, a significant relationship between grapevine yield and lethal temperature exotherm was only detected once. The 2018-2019 dormant season, characterized by severe mid-winter temperatures, incurred substantial damage on the grapevine planting going into the 2019 growing season. No relationship between previous year’s crop yield components and grapevine trunk survival were

detected; the majority of the planting succumbed to trunk death in 2019. These results cumulatively indicate ‘Frontenac’ grapevine growers in eastern North Dakota may not anticipate a negative impact on fruit acidity associated with increased crop-load, despite a slight reduction in SSC. However, based on the severe winter damage observed, these results also indicate ‘Frontenac’ grapevines may not be adequately adapted for long-term production within eastern North Dakota’s environmental conditions in the context of a turbulent, changing climate.

Introduction

Recent grapevine breeding work and reduction in prohibition laws have contributed to the establishment and expansion of grapevine growers and wine producers in North Dakota (USDA NASS, 2021). The interspecific, cold-hardy grapevine ‘Frontenac’ and its clonally derived sports, ‘Frontenac gris’ and ‘Frontenac blanc’, are among the most widely planted grapevines in North Dakota and South Dakota (Luby and Hemstad, 2006; Tuck and Gartner, 2014). The suite of ‘Frontenac’ grapevines have proven survival in many Midwestern and Northern Great Plains climate conditions, and are among the higher performing individuals in previous North Dakota variety assessments (Bavougian et al., 2012; Hatterman-Valenti et al., 2014; Stenger and Hatterman-Valenti, 2016; Wimmer et al., 2018; Schrader et al., 2019). However, ‘Frontenac’ grape wine must is frequently harvested with undesirably high levels of titratable acidity (TA) combined with a low pH (Hatterman-Valenti et al., 2014; Olson, 2016; Riesterer-Loper et al., 2019; Schrader et al., 2020). These fruit composition attributes are derived from genotype and environmental effects. The maternal, seed-parent of ‘Frontenac’, *V. riparia* #89, is a selection of *V. riparia*, a species known for high TA values. This predisposition for high TA and low pH is exacerbated in North Dakota’s short-season growing conditions where the frost-free growing season rarely extends beyond the four-to-five-month period of mid-May to late-September (NDAWN, 2021).

Wine makers demand high quality fruit with optimized harvest parameters so that the need for winemaking interventions are reduced. In North Dakota, the high acidity of musts is a key limiting factor for wine production, and this constrains winemaking styles. Because ‘Frontenac’ is one of the most widely planted grapevines in the Dakotas, multiple experiments have attempted to viticulturally drive alterations in fruit composition (Aipperspach et al., 2020; Olson, 2016). Experiments in North Dakota have focused on leaf removal of ‘Frontenac’ and ‘Frontenac gris,’ with a goal of reduced acidity. However, these experiments failed to significantly alter the fruit composition as measured by pH and titratable acidity.

Along with leaf removal, multiple cultural practices exist with potential to alter grapevine yield and fruit composition (Rolfes, 2014; Vos, 2014; Reeve et al., 2018; Smith and Centinari, 2019; Harner et al., 2020). Cluster thinning is another widely used viticultural practice that alters crop-load via the direct reduction in cluster number per vine. Cluster thinning has a direct effect on yield, and an inconsistent effect on fruit composition. Shoot-thinning is another technique combining concepts of fruit-zone leaf removal and cluster thinning. Shoot-thinning leads to a reduction in shoot number, cluster number, and overall shoot density.

Due to the fact that fruit-zone leaf removal has failed to produce impacts on the fruit composition in ‘Frontenac’ and ‘Frontenac gris’ in previous North Dakota viticultural experiments, this study sought to examine shoot-thinning as an alternative methodology with potential for impact on fruit quality, particularly as monitored via TA and pH.

Material and Methods

Experimental Design

The research was conducted in an experimental vineyard planted at the North Dakota State University Horticulture Research Farm (NDSU HRF) located near Absaraka, ND (46°59'N 97°21'W) using own-rooted, non-irrigated ‘Frontenac’ vines established in 2006 in a Warsing sandy loam, mixed, superactive, frigid Oxyaquic Hapludolls (Soil Survey Staff, NRCS-USDA, 2021). Vine spacing was 2.5 m within row and 3.0 m between rows. Vines were trained in 2010 in a randomized complete block design (RCBD) with eight replications composed of four plant replicates within the following trellis-training systems: four-arm kniffen (4AK), mid-wire bilateral cordon with a vertical shoot positioning (VSP) system (Fig. 2.1), Geneva double curtain (GDC) (Fig. 2.2), and high wire curtain (HW) (Fig 2.3).

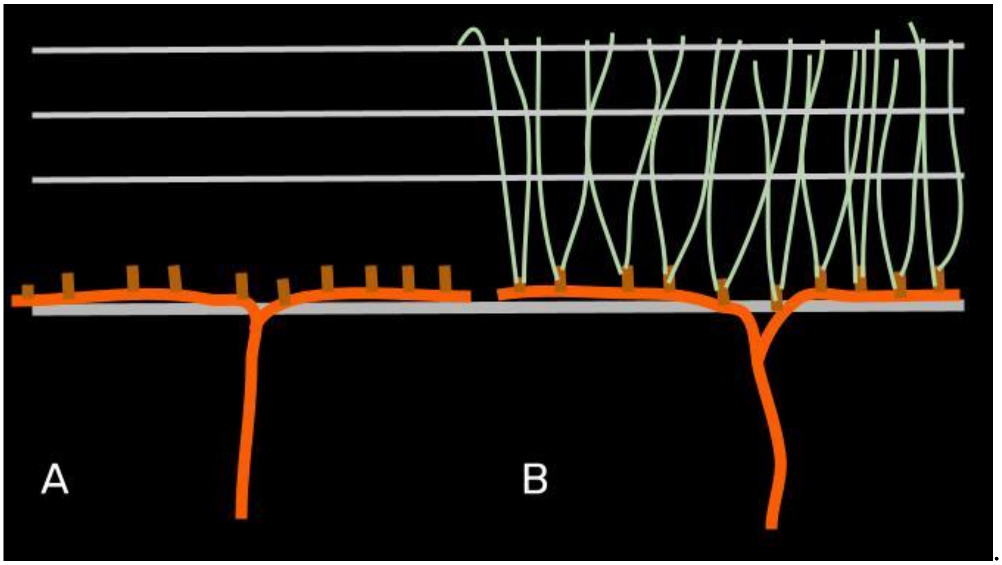

Figure 2.1. Cartoon rendering of vertical shoot positioning (VSP) trellis-training system after dormant pruning (A) and with shoots (leaves omitted for clarity) during the growing season (B).

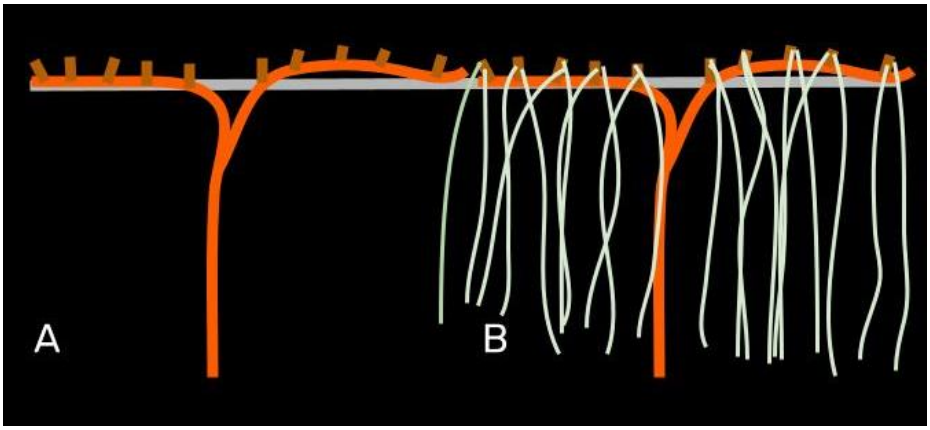

Figure 2.2. Cartoon rendering of high wire cordon, trellis-training system after dormant pruning (A) and with shoots (leaves omitted for clarity) during the growing season (B).

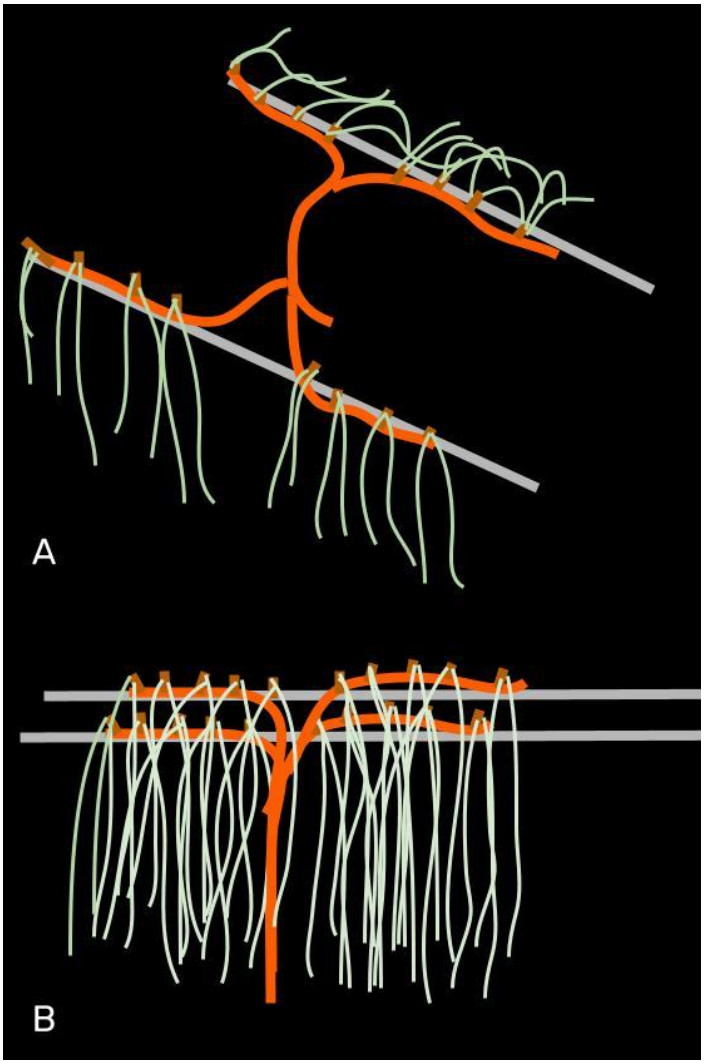

Figure 2.3. Cartoon rendering of Geneva double curtain (GDC) trellis-training system from top-view (A) and side-view (B) with shoots (leaves omitted for clarity) during the growing season.

This experiment investigated regression-based crop load manipulation within VSP, GDC, and HW trellis-training methods. In each year, individual grapevines were pruned to 40 (VSP and HW) or 80 buds per vine (GDC) during dormant pruning. After dormant pruning, individual vines were assigned to randomly selected crop-loads (dictated by shoot-number in a range of 50 to 100% of the initial bud capacity) within each trellis-training system. This was conducted to develop a gradient of potential commercial crop levels for the region. Identical bud numbers were targeted in 2017 and 2018 on each individual vine. Crop-load reduction was conducted as hand-shoot thinning approximately one-month after budburst, shortly after flowering. Shoots were removed, giving preference to the retention of shoots with clusters as opposed to blind shoots not bearing a crop.

Weather data were recorded at a weather station located near Prosper, ND, approximately 18 km from the NDSU HRF; the station is a part of the North Dakota Agriculture Weather Network (NDAWN, 2021). Minimum and maximum daily temperatures were recorded throughout the experimental period (Fig. 2.4), as well as the growing season (May 01 to Oct 31) accumulated growing degree days and rainfall (Fig. 2.5).

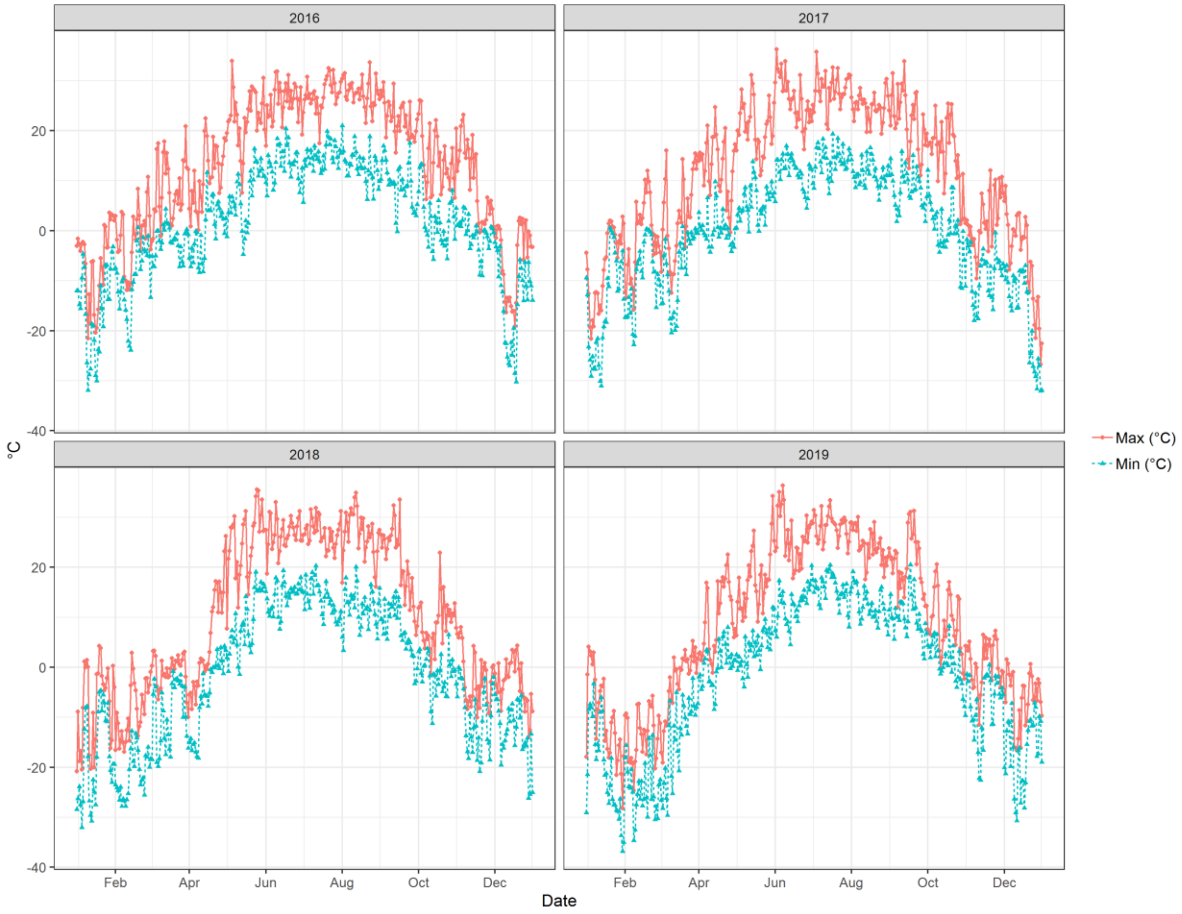

Figure 2.4. Maximum and minimum (°C) temperatures at Prosper, ND for the years of 2016 to 2019.

Vegetative Characteristics

Pruning mass (PM) for each vine was recorded to determine vine size (Table 2.1). Combining pruning mass data with yield two harvest indices were calculated: Ravaz index (RI) and Growth-yield relationships (GY). The Ravaz index was calculated by dividing the vine's yield from the current $year_n$ by the pruning mass from the following dormant season ($year_{n+1}$). Growth-yield relationships were calculated by dividing the vine's yield from the current $year_n$ by the pruning mass from the same year ($year_n$).

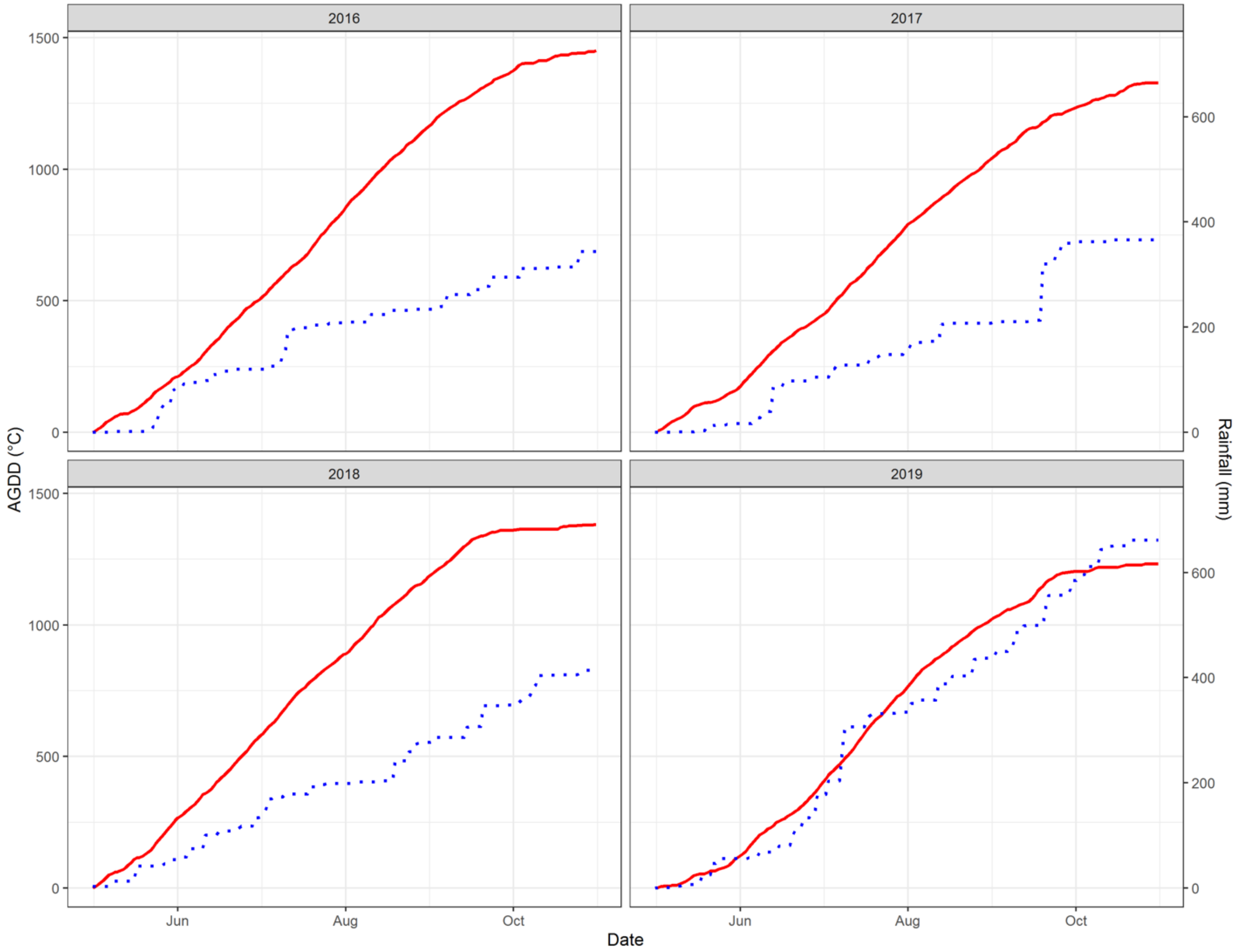

Figure 2.5. Accumulated growing degree days, base 10°C (AGDD) and accumulated rainfall (mm) at Prosper, ND for the growing seasons of 2016 to 2019.

Fruit Characteristics

At harvest, total fruit weight (yield), total cluster number, and the mass of a random six-cluster sample were recorded for each vine. From the six-cluster sample, a 100-berry subsample was collected from each experimental unit for evaluation of fruit composition. Total soluble solid content (SSC) was measured using a Pal-1 digital refractometer (Atago Co., Tokyo, Japan). Acid components of titratable acidity (TA; expressed as g tartaric acid/100 ml juice) and pH were measured with an Orion Star A111 pH meter (Thermo Fisher Scientific, Waltham, MA, USA).

The TA was measured via titration of a 5.0 mL aliquot of juice to an endpoint pH of 8.2 with 0.1 N NaOH titrant. Juice pH was measured using standard practices, via emersion in the extracted juice solution.

Differential Thermal Analysis

Through the 2017-2018 and 2018-2019 dormant season dormant buds were subjected to differential thermal analysis (DTA) to examine low temperature exotherms (LTE_{50}) on four separate dates. Cane caliper was recorded on Jan. 10, 2018 to investigate a hypothesis concerning a relationship between cane diameter and LTE_{50} values. However, this hypothesis was not further examined in 2018-2019 due to compounded technical challenges associated with sample collection at the site and time constraints.

Differential thermal analysis (DTA) was conducted similar to Mills et al. 2006 using methods described by Svyantek et al., 2020. Briefly, buds were sampled on randomly selected canes from node positions three to eight, acropetally up from the base of the cane. Two-bud spurs were retained below the sampled cane to allow for production of future fruit production. Canes were cut into six individual bud sections in the laboratory and collectively placed on moist Kimwipe tissue (Kimberly-Clark) within individual thermoelectric module cells. Lethal temperature exotherm (LTE) data were recorded through gradual (temperature decline of 4°C per hour) freezing of buds in a Tenney Model T2C programmable freezer (Thermal Product Solutions). After the freezer reached -50 °C, data collection was completed, and the median LTE values (LTE_{50}) were identified manually with Bud Processor 1.8.0 (Brock University, St. Catherines, ON, CAN).

Trunk Death Following Extreme Winter Freeze Events

Due to extensive grapevine trunk collapse and death following severe winter trunk injury experienced during the winter of 2018-2019, shoot-thinning treatments were not employed in 2019. Trunk removal was conducted in late June 2019, with additional grapevines showing symptoms of trunk collapse throughout July and Aug. 2019.

To assess the impact of trunk age on grapevine mortality, all trunks within the planting were recorded as young (1 yr), established (2-5 yr), or mature (>5 years). This was supplemented with monitoring of trunk caliper at 25 cm above the soil level using a digital caliper. Trunk circumference was calculated as:

$$\text{Trunk circumference} = \pi((D_1+D_2)/2)$$

D_1 is defined as the larger diameter of the trunk and D_2 is defined as smaller diameter of the trunk.

These data were assessed in conjunction with the binary response of trunk mortality in late June 2019. A subsequent follow-up measurement of mid and late season trunk collapse vines was conducted in Dec. 2019.

Crop of surviving grapevines in 2019 was not altered via shoot-thinning treatments, thus crop load was dictated manually only via initial pruning to target bud numbers of 40-buds per vine for single curtain trellis systems and 80-buds per vine for GDC trellis. Fruit was harvested and quality was analyzed as previously described.

Table 2.1. Abbreviations of viticultural traits monitored for shoot-thinned 'Frontenac' grapevines grown at the NDSU-HRF, located near Absaraka, ND, 2017-2019.

Abbreviation	Description	Unit
PM	Dormant pruning mass	kg/vine
GY	Growth-yield relationship;	
	yield of year_1/ pruning mass of year_2	unit less
RI	Ravaz-index;	
	yield of year_1/ pruning mass of year_1	unit less
SBM	Single berry mass	g/berry
CN	Cluster number	no./vine
SCM	Single cluster mass	g/ cluster
YLD	Single vine yield	kg/vine
SSC	Soluble solid content	Brix
pH	Potential of hydrogen	pH
TA	Titratable acidity, expressed as tartaric acid equivalents	g/L
BpH	Harvest ratio: Brix × pH^2	Brix × pH^2
B:TA	Harvest ratio: Brix: Titratable acidity ratio	Brix/ (g TA/L)

Statistical Analysis

Data were analyzed using R statistical software (R core team, 2021). Pearson correlations among phenotypic traits were generated using the rcorr function of HMISC package (Harrell and Dupont, 2020). Following evaluation of correlated traits, regression models were developed with the lme4 package. Likelihood-ratio based pseudo r-squared values of models were extracted with the MuMIn package (Magee, 1990; Barton, 2019). Root mean squared error (RMSE) was estimated using merTools (Knowles et al., 2019). Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were identified with the AIC and BIC functions, respectively (Schwarz, 1978, Sakamoto et al., 1986). Graphics were rendered using the ggplot2 package (Wickham, 2016).

Results and Discussion

Descriptive Statistics

Examining grapevine size indicators pruning mass, Ravaz index, and growth-yield relationship across the years of the study provided context for grapevine size and balance (Table 2.2). Pruning mass values ranged from a maximum of 2.16 kg to a minimum of 0.10 kg. For the 2017 and 2018, Ravaz index and growth-yield relationship averages exceeded 10, indicating potential over-cropping of grapevines across the years of the study. While excessive cropping is a logical occurrence, given the experimental scenario examining crop-load, the average for the whole experiment was not expected to land within the bounds of over-cropping. Grapevine balance is not fully understood for interspecific grapevines with diverse pedigrees. Ravaz index and growth-yield relationship values above 10 are traditionally considered out of balance for *V. vinifera* cultivars, depending on location and management. Yet, with mean pruning mass values of 0.78 and 0.50 kg in 2017 and 2018, only heavily thinned grapevines would be expected to fall below a RI or growth-yield relationship value of 10. Pruning mass may not be the only indicator necessary when accounting for grapevine size and yield potential for hybrid wine grape growers. In North Dakota, where a substantial portion of shoots fail to acclimate for winter entirely, the top portion of shoots that fails to develop periderm is potential pruning mass immediately lost in the dormant season at fall frost or through subsequent dehydration processes throughout winter.

Yield components single berry mass, single cluster mass, cluster number, and yield combined to give a depiction of harvest size (Table 2.3). The highest coefficient of variation (CV) for single berry mass (14.90) was observed in 2019 after significant grapevine death and injury. Within the healthy cropping years, the CV was substantially smaller (2017 CV=6.65 and 2018 CV=6.22). In 2018 single berry mass averaged 1.28 g; this was the largest single berry mass observed among the three years of evaluation. The single cluster mass minimums to maximums were 60.07 g to 154.39 g in 2017, 76.98 g to 162.14 g in 2018, and 12.51 g to 92.61 g in 2019. The range of single cluster mass in 2017 and 2018 both encompass normal values for ‘Frontenac’. Unhealthy grapevines in 2019 contributed to the reduction in single cluster mass observed; likewise, damage to buds and connective tissue experienced during the 2018-2019 winter may have driven single cluster mass to smaller values. Grapevines, if cropping on secondary buds, have smaller floral structures to begin with. Further, for grapevines growing with damaged conductive tissues, carbohydrate repartitioning prior, during, and after bloom may have been disrupted leading to reduced fruit-set (Antivilo et al., 2019).

A reduction in average cluster number in 2019 (44.00 clusters/vine) may be indicative of primary bud damage in the preceding winter. Despite no alteration to the retained shoot-number at prescribed shoot-thinning treatments, cluster number was also substantially reduced in 2018 (48.67) relative to 2017 (67.10). Peculiarly, in nearly all viticultural studies in North Dakota that monitored the 2017 and 2018 growing season, a reduction in cluster number was observed (data not shown). The Northern Great Plains experienced a substantial drought in July 2017 which, when combined with high average temperatures, may have reduced grapevine productivity in eastern North Dakota in 2018. Throughout the experiment, no vine retained 160 cluster number on GDC, something that would have been anticipated for the maximum, randomly selected value of 80 shoots dictated via shoot-thinning. This may also be indicative of a pre-existing lack of fruitfulness stemming from 2016 weather conditions during the growing season, or subtle, undetected damage to buds throughout the preceding dormant seasons.

Overall yield followed a similar trend, with an experimental decline across years starting with a mean of 6.85 kg/vine in 2017 and ending with a mean of 2.04 kg/vine in 2019. As previously discussed, no alteration in prescribed shoot-thinning was conducted in 2018 vs. 2017; all grapevines were shoot-thinned to identical, randomly selected shoot-number within the experimental bounds. The reduced mean values for cluster number and yield may have been driven by a specific reduction in fruiting capacity of VSP and GDC grapevines in 2018 relative to 2017. For GDC grapevines, cluster number reduced from 105 down to 68 clusters/vine and 10.28 down to 7.67 kg/vine. Likewise, VSP grapevines cluster number reduced from 49 to 32 clusters/vine and 5.26 to 3.62 kg/vine. HW grapevines had no such reduction in mean performance when comparing 2017 and 2018 cluster number or yield suggesting that HW primary buds had greater survival or were more fruitful. No VSP grapevines survived the 2018-2019 dormant season freeze events, thus were omitted entirely from evaluation of yield components.

Table 2.2. Descriptive statistics for individual vine yield components monitored for shoot-thinned ‘Frontenac’ grapevines grown at the Absaraka HRF, near Absaraka, ND, 2017-2019.

	Single berry mass (g)			Single cluster mass (g)			Clusters (no./vine)			Yield (kg/vine)		
Trait	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Min Value	0.86	1.10	0.78	60.07	76.98	12.51	21.00	13.00	11.00	2.19	1.27	0.36
Max Value	1.18	1.46	1.32	154.39	162.14	92.61	147.00	136.00	87.00	18.05	15.53	6.67
Coefficient of variation	6.65	6.22	14.90	18.95	15.79	43.03	49.32	53.10	48.31	48.75	54.30	74.00
Variance	0.00	0.00	0.02	394.79	332.47	530.40	1095.21	667.81	451.88	11.06	9.42	3.59
Standard Deviation	0.07	0.08	0.15	19.87	18.23	23.03	33.09	25.84	21.26	3.33	3.07	1.90
Confidence interval-upper (95%)	1.07	1.29	1.13	109.97	119.86	65.36	75.65	54.87	54.93	7.71	6.39	3.54
Confidence interval lower (95%)	1.03	1.26	0.92	99.71	111.10	41.68	58.55	42.46	33.07	6.00	4.92	1.59
Overall Mean	1.05	1.28	1.02	104.84	115.48	53.52	67.10	48.67	44.00	6.85	5.65	2.56
Median	1.06	1.28	0.97	102.16	114.70	55.79	59.00	40.00	36.00	6.05	4.89	2.04
GDC Mean	1.03	1.24	1.00	97.64	108.87	55.27	105.17	67.58	51.33	10.28	7.67	2.44
HW Mean	1.04	1.28	1.05	106.84	124.88	44.59	52.26	54.00	40.60	5.59	6.64	1.79
VSP Mean	1.08	1.30	---	108.82	113.00	---	48.74	32.41	---	5.26	3.62	---

Table 2.3. Descriptive statistics for pruning mass (PM), Ravaz index (RI), and growth-yield relationship (GY) monitored for shoot-thinned ‘Frontenac’ grapevines grown at the Absaraka HRF, near Absaraka, ND, 2017-2019.

	Pruning mass (kg/vine)			Ravaz index			Growth-yield relationship		
Trait	2017	2018	2019	2017	2018	2019	2017	2018	2019
Min Value	0.20	0.10	0.19	1.76	2.55	---	1.76	2.55	1.14
Max Value	2.16	1.42	1.15	56.70	41.62	---	56.70	41.62	14.62
Coefficient of variation	61.86	46.67	56.56	92.97	62.02	---	93.69	62.35	85.47
Variance	0.23	0.06	0.09	155.92	67.30	---	154.76	66.79	20.08
Standard Deviation	0.48	0.23	0.30	12.49	8.20	---	12.44	8.17	4.48
Confidence interval-upper (95%)	0.91	0.56	0.74	16.66	15.20	---	16.46	15.04	8.26
Confidence interval lower (95%)	0.66	0.45	0.33	10.21	11.26	---	10.09	11.17	2.23
Overall Average	0.78	0.50	0.53	13.43	13.23	---	13.28	13.11	5.24
Median	0.67	0.49	0.45	8.81	10.50	---	8.58	10.50	2.91
GDC Average	0.44	0.44	0.45	33.21	18.35	---	27.81	18.12	6.80
HW Average	0.79	0.55	0.64	13.26	11.64	---	8.46	16.42	3.38
VSP Average	1.05	0.51	---	10.80	4.62	---	6.20	7.45	---

Table 2.4. Descriptive statistics for fruit quality traits monitored for shoot-thinned ‘Frontenac’ grapevines grown at the Absaraka HRF, near Absaraka, ND, 2019.

	Soluble solids content (Brix)			pH			Titratable acidity (g/L)			Brix × pH^2			Brix:TA ratio		
Trait	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Min Value	18.2	22.4	10.2	2.85	3.26	2.62	13.20	9.50	17.55	147.83	243.94	75.46	1.08	1.27	0.52
Max Value	26.6	27.4	22.1	3.26	3.65	3.04	18.30	19.50	20.70	276.47	361.04	180.77	1.88	2.75	1.25
Coefficient of variation	7.4	4.2	19.1	2.63	2.61	4.46	7.37	14.28	5.81	10.89	7.64	23.61	1.04	1.48	21.93
Variance	3.1	1.1	10.2	0.01	0.01	0.02	1.32	0.30	1.19	615.33	549.58	989.25	0.02	0.94	0.04
Standard Deviation	1.8	1.1	3.2	0.08	0.09	0.13	1.15	1.80	1.09	24.81	23.44	31.45	0.16	0.31	0.20
Confidence interval-upper (95%)	24.0	25.9	18.9	3.13	3.48	2.89	15.89	13.10	19.50	234.11	312.57	154.34	1.56	2.14	1.03
Confidence interval lower (95%)	23.1	25.4	14.6	3.08	3.44	2.72	15.30	12.24	18.03	221.30	301.30	112.08	1.48	1.99	0.77
Overall Average	23.6	25.6	16.8	3.11	3.46	2.81	15.60	12.70	18.76	227.70	306.94	133.21	1.52	2.06	0.90
Median	23.6	25.6	17.8	3.11	3.45	2.84	15.60	12.50	18.60	227.76	306.17	125.62	1.51	2.04	0.88
GDC Average	23.2	25.5	17.4	3.09	3.41	2.83	14.94	12.30	18.50	221.56	296.64	140.79	1.56	2.10	0.94
HW Average	24.9	25.6	16.0	3.13	3.43	2.78	16.08	12.60	19.08	243.10	302.68	124.13	1.55	2.08	0.85
VSP Average	22.8	25.7	---	3.11	3.51	---	15.71	13.0	---	219.80	316.76	---	1.45	2.02	---

SSC ranged from 18.20 to 26.60 Brix in 2017, from 22.40 to 27.40 Brix in 2018, and 10.20 to 22.10 Brix in 2019 (Table 2.4). HW trellis grapevines had the highest average SSC in 2017 and 2018. The mean SSC was highest in 2018; the mean of other fruit composition traits were also most favorable in 2018. TA was lowest, while pH, Brix × pH^2, and BTA were greatest in 2018 relative to other years.

The year effect on fruit composition and yield was similar to values observed in other North Dakota viticultural experiments. In general, 2017 was a higher yielding year, with acceptable, but less desirable fruit composition. By contrast, 2018 was a year with lower yields, smaller, and fewer clusters. However, the fruit composition was more desirable in 2018. In 2019, fruit composition was generally poor, and yield was exceptionally low as most experimental vineyards exhibited severe winter damage. For these reasons, correlations were only examined within a given year's data to avoid skew stemming from the disparity between yield and fruit composition for years. As a result, the observed correlations and subsequent models are neither inclusive nor all-encompassing for the region; however, they provide an initial predictive framework for vineyard managers to employ when establishing on-farm production objectives.

Pearson Correlations of Viticulture Traits

In 2017, yield was negatively associated with SSC ($r=-0.310$, $p \leq 0.05$), pH ($r=-0.233$, $p \leq 0.05$), and their compound trait, BPH ($r=-0.315$, $p \leq 0.05$) (Table 2.5). For the two yield indices, GY and Ravaz index, multiple fruit composition (SSC, pH, and Brix × pH^2) traits were negatively correlated, but no correlations were identified for TA or Brix:TA ratio. No significant correlation was detected for TA except for the compound trait containing TA, Brix:TA ratio ($r=-0.694$, $p \leq 0.001$). Fruit yield components, cluster number, single cluster mass, and single berry mass varied in the correlations. cluster number did not directly correlate with single cluster mass, though subtle contributions may have arose from the negative correlation between cluster number and single berry mass (r=-0.253, $p \leq 0.05$). Yield was not significantly correlated with the single berry mass nor single cluster mass. Yet, as would be expected, cluster number and yield were strongly, positively correlated (r=0.922, $p \leq 0.001$).

Correlations in 2018 followed similar trends (Table 2.6). As anticipated, cluster number and yield were positively correlated (r=0.952, $p \leq 0.001$). However, no correlations were detected between cluster number and single cluster mass. Concerning fruit composition variables, TA was not correlated with any variable monitored except for Brix:TA ratio (r=-0.947, $p \leq 0.001$). SSC correlations were identified for pruning mass, Ravaz index, cluster number, yield, pH, and Brix × pH^2. For pH, correlations were identified with GY, Ravaz index, single berry mass, single cluster mass, cluster number, yield, Brix × pH^2, and Brix:TA ratio.

Table 2.5. Pearson’s correlation coefficient and significance estimates for 12 variables of eastern North Dakota ‘Frontenac’ in 2017.[1]

Trait	PM	GY	RI	SBM	CN	SCM	YLD	SSC	pH	TA	BPH	Brix:TA ratio
PM		-0.616 ***[1]	-0.479 ***	0.319 *	-0.341 **	0.500 ***	-0.165	-0.137	0.240 *	0.035	0.012	-0.140
GY			0.799 ***	-0.286 *	0.803 ***	-0.289 *	0.677 ***	-0.331 **	-0.440 ***	-0.119	-0.415 ***	-0.144
RI				-0.344 **	0.708 ***	-0.243 *	0.555 ***	-0.401 **	-0.489 ***	-0.123	-0.485 ***	-0.192
SBM					-0.253 *	0.466 ***	-0.001	-0.025	0.432 ***	-0.042	0.184	0.008
CN						-0.131	0.922 ***	-0.346 **	-0.356 **	-0.205	-0.396 **	-0.094
SCM							0.081	-0.057	0.304 *	-0.1	0.097	0.010
YLD								-0.310 *	-0.233 *	-0.176	-0.315 *	-0.093
SSC									0.509 ***	0.025	0.912 ***	0.699 ***
pH										-0.166	0.815 ***	0.475 ***
TA											-0.057	-0.694 ***
BPH												0.692 ***
Brix:TA ratio												

[1] PM= dormant pruning mass (kg/vine); GY= growth-yield relationship; RI= Ravaz index; SBM=single berry mass (g/berry); CN= cluster number (no./vine); SCM= single cluster mass (g/cluster); YLD= single vine yield (kg/vine); SSC= soluble solid content (Brix); TA= titratable acidity (g/L); BpH= harvest ratio, Brix × pH^2; Brix:TA ratio= harvest ratio, Brix: Titratable acidity ratio.

[2] P-value 0.05 *, 0.01**, 0.001 ***.

Table 2.6. Pearson's correlation coefficient and significance estimates for 12 variables of eastern North Dakota 'Frontenac' in 2018.[1]

Trait	PM	GY	RI	SBM	CN	SCM	YLD	SSC	pH	TA	BPH	Brix:TA ratio
PM		-0.507 ***[2]	-0.081	0.277 *	0.309 **	0.405 ***	0.434 ***	-0.346 **	0.115	0.033	-0.119	-0.086
GY			0.683 ***	-0.187	0.445 ***	-0.124	0.400 ***	-0.084	-0.369 **	-0.033	-0.206	0.012
RI				-0.188	0.754 ***	-0.164	0.685 ***	-0.340 **	-0.531 ***	-0.071	-0.477 ***	-0.032
SBM					0.055 *	0.118 ***	0.051	-0.354	0.087 ***	0.048	-0.097	-0.098
CN						0.014	0.952 ***	-0.527 ***	-0.478 ***	-0.142	-0.475 ***	-0.004
SCM							0.136	-0.041	0.129 *	-0.036	0.028	0.050
YLD								-0.509 ***	-0.404 ***	-0.128	-0.436 ***	-0.007
SSC									0.323 **	-0.205	0.685 ***	0.436 ***
pH										-0.125	0.719 ***	0.223
TA											-0.109	-0.947 ***
BPH												0.287 *
Brix:TA ratio												

[1] PM= dormant pruning mass (kg/vine); GY= growth-yield relationship; RI= Ravaz index; SBM=single berry mass (g/berry); CN= cluster number (no./vine); SCM= single cluster mass (g/cluster); YLD= single vine yield (kg/vine); SSC= soluble solid content (Brix); TA= titratable acidity (g/L); BpH= harvest ratio, Brix × pH^2; Brix:TA ratio= harvest ratio, Brix: Titratable acidity ratio.

[2] P-value 0.05 *, 0.01**, 0.001 ***.

Table 2.7. Pearson's correlation coefficient and significance estimates for 12 variables of eastern North Dakota 'Frontenac' in 2019.[1]

Trait	PM	GY	SBM	SCM	CN	YLD	SSC	pH	MAL	TA	BpH	BTA
PM		-0.138	-0.089	0.254	0.199	0.159	-0.188	-0.387	-0.283	0.291	-0.318	-0.262
GY			-0.025	0.745 **	0.811 **	0.941 ***	-0.222	0.074	-0.018	0.262	-0.110	-0.266
SBM				0.329	-0.171	0.082	0.034	-0.134	0.182	0.240	-0.023	-0.018
SCM					0.507	0.838 **	0.067	-0.065	0.151	0.261	0.050	-0.020
CN						0.872 ***	-0.637 *	-0.220	-0.388	0.345	-0.562 *	-0.652 *
YLD							-0.317	-0.045	-0.090	0.337	-0.237	-0.373
SSC								0.372	0.516	-0.383	0.931 ***	0.969 ***
pH									0.274	-0.607 *	0.682 *	0.478
MAL										-0.119	0.507	0.471
TA											-0.533 *	-0.597 *
BpH												0.947 ***
BTA												

[1] PM= dormant pruning mass (kg/vine); GY= growth-yield relationship; RI= Ravaz index; SBM=single berry mass (g/berry); CN= cluster number (no./vine); SCM= single cluster mass (g/cluster); YLD= single vine yield (kg/vine); SSC= soluble solid content (Brix); TA= titratable acidity (g/L); BpH= harvest ratio, Brix × pH[2]; Brix:TA ratio= harvest ratio, Brix: Titratable acidity ratio.

[2] P-value 0.05 *, 0.01**, 0.001 ***.

The analysis of 2019 data consisted of a limited number of surviving grapevines, following the removal of dead and collapsed grapevines. Pearson's correlation coefficients indicated a significant negative association for cluster number and SSC ($r=-0.637$, $p \leq 0.05$); however, no significance was detected for SSC and yield (Table 2.7). As anticipated, SSC was positively associated with Brix × pH^2 and Brix:TA ratio, the fruit composition traits accounting for SSC and acid components. Stemming from this, Brix × pH^2 and Brix:TA ratio were both negatively correlated with cluster number.

Best Models for SSC

Following response screening based on viticultural variables, multiple models were generated to identify predictive variables for fruit composition traits of 'Frontenac' grapevines in eastern North Dakota. Due to the lack of correlation between TA and viticultural traits, no further exploration of models was conducted for TA. Significant correlations for SSC, an important determinant of wine style, was examined in depth.

For SSC in 2017, multiple models were evaluated, and reduced to the best 15 models (Table 2.8). The selected model for SSC prediction, based on r^2, AIC, BIC, RMSE, and variable number, incorporated trellis-training type and cluster number for the equation SSC= 26.73 – 0.06 (HW) –2.30 (VSP) –0.034*cluster number. Thus, for each retained cluster, a grower may anticipate a reduction in SSC for grape must.

Based on 2018, a similar model was identified, where SSC= 27.58 – 0.35 (HW) – 0.87 (VSP) – 0.030*cluster number (Table 2.9). Examining both years, farmers may be able to retain 29-33 clusters per vine before expecting a loss of 1% SSC. In both years' models, the VSP trellis-training system resulted in a reduction of SSC relative to either HW or GDC systems. At least for ‘Frontenac’ in North Dakota conditions, VSP cannot be recommended due to this resulting reduction in sugar accumulation.

Table 2.8. Best models for total soluble solid content prediction based on basic viticultural information for ‘Frontenac’ grapevines grown in Eastern North Dakota in 2017.

	Variable included in model							
No. of Variables	Trellis	YLD	CN	PM	r^2	AIC	BIC	RMSE
1	Y	--	--	--	0.273	231.2	241.8	1.47
1	--	Y	--	--	0.096	246.4	254.8	1.64
1	--	--	Y	--	0.120	249.4	257.8	1.62
1	--	--	--	Y	0.019	247.4	255.8	1.71
2	Y	Y	--	--	0.373	228.1	240.7	1.37
2	Y	--	Y	--	0.446	225.3	238.0	1.28
2	Y	--	--	Y	0.283	232.1	244.8	1.46
2	--	Y	Y	--	0.120	253.1	263.7	1.62
2	--	Y	--	Y	0.132	245.7	256.3	1.61
2	--	--	Y	Y	0.193	246.0	256.6	1.55
3	Y	Y	Y	--	0.452	228.9	243.7	1.28
3	Y	Y	--	Y	0.373	229.7	244.5	1.37
3	Y	--	Y	Y	0.451	226.7	241.4	1.28
3	--	Y	Y	Y	0.216	248.0	260.7	1.53
4	Y	Y	Y	Y	0.465	229.3	246.2	1.26

Best regression model equation for SSC prediction in 2017.

Model	r^2	Adj. r^2
SSC= 26.73 – 0.06(HW) – 2.30(VSP) – 0.034*CN	0.446	0.455

Table 2.9. Best models for total soluble solid content prediction based on basic viticultural information for ‘Frontenac’ grapevines grown in Eastern North Dakota in 2018.

	Variable included in model							
No. of Variables	Trellis	YLD	CN	PM	r^2	AIC	BIC	RMSE
1	Y	--	--	--	0.012	221.54	232.85	1.02
1	--	Y	--	--	0.268	201.98	211.03	0.85
1	--	--	Y	--	0.289	204.17	213.23	0.84
1	--	--	--	Y	0.120	209.62	218.67	0.96
2	Y	Y	--	--	0.341	200.33	213.91	0.80
2	Y	--	Y	--	0.368	201.68	215.25	0.78
2	Y	--	--	Y	0.139	213.68	227.26	0.95
2	--	Y	Y	--	0.289	208.74	220.06	0.84
2	--	Y	--	Y	0.285	201.90	213.21	0.85
2	--	--	Y	Y	0.323	202.46	213.78	0.82
3	Y	Y	Y	--	0.371	206.00	221.84	0.78
3	Y	Y	--	Y	0.341	201.59	217.43	0.80
3	Y	--	Y	Y	0.378	202.23	218.07	0.77
3	--	Y	Y	Y	0.329	206.15	219.73	0.82
4	Y	Y	Y	Y	0.378	206.49	224.59	0.77

Best regression model equation for SSC prediction in 2018.

Model	r^2	Adj. r^2
SSC= 27.58 – 0.35(HW) – 0.87(VSP) – 0.030*CN	0.368	0.388

Observed Cold-Hardiness

Tracking winter cold-hardiness of grapevines throughout the 2017-2018 season across four testing events revealed LTE_{50} values ranging from a mean of -31°C to -26 °C (Fig. 2.6.). No differences were detected among trellis-training systems within dates. Similar results were observed in the 2018 fall and winter dormant season, for which no differences were detected among trellis-training systems (Fig. 2.7.). Overall, observed LTE_{50} values were between -23 °C and -28 °C.

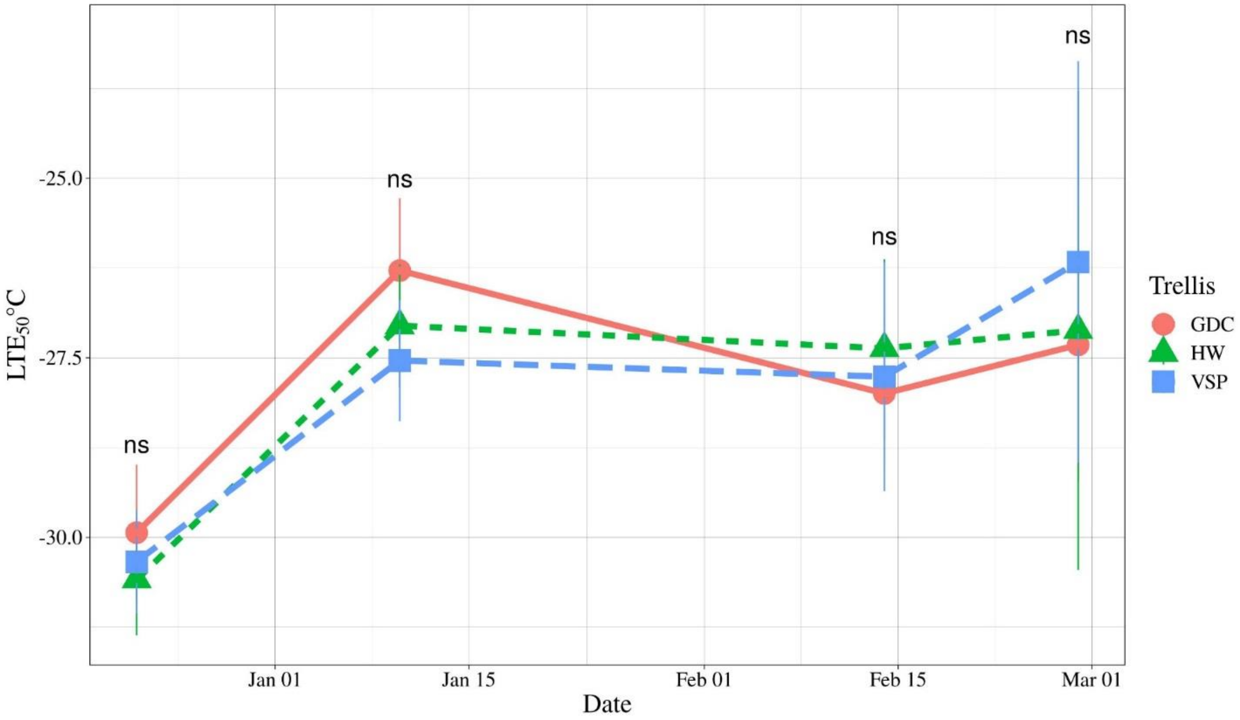

Figure 2.6. LTE_{50} values for shoot-thinned ‘Frontenac’ grapevines grown at the Absaraka HRF, near Absaraka, ND, during the winter of 2017-2018.

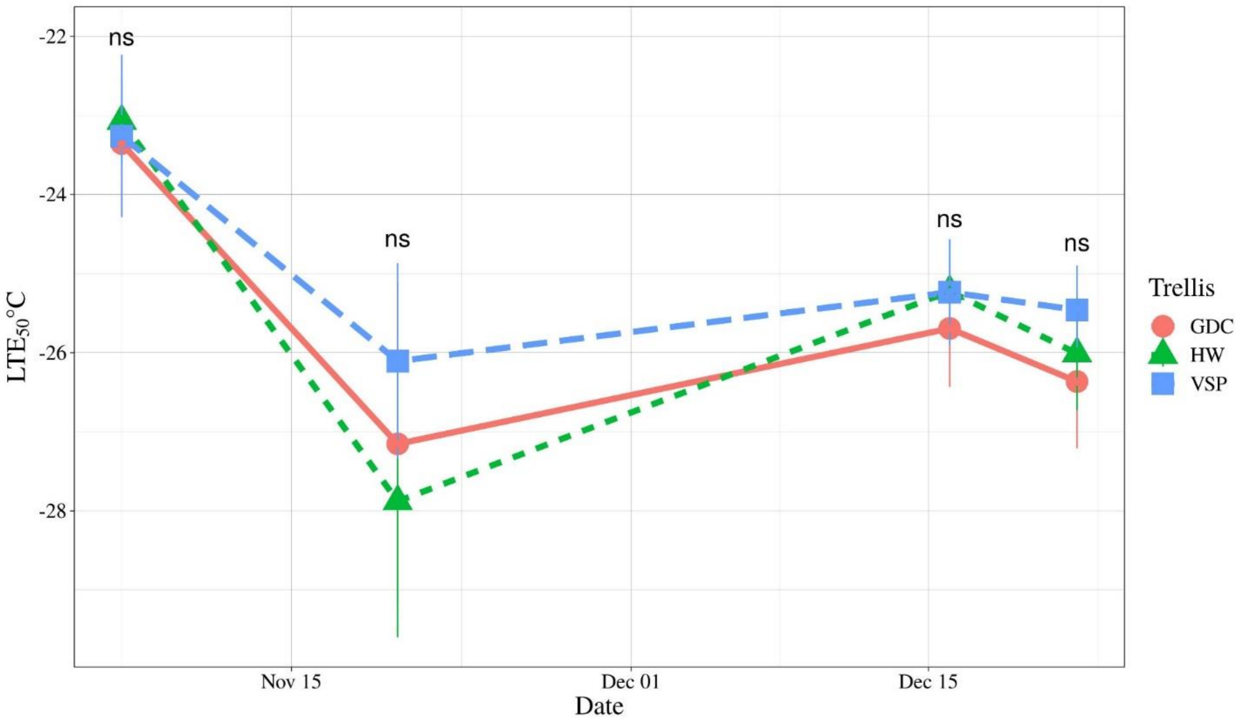

Figure 2.7. LTE_{50} values for shoot-thinned 'Frontenac' grapevines grown at the Absaraka HRF, near Absaraka, ND, during the fall and winter of 2018.

Linear Models for Cold-hardiness

Within each DTA evaluation date, we screened for linear effects of cluster number, pruning mass, Ravaz index, growth-yield relationship, and yield. The only trait with a significant response was yield (Figs. 2.8 and 2.9); however, this response was only detected on one testing event throughout the entire experimental period, Jan. 10, 2018. All remaining testing events failed to produce a significantly observable response. This indicates that grapevine cold-hardiness of 'Frontenac' in eastern North Dakota, as expressed by LTE_{50} values, may not be linearly correlated in this laboratory based assay of buds. Cold-hardiness and yield may be correlated for other genotypes, tissues (xylem, phloem), or environmental conditions, yet for 'Frontenac' no consistent relationship was detected within these evaluations.

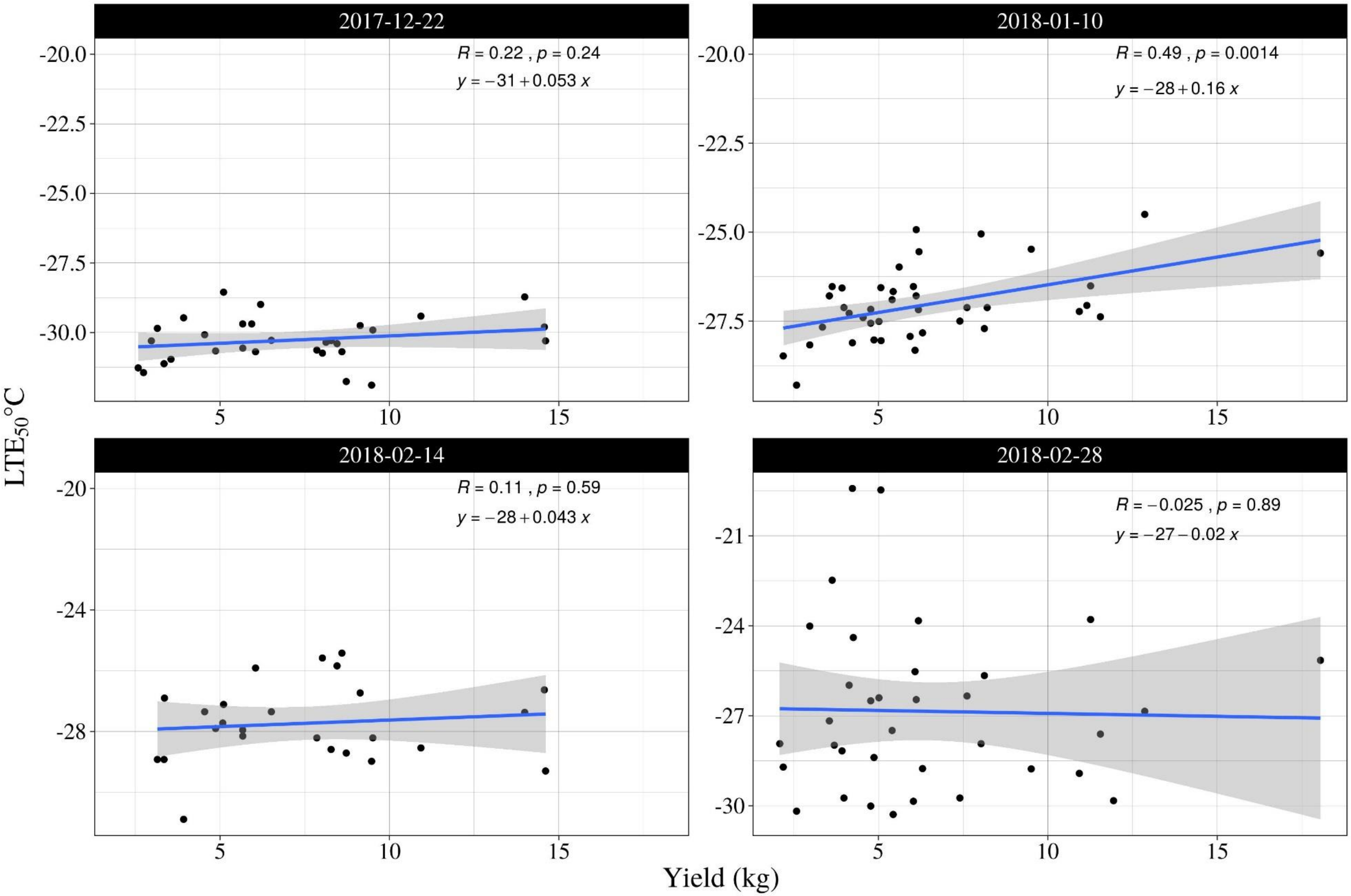

Figure 2.8. Linear relationship of LTE_{50} values and yield for shoot-thinned ‘Frontenac’ grapevines grown at the NDSU HRF, near Absaraka, ND, during the 2017-2018 dormant season.

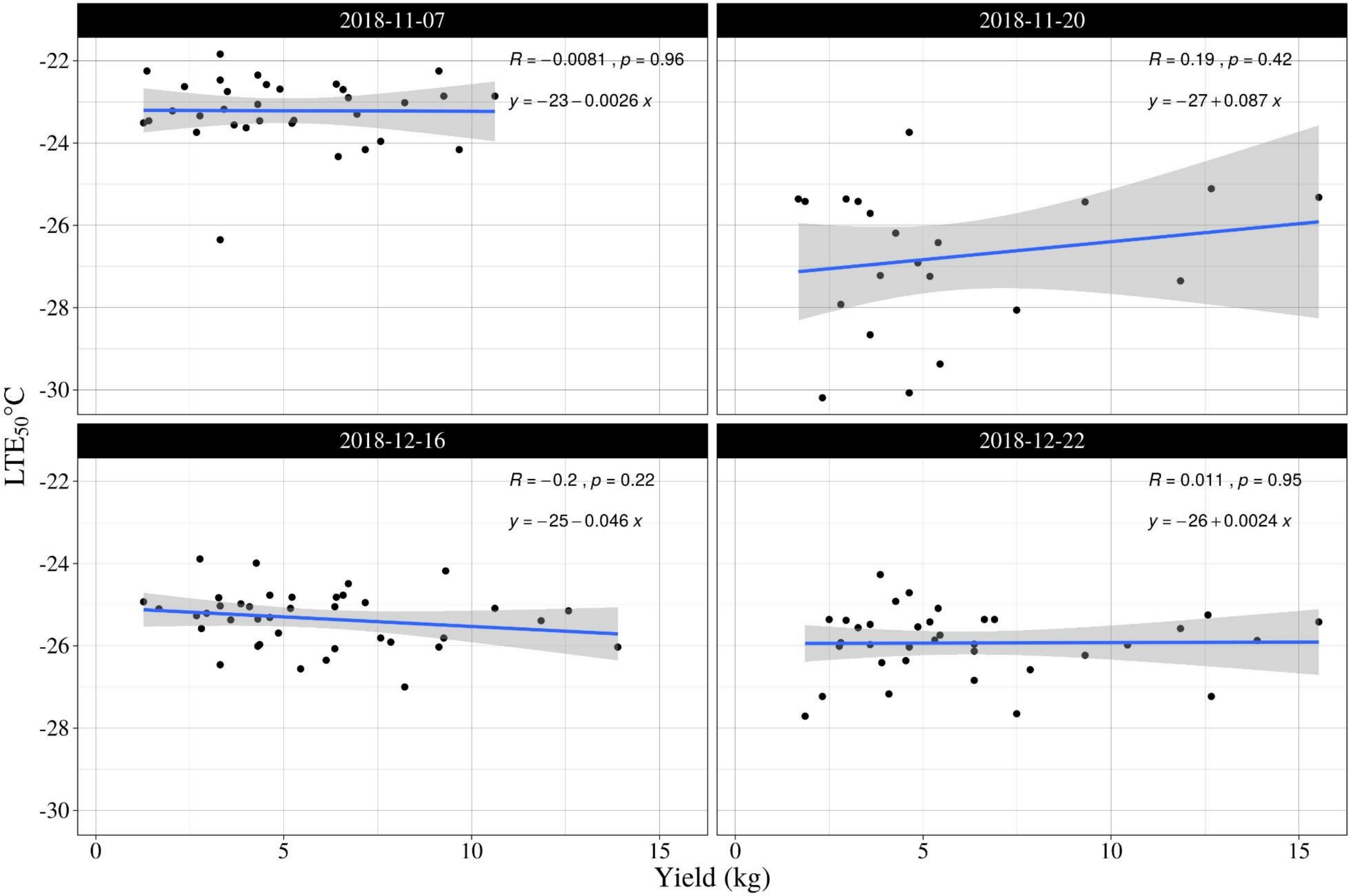

Figure 2.9. Linear relationship of LTE$_{50}$ values and yield for shoot-thinned 'Frontenac' grapevines grown at the NDSU HRF, near Absaraka, ND, during fall and winter of the 2018 dormant season.

Grapevine Trunk Survival Screening

The winter of 2018-2019 offered a unique opportunity for phenotypic evaluation of grapevine trunk survival. Due to extreme weather events described by Svyantek et al. 2020, the majority of grapevine plantings at the NDSU HRF succumbed to cold damage (bud death, trunk death, and mid-season trunk collapse). More than 90% of the trunks in the experimental ‘Frontenac’ vineyard died outright or experienced trunk collapse mid-season (Figs. 2.10, 2.11); this led to vineyard management objectives shifting from research to re-establishment. After trunk removal (Fig. 2.12), new suckers were retained for future trunk establishment evaluations (Fig. 2.13).

Figure 2.10. Initial symptoms of shoot collapse for an individual spur of ‘Frontenac’ grown in at the NDSU HRF near Absaraka, ND (image captured on June 26, 2019).

Figure 2.11. Additional symptoms of shoot collapse on spurs of ‘Frontenac’ grown in at the NDSU HRF near Absaraka, ND (image captured on June 26, 2019); note the abscission of leaves and general lack of budburst on multiple spurs.

Figure 2.12. Trunk removal of dead and collapsing ‘Frontenac’ trunks at the NDSU HRF near Absaraka, ND (image captured on June 26, 2019).

Figure 2.13. Establishing new ‘Frontenac’ trunks from sucker shoots following removal of dead grapevine trunks at the NDSU HRF near Absaraka, ND (image captured on July 08, 2019).

As trunks were removed, they were monitored for relative age and trunk diameter to evaluate the hypothesis that younger grapevine trunks and trunks of reduced caliper (smaller diameter, circumference, less vigor) may more successfully survive severe winter events (Figs. 2.14-16). While no trends were detected between grapevine yield and survival, one interesting observation was that no VSP vines survived the winter events. Although multiple two- to four-year-old VSP vines collapsed midseason, the majority of all VSP vines failed to break bud in the spring of 2019, having died outright during the preceding winter. While GDC and HW had higher survival rates, the surviving vine’s cropping performance in 2019 was severely diminished. No trellis-training system had commercially acceptable survival for ‘Frontenac’ grapevines. Further, no clear association was detected between trunk age or size and survival following the catastrophic winter events.

In cold-climates, “spare-parts” viticulture is regularly practiced to avoid or overcome potential winter damage. “Spare-parts” viticulture refers to the orchestrated retention of grapevine parts (trunks, cordons, spurs) of various ages to diversify the risk associated with winter freeze events. While this is a logical, viticultural solution to challenging environments, the evaluation of trunk survival provided no evidence of variation in survival under North Dakota conditions. Considering ‘Frontenac’ is one of the most widely grown cold-hardy interspecific hybrid wine grapes in northern climates with minimal damage observed under other environmental conditions, the lack of survival and productivity buffering provided by “spare-parts” practices is likely localized to the extreme challenges of North Dakota’s specific winter conditions. While “spare-parts” viticulture should continue to be practiced, especially in light of risks associated with debilitating trunk disease complexes, in North Dakota, more protective and preventative efforts may be necessary for consistent, profitable production of ‘Frontenac’ grapevines.

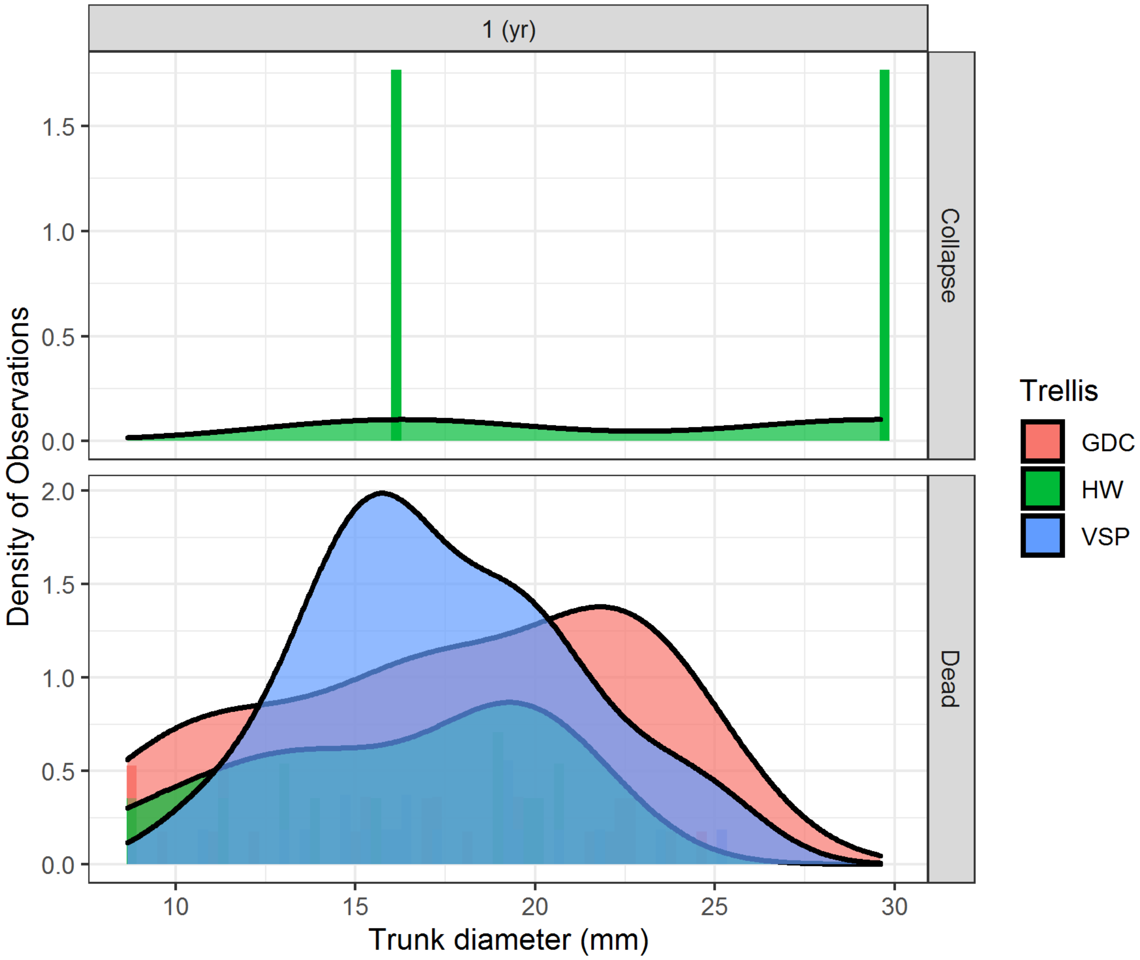

Figure 2.14. Distributions of dead and collapsed one year old ‘Frontenac’ grapevine trunks at the NDSU HRF near Absaraka, ND in 2019.

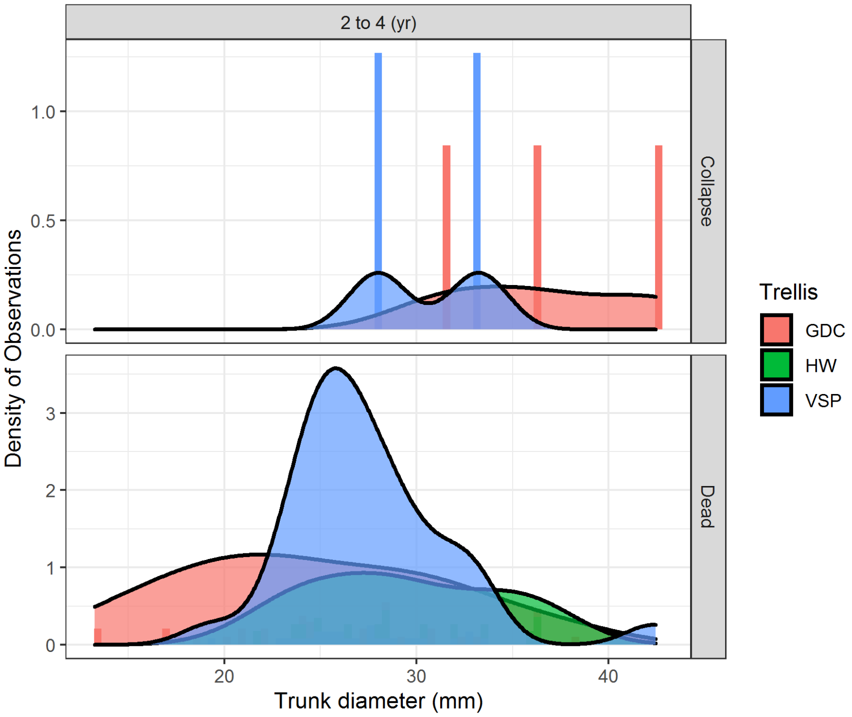

Figure 2.15. Distributions of dead and collapsed two- to four-year-old ‘Frontenac’ grapevine trunks at the NDSU HRF near Absaraka, ND in 2019.

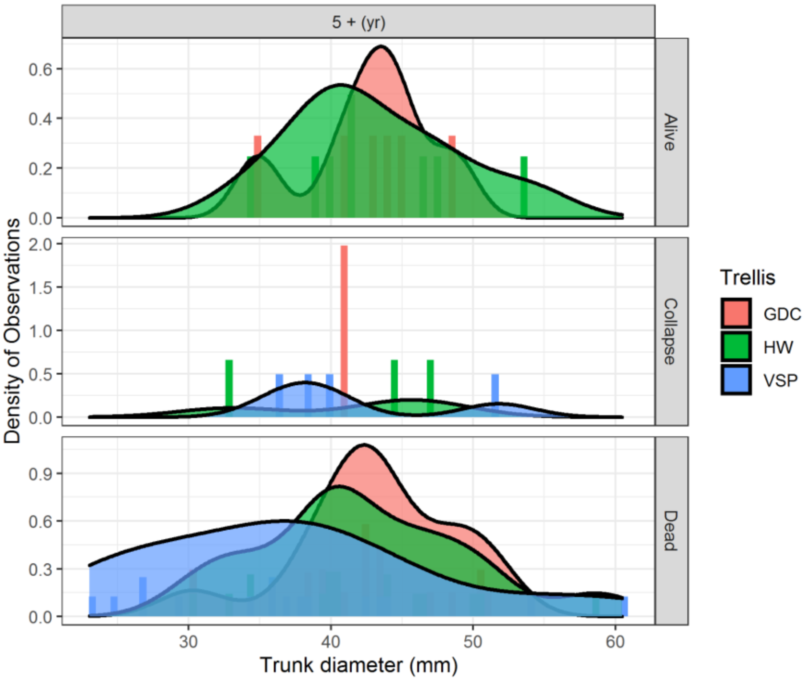

Figure 2.16. Distributions of dead and collapsed mature (>five-year-old) ‘Frontenac’ grapevine trunks at the NDSU HRF near Absaraka, ND in 2019.

Future Work

Improving Fruit Composition

Shoot-thinning is a cultural practice that reduces crop-load while simultaneously altering canopy dynamics. Through a reduction in shoot number, a vine's source-sink carbohydrate allocation is altered and further impact on fruit composition might be anticipated due to changes in sunlight penetration and fruit exposure. Typically, these conjoined effects confound the interpretation of results making it difficult to ascertain whether a given grapevine's performance stems from reduced yield or increased fruit exposure.

In this experiment, the treatment effects, as monitored via regression, had minute impacts on 'Frontenac' fruit composition. These results combined with prior canopy management research on 'Frontenac' and 'Frontenac gris', lead to the realistic concession that neither crop-load nor canopy management drive sugar accumulation or acid degradation in this unique, interspecific hybrid, cold-hardy wine grapevine (Aipperspach et al. 2020, Olson, 2016). From a production standpoint, this is beneficial to acknowledge; farmers can target higher yields without an economic penalty to fruit composition. However, this also indicates that there may be few cultural practices that farmers can utilize to improve the initial grape must composition prior to fermentation. Thus, the emphasis for deacidification of 'Frontenac' musts is placed on the shoulders of winemakers who must further explore deacidification processes both biological (*Saccharomyces* spp. yeast selections, *Oenococcus oeni* bacteria selections) and chemical in order to reduce the high acid levels of 'Frontenac' derived wines in North Dakota (Olson, 2016).

There are additional methods to increase ripening within current plantings of 'Frontenac' that have yet to be explored. Chiefly, nutrient supplementation (phosphorous) may induce a reduction in titratable acidity at harvest. Yet, this is likely to alter only the tartaric acid component of the grape must, an obstacle for which winemakers already have a tool (cold-stabilization of potassium tartrate). The malic acid content is unlikely to be directly altered through nutritional supplementation; however, a healthier grapevine may more effectively metabolically breakdown malic acid. Depending on the extent to which potassium supplementation alters fruit pH, potassium treatment effect on fruit composition may be viewed as either a positive or negative outcome. Despite the fact that fruit may be harvested and crushed with a lower initial TA, winemakers may still require use of malolactic degrading yeasts or bacteria due to high malic acid content, thus restricting targeted wine styles.

Reflective mulch is used in peach cultivation and has been explored in other crops, such as okra and banana (Layne et al., 2001; Gordon et al., 2010; Vinson, 2016). The use of reflective mulch creates a one-time expense for farmers rather than a recurrent labor cost for manual leaf removal or shoot-thinning. Its benefits include weed suppression and increased light reflected into the underside of grapevine canopies that are frequently overgrown with weeds when left unchecked.

The results of reflective mulch use in grapevines are largely preliminary; it is not utilized on a commercial scale to-date. In grapevines, Reynolds et al. (2008) found little to no effect of reflective mulching on must TA or SSC of ‘Riesling’, ‘Pinot Meunier’, ‘Pinot noir’, ‘Cabernet Franc’, or ‘Cabernet Sauvignon’. However, for final wine compositions, mulched ‘Pinot noir’ had lower TA. In general, the examined reflective mulches had a subtle-to-undetected effect relative to grapevine age except for impacts on increased color content (hue and intensity) of musts. For ‘Cabernet Sauvignon’, Wang et al. (2020) noted losses of total norisoprenoids and terpenoids when black geotextiles were employed, a potential negative impact of geotextiles on final wine matrix chemical composition. In New York state, Hostetler et al. (2007a) did not detect TA or SSC differences associated with black or white geotextiles for ‘Cabernet Franc’. Vine size was increased due to geotextiles, potentially owing to weed suppression relative to the herbicide strip controls. Hostetler et al. (2007b) completed a similar study examining under vine geotextile mulches in ‘Pinot noir’ grown in the Finger Lakes region of New York. While no fruit composition alterations were noted, white geotextiles resulted in higher yields and larger cluster mass.

Crop-load reduction via cluster thinning and shoot-thinning has an inconsistent effect on fruit composition across locations and cultivars. A five-year evaluation of ‘Vidal blanc’ noted a 0.7 Brix reduction in SSC when retained cluster number increased from 40 to 60, although no TA effect was observed (Dami et al., 2013). Likewise, no differences were detected with varying crop-load for LTE_{50} values of buds nor primary bud injury. Similar work on ‘Chambourcin’ by Dami et al. (2005) demonstrated cluster thinning effect on ripened wood nodes and bud cold-hardiness; their observations were that a reduced crop-load was associated with more desirable number of nodes and increased hardiness.

For many traits, the impact of vintage (year of production) was definitively observed, this is a trend common in grapevines and numerous other crops due to environmental factors (Martin et al., 2020; Zhu et al., 2020). In grapevines, the driving factors affecting within-year performance may be difficult to discern due to accrued impact of previous seasons. More work evaluating genotype by environment interactions for grapevines’ yield, fruit composition, and survival across years and sites will likely provide greater insight into sustainable grapevine performance for North Dakota.

Not examined within this study is economic impact of crop-load reduction via shoot-thinning. From a producer perspective, following the conclusion that TA is not beneficially reduced through crop-load or canopy practices for ‘Frontenac’, a further conclusion is that there is no potential increase in value of a reduced crop-load based on sale price. Observed in other growing regions, crop-load management on grapevines may rarely be economically justified for growers (Berkey, et al., 2011). Further studies may be necessary to fine tune an understanding of crop-load and other viticultural management practices impact on survival of ‘Frontenac’, because this study demonstrated no consistent relationship between vine yield components and overwintering ability. Ultimately, grapevine survival dictates yield capacity more than any other trait observed in North Dakota’s conditions. Survival must become the top priority in breeding, management, and maintenance of grapevines and vineyards because, from an economic stand-point, any high TA, low SSC, low pH fruit is worth infinitely more financial compensation towards the success of grapevine growers and wineries than no fruit at all.

Improving Grapevine Survival

Beyond the target objective of reducing titratable acidity in ‘Frontenac’ grape musts, this research also failed to identify any trends between ‘Frontenac’ trunk survivals and the previous year’s crop load. Based on our data, it is unlikely that all grapevines were in an over-cropped state, thus, we would have anticipated some survival following a harsh, but not uncommon, series of winter freeze events in 2018-2019. This was not the case, throughout the entire experimental vineyard, the majority of ‘Frontenac’ grapevines experienced entire trunk death or mid-summer collapse. Further, no survival trends were identified for different trellis type. While ‘Frontenac’ is a proven cultivar in most climates with severe mid-winter temperatures, the 2018-2019 winter freeze events crippled it at our experimental site. The freeze events occurred in late January-early February, when grapevines would be anticipated to approach maximal hardiness (Wolf and Cook, 1994; Clark et al., 1996; Yilmaz et al., 2021).

On-going breeding efforts at NDSU through the NDSU-GGEP are striving to address cold-hardiness for the Northern Great Plains, but for rapid impact for producers committed to growing ‘Frontenac’, a deeper exploration of its cold-hardiness dynamics and potential winter protection methodology is required. More extensive work in ‘Frontenac’ and other hybrid grapevines under diverse annual climate conditions is likely to reveal a deeper understanding of vine survival under varying abiotic and biotic stressors. As suggested by Howell (2001), for short-season, cool-climate viticulture a carbon-budget approach may be useful and necessary in fully elucidating stress tolerance and consistent performance.

Targeting an early ripening crop might be one approach to increase grapevine acclimation time, yet harvest date and crop-load have been inconsistent in association with bud and stem cold-hardiness. ‘Cabernet Sauvignon’ LT_{10} values for xylem and phloem tissue showed a variable gain of cold-hardiness (range 1 to 2 °C increase) when the entirety of the crop was removed at or before veraison. This increase in cold-hardiness associated with crop removal was observed less than 75% of all evaluated time point. Wample and Bary (1992) also observed minimal differences in cold-hardiness among harvest date treatments for ‘Cabernet Sauvignon’, with negligible differences in soluble carbohydrate and starch reserves of cane and bud tissues. Likewise, for eastern Washington ‘Concord’ grapevines, yield was negatively correlated with lignified nodes/shoot on only three out of ten possible evaluated year/site combinations. Contrastingly, Kentucky grown ‘Vidal blanc’ increased in node maturation and bud cold-hardiness with reduced crop loads (Wilson et al., 2014).

The cumulative results indicate vineyard management practices can be used to alter fruit composition as measured by SSC but not as measured by TA. Increased SSC may be desirable, but SSC is not a major obstacle in most years for ‘Frontenac’. Further, while increased SSC may be attainable, it may not be economical for growers unless substantial investment is made by wineries to compensate for reduced crop-loads.

Beyond a focus on improving ‘Frontenac’ fruit composition, researchers must strive to identify management practices that ensure ‘Frontenac’ grapevines yield consistently. In perennial fruit crops, like grapevines, yield is a function of survival, and in North Dakota, grapevine survival is not a given. Viticultural practices and environmental conditions that improve grapevine health and winter survival in North Dakota require further evaluation and include irrigation, soil-type, vineyard slope and aspect, nutrient management, and winter protection. Anecdotally, two commercial vineyards located nearby (Buffalo, ND and Casselton, ND) experienced minimal trunk loss for ‘Frontenac’ following the 2018-2019 winter freeze events. This evidence points to potential effects of micro-climate, vine age, propagation material, or nutrient practices on winter survivability.

Improvement of overwintering techniques must be investigated, such as geotextiles, trunk burial, protective paints, and trunk wraps. These techniques may provide more consistent survival for ‘Frontenac’ and potentially other wine grapevines, thus expanding the potential cultivation candidate grapevines for the region.

Nutrient supplementation may be necessary to increase grapevine health going into dormant seasons. Calcium and potassium are two nutrients associated with cold-hardiness processes with observed effects on grapevines (Sarikhani et al., 2014; Karimi, 2017; Mirbagheri et al., 2018; Haghi et a., 2019; Karimi, 2019). Nitrogen application, within reasonable limits, may not be detrimental to grapevine bud cold-hardiness (Wample et al., 1993). Further evaluation on fertility of vine’s and its impact on cold-hardiness is necessary to develop on-farm recommendations in North Dakota.

Despite grapevine trunk drastic losses in this experiment, ‘Frontenac’ retains a seat near the head of the table for North Dakota grapevine genotypes. In an adjacent replicated variety trial, trunk survival for ‘Frontenac’ and ‘Frontenac gris’ at the end of 2019 was calculated as 7% and 4%, respectively (Svyantek, et al. 2020). Though not exceptional, this was higher than 23 other replicated genotypes. Protective measure may be necessary for consistent ‘Frontenac’ production and to avoid high labor costs associated with retraining in the vineyard.

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CHAPTER 3. EFFECT OF FRUIT-ZONE LEAF REMOVAL ON ‘MARQUETTE’ GRAPEVINES

Abstract

High levels of titratable acidity (TA) in grapevine musts plague winemakers in northern, cool-climate viticultural regions; however, canopy management practices have demonstrated capacity to alter fruit composition. Thus, examining ‘Marquette’, one of the regions’ most important red wine grapevines, planted at a commercial vineyard in eastern North Dakota, a total of seven treatments, six fruit-zone leaf removal (FZLR) treatments and a control, were applied in three consecutive growing seasons (2017-2019). The FZLR was conducted at trace-bloom, fruit-set, and veraison at two levels (50% and 100%) and compared to nontreated, control vines. Fruit soluble solid content was altered by FZLR treatments, while pH was not. Fruit TA was only altered in 2018. Whole berry phenolic content was higher with early FZLR treatments, while anthocyanin content was inconsistently altered. Yield was lowest for early FZLR treatments, this resulted from lower cluster mass and reduced berry number per cluster. The results of this study indicate FZLR is not a useful management technique for reducing TA of ‘Marquette’ in eastern North Dakota. While benefits were observed (increased soluble solid content and reduced cluster compactness), they may be insufficient to justify widespread use of FZLR in eastern North Dakota where TA is a major impediment to consumer acceptability of final wine composition.

Introduction

Leaf removal and canopy management practices have been shown to impact grapevine health and disease incidence. Powdery mildew (*Erysiphe necator* Schw.) infection can be reduced through proper canopy management in conjunction with improved spray penetration (Austin et al., 2011; Moyer et al., 2016). Botrytis bunch rot (*Botrytis cinerea* Pers.), sour rot, and other diseases may also be reduced via canopy management practices with varied levels of success (English et al., 1993; Ferree et al., 2003; Hed and Centinari, 2018; Hall et al., 2018; Sabbatini and Howell, 2010; Zoecklein et al., 1992).

Along with disease management, leaf removal promotes sun exposure that can alter grapevine performance and fruit and wine composition (Bergqvist et al., 2001; Wang et al., 2018; Sanchez and Dokoozlian, 2005). Leaf removal can also be used to modulate aroma components such as methoxypyrazines, norisoprenoids, sesquiterpenes, as well as final wine composition and sensory attributes (Geoffrey et al., 2019; Harner et al., 2019; Homich et al., 2017; Kwasniewski et al., 2010; Marais et al., 1992; Scheiner et al., 2010, 2012; Silvilotti et al., 2016; Tardáguila et al., 2008). Lastly, monoterpene content may be altered via canopy and crop management practices (Kok, 2011; Skinkis et al., 2010; Yue et al., 2020).

Canopy management that changes cluster exposure leads to alterations in sunlight and temperature (Spayd et al., 2002; Chorti et al., 2010; Ristic et al., 2007). This exposure and altered microclimate drives resulting effects on fruit composition (Hickey and Wolf, 2019; VanderWeide et al., 2020; Vogel et al., 2020). Research has evaluated the effect of leaf removal timing, severity and canopy side for its impact on fruit composition, disease, and other viticultural characteristics (Hickey and Wolf, 2019; Smith and Centinari, 2019; Tarricone, et al., 2020).

With increasing production of hybrid winegrapes in the US, recent research has expanded to evaluate leaf removal practices and canopy shading on the fruit composition of diverse winegrapes. In ‘Norton’, an interspecific hybrid grapevine, malic acid content reduction is associated with increased sun exposure (Jogaiah, et al., 2012). Similar, year dependent acid reductions have been observed for ‘Cynthiana’ (Main and Morris, 2004).

Less consistent acid management via canopy management has been observed in cool climate, *V. riparia* based hybrid wine grape cultivars. North Dakota's short growing season and extreme winter temperatures limit the number of grapevine cultivars adapted for production. 'Marquette', a 2006 release from the University of Minnesota grapevine breeding program, is one of the most widely planted grapevines in North and South Dakota (Hemstad and Luby, 2008; Tuck and Gardner 2014). 'Marquette' is the result of a 1989 cross between MN 1094 and 'Ravat noir' (syn. Ravat 262) (Hemstad and Luby, 2008). Despite having low tannin content, 'Marquette' is widely used in the Upper Midwest for red wine production (Rice et al., 2017; Rice et al., 2018).

Relative to 'Frontenac', 'King of the North', and 'Valiant', three widely planted red wine grapevines in North Dakota, 'Marquette' has lower titratable acidity (TA) and minimal off-flavors. Thus, it is desirable for wine production. However, despite having lower TA than grapevines like 'Frontenac' and 'King of the North', the TA of 'Marquette' remains exceptionally high when compared to traditional *V. vinifera* lines grown in warmer climates. The TA content of wine grapes in cool and cold climates is a compounded result of climate conditions (short-season and low growing degree day accumulation) and genotype (many cold-hardy interspecific wine grapes draw heavily on *V. riparia* backgrounds). Thus, TA values in short-season hybrid grapevine growing conditions exceeding 10 g/L are normal and expected, despite breeding and management efforts (Hatterman-Valenti et al., 2016; Maante et al., 2016; Maante-Kuljus et al., 2019).

As a result of its high TA, viticulture efforts in North Dakota, and other 'Marquette' growing regions of the Midwestern and Upper-Midwest USA have evaluated methods to improve ripening potential. Aipperspach et al. (2020) identified a reduction in TA for

‘Marquette’ following fruit zone leaf removal (FZLR) practices in eastern North Dakota. Interestingly, Aipperspach et al. failed to identify any response of ‘Frontenac gris’ to FZLR within the same study, indicating ‘Marquette’ fruit composition may be more viticulturally responsive than ‘Frontenac gris’. This is similar to the observations of Olson (2016), who noted no response as measured by fruit composition for ‘Frontenac’ grapevines grown on different trellis-systems despite varied FZLR treatments.

In Wisconsin, FZLR resulted in a more favorable tartaric: malic acid ratio for ‘Marquette’ compared to nontreated vines owing to a reduction in malic acid content (Riesterer-Loper et al., 2019). Similar work by Scharfetter observed exposed ‘Marquette’ fruit to have an increase in monomeric anthocyanins and polymeric color in wine, increased total phenolic concentration of exposed fruit, and reduced TA of fruit and wine. McCabe et al. (2017), observed no reduction in ‘Marquette’ TA following cluster thinning under Iowa conditions. This is similar to the observations of Vos (2014) who noted a reduction in malic acid content with reduced shoot density but no alteration stemming from cluster reductions. Working with ‘Marquette’ in Adel, IA, Rolfes (2014) noted no statistical impact of canopy management on fruit TA or acid components (malic and tartaric acid).

Previous studies indicate variable impact of canopy and crop load management on ‘Marquette’ grapevines. To enable practical vineyard management advice for growers, our research sought to identify the optimal timing of FZLR, the management practice with the most perceived potential for TA reduction.

Material and Methods

Planting Information

The viticultural treatments were evaluated at a commercial vineyard located near Buffalo, ND (46°54'N 97°29'W) on mature, own rooted, non-irrigated vines planted in 2006 with a soil of Barnes-Buse fine-loamy, mixed, superactive, frigid Calcic Hapludolls. Treatments were established on vines in a randomized complete block design composed of six blocks. Each experimental unit was composed of three contiguous individual vines; treatments were applied to the same vines in consecutive years. Seven experimental treatments were applied during the growing season, a nontreated control and a combination of three defoliation timings and two defoliation intensities (Table 3.1). Defoliation timings were at trace-bloom, the initiation of floraison at which 10-40% flower caps were fallen, fruit-set, approximately 2 weeks following bloom, and at the onset of veraison (0-20% berry color throughout experimental units). Defoliation intensity treatments consisted of removal of 50% or 100% of leaves within the fruit zone. The 50% fruit zone leaf removal was defined as the removal of leaf numbers 1, 3, and 5 from the base of each shoot. The 100% fruit zone leaf removal was defined as the removal of the first six leaves acropetally from the base of each shoot. All shoots of each treated vine, fruiting and non-fruiting, received the prescribed defoliation treatments.

Table 3.1. Treatment description for seven fruit-zone leaf removal treatments applied to 'Marquette' grapevines in Buffalo, ND, 2017-2019.

Treatment	Leaf removal timing	Leaf removal severity
TB50	Trace-bloom	50%
TB100	Trace-bloom	100%
FS50	Fruit-set	50%
FS100	Fruit-set	100%
VR50	Veraison	50%
VR100	Veraison	100%
Control	NA	0%

Vines were maintained as bilateral-cordon-trained to a mid-wire trellis. Vines were spur pruned and supported with three sets of catch wires in a vertical shoot positioning system. Pruning occurred annually to retain 40 buds per vine (targeting 80 clusters per vine), and renewal pruning (retention of new canes for cordon establishment) was implemented as necessary. Shoot-positioning was conducted approximately four times per growing season to reduce sprawl and promote upwards growth of vegetation. For shoots extending beyond the allocated trellis area, pallisaging of shoot tips was conducted along the top trellis wire, rather than shoot-topping.

Vegetative and Yield Components

Light exposure of fruit was measured as photosynthetic photon flux density (PPFD) using a Line Quantum Sensor (Apogee Instruments, Logan, UT); measurements were recorded between veraison (berries at 50% of mature color) and harvest. The PPFD within the fruit zone, measured in micromoles per square meter per second, was calculated by dividing the reading taken from within the fruit zone by an average of two ambient measurements taken directly within the unshaded row middles before and after the measurement within the vine canopy. Fruit zone PPFD for each vine was expressed as a percent of irradiance transmitted to the fruit zone.

Fruit Composition and Cluster Morphology

At harvest, total yield, total cluster number, and the mass of a random 6-cluster sample was recorded for each vine. From the 6-cluster sample, a 100-berry subsample was collected from each experimental vine for evaluation of fruit composition. Total soluble solid content (SSC) was measured using a Pal-1 digital refractometer (Atago Co., Tokyo, Japan). Acid components such as titratable acidity (TA; expressed as g tartaric acid/100 ml juice) and pH were measured with an Orion Star A111 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). The TA was measured via titration of a 5.0 mL aliquot of juice to an endpoint pH of 8.2 with 0.1 N NaOH titrant. Juice pH was measured using standard practices, via emersion in the extracted juice solution.

Analysis of color and phenolics within berries was conducted according to Iland et al., (2004). Following homogenation of berry samples with a stand mounted homogenizer (Brinkmann Kinematic Polytron PT 10-20-3500, Luzern, Steinhofhalde,CH), 1 g of homogenate was transferred to a centrifuge tube for a 1 hr extraction in 50% ethanol acidified to pH 2.0 with 1M HCl. Throughout the 1 hr extraction, tubes were placed on a laboratory shaker and manually agitated every 5 min. Following the 1 hr extraction, tubes were centrifuged at 3500 rpm for 5 min. From the centrifuged samples, 1 mL of extract was transferred into 10 mL of 1M HCl. After a 3 hr immersion time, samples were pipetted into 10 mm path length, disposable, polycyclical olefin, UV-cuvettes (Brand ® UV cuvette semi-micro, Brand GMBH + CO KG, Otto-Schott-Straße,Wertheim, DE). Absorbances at 280, 520, and 700 nm were then read through a UV-Vis spectrophotometer (Genesys^{Tm} 10S UV-Vis Spectrophotometer, ThermoFisher Scientific, Waltham, MA, USA).

A four-cluster subsample per experimental vine was frozen following harvest in 2017 and 2018. Cluster compactness was measured according to Hed et al. (2009). In addition to cluster compactness, individual berries damaged by sunscald or fungal disease were identified and enumerated at this stage.

Winter Damage

The Upper Midwest experienced extreme winter temperatures during the 2018-2019 dormant season causing notable damage to regional vineyards. The experimental vines were evaluated for trunk death (a binary response). Grapevine health of remaining trunks was also monitored following the winter events using a 1-7 scale where 1= trunk alive, but majority of cordons dead, 3= one cordon dead, 5= both cordons alive, but compromised, 7= whole grapevine in-tact.

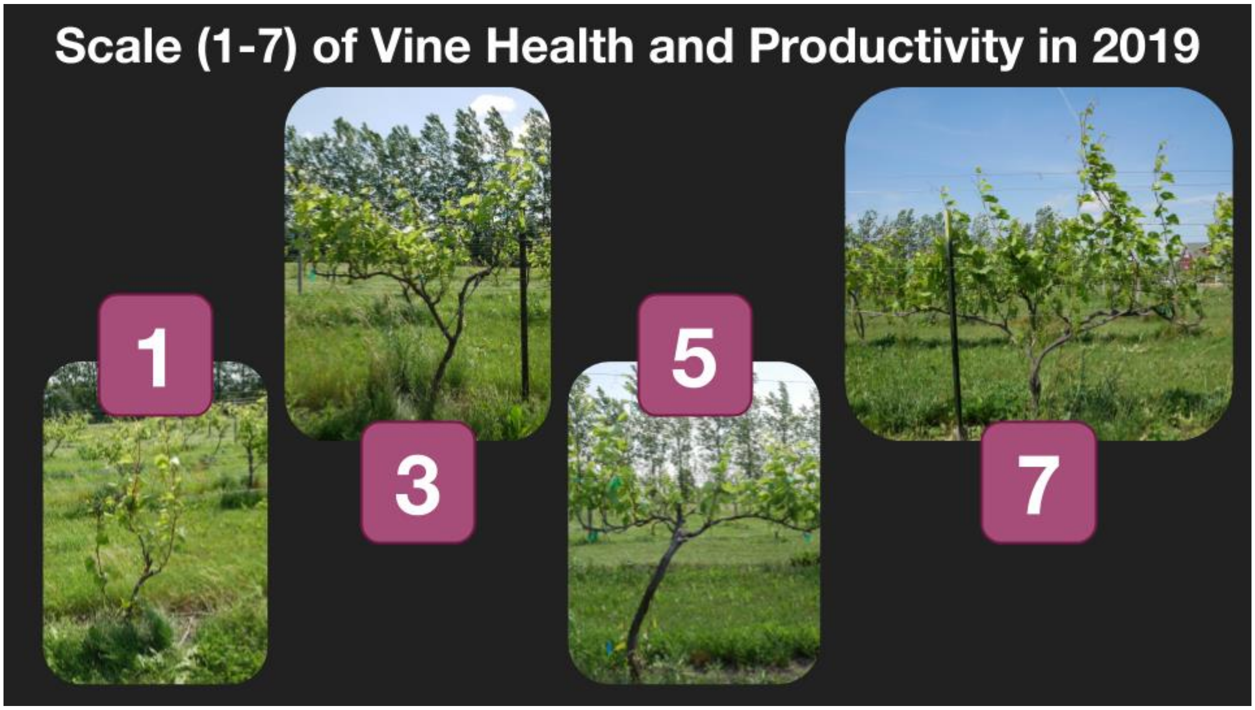

Figure 3.1. Visual scale used in assessing winter injury of ‘Marquette’ grapevines in 2019 following severe winter; 1= trunk alive, but majority of cordons dead, 3= one cordon dead, 5= both cordons alive, but compromised, 7= whole grapevine in-tact.

Statistical Analysis

Statistical analysis was conducted in JMP Pro 15.0.0 as a mixed-model. In the model statement, treatments were designated as fixed-effects, and replicates were designated as random-effects. Figures were generated using ggplot2 in R version 3.6.1.

Results and Discussion

Phenology

In 2019, budbreak was delayed by approximately two weeks compared to the previous years of study (Table 3.2). Accumulated GDD from May 1 to budbreak was similar for all three years despite the difference in date of occurrence. Bloom was delayed in 2019 by 12 days relative to 2017, and 16 days relative to 2018. Veraison occurred in early August in all three years. Harvest was conducted in the final days of Sept. for the 2017 and 2019 crops, while the 2018 harvest was conducted approximately one week earlier on Sept. 21.

Phenological development across the 2017 and 2018 growing seasons were generally similar, as monitored by calendar dates of events. Budbreak occurred by the second week in May, and bloom approximately one month later. Following bloom, color accumulation did not begin until early Aug., with harvest arriving in late Sept. Harvest was earlier in 2018, with more heat accumulated than in 2017 (Table 3.2). The 2019 season was characterized by less heat accumulation. This resulted in a delayed phenology of budbreak (May 27) and bloom (Jun. 27). However, veraison occurred in a similar time of the year (early Aug.). Harvest occurred on Oct. 06 in 2019.

GDD accumulation for budbreak phenology in this study was relatively lower than the observed GDD accumulation prior to budbreak in central Iowa which ranged from 79-128 AGDD (base 10°C) (Schrader et al., 2019). This may be due to the designation of Jan. 1 for

GDD accumulation initiation by Schrader et al. (2019), while our study utilized a May 1 start date based on prior observations of budbreak and general absence of AGDD prior to May under most annual climate circumstances. Similarly, the observed budbreak for North Dakota ‘Marquette’ was also earlier than Michigan ‘Marquette’ described by Frioni et al. (2017) who noted budburst to occur at 94.2 AGDD with a start date of Mar 01. Observations of veraison timing were in range of Frioni et al. who noted fruit of primary buds reach veraison by 806.5 AGDD and fruit of secondary buds reach veraison by 964.6 AGDD. In our evaluation, veraison was reached by 847 to 934 AGDD (Table 3.2). Under both climates, Michigan and North Dakota, veraison occurred at fewer AGDD than the average for central Iowa (1040) (Frioni et al., 2017; Schrader et al., 2019).

Table 3.2. Dates and accumulated growing degree days (base 10°C) for major phenological events for ‘Marquette’ grapevines grown near Buffalo, ND 2017-2019.

	Budbreak			**Bloom**			**Veraison**			**Harvest**		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Date	May 13	May 12	May 27	Jun. 15	Jun. 11	Jun. 27	Aug. 08	Aug. 05	Aug. 09	Sept. 30	Sept. 21	Oct. 07
AGDD (°C)	77	78	82	330	361	351	847	934	851	1230	1342	1210
Norm. AGDD (°C)	57	52	139	282	248	398	875	841	886	1291	1247	1317

Light Interception

The lowest PPFD (%) in both 2017 and 2018 was in Control vines (Fig. 3.2 and 3.3). VR50 resulted in the lowest light interception among treated vines in both years. VR100 had the highest. While all leaf removal treatments involved similar removal of leaf number as well as any lateral shoots that developed at the time of treatment, the VR treatments occurred approximately two weeks before interception readings. These more recently treated plots would have had less time to recover leaf area through lateral development. This is likely why VR100 had the greatest numerical light interception in each year. However, it was never statistically different from TB100 nor FS100. Fruit-zone PPFD was not recorded in 2019 due to the reduction in vine health leading to relatively open canopies stemming from reduce shoot number and decline in health of shoots.

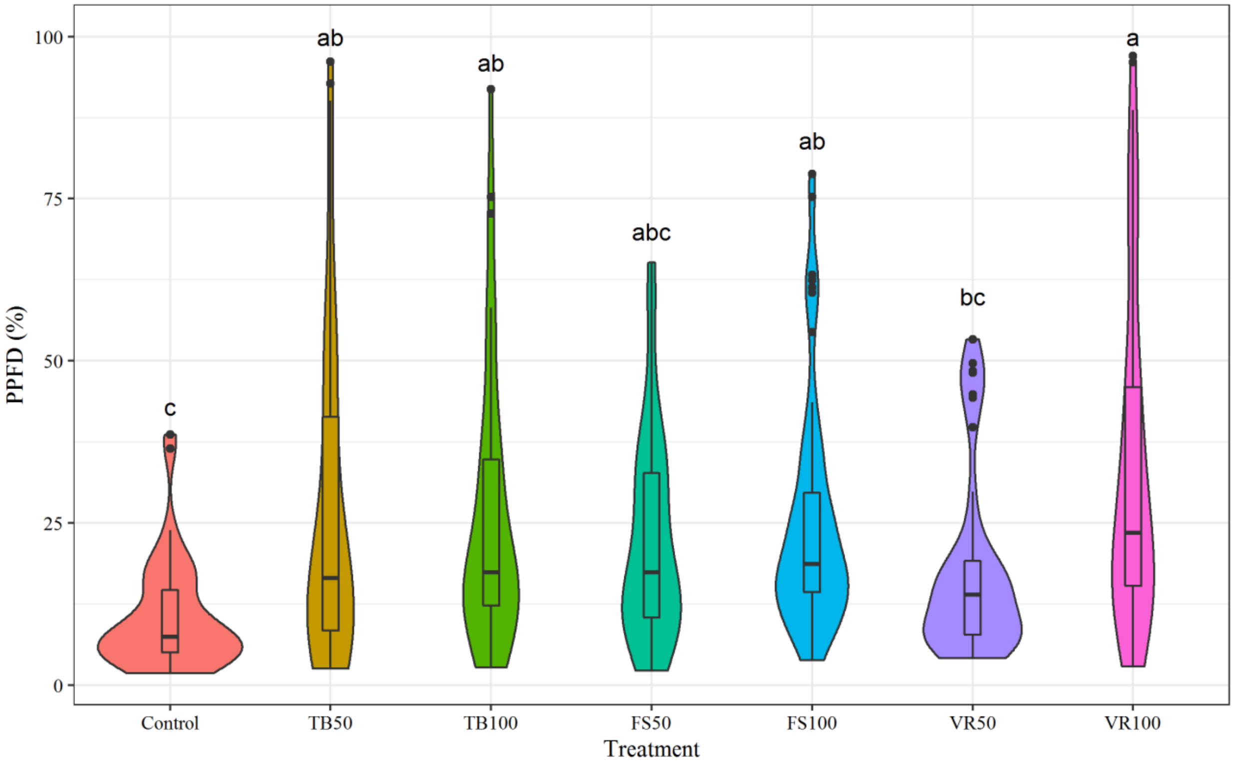

Figure 3.2. Fruit zone photosynthetic photon flux density (PPFD) percent relative to ambient PPFD for ‘Marquette’ grapevines with different leaf removal treatments near Buffalo, ND, Aug. 19, 2017. TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

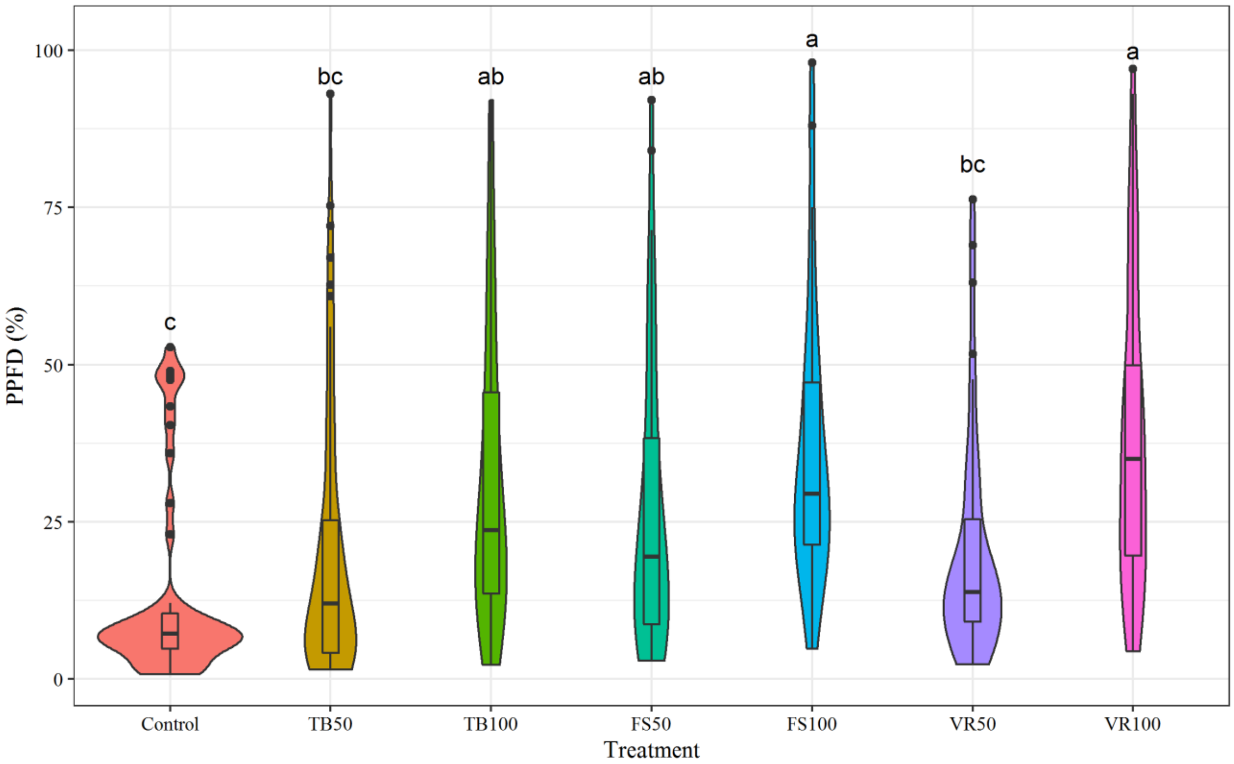

Figure 3.3. Fruit zone photosynthetic photon flux density (PPFD) percent relative to ambient PPFD for ‘Marquette’ grapevines with different leaf removal treatments near Buffalo, ND, Aug. 12, 2018. TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

Yield Components

In 2017 single berry mass ranged from 1.01 to 1.10 g and was smallest for fruit from FS50 and FS100 vines, though not different from the nontreated control (Table 3.3). In 2018 berry mass was between 1.14 and 1.27 g with fruit from VR50, VR100, and nontreated control vines having the largest berry mass. In 2019 single berry mass was between 1.15 and 1.26 g with fruit from VR100 vines having the largest berry mass (1.26 g) and fruit from FS100 vines having the smallest berry mass (1.15 g).

Throughout the experimental period, there were only two instances in which single cluster mass exceeded 50 g, for 2018 nontreated control vines and 2017 VR50 vines. Average cluster mass was 42.8 g in 2017, 46.1 g in 2018, and 35.5 g in 2019. For every year evaluated, TB100 vines produced the smallest clusters.

Cluster number per vine and total yield per vine decline within each year of the study. In 2017 grapevines averages 84 clusters and 3.62 kg per vine, and in 2019 vines averaged 45 clusters and 1.51 kg per vine. No differences were detected for cluster number per vine, but yield was impacted by FZLR treatments. Like the observations of reduced cluster mass for TB100 vines, these vines also produced the lowest yield per vine in each year. In 2019, TB100 vines yielded only 0.96 kg, 45% of the top yielding, nontreated control vines (2.15 kg).

Table 3.3. Yield components under differing fruit-zone leaf removal timings and severities for 'Marquette' grapevines grown near Buffalo, ND 2017-2019.

Treatment[z]	**Single berry mass (g)**	**Single cluster mass (g)**	**Cluster no.**	**Yield (kg/vine)**
2017				
TB50	1.10 ±0.02 a[y]	43.1 ±2.1ab	87.8 ±4.1ns	3.78 ±0.25ab
TB100	1.09 ±0.02 a	37.0 ±2.1b	79.7 ±4.1	2.98 ±0.25b
FS50	1.02 ±0.02 b	40.1 ±2.1b	79.9 ±4.1	3.14 ±0.25b
FS100	1.01 ±0.02 b	40.4 ±2.1b	78.1 ±4.1	3.17 ±0.25b
VR50	1.08 ±0.02 a	50.1 ±2.1a	87.9 ±4.1	4.46 ±0.25a
VR100	1.10 ±0.02 a	46.0 ±2.1ab	92.6 ±4.1	4.25 ±0.25a
Control	1.04 ±0.02 ab	43.1 ±2.1ab	82.7 ±4.1	3.54 ±0.25ab
Significance	0.0054	0.0010	0.1194	<0.0001
2018				
TB50	1.21 ±0.02 ab	42.5 ±2.2 b	60.4 ±3.3 ns	2.61 ±0.20 ab
TB100	1.14 ±0.02 b	32.5 ±2.2 c	53.5 ±3.3	1.77 ±0.20 b
FS50	1.19 ±0.02 ab	47.0 ±2.2 ab	52.7 ±3.3	2.43 ±0.20 ab
FS100	1.21 ±0.02 ab	49.6 ±2.2 ab	61.7 ±3.3	3.08 ±0.20 a
VR50	1.25 ±0.02 a	49.3 ±2.2 ab	61.8 ±3.3	3.11 ±0.20 a
VR100	1.26 ±0.02 a	49.3 ±2.2 ab	53.0 ±3.3	2.58 ±0.20 ab
Control	1.27 ±0.02 a	52.5 ±2.2 a	51.5 ±3.3	2.73 ±0.20 a
Significance	0.0032	<0.0001	0.0730	0.0002
2019				
TB50	1.19 ±0.02 abc	35.6 ±2.6 a	42.9 ±6.6	1.42 ±0.24ab
TB100	1.16 ±0.02 bc	24.6 ±2.6 b	47.4 ±6.6	0.96 ±0.24b
FS50	1.16 ±0.02 bc	40.6 ±2.6 a	35.4 ±6.6	1.32 ±0.24ab
FS100	1.15 ±0.02 c	32.4 ±2.6 ab	53.5 ±6.6	1.69 ±0.24ab
VR50	1.19 ±0.02 abc	34.2 ±2.6 ab	41.8 ±6.6	1.42 ±0.24ab
VR100	1.26 ±0.02 a	42.0 ±2.6 a	41.1 ±6.6	1.62 ±0.24ab
Control	1.24 ±0.02 ab	39.3 ±2.6 a	53.6 ±6.6	2.15 ±0.24a
Significance	0.0012	<0.0001	0.3896	0.0356

[z] TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal. [y] LSmeans comparisons between treatments within year using the Tukey's HSD method at α = 0.05.

Cluster Morphology

Rachis length was not altered by treatment in either year, however FZLR treatments altered total berry number per cluster (Table 3.4). In 2017, FS100 vines had the fewest berries per cluster while in 2018 TB100 vines had the fewest berries per cluster. In 2017 there were more berries that failed to ripen (shot-berries) for VR100 vines, and less for TB50, TB100, and FS50 vines.

Overall cluster compactness as measured by the number of berries per cm of rachis was reduced with earlier FZLR treatments in 2017 and 2018. Clusters had a higher number of berries per cm rachis in 2018 with the greatest compactness observed for the nontreated control vines, 8.0 berries per cm, compared to 6.1 berries per cm rachis for TB100 vines.

Minimal black rot (*Guignardia bidwellii* (Ellis) Viala and Ravaz) infections were observed on berries in the experimental period (data not shown). Likewise, while other cluster rot complexes were observed on more compact clustered genotypes in a locally planted grapevine variety trial in 2018, no sour-rot, botrytis bunch rot, or other rot complexes were observed on ‘Marquette’ fruit evaluated in this study. Berries exhibiting sunscald and shriveling were not explained by treatment; however, they were rarely noted (data not shown).

Cluster morphology was not monitored in 2019 due to expansive damage to grapevines resulting in a considerable lack of yield and poor fruit-set.

Examining correlations among cluster compactness, disease, and abiotic disorders identified no observed significant correlation with black rot, sunscald, or berry shrivel incidence (Fig. 3.4 and 3.5). No symptoms of botrytis bunch rot nor sour rot were observed during the duration of this study; however, during the 2018 growing season, sour rot was observed on multiple, densely clustered grapevine cultivars grown at the nearby North Dakota State

University Horticulture Research Farm. As such, these leaf removal practices, such as early season FZLR at a high severity, which result in a lower cluster compactness may be explored for their potential to reduce rot risks in other more rot prone genotypes.

Reduction in cluster compactness following earlier FZLR treatments was anticipated based on prior research involving timing of source-sink limitations in grapevines (Intrigliolo et al., 2014; Palliotti et al., 2012; Poni et al., 2006). This cluster compactness reduction and yield reduction may be desirable in other cultivars or climates; however, the yield reduction observed in ‘Marquette’ was an undesirable effect considering the overall low yield for the vineyard.

Table 3.4. Cluster morphology under differing fruit-zone leaf removal timings and severities for ‘Marquette’ grapevines grown near Buffalo, ND in 2017 and 2018.

Treatment[z]	**Rachis length (cm)**	**Total berries (no.)**	**Failure to ripen berries (no.)**	**Cluster compactness (berry no./cm)**
2017				
TB50	9.0 ±0.5 ns[y]	46.2 ±3.0 ab	0.4 ±0.2 b	5.1 ±0.3 ab
TB100	10.4 ±0.5	44.4 ±3.0 ab	0.4 ±0.2 b	4.6 ±0.3 b
FS50	9.2 ±0.5	45.3 ±3.0 ab	0.4 ±0.2 b	4.9 ±0.3 b
FS100	8.8 ±0.5	41.9 ±3.0 b	0.6 ±0.2 ab	5.0 ±0.3 b
VR50	9.8 ±0.5	51.3 ±3.0 ab	0.9 ±0.2 ab	5.4 ±0.3 ab
VR100	9.0 ±0.5	49.7 ±3.0 ab	1.3 ±0.2 a	5.6 ±0.3 ab
Control	8.8 ±0.5	56.3 ±3.0 a	0.9 ±0.2 ab	6.5 ±0.3 a
Significance	0.1668	0.0226	0.0019	0.0041
2018				
TB50	8.5 ±0.3 ns	45.5 ±2.7 ab	2.2 ±0.4 ns	7.1 ±0.2 b
TB100	8.2 ±0.3	37.7 ±2.7 b	0.8 ±0.4	6.1 ±0.2 c
FS50	8.2 ±0.3	45.8 ±2.7 ab	1.7 ±0.4	7.5 ±0.2 ab
FS100	8.7 ±0.3	49.8 ±2.7 a	1.3 ±0.4	7.5 ±0.2 ab
VR50	8.5 ±0.3	48.9 ±2.7 a	1.6 ±0.4	7.5 ±0.2 ab
VR100	8.0 ±0.3	48.1 ±2.7 a	1.9 ±0.4	7.8 ±0.2 ab
Control	8.6 ±0.3	53.2 ±2.7 a	2.4 ±0.4	8.0 ±0.2 a
Significance	0.7300	<0.0001	0.0618	<.0001

[z] TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

[y] LSmeans comparisons between treatments within year using the Tukey’s HSD method at α = 0.05; ns= not significant.

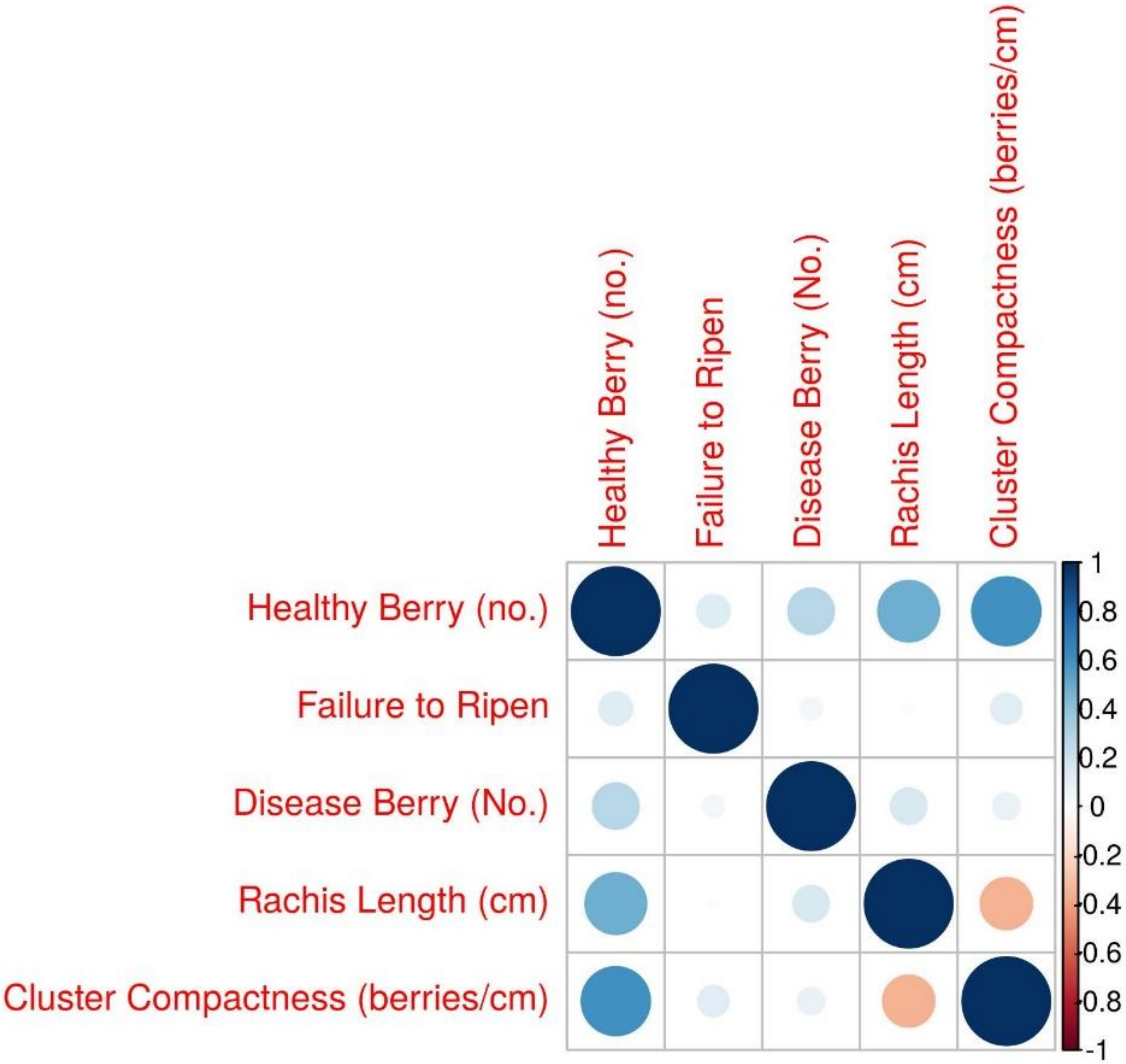

Figure 3.4. Correlations among berry health and compactness cluster metrics monitored on ‘Marquette’ clusters from Buffalo, ND in 2017; correlation by color and size (blue= positive, red=negative; size relative to correlation coefficient).

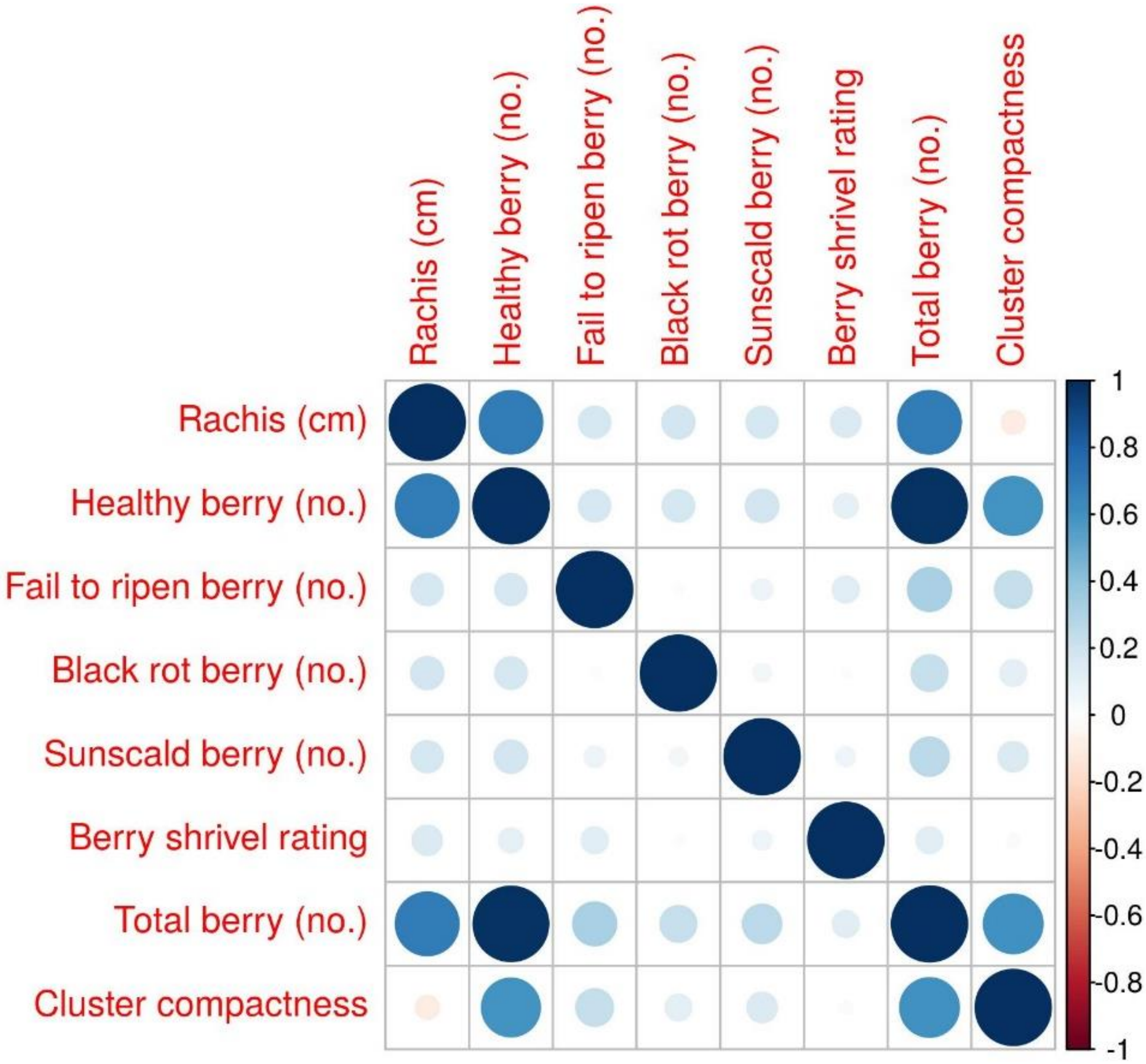

Figure 3.5. Correlations among berry health and compactness cluster metrics monitored on ‘Marquette’ clusters from Buffalo, ND in 2018; correlation by color and size (blue= positive, red=negative).

Fruit Composition

The SSC was impacted by FZLR treatments in each year of the study, while fruit pH was never altered (Table 3.5). The SSC was lowest for VR100 vines in 2017, though not different from VR50 vines and the nontreated control vines. In 2018, the only separable difference for SSC was between the lowest, VR50 vines (25.7), and the highest treatments, TB100 vines (26.9). In 2019, following severe winter damage the SSC was least consistent, ranging from 19.3 to 24.2, with nontreated control vines having the lowest SSC.

Fruit acidity as measured by TA was only affected in 2018 when FS100 vines had a lower TA (9.8) than TB50 vines (10.8). The TA ranged from 9.8 to 14.6 g/L throughout the experimental period. These TA values were well within the described range for northern climate grown ‘Marquette’ (Hatterman-Valenti et al., 2016; Atucha et al., 2018; Wimmer et al., 2018).

Although titratable acidity decreased for fruit from FS100 vines by over 9% relative to the highest TA in 2018, the percent reduction while statistically significant, may not be substantial enough to warrant a local producer’s adoption of these management techniques. The inconsistent to non-substantial TA alteration is consistent with previous research (Zoecklein et al., 1992; Bledsoe et al., 1998; Main and Morris, 2004; Tardaguila et al., 2010). Smith and Centinari (2019) examined FZLR at trace bloom and fruit set for ‘Grüner Veltliner’ and observed inconsistent alterations to fruit chemistry, with no TA reduction compared to nontreated control vines. With ‘Sauvignon blanc’ Mosetti et al. (2016) observed consistent reductions of methoxypyrazine compounds (3-isobutyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine) with leaf removal practices; this observation came in conjunction with reductions in malic acid content, and rot incidence.

Screening leaf removal's applicability towards improving fruit chemistry in other regionally adapted cultivars may be necessary before encouraging farmer utilization. Similarly, for North Dakota's newly formed viticulture sector, advances in mechanization of vineyard tasks may be necessary in order to enable economic implementation of beneficial techniques.

Total anthocyanins were only statistically different in the first year of study, 2017 (Table 3.6). Fruit from VR100 vines had the lowest anthocyanin content (1.25 mg per g berry). Similarly, fruit from VR100 vines had the lowest total phenolic content in 2017 (1.15 AU [absorbance units]). In 2019 there were no differences for fruit total phenolic content; however, in 2017 and 2018, fruit from TB100 vines had the greatest total phenolics (1.39 AU in both years). Berry anthocyanins ranged from 1.25 to 2.11 mg per g berry throughout the experimental period. Both 2017 and 2018 average 1.54 mg per g berry while the 2019 crop averaged 1.93 mg anthocyanins per g berry. Total phenolics were also greatest in 2019, with an average of 1.35 AU compared to 1.29 and 1.23 AU for 2017 and 2018, respectively.

The observed total anthocyanins and total phenolics in North Dakota were greater than those of 'Marquette' grown in Michigan following damaging spring frost events (Frioni et al., 2017a). Michigan grown 'Marquette' ranged from 1.22 to 1.23 mg anthocyanin per g berry wt and 0.805 to 0.854 AU per g berry wt for total phenolics. In North Dakota, 'Marquette' anthocyanin content was comparable and phenolic content was typically two or more times the values observed in Michigan. These differences likely stem from environmental conditions, a major driving force in cool climate viticulture that is difficult to predict and model (Frioni et al., 2017b; Maante-Kuljus et al., 2020; Scharfetter et al., 2020).

Under certain circumstances, such as late season treatment application, leaf removal may temporarily stunt and delay fruit technological ripening parameters (Poni et al., 2013). North

Dakota’s grape growing season is likely too short to consistently observe trends related to late season stunting of fruit maturation; however, in 2017, the reduced SSC for fruit from VR100 may be explained by this.

In many evaluations of red wine grapevines, early FZLR leads to increased phenolic acid content, total polyphenol content, and anthocyanin content within berries and wines, although these results vary by region, cultivar, and other factors. Hickey et al. (2018) reported no alterations to berry anthocyanin content for Virginia grown ‘Petit Verdot’ and ‘Cabernet Sauvignon’; however, in half of the year-cultivar combinations evaluated, total berry phenolics were increased by pre-bloom FZLR. In the cool climate of Szczecin, Poland, early defoliation prior to flowering with ‘Rondo’, early defoliation prior to flowering led to increased anthocyanins, flavonols, and total polyphenols (Mijowska et al., 2016). In the warm, continental climate of eastern Serbia, ‘Cabernet Sauvignon’ berry and wine composition were shown to benefit from early FZLR as monitored by SSC, ethanol, anthocyanins, flavonols, phenols, and more (Stefanovic et al., 2021). The increase in numerous chemical components in the early FZLR treatment was attributed to an increased proportion of skin relative to mesocarp on a per berry basis. For ‘Sangiovese’, defoliation reduced berry weight while increasing skin-to-berry ratio, seed-to-berry ratio, total phenols, and total anthocyanins relative to control treatments (Gatti et al., 2012). Although not monitored in our North Dakota grown ‘Marquette’, a deviation in skin proportion relative to other berry components is a logical factor that likely contributed to observed differences of phenolic and anthocyanin content among treatments.

Table 3.5. Fruit technological maturity under differing fruit-zone leaf removal timings and severities for ‘Marquette’ grapevines grown near Buffalo, ND 2017-2019.

Treatment	**SSC**	**pH**	**TA**
2017			
TB50	24.0 ±0.5ab	3.15 ±0.02ns	14.1 ±0.4ns
TB100	24.6 ±0.5a	3.14 ±0.02	13.8 ±0.4
FS50	23.1 ±0.5ab	3.18 ±0.02	13.9 ±0.4
FS100	24.5 ±0.5a	3.20 ±0.02	13.4 ±0.4
VR50	22.2 ±0.5bc	3.16 ±0.02	13.7 ±0.4
VR100	20.7 ±0.5c	3.21 ±0.02	13.4 ±0.4
Control	22.3 ±0.5bc	3.16 ±0.02	13.9 ±0.4
Significance	<0.0001	0.3016	0.8043
2018			
TB50	26.4 ±0.2ab	3.28 ±0.02 ns	10.8 ±0.2 a
TB100	26.9 ±0.2a	3.30 ±0.02	10.2 ±0.2 ab
FS50	26.1 ±0.2ab	3.28 ±0.02	10.3 ±0.2 ab
FS100	26.2 ±0.2ab	3.34 ±0.02	9.8 ±0.2 b
VR50	25.7 ±0.2b	3.30 ±0.02	10.8 ±0.2 a
VR100	25.9 ±0.2ab	3.30 ±0.02	10.5 ±0.2 ab
Control	26.4 ±0.2ab	3.27 ±0.02	10.6 ±0.2 ab
Significance	0.0344	0.4747	0.0128
2019			
TB50	24.2 ±0.9a	3.02 ±0.03ns	13.7 ±0.8ns
TB100	22.7 ±0.9ab	3.04 ±0.03	12.7 ±0.8
FS50	21.6 ±0.9ab	2.95 ±0.03	14.6 ±0.8
FS100	21.0 ±0.9ab	3.00 ±0.03	11.7 ±0.8
VR50	20.3 ±0.9ab	3.00 ±0.03	12.6 ±0.8
VR100	24.0 ±0.9a	3.00 ±0.03	13.9 ±0.8
Control	19.3 ±0.9b	2.99 ±0.03	13.5 ±0.8
Significance	0.0105	0.3772	0.2546

[z] TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

yLSmeans comparisons between treatments within year using the Tukey’s HSD method at α = 0.05; ns= not significant.

Table 3.6. Fruit anthocyanins and total phenolics under differing fruit-zone leaf removal timings and severities for ‘Marquette’ grapevines grown near Buffalo, ND 2017-2019.

Treatment[z]	**Anthocyanins (mg/g berry)**	**Total phenolics (AU)**
2017		
TB50	1.52 ±0.07ab	1.33 ±0.05ab
TB100	1.71 ±0.07a	1.39 ±0.05a
FS50	1.64 ±0.07a	1.34 ±0.05ab
FS100	1.63 ±0.07a	1.37 ±0.05ab
VR50	1.56 ±0.07ab	1.22 ±0.05ab
VR100	1.25 ±0.07b	1.15 ±0.05b
Control	1.50 ±0.07ab	1.26 ±0.05ab
Significance	0.0030	0.0268
2018		
TB50	1.61 ±0.06ns	1.28 ±0.06ab
TB100	1.64 ±0.06	1.39 ±0.06a
FS50	1.50 ±0.06	1.14 ±0.06ab
FS100	1.52 ±0.06	1.25 ±0.06ab
VR50	1.38 ±0.06	1.05 ±0.06b
VR100	1.57 ±0.06	1.28 ±0.06ab
Control	1.58 ±0.06	1.22 ±0.06ab
Significance	0.0721	0.0085
2019		
TB50	2.05 ±0.11ns	1.43 ±0.07ns
TB100	2.08 ±0.11	1.45 ±0.07
FS50	1.83 ±0.11	1.40 ±0.07
FS100	1.86 ±0.11	1.38 ±0.07
VR50	1.95 ±0.11	1.29 ±0.07
VR100	2.11 ±0.11	1.34 ±0.07
Control	1.60 ±0.11	1.18 ±0.07
Significance	0.0543	0.1013

[z] TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

yLSmeans comparisons between treatments within year using the Tukey’s HSD method at α = 0.05; ns= not significant.

Labor Time

In 2017 and 2018, labor and associated costs for implementing manual FZLR treatments was estimated between $1,131 and $2,547 per ha (Table 3.7). In 2017, VR100 vines were the most expensive treatment while in 2018 it was FS100 vines. Following winter damage, labor for FZLR was substantially lower in 2019, yet still averaged over $980 per ha for treatments. The high labor requirements represent a major portion of the fruit's potential revenue for producers. In 2017 and 2018, average yields were 6.1 and 4.4 ton per ha; these yields equate to a value of $10,759 and $7,760 at regional average prices of $1.76 per kg fruit (Brederson, pers. comm. 2019). Thus, applying FZLR treatments equated to approximately 15% to 18% of total revenue in years when vines were fully healthy and producing. In 2019, after severe winter injury likely contributed to yield reductions, even with the reduced labor per vine, FZLR treatments represented 27% of anticipated revenue from fruit sales. Farmers considering utilizing FZLR practices will need to consider these costs and other factors before deciding to use these techniques on a large scale.

Table 3.7. Labor required for differing fruit-zone leaf removal timings and severities for ‘Marquette’ grapevines grown near Buffalo, ND 2017-2019.

Treatment	**Labor time per vine (s)**	**Labor hours per ha**	**Labor cost per ha at $15 per hr**
2017			
TB50	232 ±11cd	86.6	$1299
TB100	293 ±23bc	109.6	$1644
FS50	190 ±9d	70.9	$1065
FS100	298 ±16bc	111.3	$1669
VR50	305 ±17b	114.2	$1712
VR100	454 ±25a	169.8	$2547
Control	0 ±0e	0.0	$0
Significance	<0.0001		
2018			
TB50	216 ±8cd	80.8	$1212
TB100	289 ±15ab	108.1	$1622
FS50	216 ±15cd	80.9	$1213
FS100	322 ±15a	120.6	$1808
VR50	202 ±12d	75.4	$1131
VR100	266 ±11bc	99.6	$1494
Control	0 ±0e	0.0	$0
Significance	<0.0001		
2019			
TB50	163 ±19a	60.8	$912
TB100	190 ±15a	71.0	$1066
FS50	162 ±17a	60.4	$906
FS100	186 ±16a	69.4	$1041
VR50	171 ±15a	63.9	$959
VR100	183 ±14a	68.5	$1028
Control	0 ±0b	0.0	$0
Significance	<0.0001		

[z] TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

yLSmeans comparisons between treatments within year using the Tukey’s HSD method at α = 0.05; ns= not significant.

Winter Damage

At the commercial vineyard planting near Buffalo, ND, very few experimental grapevines succumbed to winter injury related death in 2019 (Figure 3.6). There were six total dead vines in the experimental plot, with no trend based on treatment. One dead grapevine belonged to the nontreated control treatment, one was FS50, one was TB100, one was VR100, and two were the VR50 treatment. The median rating of damage indicated FS50 (3), TB50 (3), and VR50 vines (3) experienced greater damage than TB100 (5), FS100 (5), VR100 (5), and nontreated control (6) grapevines. In total, only 25% of the planting received a rating of 7, while 60% of the planting received a rating of 5 or less, indicating extensive damage ranging from cordon compromise to trunk death.

Damage at this vineyard was less severe than observed injury at the North Dakota State University Horticulture Research Farm (NDSU HRF), located approximately 14 km away, near Absaraka, ND. At the NDSU HRF, all 16 ‘Marquette’ grapevines within the NDSU grape variety trial experienced entire trunk death during the 2018-2019 dormant season (Svyantek et al., 2020). Similarly, 77% of ‘Marquette’ trunks were removed in an adjacent planting of ‘Marquette’ grapevines at the NDSU HRF (Tatar, 2020). While the observed death at the Buffalo, ND experimental vineyard did not approach that observed elsewhere, with only <5% grapevine trunks dead, the damage experienced during the 2018-2019 dormant season contributed heavily to a drastic reduction in yield in the 2019 yield. Although the experiment concluded in 2019, recovery was not complete in 2020, and the commercial ‘Marquette’ planting reported extremely low yields in 2020 (Hogen, pers. comm. 2020). To encourage re-establishment of healthy wood, extensive renewal pruning was conducted in the spring of 2020 following the conclusion of the experimental period.

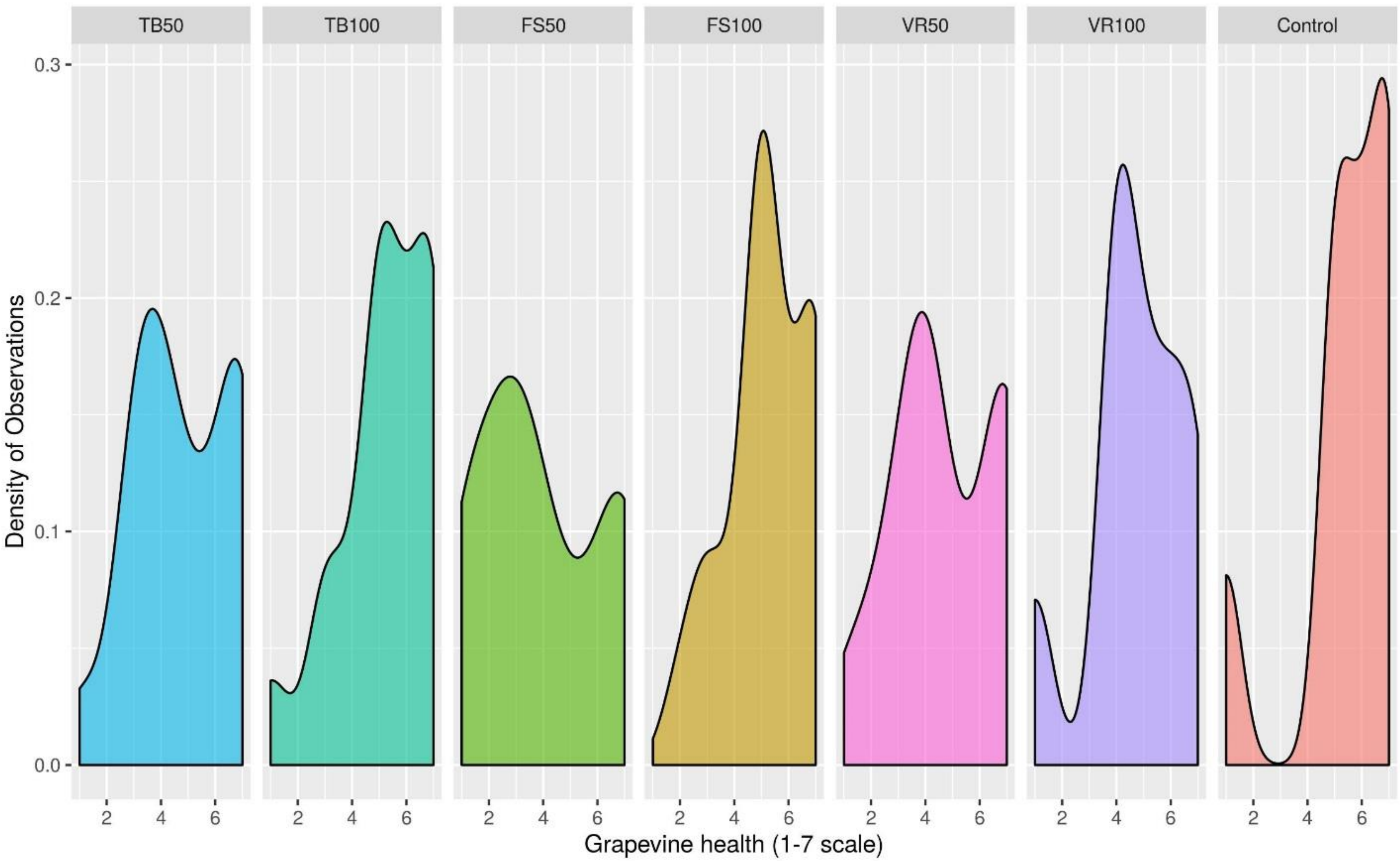

Figure 3.6. Health of 'Marquette' grapevines in midsummer 2019 following severe winter events of 2018-2019, Buffalo, ND. TB50 = 50% fruit-zone leaf removal at trace-bloom, TB100 = 100% fruit-zone leaf removal at trace-bloom, FS50 = 50% fruit-zone leaf removal at fruit-set, FS100 = 100% fruit-zone leaf removal at fruit-set, VR50 = 50% fruit-zone leaf removal at veraison, VR100 = 100% fruit-zone leaf removal at veraison, Control= untreated, no leaf removal.

Future Work

Fruit-zone leaf removal has been explored in ‘Marquette’ and other cold-hardy interspecific hybrid wine grapes. Within this study, FZLR on ‘Marquette’ in eastern North Dakota did not consistently achieve the viticultural goal of altering fruit chemistry towards TA reduction. Benefits observed in our study included reduced cluster compactness and increased fruit SSC; however, for ‘Marquette’ fruit SSC is consistently acceptable and cluster compactness was not correlated with disease incidence.

Fruit-zone leaf removal may remain a useful technique for alternative genotypes depending on the viticultural goals. It may be specifically useful for large-berried individuals with high amounts of *V. labrusca* in their pedigree, such as from Elmer Swenson’s breeding efforts and subsequent derived crosses. Example genotypes that may benefit from cluster compactness reduction via early season FZLR include ‘Brianna,’ which experienced cluster rot complexes in 2018 at NDSU HRF, and ‘Petite Pearl’, which regularly shears berries off its clusters following fruit-set due to intense compaction. Under North Dakota conditions, the berry compactness of ‘Petite Pearl’ has not been associated with rot; however, under more humid conditions conducive to fungal and bacterial infection, this may predispose ‘Petite Pearl’ clusters to infection and build-up of inoculum for future seasons.

Beyond fruit chemistry, the effect of canopy management practices on wine aroma and composition are additional, important traits requiring further evaluation before commercial recommendations can thoroughly endorse these practices for the region (Bubola et al., 2020; Hickey and Wolf, 2018; Hickey et al., 2018).

Equally important for educated implementation of canopy management in the upper-Midwest is the need for thorough evaluations of long-term effects of leaf removal on grapevine health, yield, and fruit chemistry (Hed et al., 2015; Lopes et al., 2020; Percival et al., 1994). Any long-term trend beginning to unfold during this experimental period with ‘Marquette’ was obliterated by the substantial winter injury in the 2018-2019 winter.

If FZLR was found beneficial in other cultivars, realistic approaches towards mechanization of leaf removal tasks for small growers must be developed. Mechanization of leaf removal has been evaluated for multiple *V. vinifera* cultivars, widely supporting its commercial utilization (Intrieri et al., 2008; VanderWeide et al., 2020).

Fertility at the Buffalo, ND site requires thorough investigation. Yield in 2017 and 2018 seasons were comparable to the results observed by Aipperspach et al. (2020), despite vines being multiple years more mature. In general, yield and yield components, especially cluster size for North Dakota grown ‘Marquette’ are far from the patent reported range (65.4-124.8 g per cluster with a mean of 85.3 g) and those of other prior viticultural research (Aipperspach et al., 2020; Hemstad and Luby, 2006; Frioni et al., 2017b; Wimmer et al., 2018). Our observations of ‘Marquette’ cluster mass are similar to the observations in Iowa conditions with single cluster mass ranging from an average of 32 g, 49 g, and 51 g depending on study (Schrader et al., 2019; Rolfes, 2014; Vos 2014). Management methods to increase grapevine health and cluster mass need to be investigated for small clustered, inconsistent grapevines like ‘Marquette’ in North Dakota.

Under extreme environmental conditions, protected culture of grapevines using greenhouses or high tunnels is practiced. This protection can lead to increased survival and altered ripening. Protected culture in Estonia is observed to contribute to increased phenolic and anthocyanin content in hybrid grapevines depending on interannual conditions (Maante-Kuljus et al., 2020). For North Dakota growers, protected culture of small plots of grapevines may be a beneficial practice towards obtaining consistent yields and/or quality from unadapted grapevine cultivars. In many climates, ‘Marquette’ is proven to be winter hardy with high levels of freezing tolerance (Atucha et al., 2018; Schrader et al., 2019; Schrader et al., 2020; Yilmaz et al., 2021). Yet, in North Dakota, ‘Marquette’ may be defined as moderately to highly cold-tender based on previous observations of yield reduction following winter damage and observations of dormancy acclimation tendencies (Hatterman-Valenti et al., 2016; Stenger and Hatterman-Valenti, 2016; Svyantek et al., 2020; Tatar, 2020).

The definition of adapted, cold-hardy grapevines for North Dakota conditions is under-development; however, many regionally grown grapevines have inconsistent and low yields (Hatterman-Valenti et al., 2016). Furthermore, most grapevines evaluated to-date have exhibited greater than 50% trunk mortality under North Dakota conditions when temperatures dropped below -36 °C (Svyantek et al., 2020). For these reasons, protected culture of grapevines, like ‘Marquette’ may be advisable despite their traditionally deemed cold hardy status.

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CHAPTER 4. HETEROBLASTIC LEAF SHAPE DEVELOPMENT ALONG THE DEVELOPMENTAL AXIS OF INTERSPECIFIC HYBRID GRAPEVINE SEEDLINGS DERIVED FROM ‘CHASSELAS CIOUTAT’

Abstract

Grapevines (*Vitis* spp.) exhibit heteroblastic development; following germination, they transition from the juvenile stage to a mature spiral phyllotaxis accompanied by sexual structures (tendrils and eventually true flowers) and changes in foliar morphology. To examine the transitional heteroblasty in grapevine progeny with unique foliar shapes, three crosses were conducted using the compound, lace-leaf bearing *V. vinifera* ‘Chasselas Cioutat’ as the pollen parent. The presence or absence of lacinate, lacey leaf morphology was rated in 427 outcrossed seedlings with only one anomaly exhibiting the lacinate trait. Of these progeny, the first thirteen true leaves to emerge following the cotyledon were collected and digitized from 110 individual grapevine seedlings and an additional 26 presumed selfed ‘Chasselas Cioutat’ seedlings. Landmark morphometric analysis was applied to a total of 1590 leaves sampled, and developmental changes were assessed via traditional ampelographic measurements (lengths and angles) and generalized Procrustes analysis (GPA). Principal component analysis of GPA results identified that nearly half of the variation among samples was driven by lobiness of the leaf blades; this developmental transition was visualized and noted a steady, heteroblastic increase in lobiness of samples after the first true leaf with increasing lobiness as new leaves emerged. These grapevine seedling populations were developed to assess segregation of the lacinate leaf trait derived from ‘Chasselas Cioutat’; while they failed to segregate, they produced the first informative evaluation of heteroblastic developmental leaf morphology changes in grapevine seedlings.

Introduction

Heteroblasty

Heteroblasty describes the variable morphological, physiological, and sexual changes within plant species across successive growth stages of an individual plant in time. Characteristics affected by heteroblastic variation include leaf shape and size, phyllotaxis patterning, stem internode length and structure, woodiness, and presence or absence of sexual structures. In grapevines, a perennial plant with an extended juvenile period, the appearance of tendrils, a modified floral structure, is suppressed in early plant growth until vines transition to spiral phyllotaxis, a state accompanied by regular phyllotaxis patterning.

The phenomena of heteroblasty is described by numerous authors and is summarized by the title of Hildebrand's 1875 work, '*Ueber die Jugendzustände solcher Pflanzen, welche im Alter vom vegetativen Charakter ihrer Verwandten abweichen*', translated from the German, 'About the youth of such plants, which in old age differ from the vegetative character of their relatives.' Hildebrand describes variation of *Acacia* spp., *Lathyrus* spp., *Oxalis* spp., *Ulex* spp., and other species to demonstrate the vegetative changes within different plants.

While Goethe (1790) was among the first to hypothesize about the metamorphosis of plants and their structures, Goebel (1889) first used the term heteroblastic (*heteroblastiche*). Goebel used heteroblastic to describe the development of plants in his work '*Über die Jugendzustände der Pflanzen*', translated from the German, 'About the Youth of Plants'. It describes heteroblastic plants as variable in shape between their juvenile form (*jugendform*) and subsequent stages (*folgestadium*); this is in direct contrast to plants which can be described as homoblastic (*homoblastiche*) that undergo minimal changes across developmental stages. The concepts of heteroblasty and its specific terminology is recently reviewed in depth by Zotz et al. (2011).

***Vitis* Leaf Shape**

Description of grapevine varieties' leaf shape and plant form is a botanical practice known as ampelography. Early work in ampelography set the foundation for modern grapevine descriptions (Rendu, 1854; Viala and Vermorel, 1909). In the 20th century Pierre Galet systematicized the description of grapevines (Galet, 1952; Galet 1979). The use of ampelography to characterize grapevines as true to type for cultivar descriptions is still in use within vineyard and nursery production (Galet, 1979; Preiner et al., 2014; Chitwood 2020). However, modern genetic identification technology has largely replaced ampelography in practical applications.

Ampelography and leaf shape investigations is a growing focus within grapevines due to the combination of traditional measurement techniques with new digital image analysis technologies (Alessandri et al., 1996; Soldavini et al., 2006; Bodor et al., 2012; Chitwood et al., 2014a). Recent research by Welter et al. (2007) and Demmings et al. (2019) identified quantitative trait loci (QTL) of leaf shape in grapevines indicating genetic control of morphology across multiple chromosomes.

Investigations of leaf shape have also focused on developmental and environmental effects (Bodor et al., 2014; Baumgartner et al., 2020). Leaf shape in grapes is kinetic, changing slightly from node to node in multiple features, including the degree of sinus depth. This has been readily observed in grapevines when quantifying discrete leaf shape characteristics, such as compound leaflets of *V. piasezkii* (Cousins and Prins, 2008; Min et al., 2018). Quantitative approaches have examined variation within and among individual plant samples (Bodor et al., 2014; Bodor et al., 2018; Chitwood et al., 2016a; Chitwood et al., 2016b; Klein et al., 2017; Bryson et al., 2020).

Research Gap

Research on developmental variability of leaf shape within grapevines has focused on clonal propagules, rather than seedlings. Examining seedlings offers a glimpse into heteroblastic variation of *Vitis* spp. and the progression and development of leaf lobes, sinuses, and other features. Progeny from crosses with parents' having distinct, complex leaf shape, like that of 'Chasselas Cioutat' or *V. piasezkii*, would be anticipated to segregate for quantitative and qualitative leaf descriptors. Previous work with 'Chasselas Cioutat' progeny has been unclear in defining genetic control. Initial evaluations of selfed progeny of 'Chasselas Cioutat' produced contrasting findings concerning the allelic state necessary for expression of compound, lacinate leaves (Snyder and Harmon, 1939; Galet, 2000). More recent work by Boyden (2005) proposed a two-allele control of the lacinate leaf phenotype with a dominant and a mutant allele for which only A_bb individuals exhibited the trait.

The populations formed for this study were created to develop an understanding of genetic control of the unique leaf shape exhibited by 'Chasselas Cioutat'. Although they failed to segregate for compound, lacinate leaves in the S_0/F_1 progeny, we seized the opportunity to observe foliar leaf shape developmental changes in seedling grapevines, for the first time, from a landmark morphometric standpoint.

Material and Methods

Seedling Production

A total of four grapevine populations were developed (Table 1). Dried pollen, collected from 'Chasselas Cioutat', was applied to pistillate *Vitis* accessions (E.S. 5-8-17 and B.27) and to emasculated ‘Frontenac gris’ flowers. Additionally, the fruit from ‘Chasselas Cioutat’ flowers used for pollen collection were retained as a source of open-pollinated, presumed selfed seeds. The timing of the ‘Chasselas Cioutat’ flowering under greenhouse conditions did not intersect with the flowering of any other *Vitis* accessions, thus the seeds are assumed to be self-pollinated in their origin.

Table 4.1. Number of grapevine seedlings from crosses with *V. vinifera* line ‘Chasselas Cioutat’ assessed for mutant, lacinate leaf form and scanned for developmental, morphometric measurements.

Female	**Male**	**Pseudonym**	**Total seedlings (no.)**	**Total seedlings WT (no.)**	**Total seedlings CC (no.)**	**Scanned seedlings (no.)**
V. spp. E.S. 5-8-17	*V. vinifera* Chasselas Cioutat	ESxCC	78	78	0	37
V. spp. Frontenac gris	*V. vinifera* Chasselas Cioutat	FGxCC	123	123	0	20
V. riparia ND B.27	*V. vinifera* Chasselas Cioutat	B27xCC	226	225	1	53
Total S_0/F_1			427	426	1	110
V. vinifera Chasselas Cioutat	Open Pollinated; Presumed Self	CCxCC	51	2	49	26

OIV Descriptors and Lengths

A total of 17 landmarks were placed on leaves using GRALED v2.04 (Bodor et al., 2012). This allowed for calculation of 11 OIV (International Organisation of Vine and Wine) and modified OIV descriptors (Table 4.2) and 15 additional landmark lengths (Table 4.3) (OIV 2009; Bodor et al., 2012).

Table 4.2. OIV and modified OIV descriptors used in monitoring grapevine leaf shape adapted from OIV 2009 and Bodor et al., 2012.

OIV Descriptor	Description	Unit
601	length of vein N_1	mm
602 (L/R)	length of vein N_2	mm
603 (L/R)	length of vein N_3	mm
604 (L/R)	length of vein N_4	mm
605 (L/R)	length from petiole sinus to upper lateral leaf sinus	mm
606 (L/R)	length from petiole sinus to lower lateral leaf sinus	mm
607 (L/R)	angle between N_1 andN_2	angle (°)
608 (L/R)	angle between N_2 andN_3	angle (°)
609 (L/R)	angle between N_3 and N_4	angle (°)
618aR	angle of petiole sinus	angle (°)
618R	length of petiole sinus	mm

Table 4.3. Landmark lengths used in monitoring grapevine leaf shape adapted from Bodor et al., 2012.

Landmark distances	Description	Unit
01 to 02	length between landmark 01 and 02	mm
01 to 03	length between landmark 01 and 03	mm
01 to 04	length between landmark 01 and 04	mm
01 to 05	length between landmark 01 and 05	mm
01 to 06	length between landmark 01 and 06	mm
01 to 07	length between landmark 01 and 07	mm
01 to 17	length between landmark 01 and 17	mm
07 to 08	length between landmark 07 and 08	mm
08 to 10	length between landmark 08 and 10	mm
08 to 16	length between landmark 08 and 16	mm
10 to 12	length between landmark 10 and 12	mm
10 to 14	length between landmark 10 and 14	mm
12 to 14	length between landmark 12 and 14	mm
14 to 16	length between landmark 14 and 16	mm
16 to 17	length between landmark 16 and 17	mm

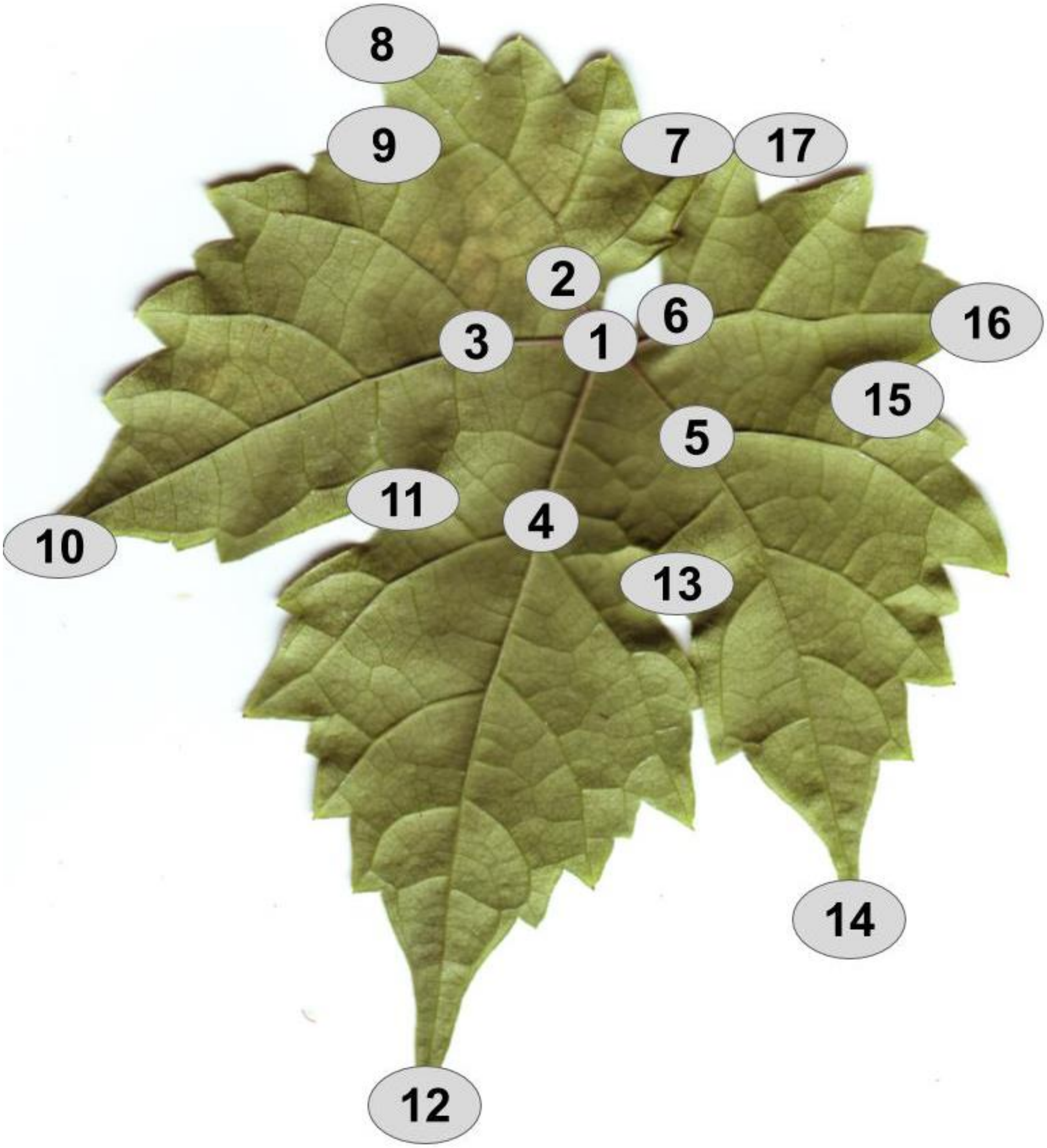

Figure 4.1. Example landmark locations placed on grapevine leaves.

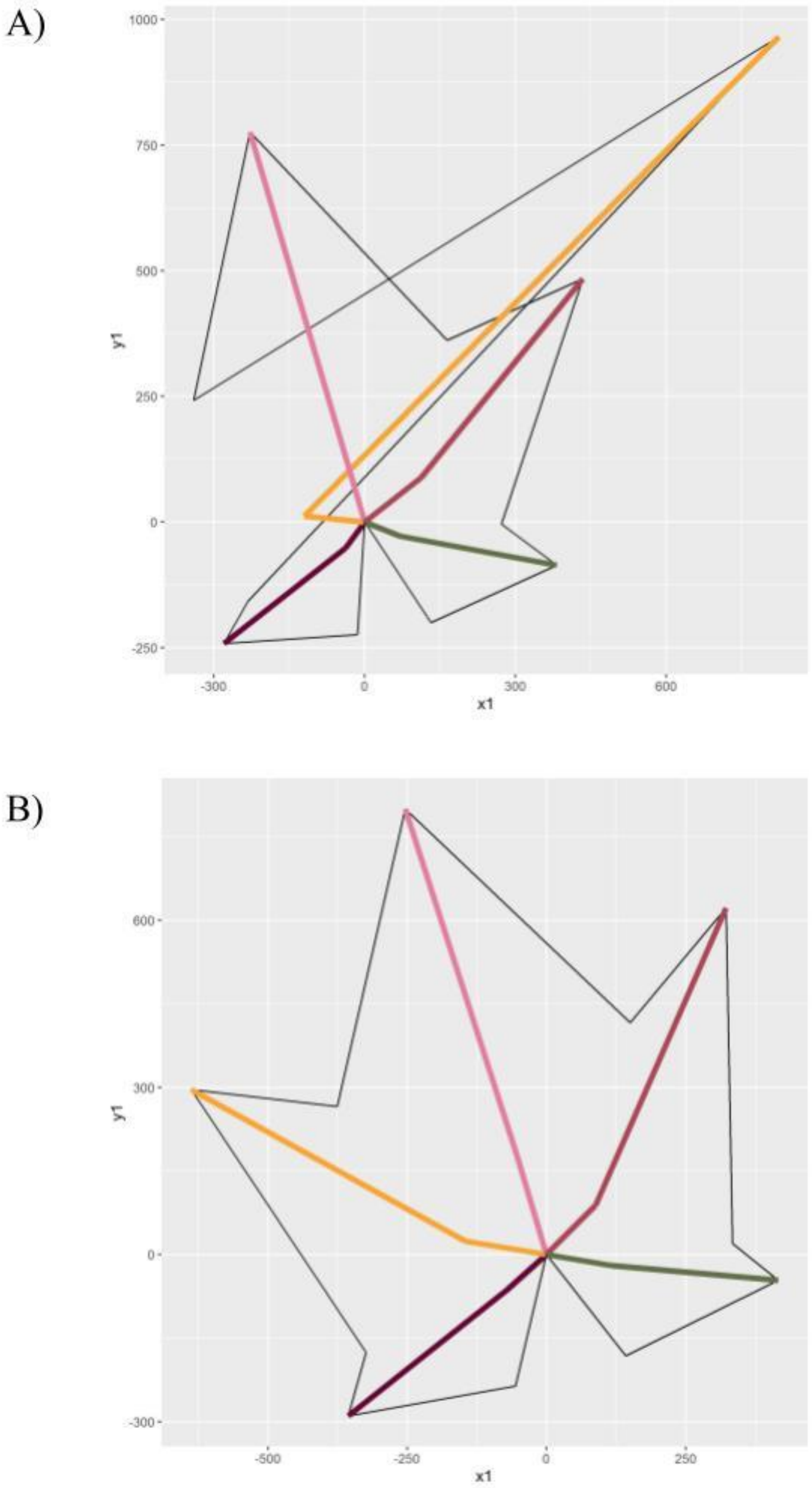

Figure 4.2. Output of leaf scan landmarking evaluation where (A) is an incorrectly landmarked leaf and (B) is a correctly landmarked leaf.

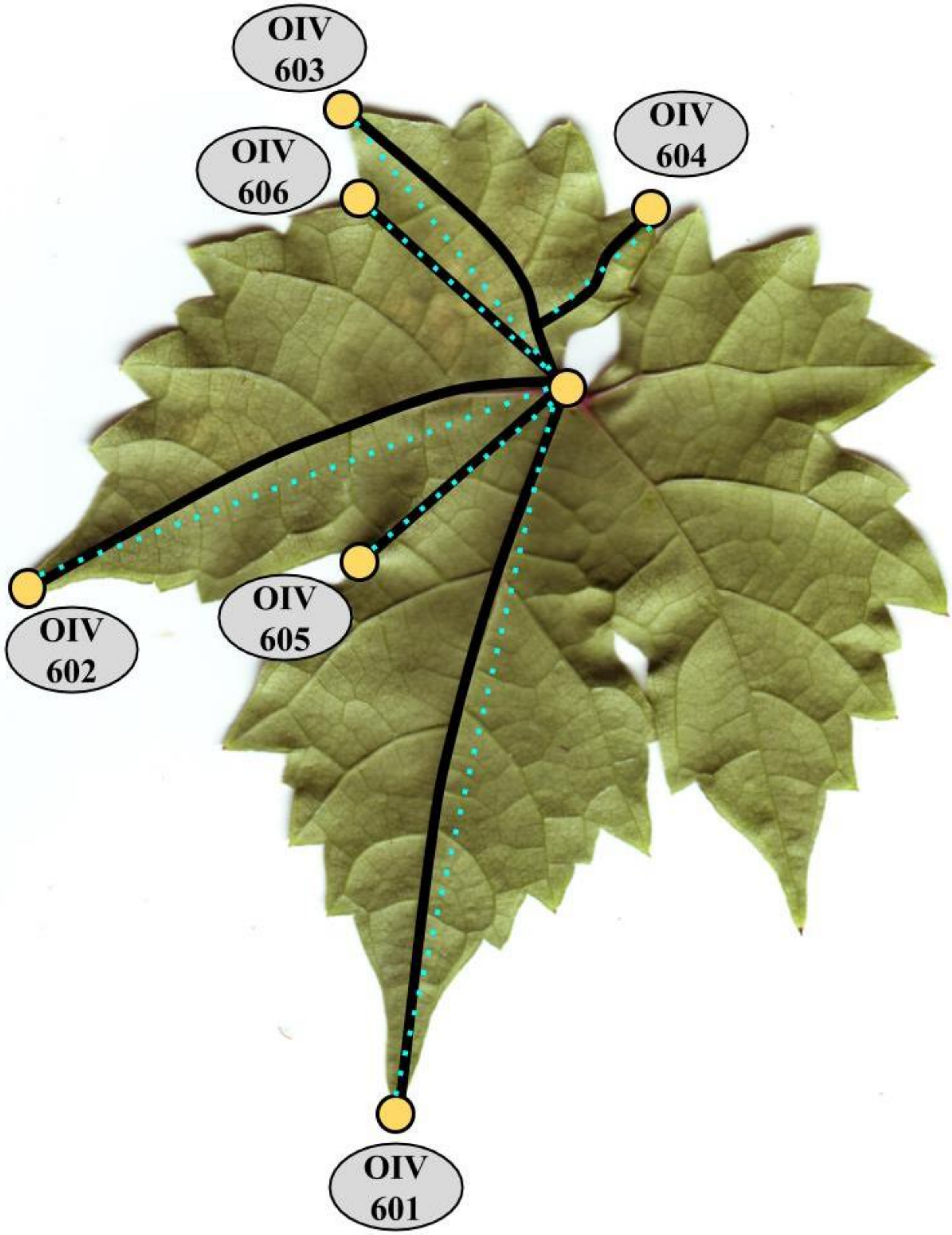

Figure 4.3. OIV descriptors 601-606 measured along each leaf sample.

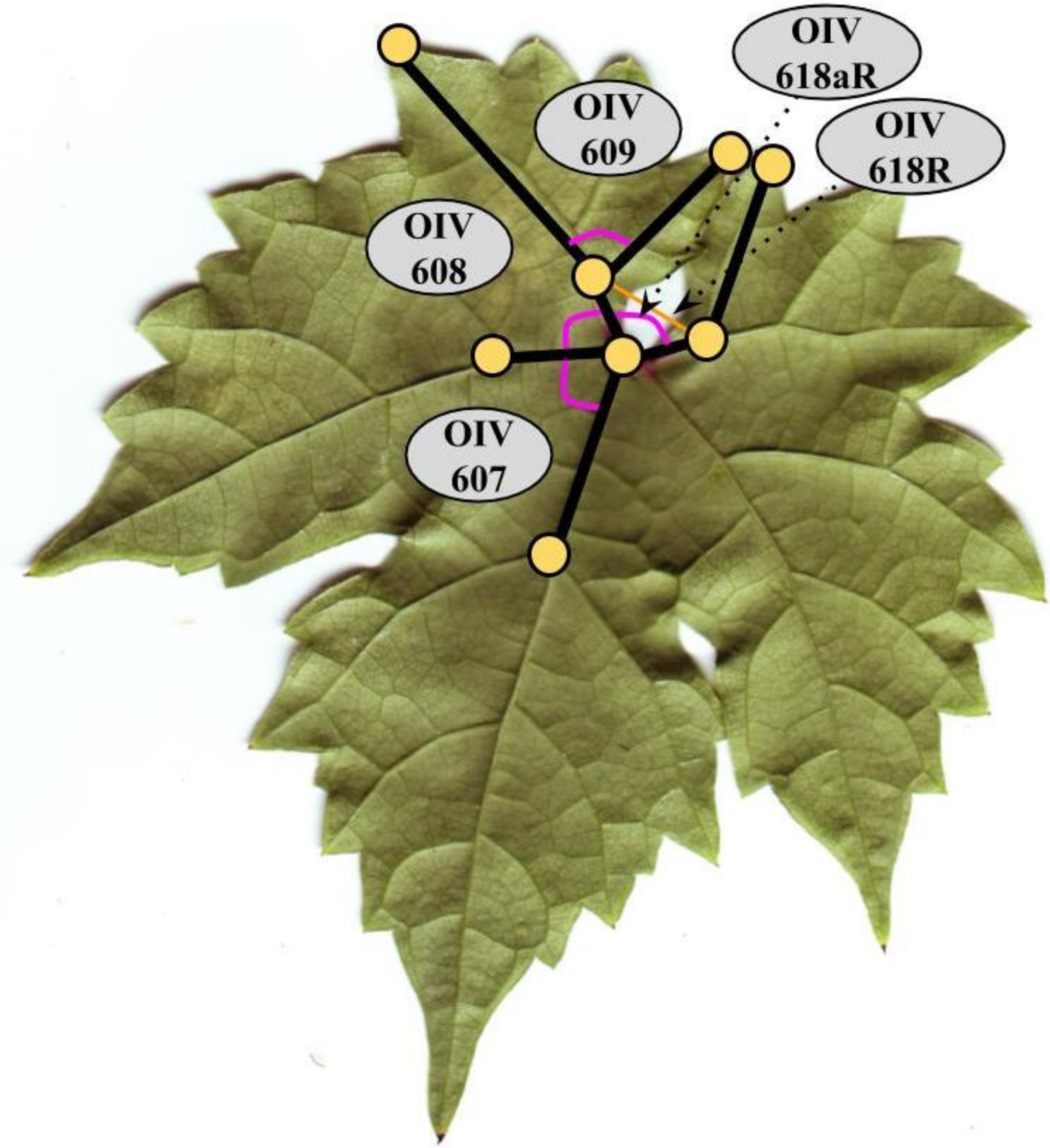

Figure 4.4. OIV descriptors 607, 608, 609 and modified OIV descriptors 618R and 618aR measured for each leaf.

Landmark and Morphometric Analysis

In addition to the mean values of individual OIV descriptors and lengths, overall principal component analysis was conducted on the collected traits using prcomp in R (R Core

Team, 2021). Visualization of PCA results were conducted using the the factoextra package (Kassambara and Mundt, 2017).

Using the shapes package in R, generalized Procrustes analysis was performed on landmarks with procGPA (Dryden, 2018). This allowed for principal component analysis of global shape which was then graphically rendered using ggplot2 (Wickham, 2009).

Results and Discussion

OIV Descriptors and Lengths

The plotting of principal component traits, based on OIV descriptors and specific lengths, along PC1 and PC2 depicts how PC1 was driven by length measurements, and can be viewed as a function of leaf age and developmental stage relative to sampling date (Fig. 4.5). The developmental landscape of leaf shape was further visualized by plotting the individual leaf PC values (Fig. 4.6). The eigenvalues of the top five PCs ranged from 61.8% to 2.7%, only the top three PCs accounted for more than 5% of variation individually (Fig. 4.7). PC1 was driven by OIV 601, OIV 602, OIV 603, OIV 604, and all non-OIV length measurements (Fig. 4.8). Most length measurements contributed nearly equal variance from each of the left and right version of the metric. PC2 variance is composed of OIV 607, OIV 608, and OIV 609 (angles between main veins) and the petiole sinus OIV 618aR and OIV 618 (Fig. 4.9). PC3 variation was also derived from OIV 608 and OIV 609, as well as OIV 605 and OIV 606, which are measurements of the petiole sinus to lateral leaf sinuses (Fig. 4.10).

Considering that PC1 accounts for leaf size, the PC measurements may be driven by leaf emergence time relative to sampling. Sampling occurred when vines had between 16 and 20 unfolded leaves. Leaves 01 and 02 rarely expanded much and leaves 10 to 13 were generally still in the process of expanding, thus quite small. As a result, the highest PC1 values generally belonged to leaves 05 to 09, because they were largest at sampling. From negative to positive PC1 values there is a general transition from initial emerged leaves (leaves 01 to 03), to recently emerged leaves (10 to 13), followed by the most expanded leaves at time of sampling. PC2 gives rise to separation among sampled leaves, with the earliest emerging leaves having the greatest values and the most recently emerged leaves generally having the lowest values. However, the OIV PCs examined have a high amount of overlap between groups of leaves and are not crisply defined based purely on OIV and length metrics.

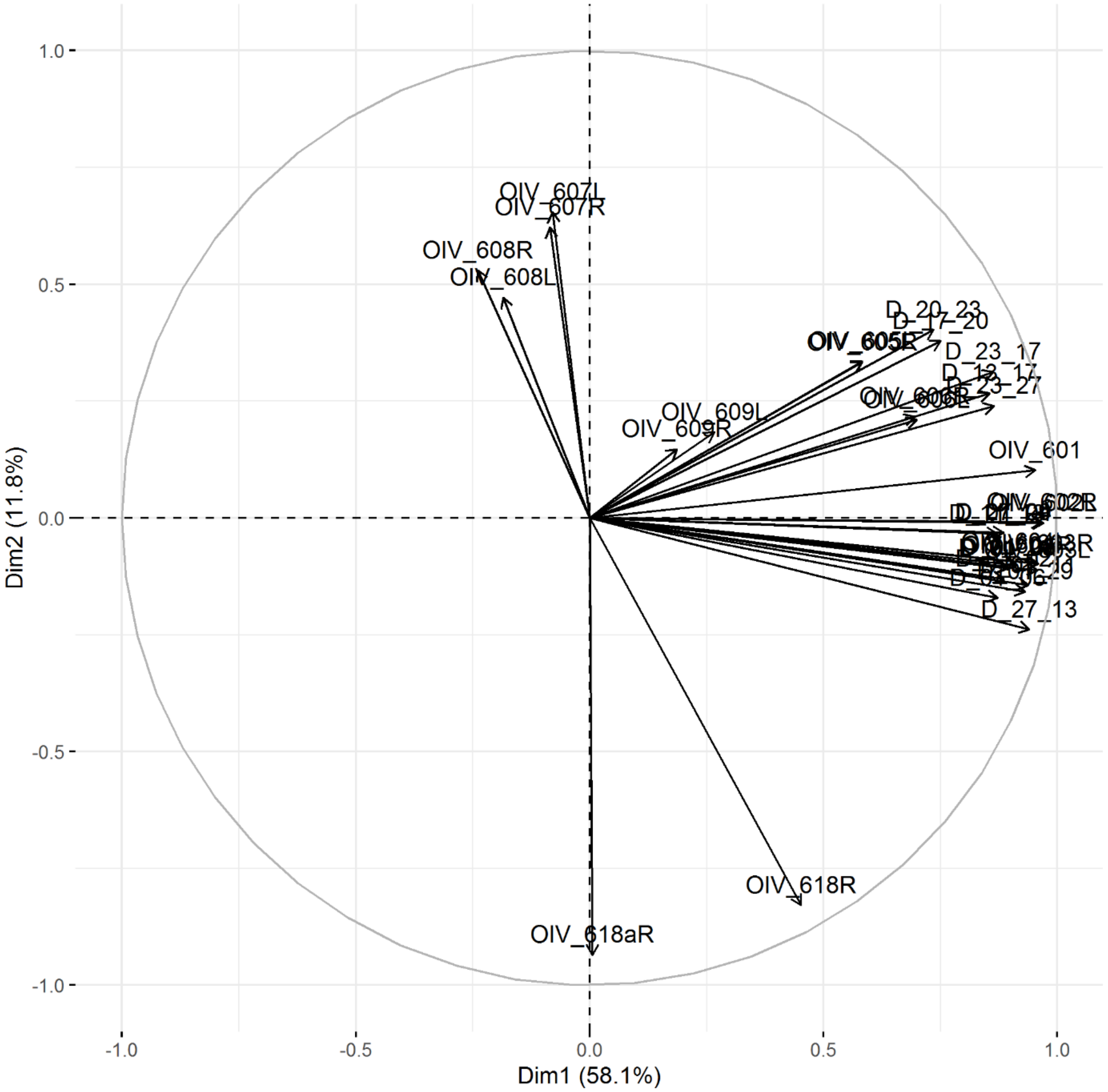

Figure 4.5. Plotting of calculated traits along the axis of PC1 and PC2 for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

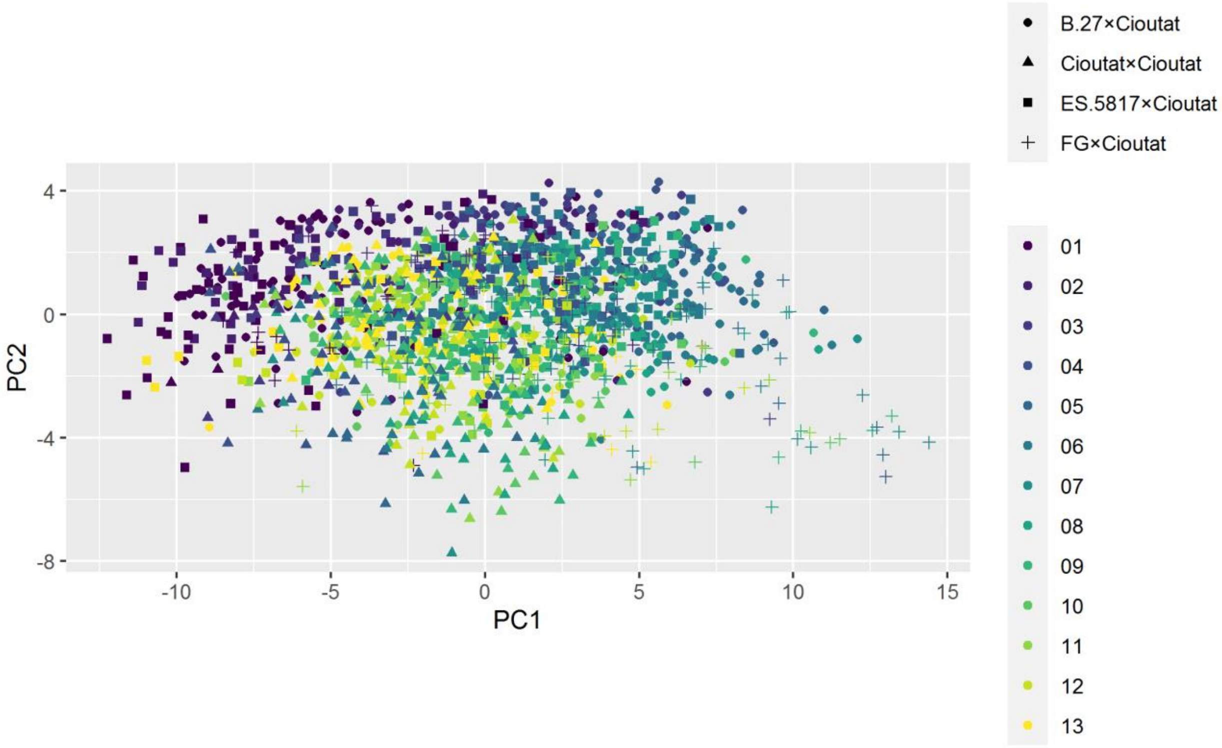

Figure 4.6. Plotting of individual leaf PC1 and PC2 values for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

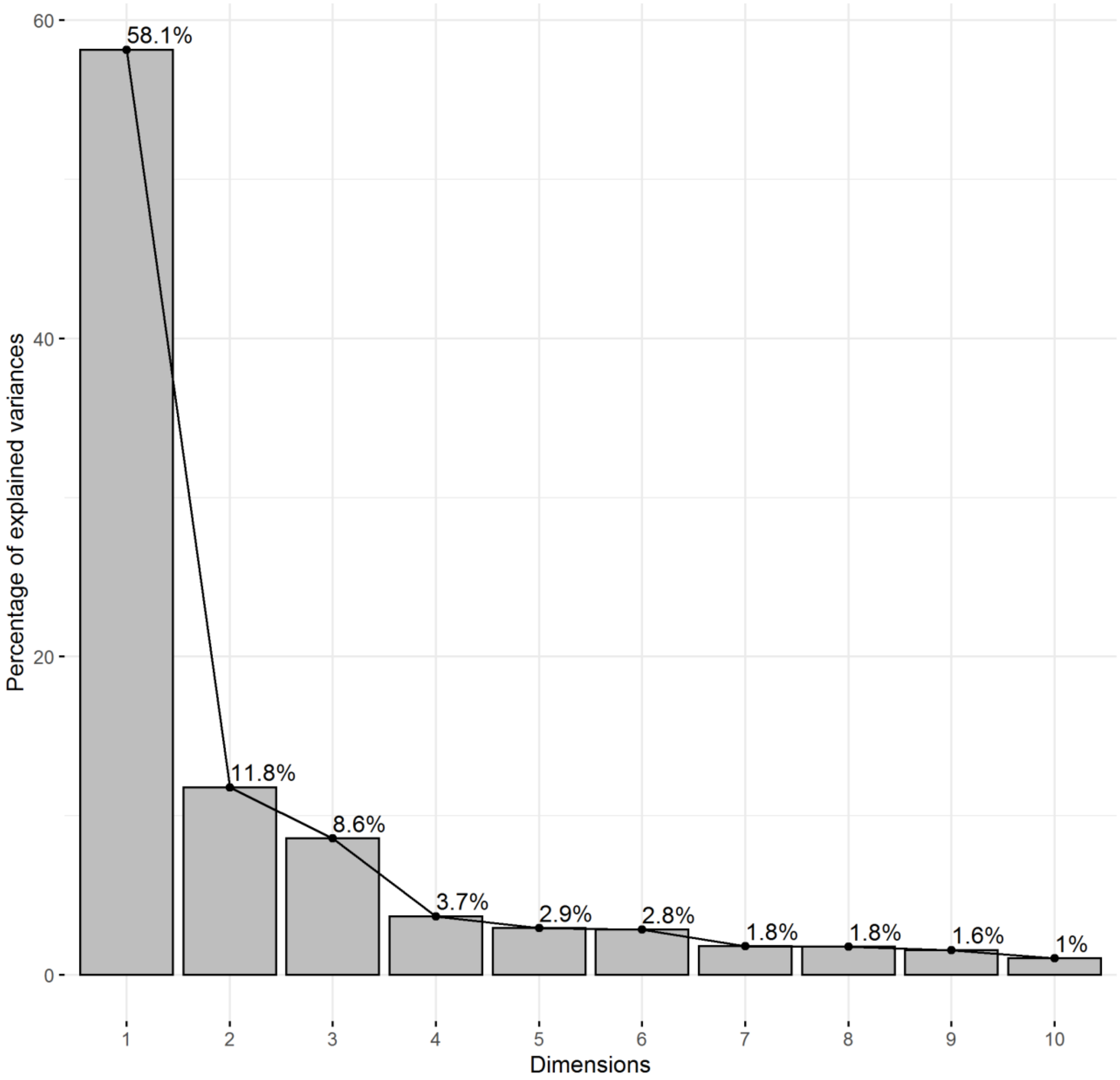

Figure 4.7. Plotting of eigenvalues for the first ten PC for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

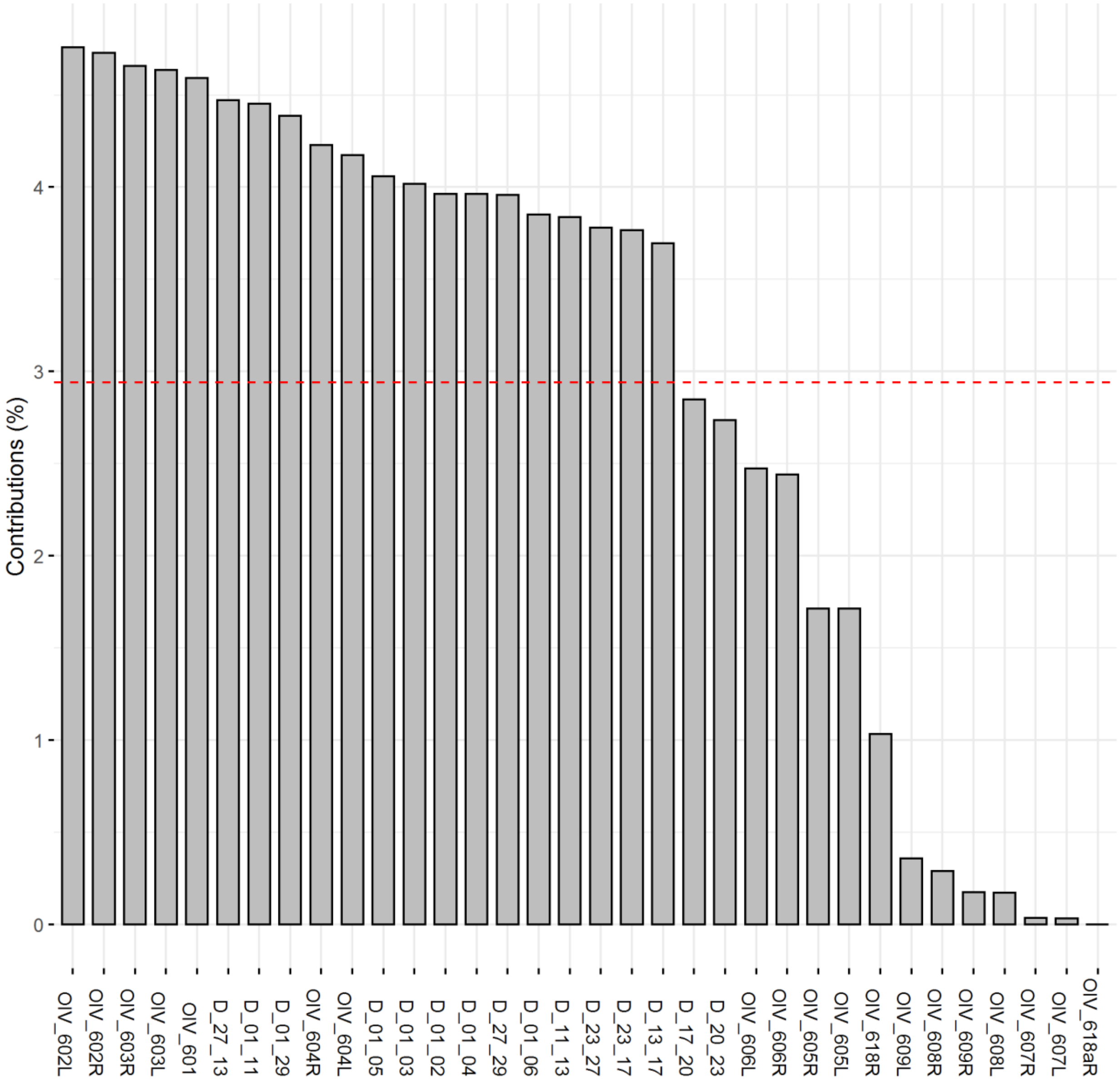

Figure 4.8. Trait contribution to PC1 for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

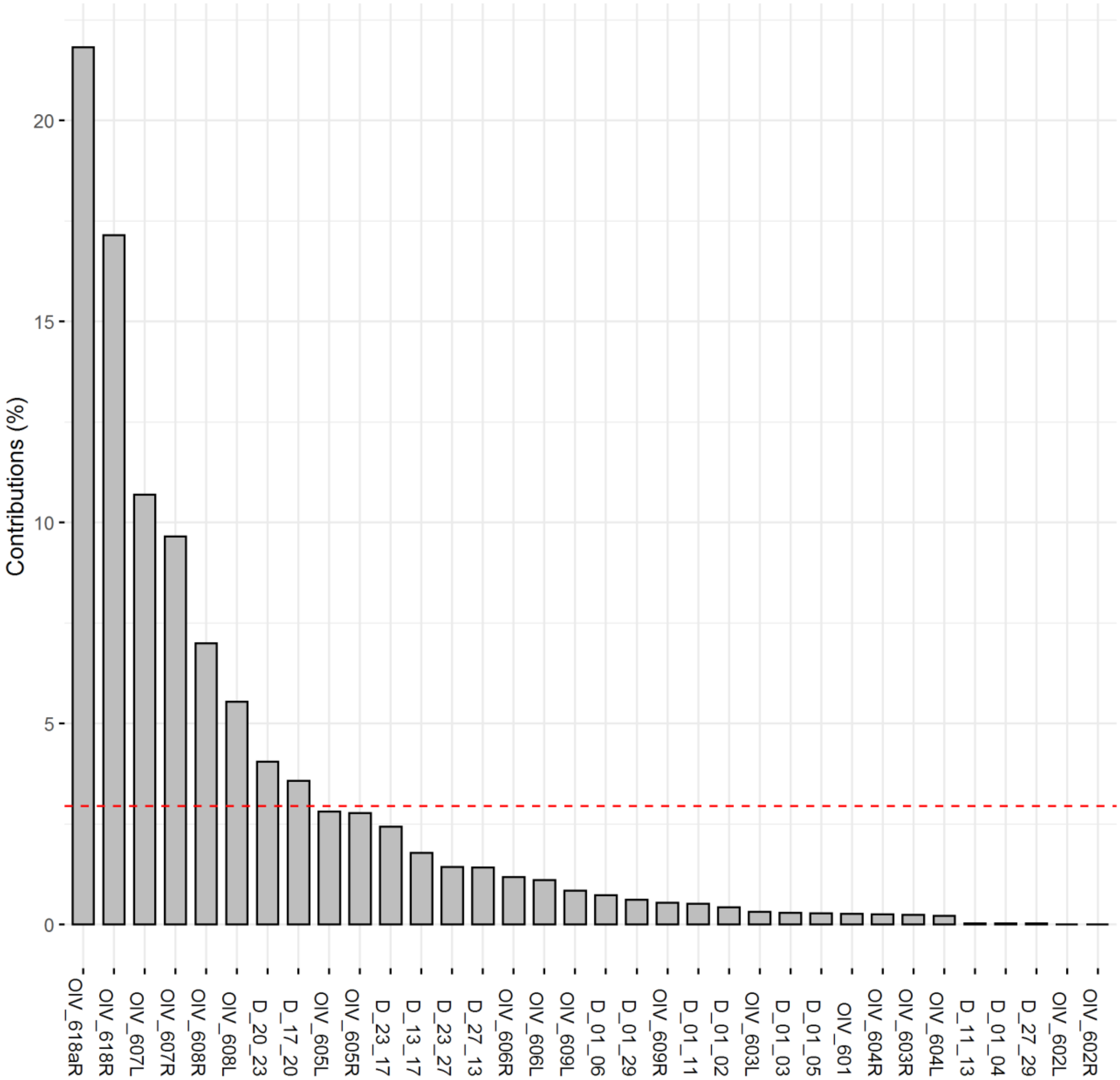

Figure 4.9. Trait contribution to variation of PC2 for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

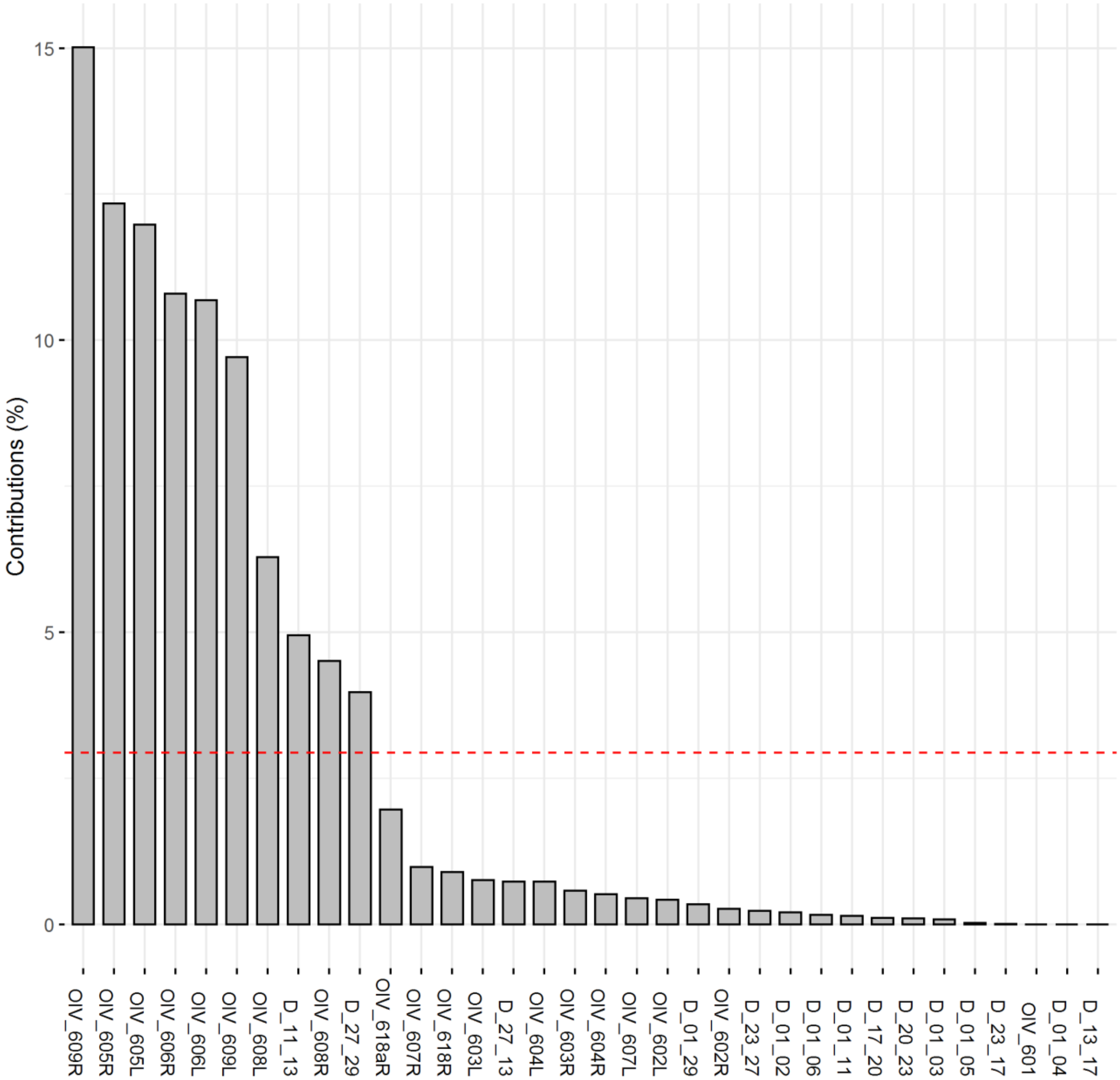

Figure 4.10. Trait contributions to variation of PC3 for OIV and distance measurements of leaves 01 to 13 of seedlings from four grapevine populations.

Landmark Morphometrics

Generalized Procrustes analysis gave a visual interpretation of landmark morphometric approaches. The PC GPA1 accounted for 43.8% of variation, it was driven by sinus depth and opening of the petiole sinus (Fig. 4.11). The PC GPA2 accounted for 19% of variation and again was driven by sinus depth and relative opening, while PC GPA3 accounted for 6.5% of variation and appears to describe the opening of the petiole sinus.

The distribution of individuals along the PC GPA plot yielded an evident and clear separation among leaf number (Fig. 4.12). The earliest to emerge leaves are clearly separated from the most recently emerged leaves with separation along PC GPA1 and PC GPA2; however, a mixture occurs around leaves 03-06 indicating a developmental transition. The separation of developing leaves became clearer when ‘Chasselas Cioutat’ selfed progeny were removed from plots (Fig. 4.13). This was attributed to their influence as an extreme case for leaf shape variation.

This developmental transition was best visualized in the mean leaves of each cross (Fig. 4.14). The increase in sinus depth was clearly progressing in mean leaves 04, 05, and 06. After this point the overall mean leaf shape shifts towards a more stable, mature leaf form.

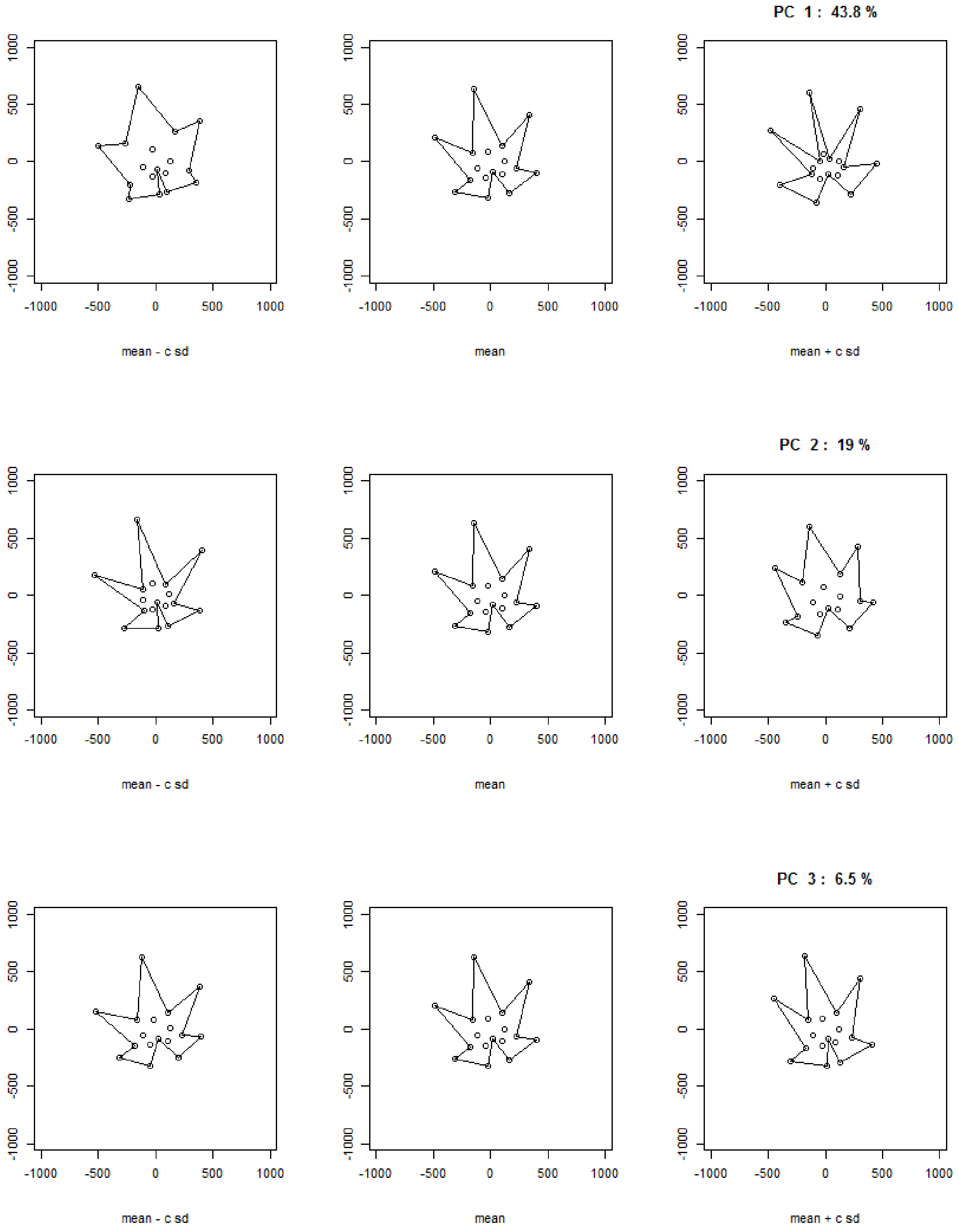

Figure 4.11. Plotting mean leaves plus/minus one standard deviation for GPA PC1-3 based on landmark morphometric analysis of leaves 01 to 13 of seedlings from four grapevine populations.

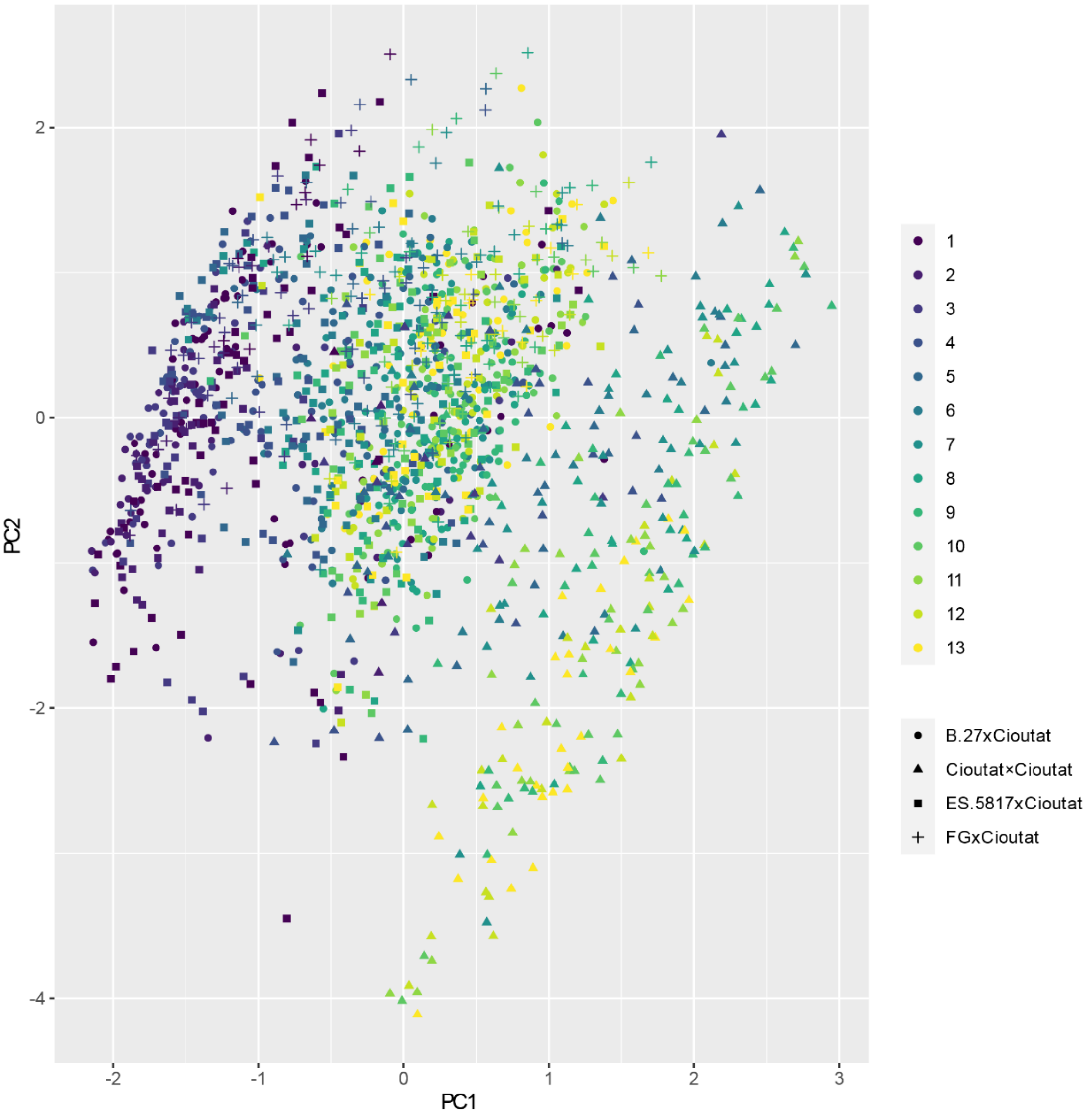

Figure 4.12. Plotting of individual leaf PC1 and PC2 values based on landmark morphometric analysis of leaves 01 to 13 of seedlings from four grapevine populations.

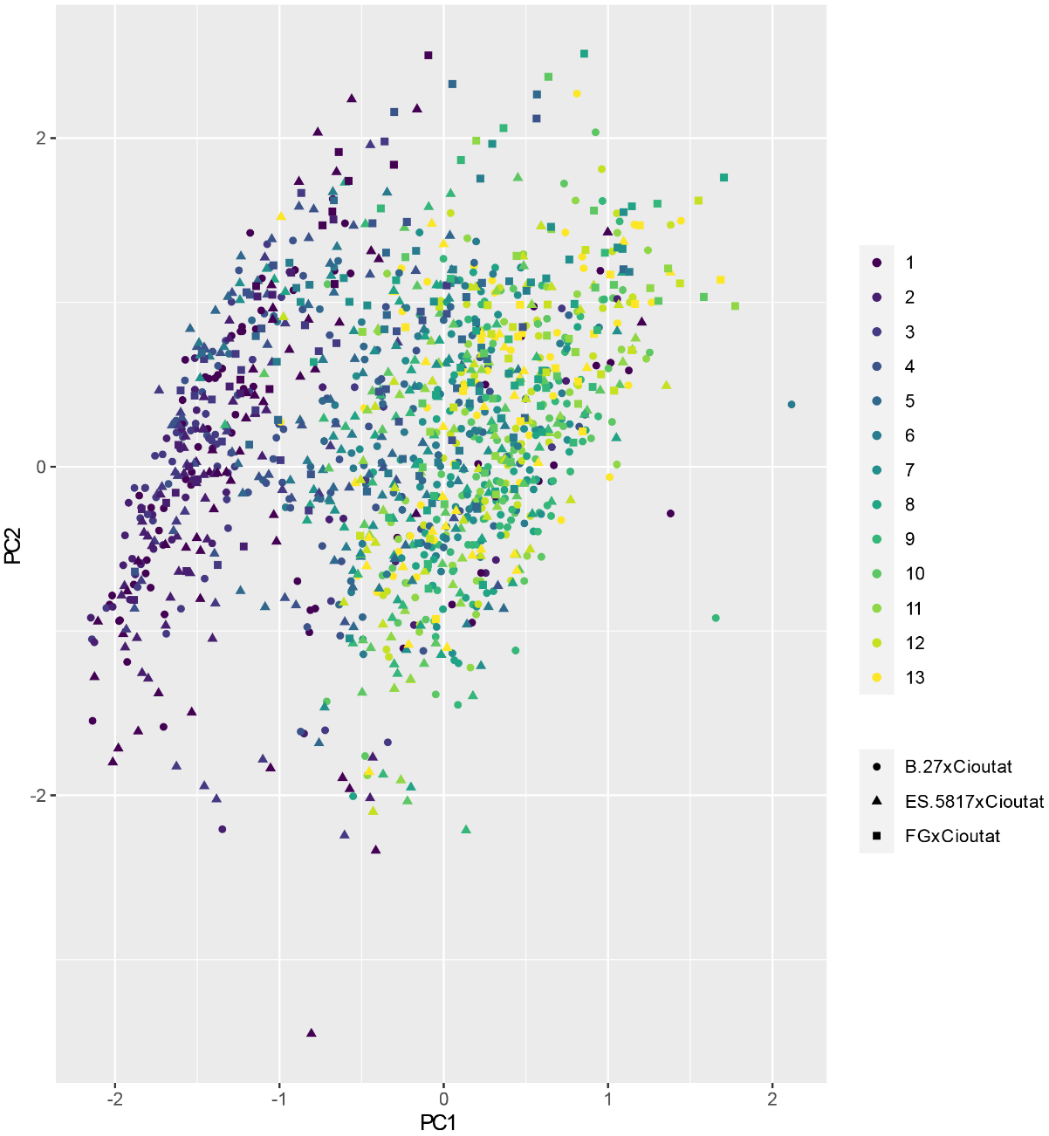

Figure 4.13. Plotting of individual leaf PC1 and PC2 values based on landmark morphometric analysis of leaves 01 to 13 of seedlings from four grapevine populations with ‘Chasselas Cioutat’ presumed-self progeny omitted.

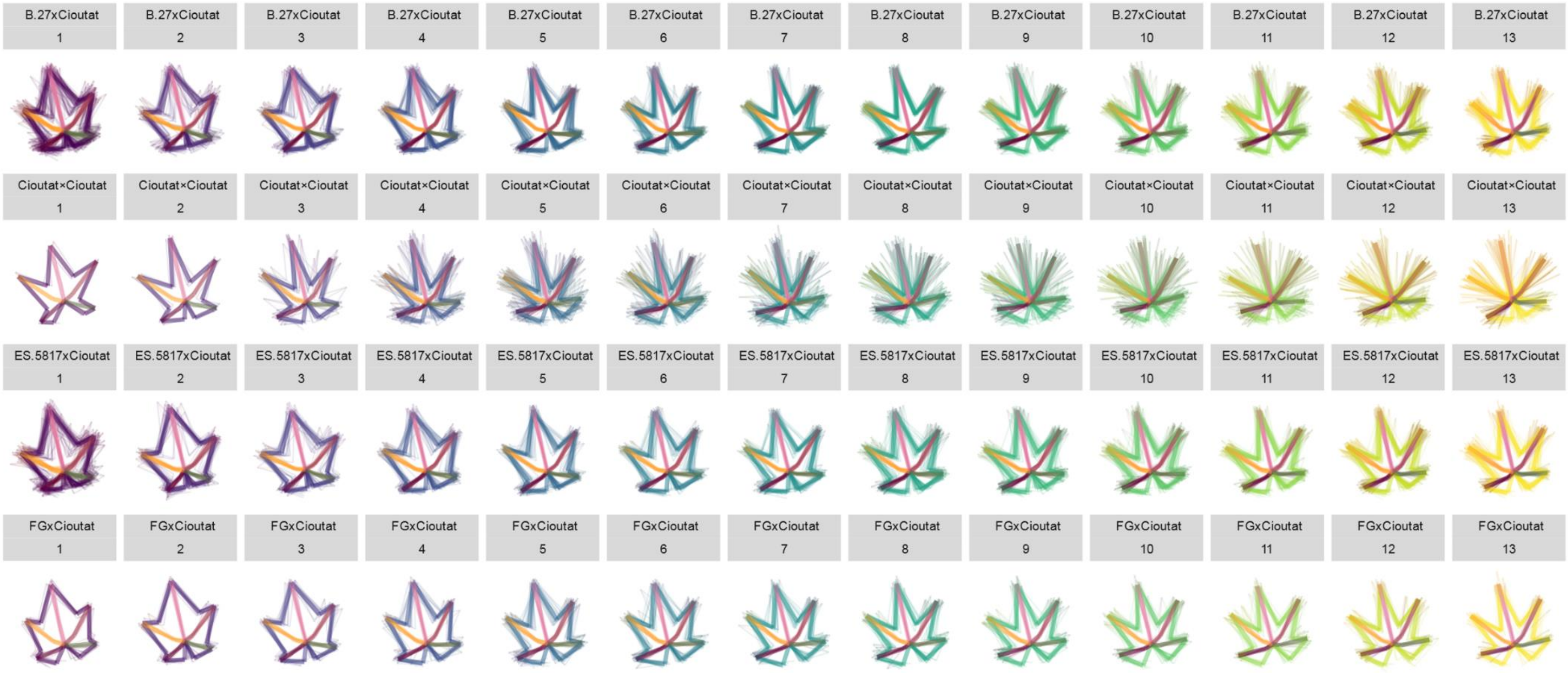

Figure 4.14. Mean leaf shapes of leaves 01 to 13 of seedlings from four grapevine populations.

Conclusion

Leaf shape changes were examined along the developmental axis of grapevine seedlings starting with the first true leaf (leaf 01) after cotyledon emergence (leaf 00). Except for one individual, ND.19.001.1.012, no transition to ‘Chasselas Cioutat’, mutant, lacinate leaf shape was observed in S_0/F_1 hybrid progeny.

Most of the observed ‘Chasselas Cioutat’ presumed-self S_1 progeny had leaves exhibiting the lacinate trait, and all scanned samples exhibited the lacinate, compound leaf trait. However, potentially greater variance for this leaf trait was observed in a larger population of inbred and presumed-inbred, direct seedlings from ‘Chasselas Cioutat’ (data not shown). Boyden (2005) and Snyder and Harmon (1939) also observed simple leafed progeny within ‘Chasselas Cioutat’ derived selfed progeny. Possible explanations for the observations found in this study and those of Boyden (2005) and Snyder and Harmon (1939) include potential inconsistency in defining the compound leaf trait as a binary trait or classification system, inadvertent open-pollination by non-intended parental genotypes, or compounded effects of heteroblasty and heterophylly on leaf shape expression. Within S_1/F_2 progeny, observed variation in lacinate leaf shape expression occurred within a single plant (data not shown). This pre-existing potential for variation within a plant when combined with variable growth conditions (shade, heat, age, fertilizer, humidity, watering status, etc.) may obscure visual phenotyping of specific characteristics prone to fluctuate within sample plants (Jones, 1995; Jones et al., 2013; Chitwood et al., 2016a; Spriggs et al., 2018; Baumgartner et al., 2020; Bryson et al., 2020).

Leaf shape changed drastically with newly emerging leaves as monitored by generalized Procrustes analysis of landmark morphometrics and traditional leaf variation metrics (Fig. A1-A34). Leaf size and shape and plant morphology are fluid and environmentally, genetically, and heteroblastically dependent (Wolf et al., 1986; Kaplan, 2001; Tsukaya, 2003; Chitwood et al., 2016a; Baumgartner et al., 2020; Bryson et al., 2020; Li et al., 2021).

Additionally, another unexamined facet was the effect of leaf growth and deformation. Due to the constraints of leaf measurement, which requires entire leaf removal for scanning, our sampling scheme was destructive for the first 13 leaves sampled along a plant with a single collection timepoint. Non-destructive measurement techniques in future research may enable researchers to monitor leaf development, growth, and the effect of growth on morphological characteristics (Kuchen et al., 2012; Rolland-Lagan et al., 2014; Wolf et al., 1986).

An example of environmental driven leaf shape responses was found in the aquatic species *Rorippa aquatica*, with drastically different leaf shapes within different environmental contexts (Nakayama et a., 2014). Lower temperatures or submergence in water drives leaf complexity while warmer temperatures and lack of submergence lead to simple leaf shapes. Leaf size and leaf shape are dependent on independent genetic and environmental factors (Baker et al., 2015). Like temperature and moisture availability, light also plays a role in leaf characteristics (Chitwood et al., 2015).

Limited work has focused on the inheritance of leaf shape traits in grapevines (Bešlić et al., 2005; Boyden, 2005; Welter et al., 2007; Nikolić, 2015; Demmings et al., 2019). However, leaf shape, physiological function, and other morphological characteristics of grapevines remain an important point of future research focus. Altering grapevine physiology and morphology through selective, informed breeding provides an opportunity for more sustainable grapevine cultivar development in the face of new pests, pathogens, and climatic challenges (Cousins and Prins, 2008). Within tomatoes, leaf shape has been linked to fruit quality and crop suitability (Rowland et al., 2020). Within grapevines, more work is necessary to identify any genetic or physiological correlations between fruit composition, plant productivity, and overall plant health with leaf shape traits.

Future Work

This work focused on three crosses with a unique pollen parent, ‘Chasselas Cioutat’. The leaf shape transitions observed followed a similar pattern and may be driven by ‘Chasselas Cioutat’ and the heavy *V. riparia* background of the seed parents; thus, they may not be entirely representative of grapevine developmental leaf shape changes. To understand more about the morphological development of foliar anatomy in seedlings of *Vitis* spp., similar work could be conducted within more diverse populations using different parents within a quantitative genetic approach to further elucidate dominant, additive, and epistatic effects.

The ultimate research focus should strive towards the goal of understanding the inheritance of ‘Chasselas Cioutat’ lacinate leaf anatomy in grapevines. Towards this goal, a subset of the phenotyped seedlings from this experiment were maintained under greenhouse conditions. Following sexual maturation and flower production, these individuals were either selfed (when hermaphroditic, perfect flowered), backcrossed to ‘Chasselas Cioutat’ (when female, pistillate flowered), or crossed with half-sibs from other S_0/F_1 populations. On-going segregation analysis of leaf morphology is anticipated to yield insight into the inheritance of the unique, lacinate leaf trait.

Within the examination of heteroblastic and heterophyllic leaf shape variation in *Vitis* spp. it is important for researchers to combine genetic maps and expression analysis to identify genes involved in the process of grapevine phase change transition from juvenility to maturity, and to better understand leaf shape maintenance and expression. When examining future progeny for effect of alleles, major genes likely involved in leaf shape expression based on their relevance in other plant species include PHANTASTICA (PHAN), LEAFY (LFY), FLORICAULA (FLO), Class I KNOTTED1-LIKE HOMEOBOX (KNOX1), CUP-SHAPED COTYLEDON (CUC), and JAGGED (JAG) (Kim et al., 2003a; Tsukaya, 2004; Champagne et al., 2007; Kimura et al., 2008; Koenig and Sinha, 2010). Regulation of adaxial identity is conducted by PHAN, with overexpression driving formation of simple leaf blades (Kim et al., 2003a; Kim et al., 2003b). Additional overexpression leads to ectopic blade formation. Additionally, PHAN dictates two different forms of palmately compound leaves (peltately and non-peltately palmate), and plays a role in determining placement of leaflets; this is combined with CUC and JAGGED regulation of leaf dissection (Kim et al., 2003b; Barkoulas et al., 2007; Koenig and Sinha, 2010; Bar and Ori, 2015).

Formation of compound leaf primordia requires a more intricate developmental differentiation process than simple leaves, and the presence of germplasm with compound leaves has implications to evolutionary adaptation and applied breeding (Blein et al., 2008; Blein et al., 2010; Nicotra et al., 2011; Ogden and Lacroix, 2017). A blastozone forms at primordium margins before yielding the primordia of leaflets (Hageman and Gleissberg, 1996). Harevan et al. (1996) examined the expression and phenotypic effect of a maize homeobox-containing Knotted-1 (Kn1) gene in tomato, with observed intensification of compound leaf phenotypes. Consequences of transgenic tomato plants expressing the Kn1 gene included super compound phenotypes, bushy growth, and loss of apical dominance. Similar results with Kn1, a KNOX gene, observed increasingly lobed leaves with overexpression; this was combined with abnormal leaf morphology (Lincoln et al., 1994). From the perspective of leaf serrations and lobiness, these traits also warrant extended investigation, as they also contribute to the variation in leaf shape observed.

Lobed leaf expression is triggered by KNAT1 in arabidopsis, *Arabidopsis thaliana* (L.) Heynh. (Chuck et al., 1996). Similarly, KNOX protein expression leads to dissected leaves in hairy bittercress (*Cardamine hirsuta* L.), a close relative of arabidopsis (Hay and Tsiantis, 2006). Further work refined understanding of developmental cues and leaflet formation in hairy bittercress focused on SIMPLE LEAF3 and KNOX1 (Barkoulas et al. 2008; Kougiousmoutzi et al., 2013; Vlad et al., 2014). Leaf developmental processes in Brassicaceae species have supported the evidence of KNOXI proteins in expression of complex leaves (Bharathan et al., 2002). Additional work with a recessive mutant allele at the BLADE-ON-PETIOLE1 locus (bop1-1) is proposed to determine regulation of class I KNOX genes, with mutants producing ectopic blades along the sides of petioles in *Arabidopsis* (Ha et al., 2003).

Within Fabaceae, the KNOX1 genes, FLO, and LFY genes have been shown to influence leaflet number and compound expression of leaves in soybean (Glycine max) and alfalfa (Medicago sativa), with FLO and LFY playing major roles (Champagne et a., 2007). Specifically, within the pea clade, LFY is a major regulator of foliar complexity. The expression of UNIFOLIATA (UNI) has also been implicated with control of leaf and flower formation in pea, *Pisum sativum* L. (Hofer et al., 1997).

Mungbean (*Vigna radiata* L.) is another example of a plant which transitions from simple, juvenile leaves to compound, adult leaves (Jiao et al., 2019a; Jiao et al., 2019b). Jiao et al. (2019b) identified epistatic interactions between heptafoliate leaflets1 (hel1-1) and small-pentafoliate leaflets1(smp1-1) allele mutants leading to increased leaflet number with reduced size relative to hel1-1 and smpl1-1 mutants alone. Again, these traits do not express in juvenile leaves, similar to the WT trifoliate compound leaf expression.

Hormonal regulation is an important point to investigate further in progeny. Within compound leaf primordia, auxin has been shown to determine site of developing leaflets (Koenig et al., 2009). Expression of LYRATE genes occurs in the auxin determining sites, helping with early events at the shoot apex (David-Schwartz et al., 2009). Auxin has been further explored in tomato leaf shape dynamics recently by Wu et al., (2018). Cytokinin has also been implicated in developmental processes for tomato compound leaf development (Shani et al., 2010).

In tomato, sampling leaves of varying ages showed that as leaves become increasingly complex there is an increased expression of CUC genes and decreased expression of TCP genes (Chitwood et al., 2012). Simultaneously, this demonstrated that KNOX was not involved in increasing heteroblastic complexity of tomato leaves. The CUC genes have an important role in both heteroblasty and heterophylly by converging, but different means (Chitwood and Sinha, 2016).

Despite the lack of KNOX genes' role in heteroblastic expression of tomato leaf shape, the KNOX genes are critical in evolutionary leaf shape variation in tomato and remain important to heterophyllic responses such as leaflet production for shade avoidance (Chitwood et al., 2015).

MicroRNA expression analysis will be useful to understand more about the transition from juvenile to sexually mature tissue in grapes as it relates to heteroblastic form (Poethig, 2013; Lawrence et al., 2020). Multiple microRNAs are involved in the regulation of phase change transitions from juvenile to adult shoot characteristics (Huijser and Schmied, 2011; Poethig, 2013). In addition to miR156, important protein-protein interactions are involved in increased leaf complexity associated with plant maturation (Rubio-Somoza et al., 2014).

Leaf number and plant carbohydrate status may work to regulate miR156 levels (Yang et al. 2011). This may contribute to the phenomena of leaf shape discrepancies between spur- and cane-produced leaves in *Vitis* spp. compared to the leaves of suckers; these are often morphologically different based on visual assesment. Along with relative moisture available due to proximity to soil and timing of growth in relation to annual heat cycles, the level of miR156 may also play a role in grapevine sucker growth characteristics compared to cane- and spur-pruned shoots from established trunks considering the importance of miR156in shade avoidance (Xie et al., 2017).

In tree species, Wang et al. (2011) demonstrated the importance of miRNA for vegetative phase changes, indicating the importance of miR156 as a conserved regulator pathway in dicotyledonous plants for maintenance of juvenility. As trees from diverse woody species transitioned out of their juvenile phase miR156 abundance decreased while miR172 increased. In *Passiflora edulis,* miR156 and miR172 follow a similar pattern with miR156 correlated with juvenile leaf traits and miR172 correlated with adult leaf traits (Silva et al., 2019).

Along with microRNA expression, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors, NAC, and YUCCA genes are also likely involved in the developmental transition of some woody species based on investigations in *Gevuina avellana* (Ostria-Gallardo et al., 2016). NAC transcription factors are among the largest families of plant transcription factors encompassing (NAM, ATAF, and CUC) (Wang and Dane, 2013). They have demonstrated involvement in stress responses including light, drought, and salt (Wang et al., 2014a; Wang et al., 2014b, Wang et al., 2014c, Wang et al., 2017). The understanding of relationships between leaf morphology and stress responses is expanding, and the overlap of responsible genes and transcription factors likely reduces the ease of discernibility for certain heteroblastic and heterophyllic responses (Hwang et al., 2010; Song et al., 2012; Zhang et al., 2012; Fambrini and Pugliesi, 2013; Belluau and Shipley, 2018).

Monitoring developmental physiology transitions in grapevine seedlings may also yield insight into heteroblasty as has been investigated in *Passiflora edulis* using hyperspectral techniques, moisture stress index (MSI), normalized difference vegetation index (NDVI), and photochemical reflectance index (PRI) (Fernandes et al., 2020).

Okra leaf upland cotton (*Gossypium hirsutum*) is an example of a similar mutant type in which a traditionally simple leaf is now complex (Andres et al., 2014; Kaur et al., 2016; Andres et al., 2016; Andres et al., 2017). In the case of okra leaf upland cotton, a HD-Zip transcription factor has been demonstrated as the causal gene of the okra allele (Andres et al., 2016). This gene is homologous to the LATE MERISTEM IDENTITY1 (LMI1) gene of *Arabidopsis* and its mutation has a 133-bp tandem duplication within the promoter that is correlated with the phenotype of okra leaf shape in upland cotton. Andres et al. (2016) further identified increased transcription of photosynthesis related regulators; this up-regulation may be useful for breeding applications, and it is similar to observed trends within tomato (Chitwood et al., 2013). Further akin to upland cotton, Zhou et al. (2019) showed that patterns of compound leaves in *Medicago truncatula* are altered by mutations to a HD-ZIPIII gene, termed REVOLUTA (MtREV1). Mutations in similar homeodomain-leucine zipper gene families may be important to the compound leaf expression in 'Chasselas Cioutat', and their expression may be mediated by miRNA.

Ultimately, aggressive investigation of leaf shape combined with genetic maps and expression data will enable elucidation of genetic factors controlling compound leaf characteristics in *Vitis* spp. The compound, lacinate leaf shape observed in 'Chasselas Cioutat' is technically compound, yet the traits genetic regulation may differ from the demonstrated control of compound leaf expression in other dicotyledonous plant. While 'Chasselas Cioutat' leaves are technically compound, this is visibly due to an intensification of dissection rather than distinct leaflet formation. Thus, the developmental control may vary relative to the complex control of compound leaves observed elsewhere (Jones et al., 2013; Du et al., 2020; Israeli et al., 2020). It is likely that more than one gene is responsible for control of lacinate, compound leaf expression, as proposed by Boyden (2005). Further work with mapping populations derived from the individuals described here are anticipated to enable identification of critical genetic components of leaf shape in *Vitis* spp. and the transition from simple to complex.

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APPENDIX

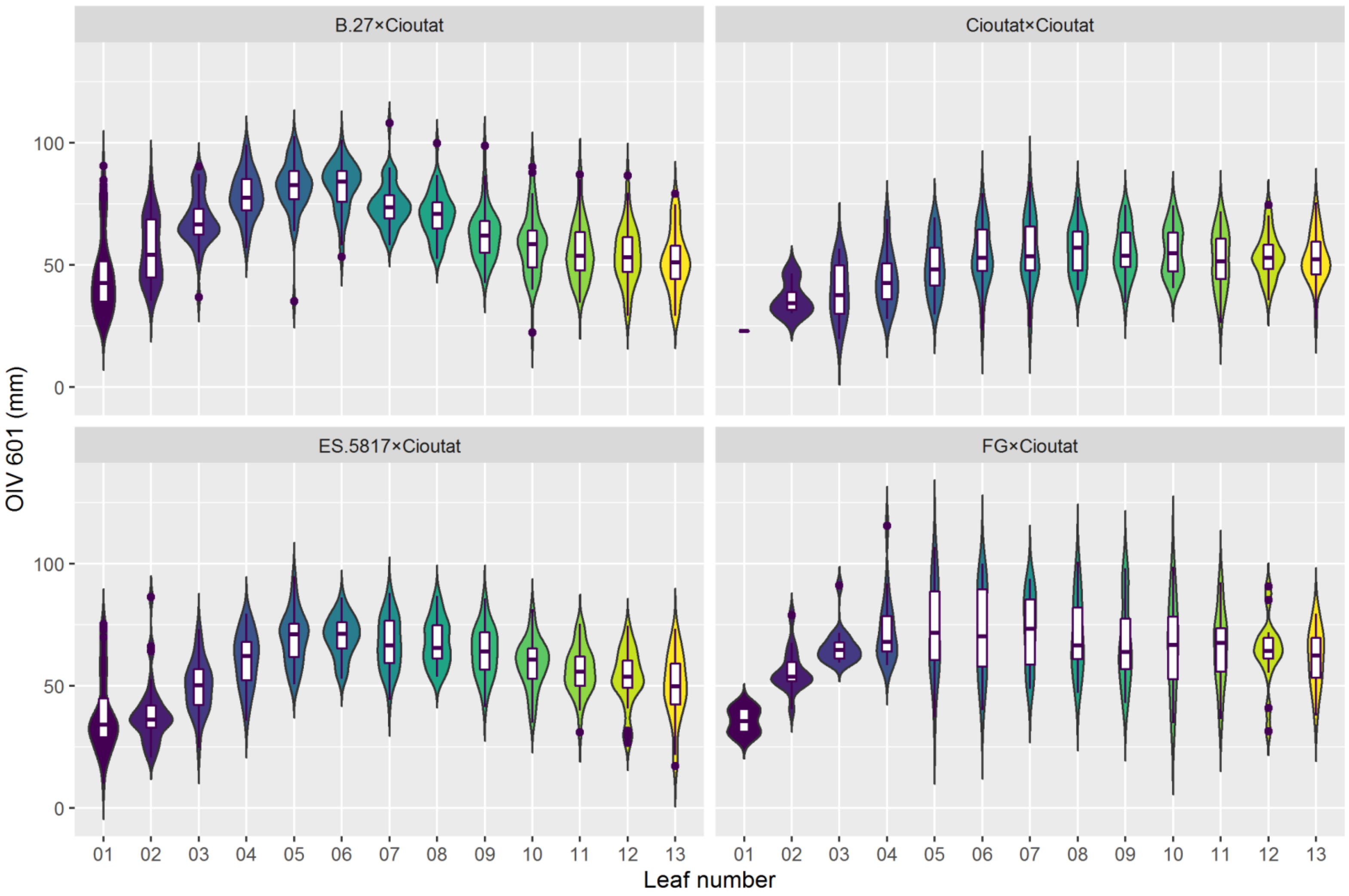

Figure A1. OIV 601, length of main vein N_1 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

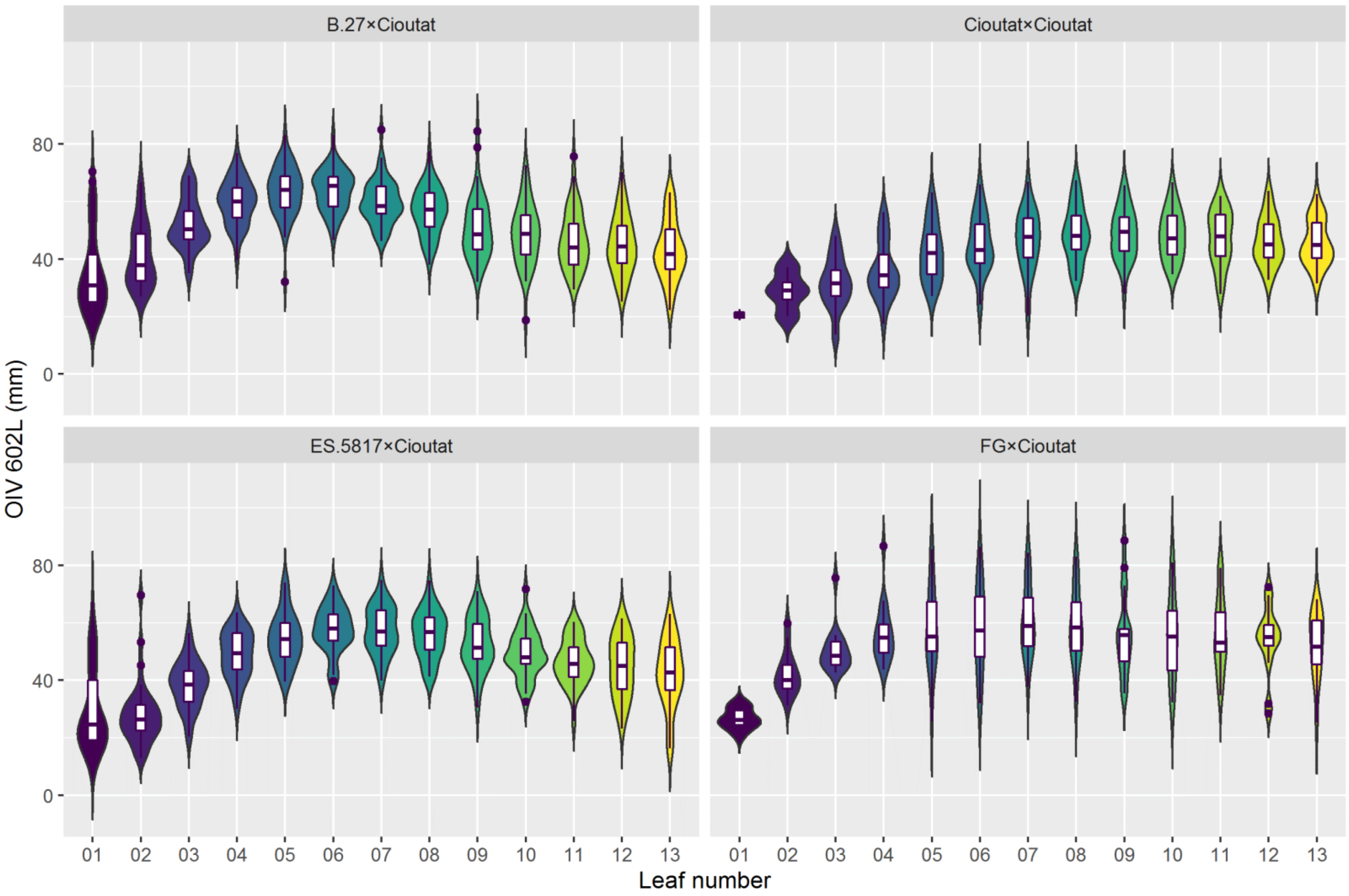

Figure A2. OIV 602L, length of left vein N2 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

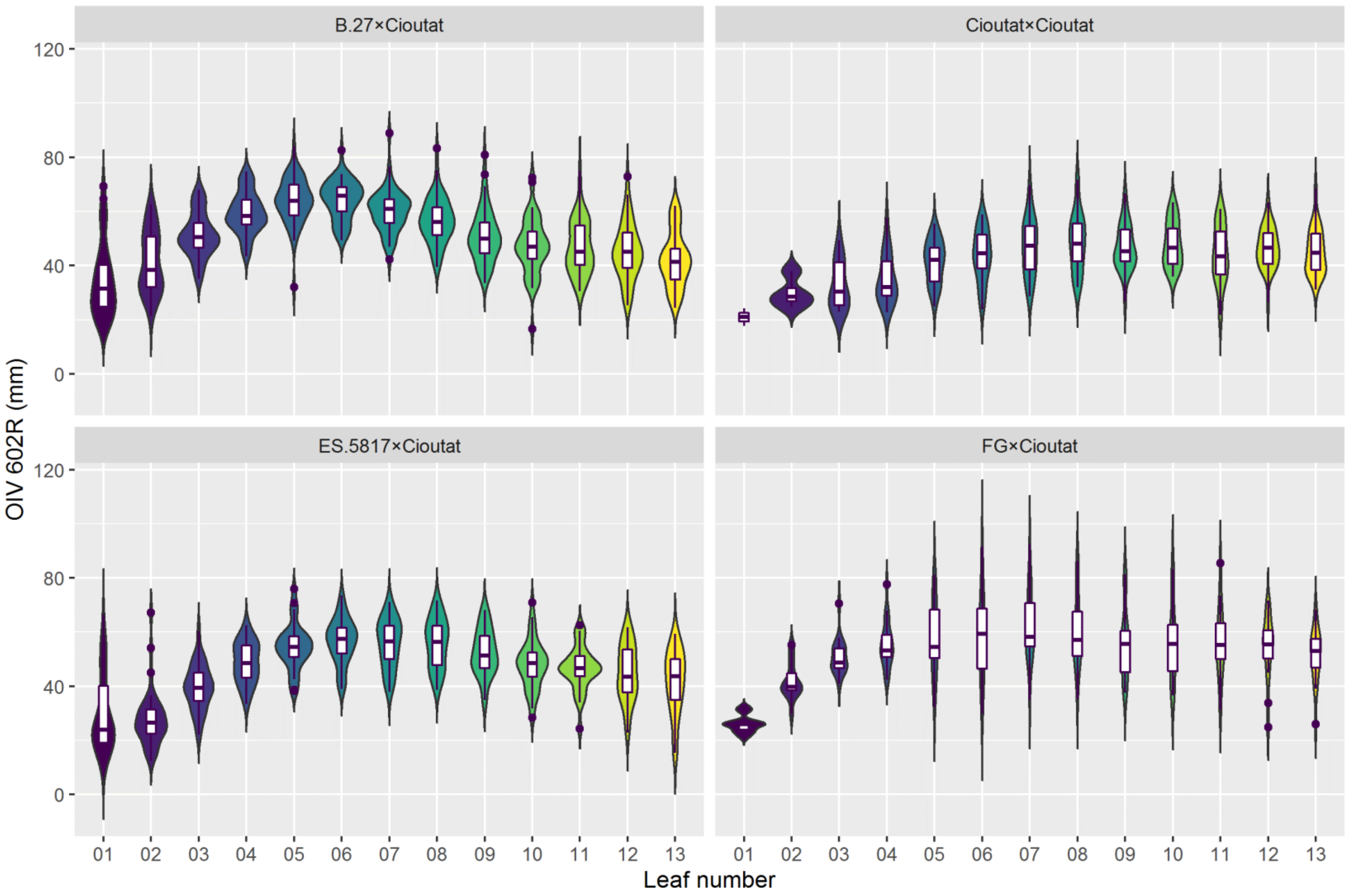

Figure A3. OIV 602R, length of right vein N2 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

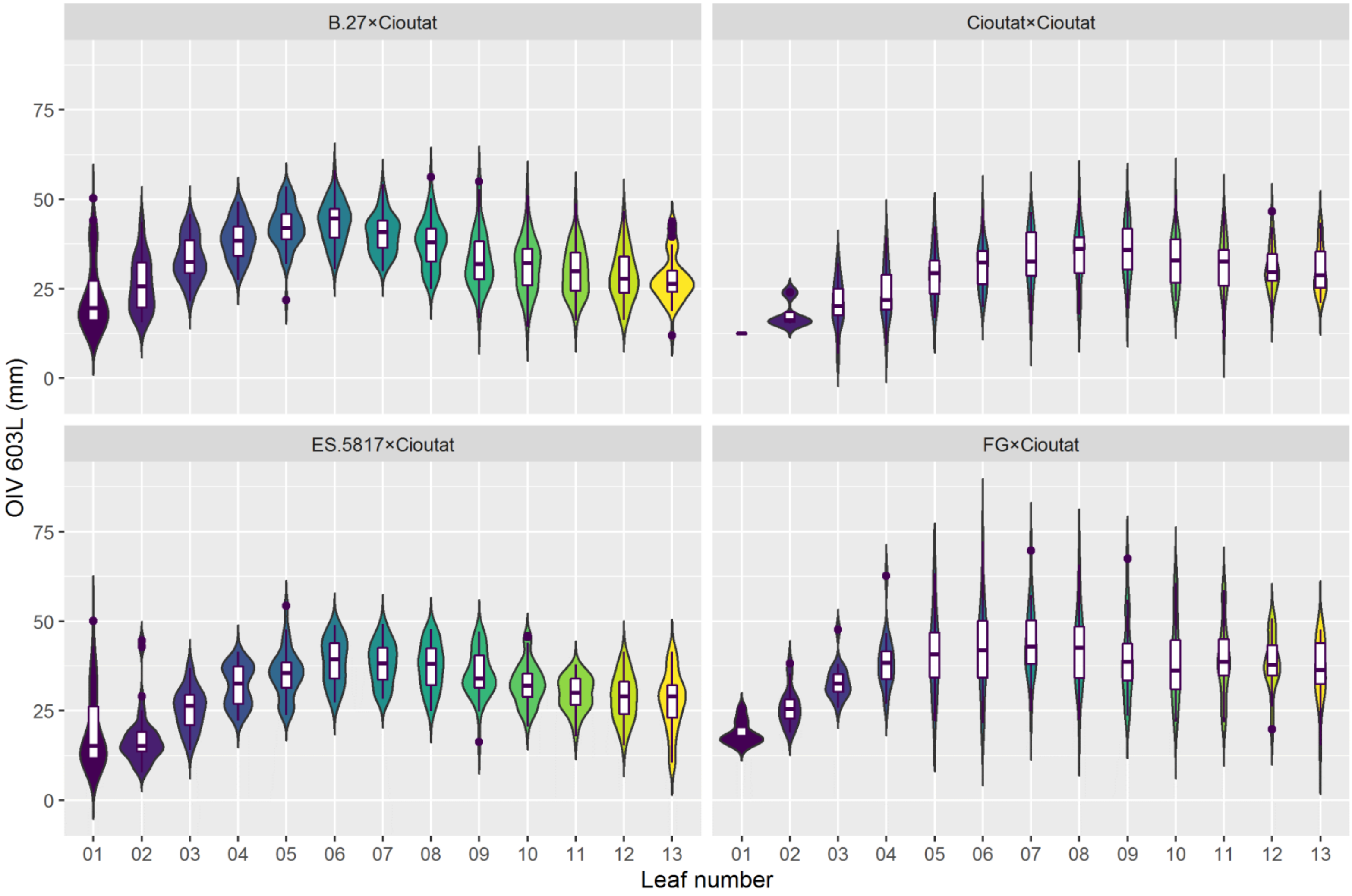

Figure A4. OIV 603L, length of left vein N3 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

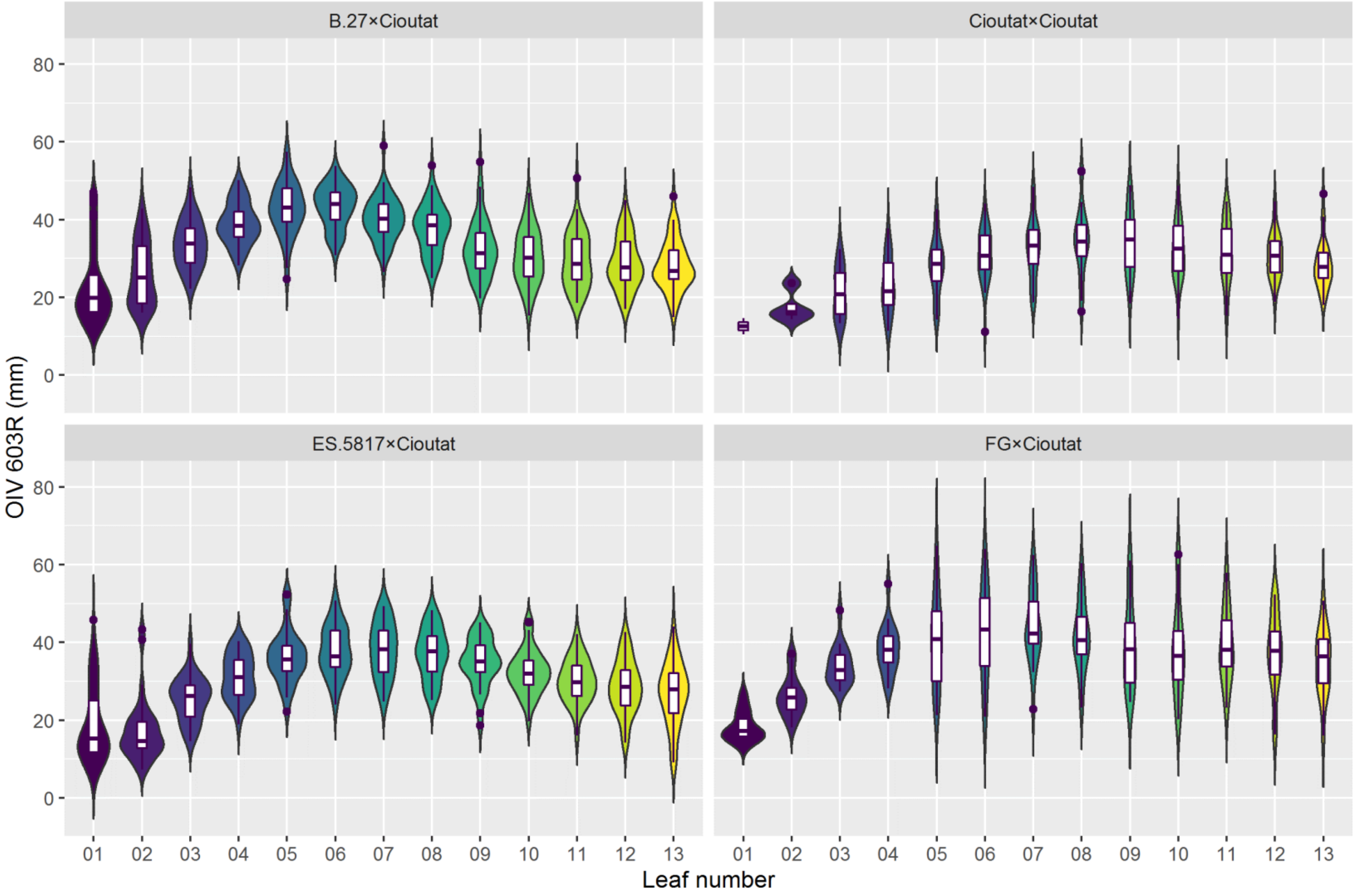

Figure A5. OIV 603R, length of right vein N3 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

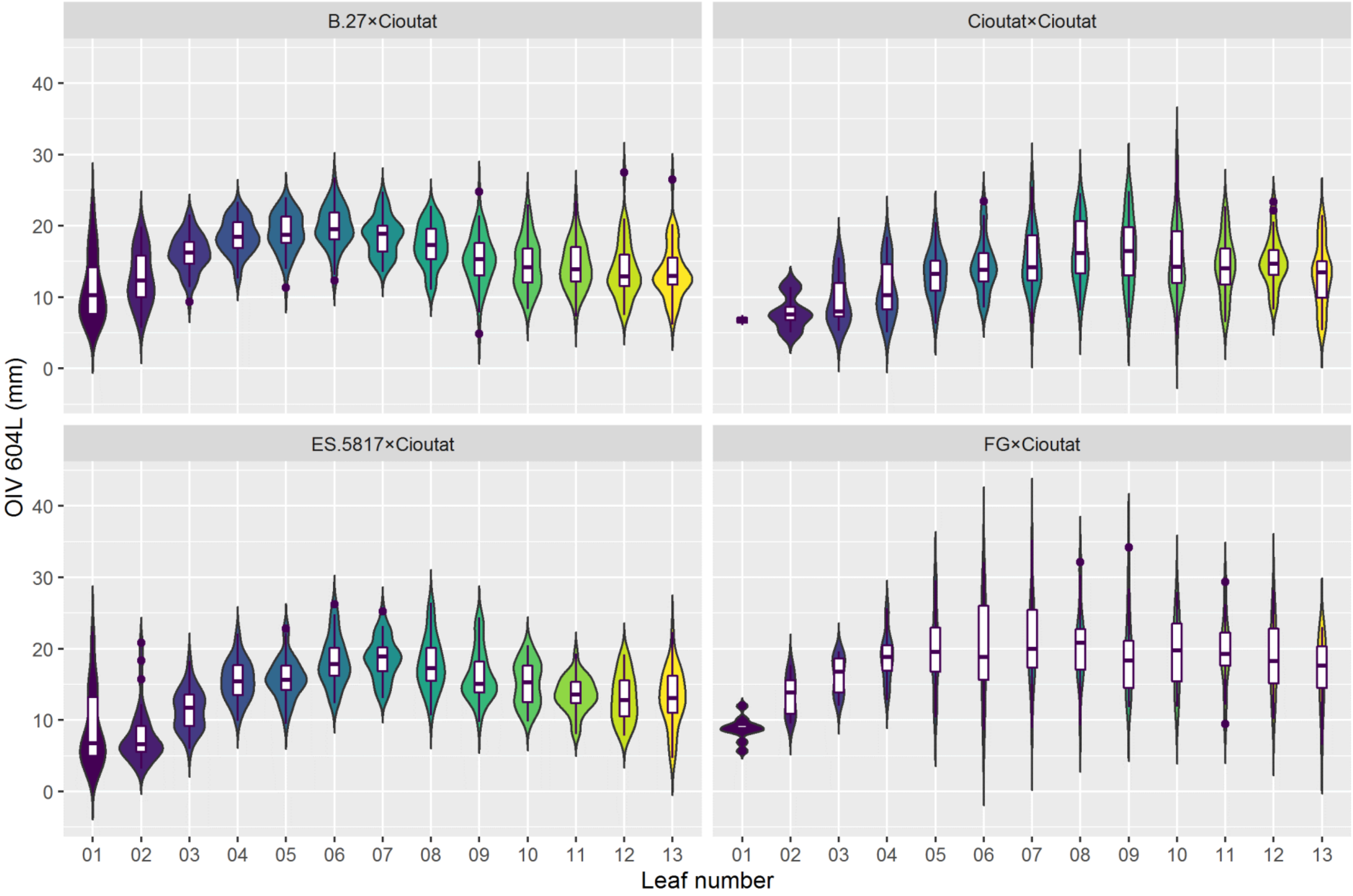

Figure A6. OIV 604L, length of left vein N4 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

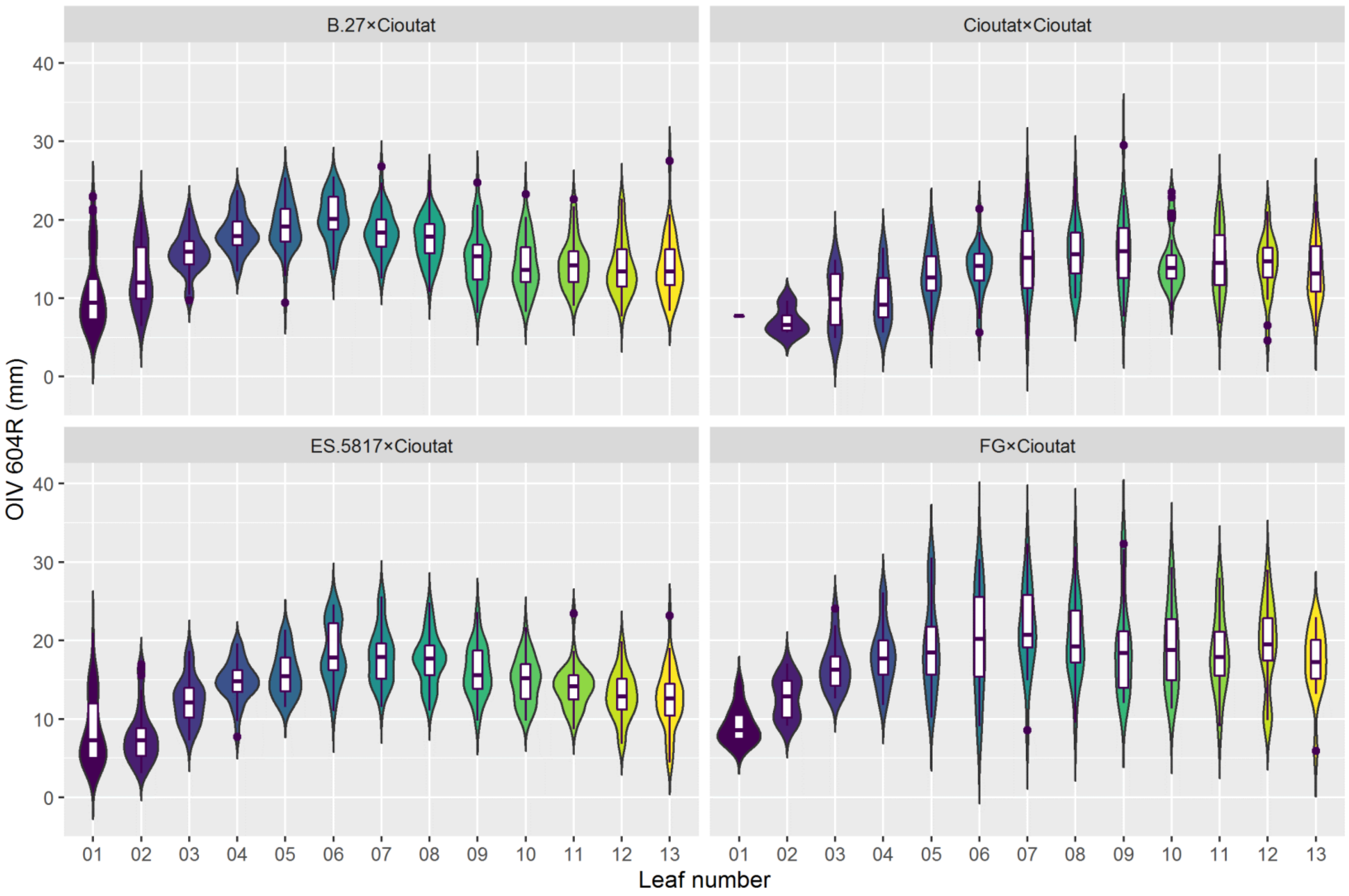

Figure A7. OIV 604R, length of right vein N4 (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

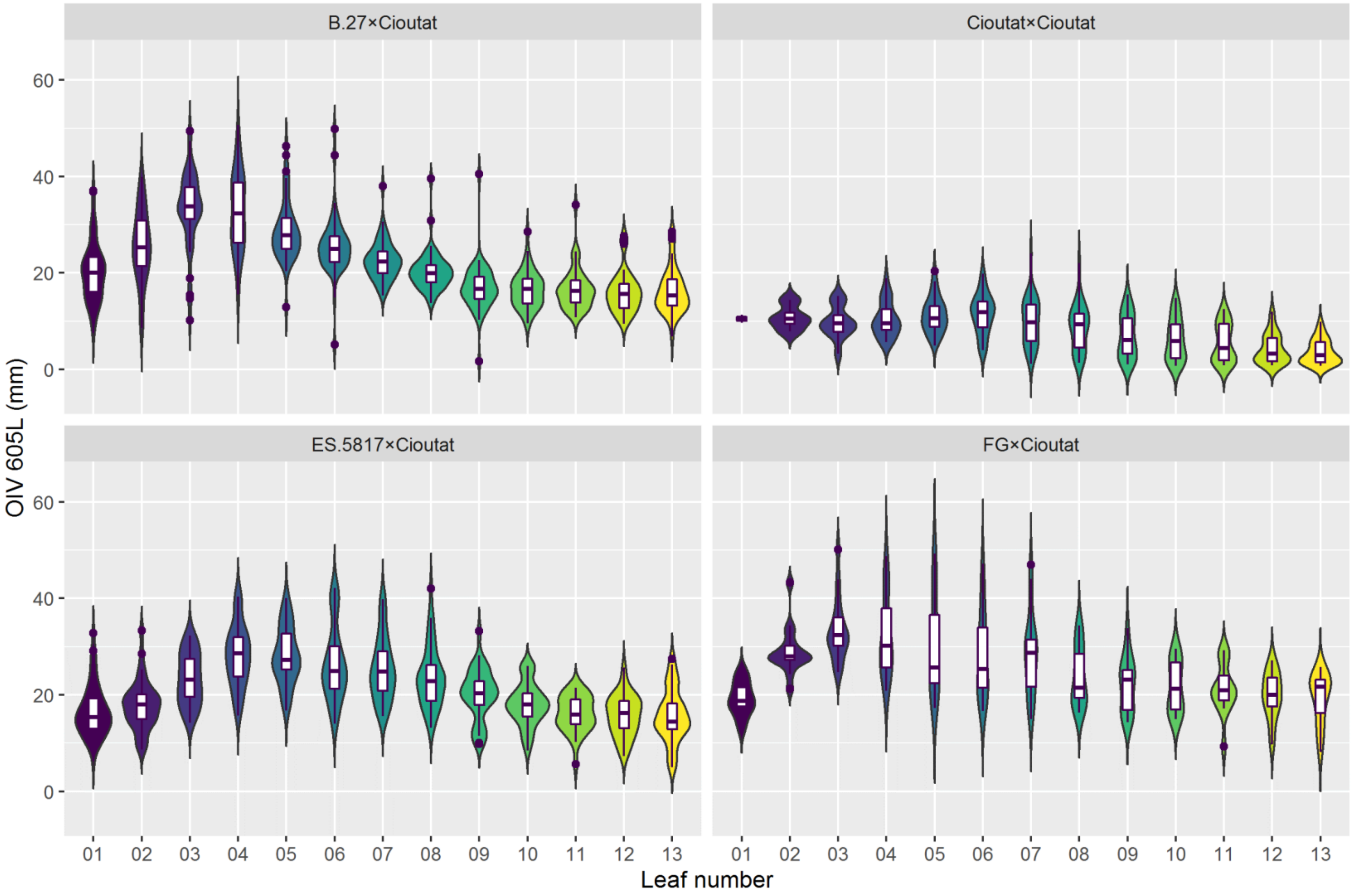

Figure A8. OIV 605L, length from petiole sinus to left upper lateral leaf sinus (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

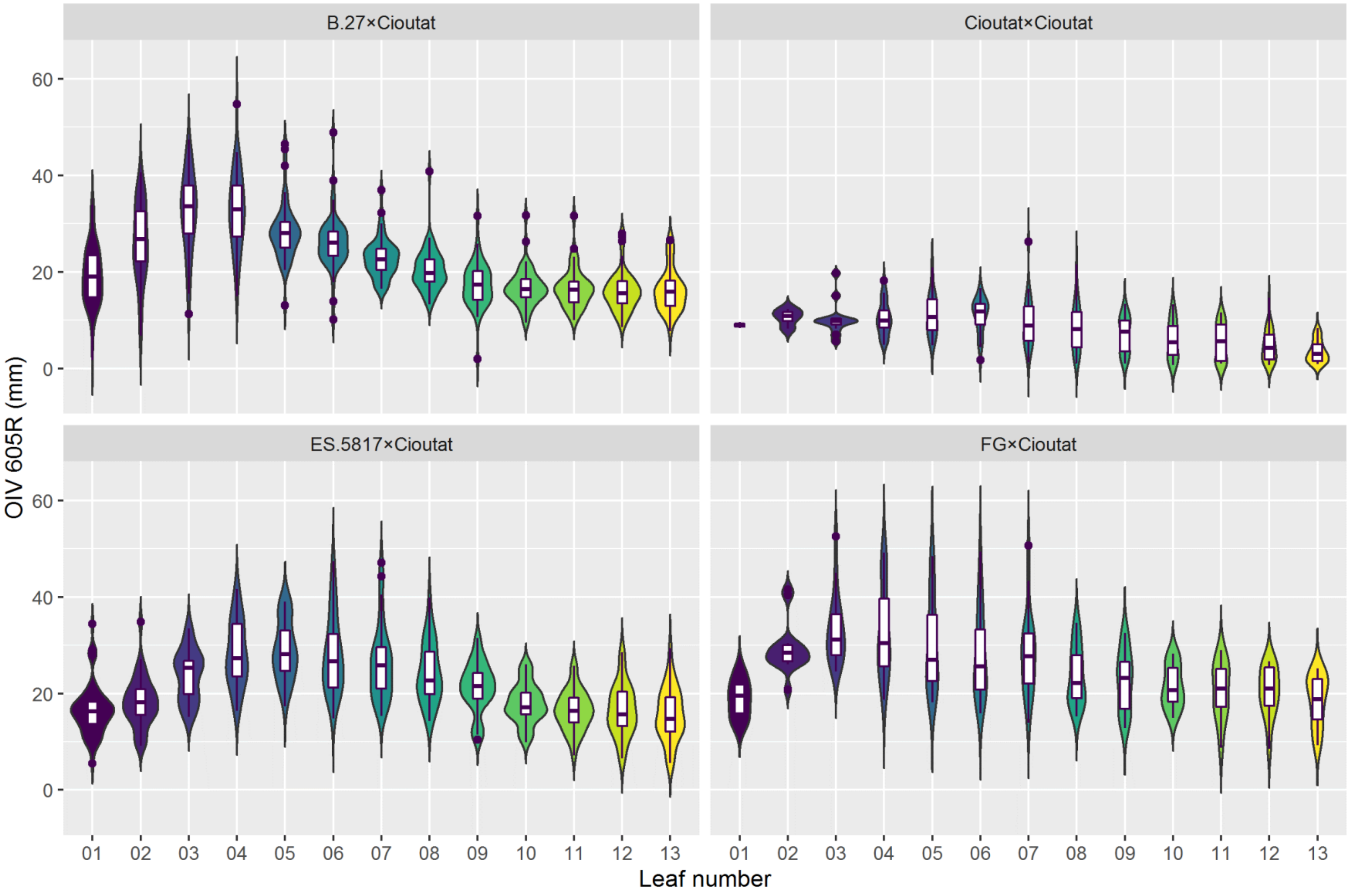

Figure A9. OIV 605R, length from petiole sinus to right upper lateral leaf sinus (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

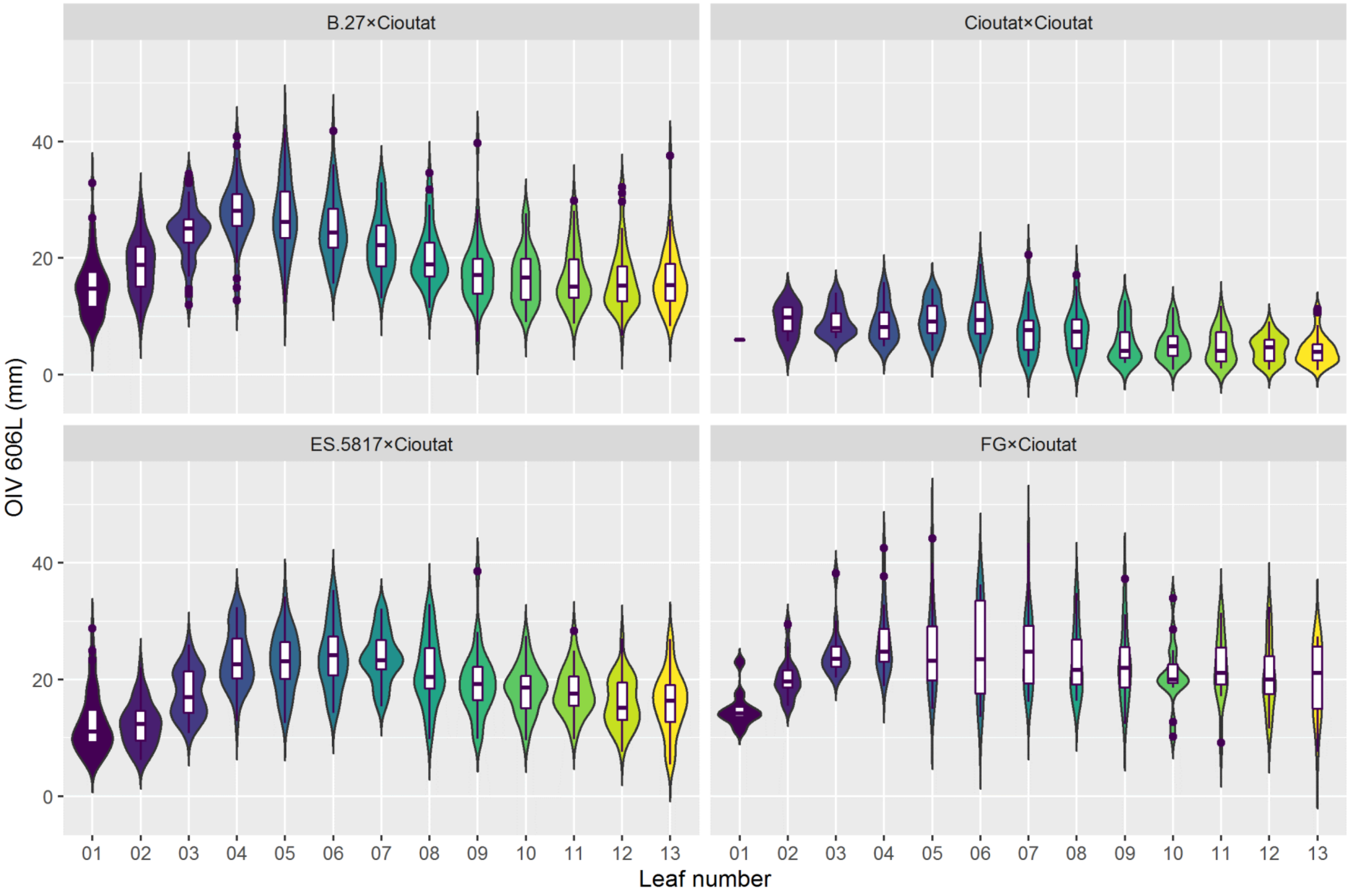

Figure A10. OIV 606L, length from petiole sinus to left lower lateral leaf sinus (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

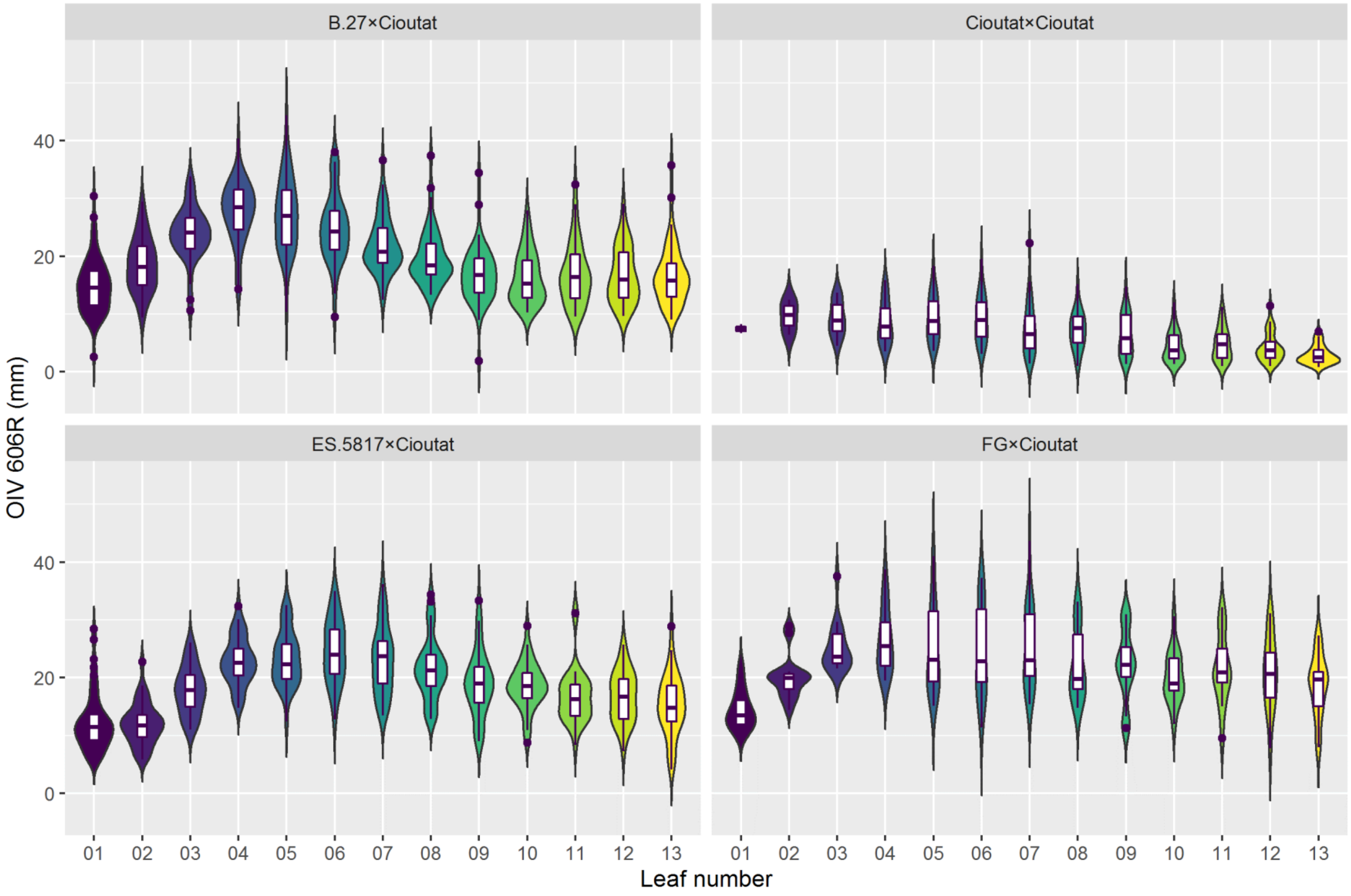

Figure A11. OIV 606R, length from petiole sinus to right lower lateral leaf sinus (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

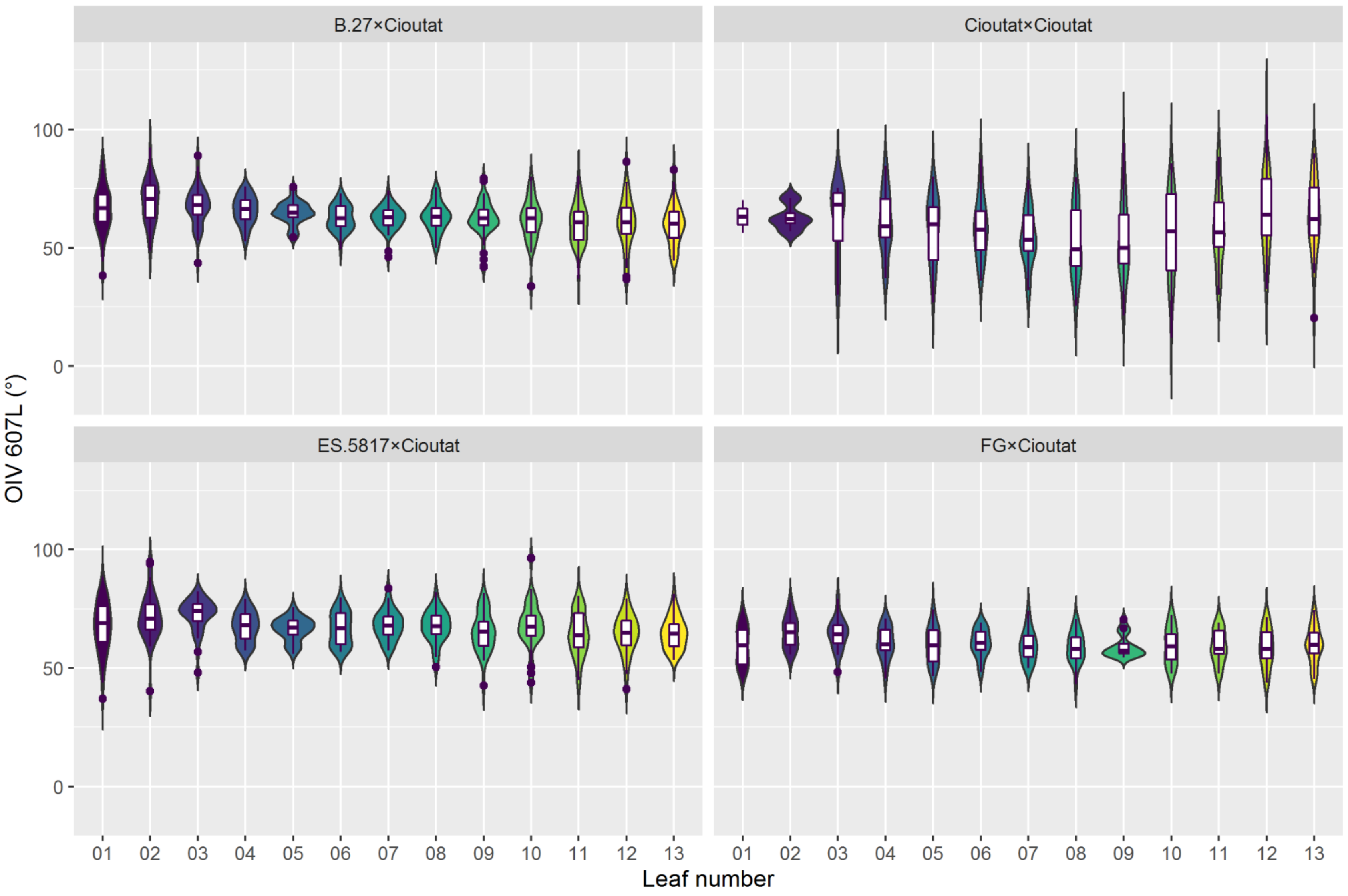

Figure A12. OIV 607L, angle (°) between N1 and left N2, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

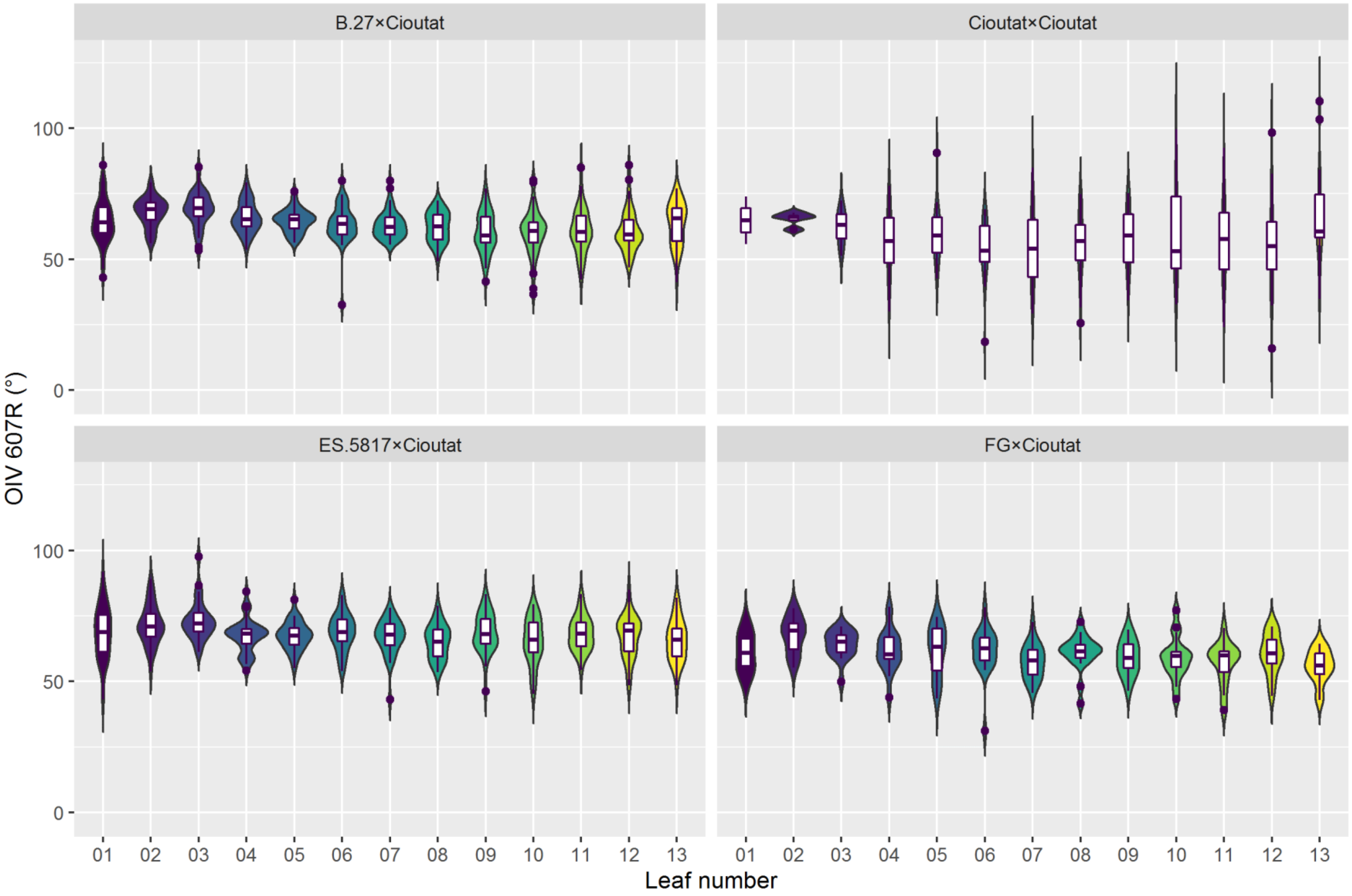

Figure A13. OIV 607R, angle (°) between N1 and right N2, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

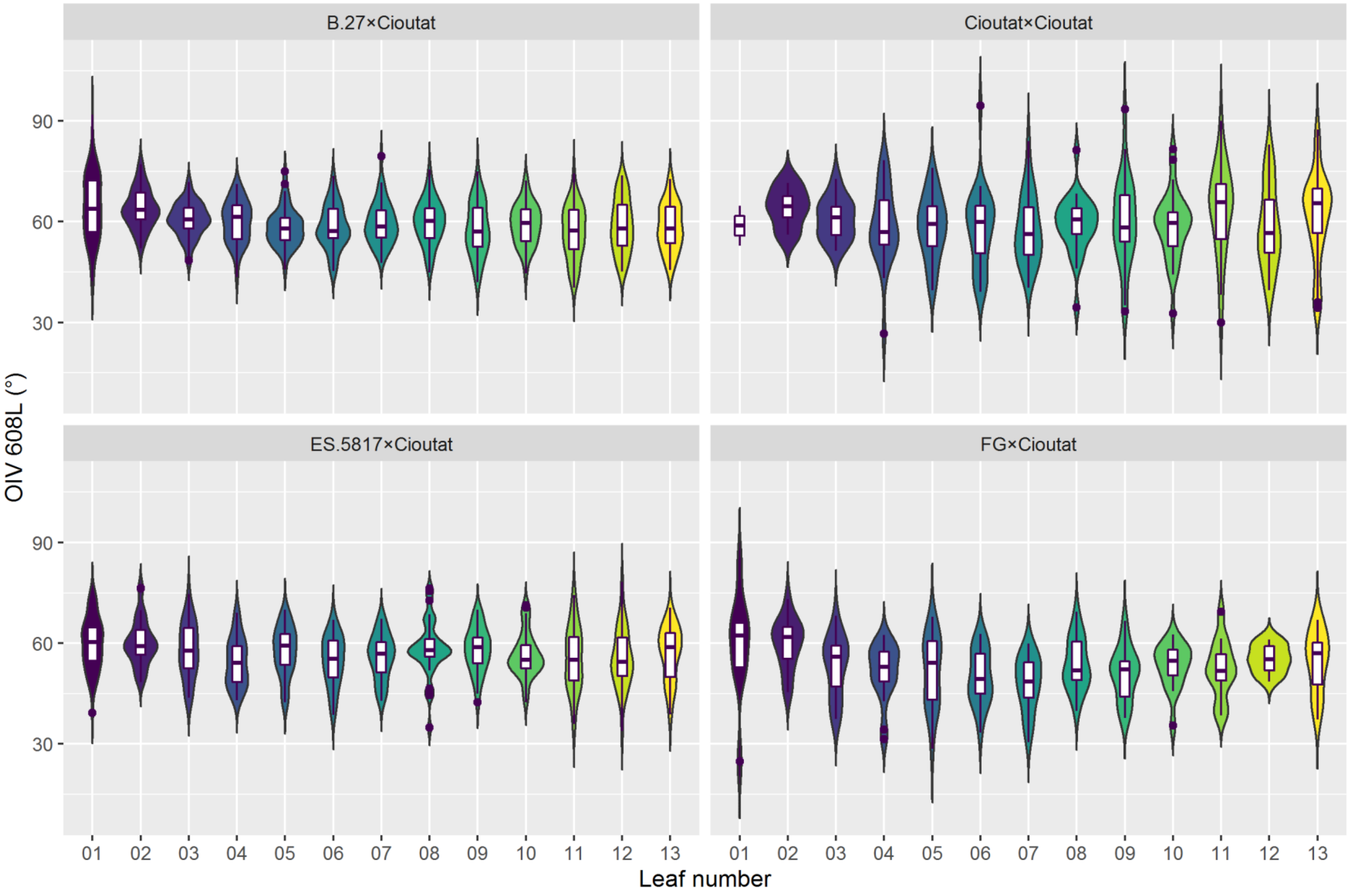

Figure A14. OIV 608L, angle (°) between N2 and left N3, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

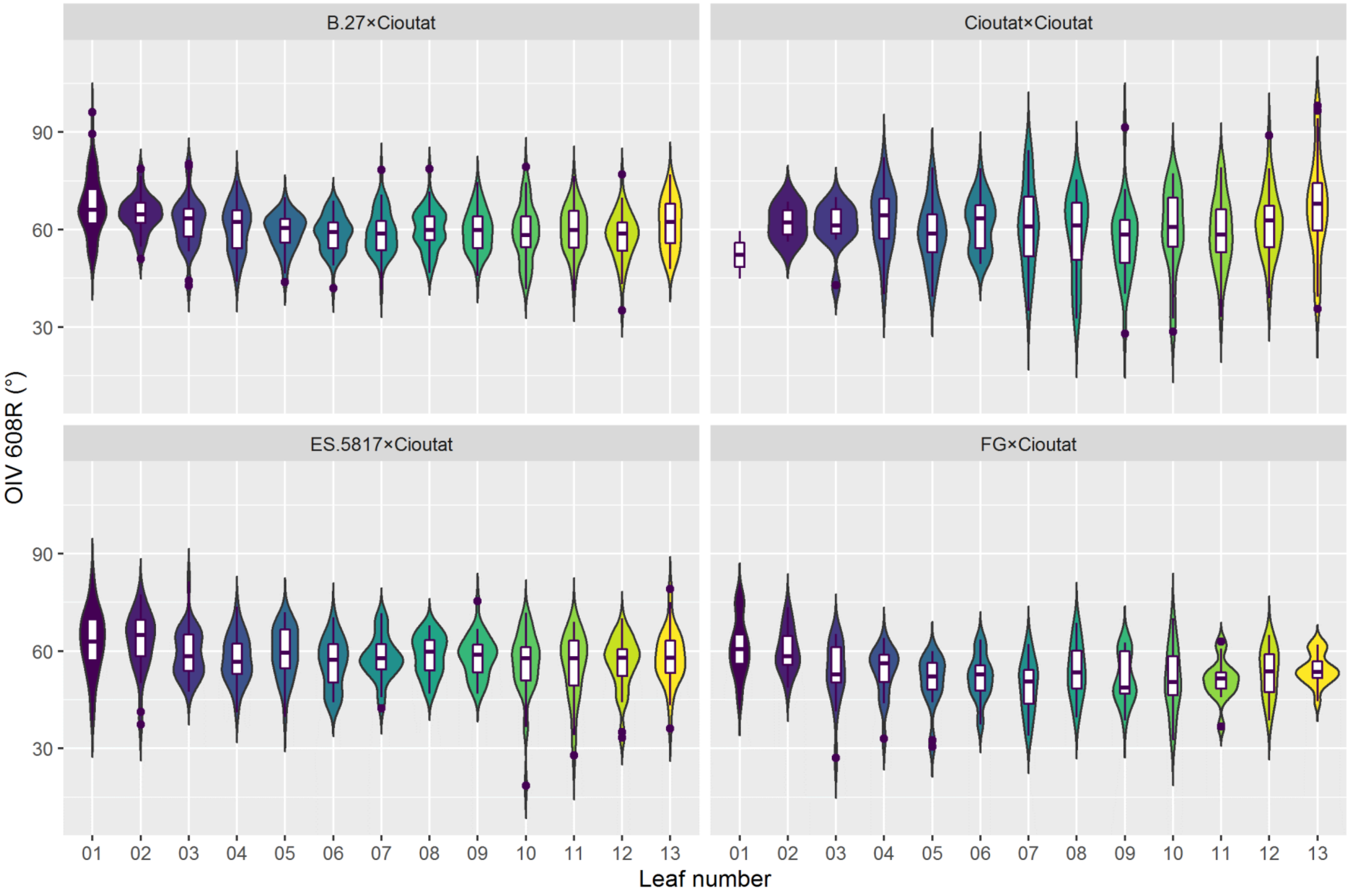

Figure A15. OIV 608R, angle (°) between N2 and right N3, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

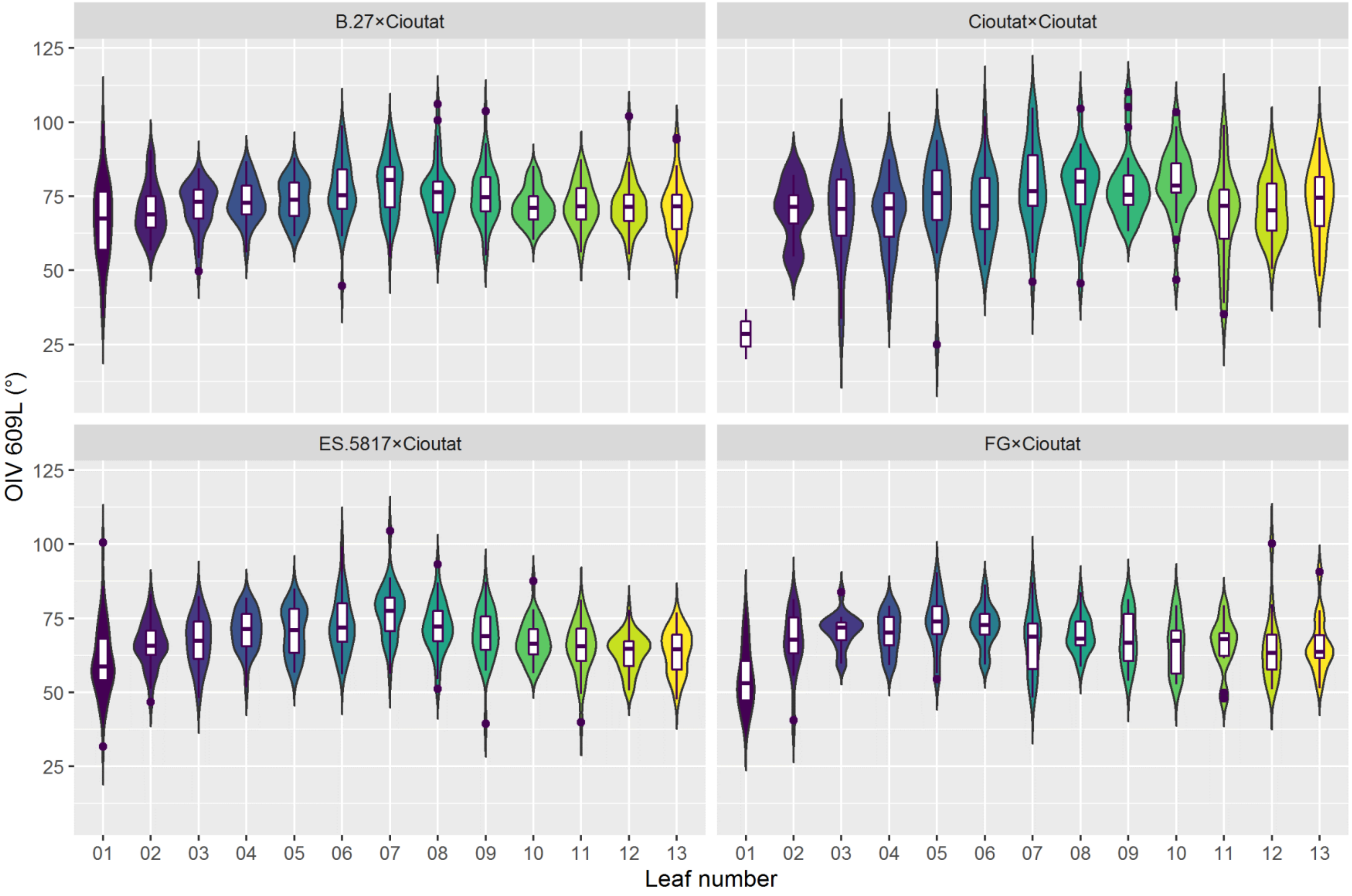

Figure A16. OIV 609L, angle (°) between left N3 and left N4, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

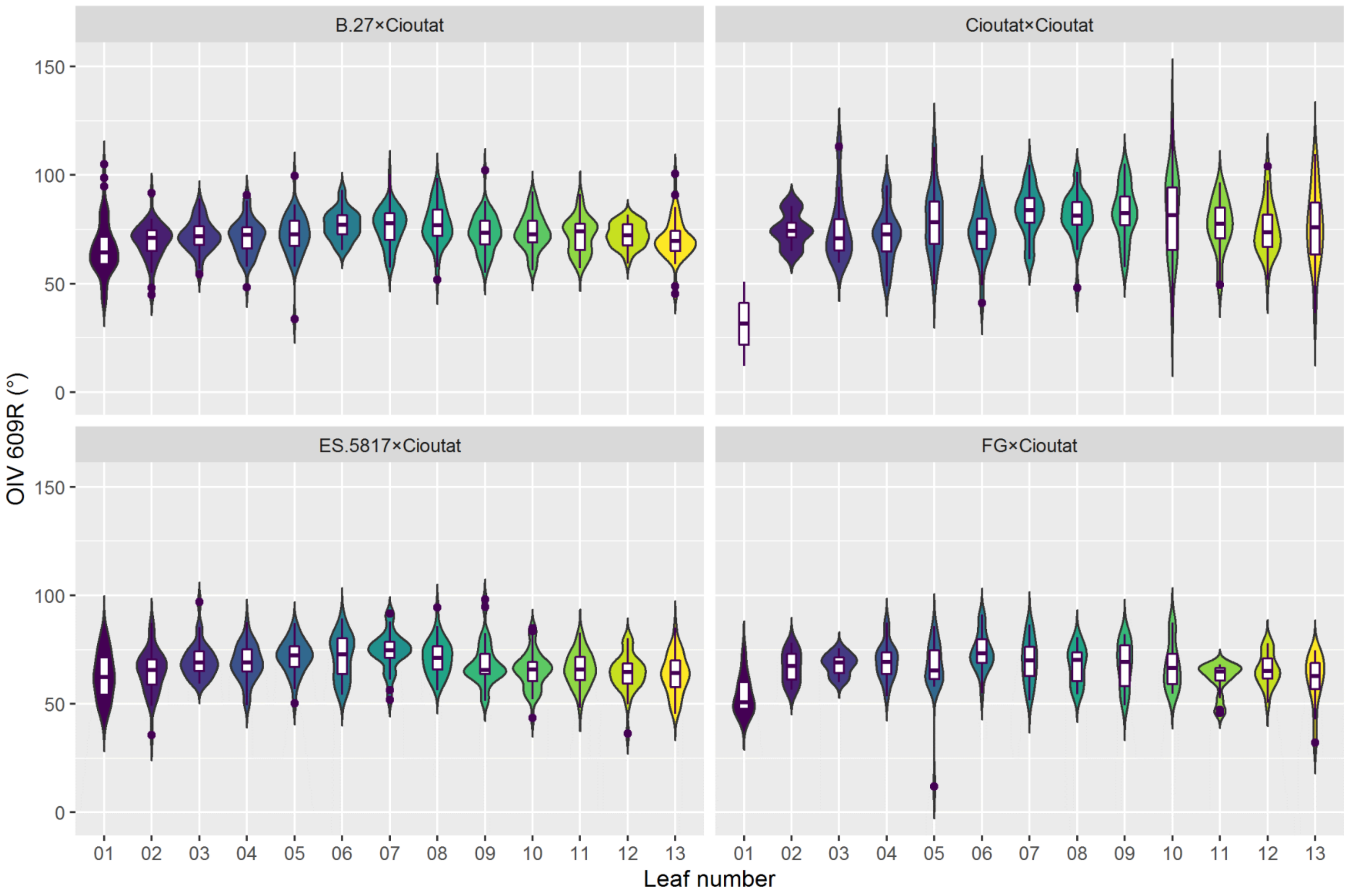

Figure A17. OIV 609R, angle (°) between right N3 and right N4, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

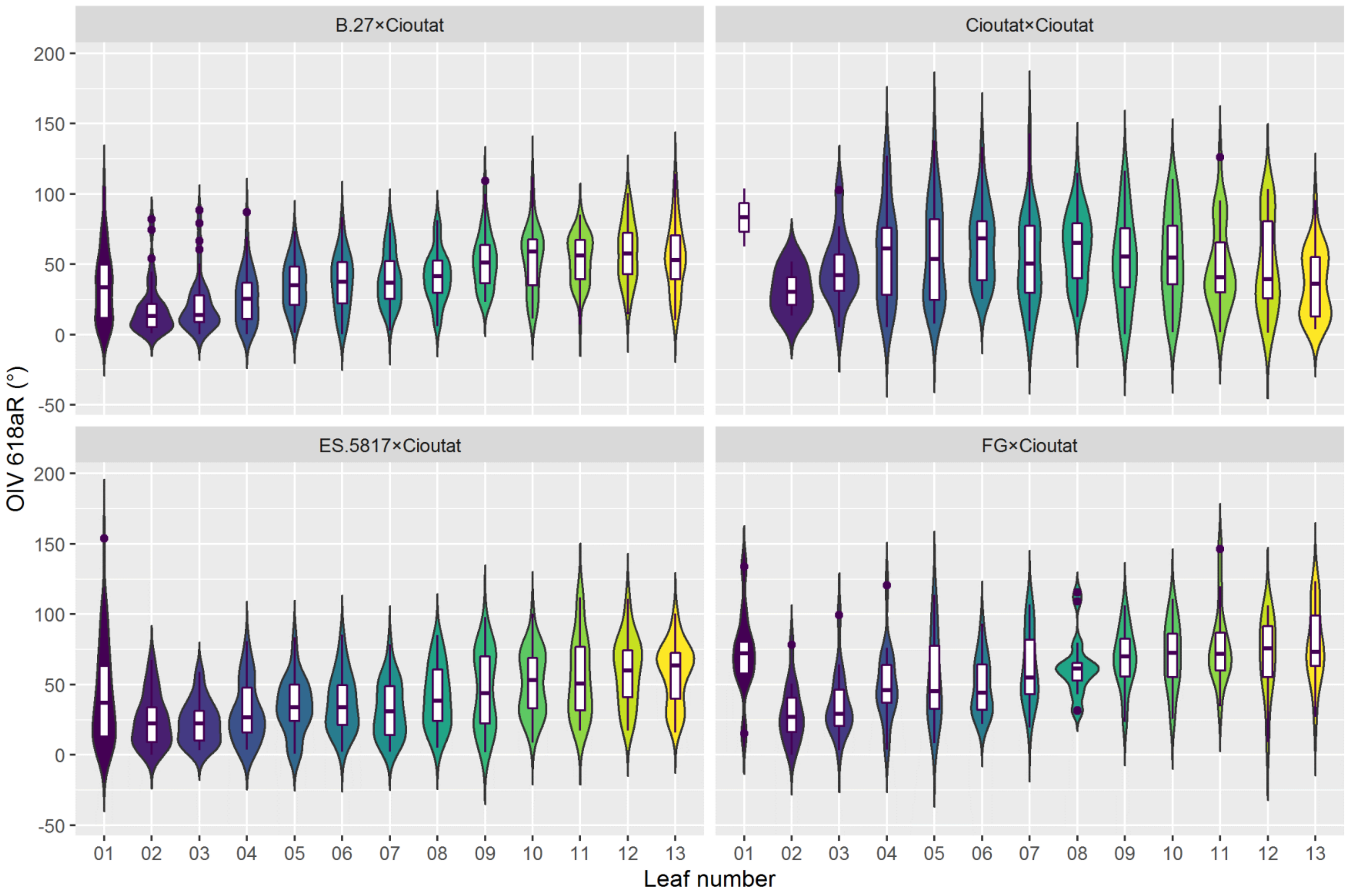

Figure A18. OIV 618aR, angle (°) of petiole sinus, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

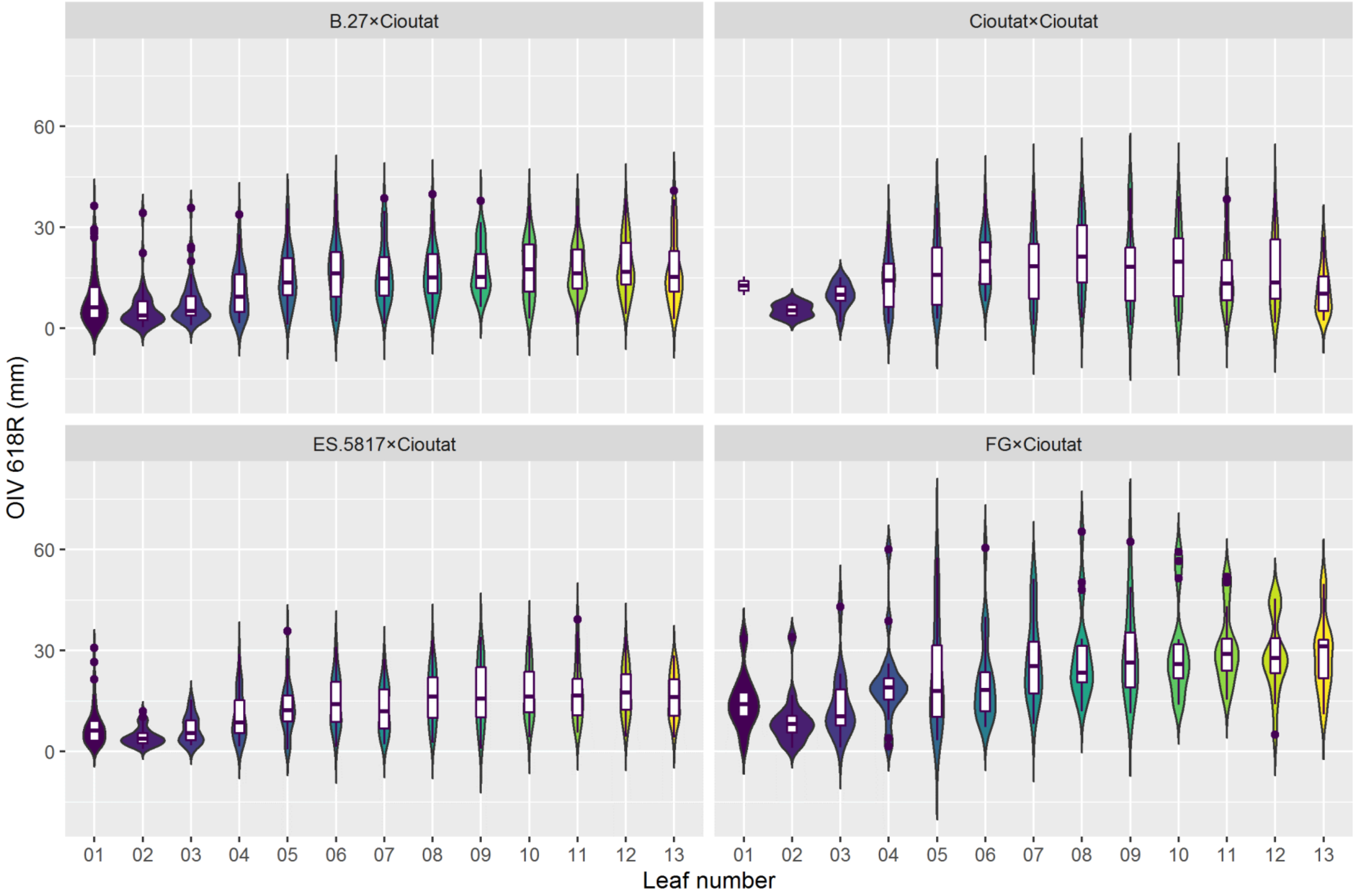

Figure A19. OIV 618R, length of petiole sinus (mm), across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

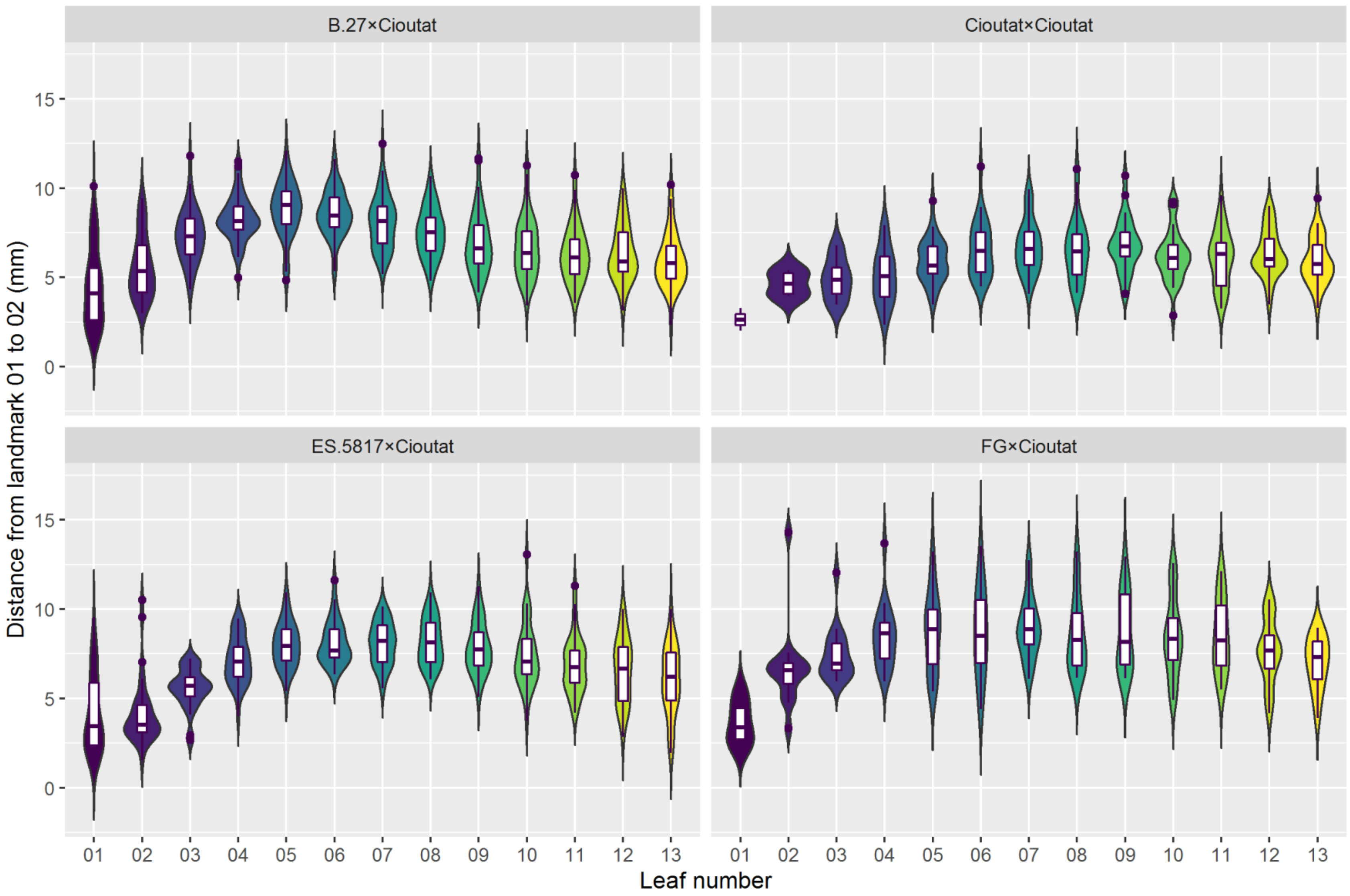

Figure A20. Length (mm) between landmark 01 and 02, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

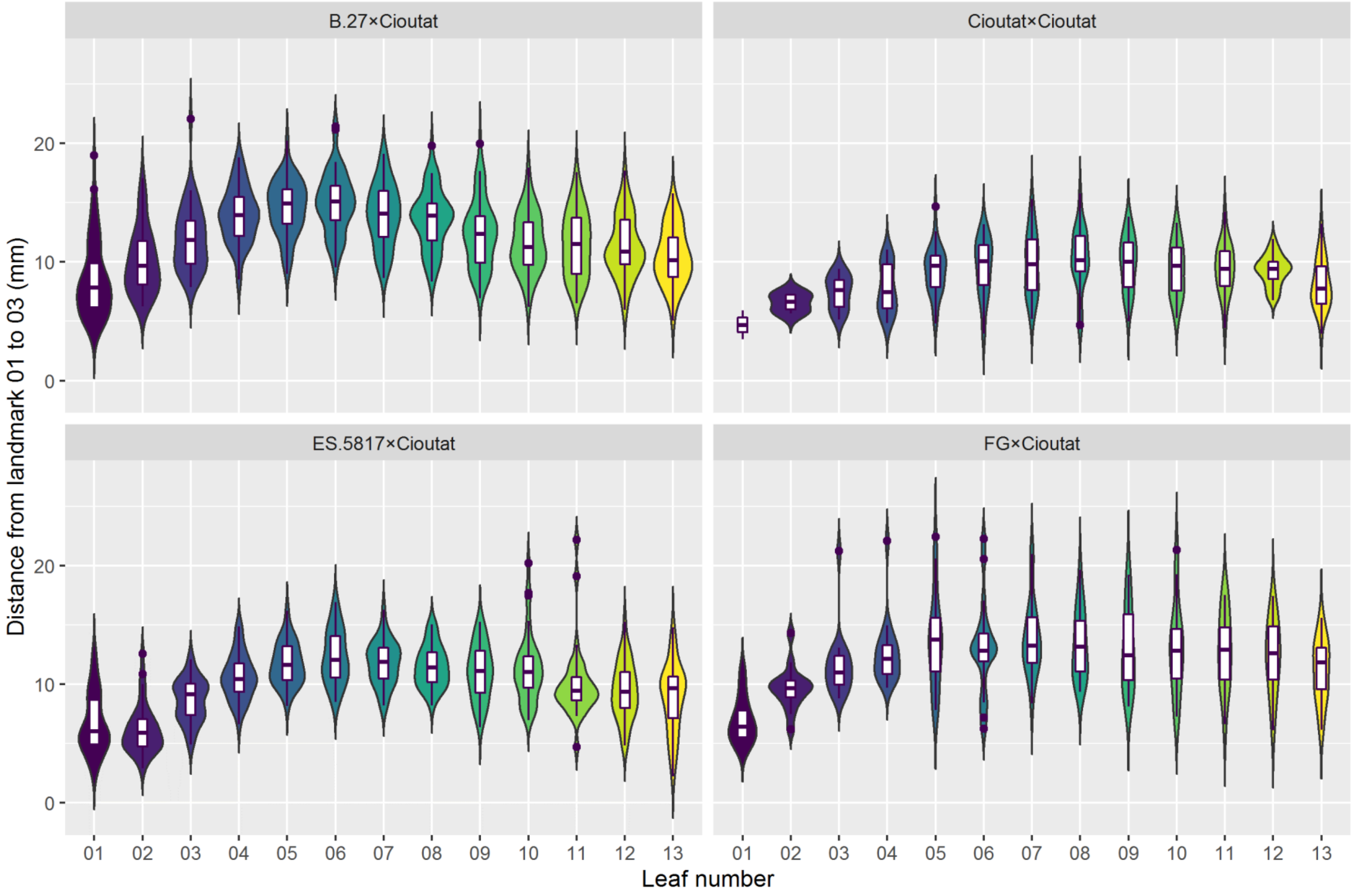

Figure A21. Length (mm) between landmark 01 and 03, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

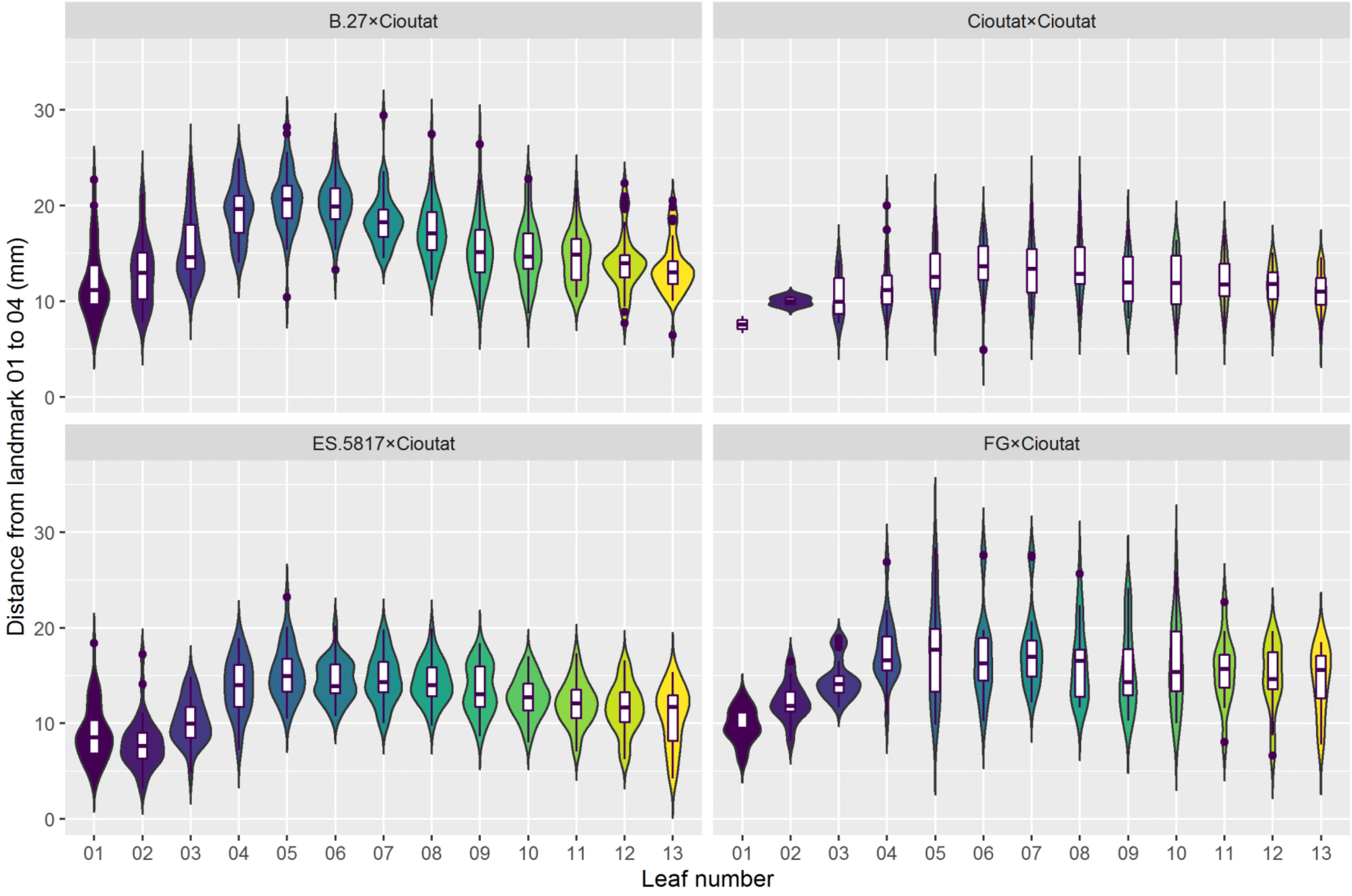

Figure A22. Length (mm) between landmark 01 and 04, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

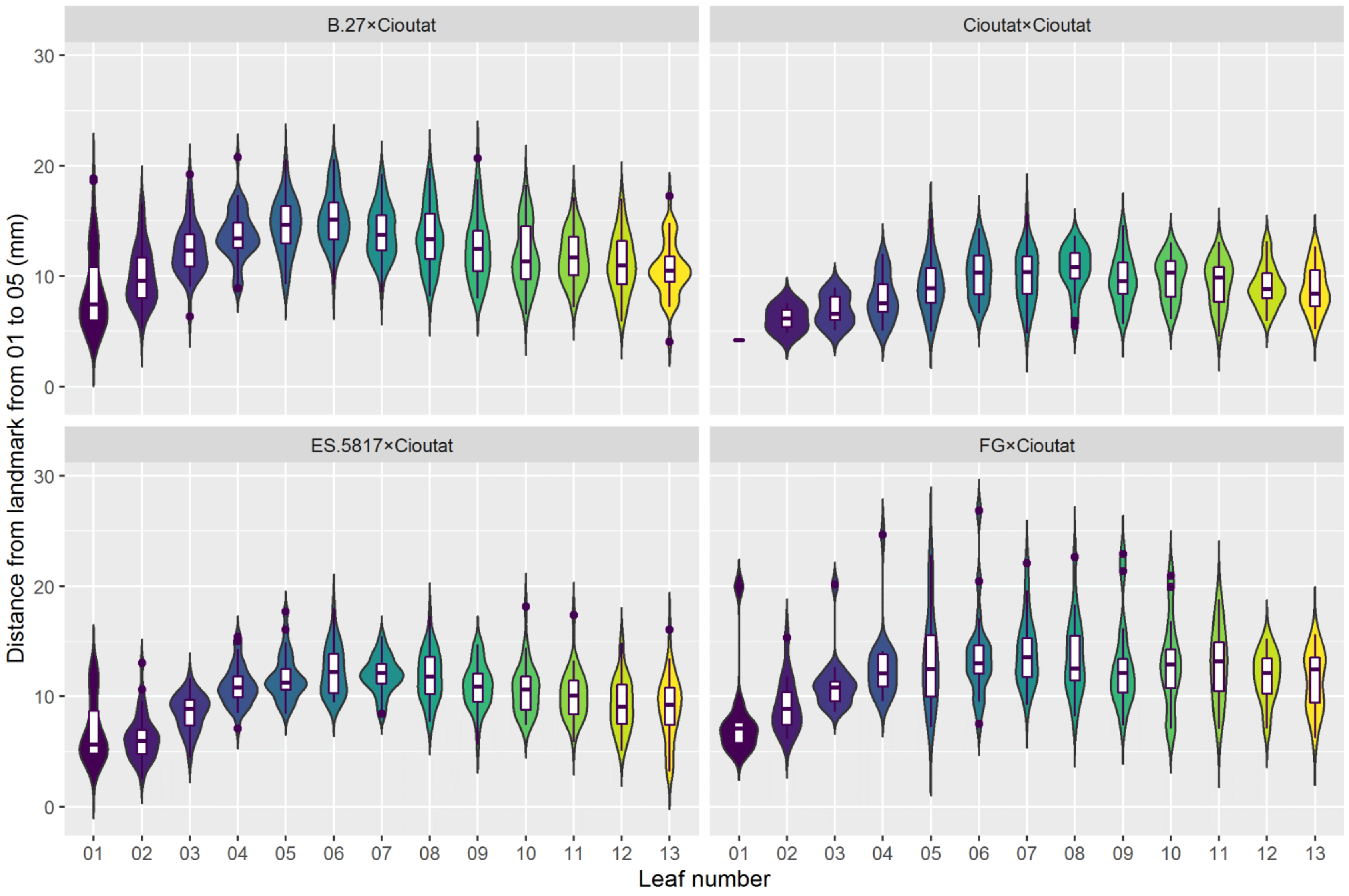

Figure A23. Length (mm) between landmark 01 and 05, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

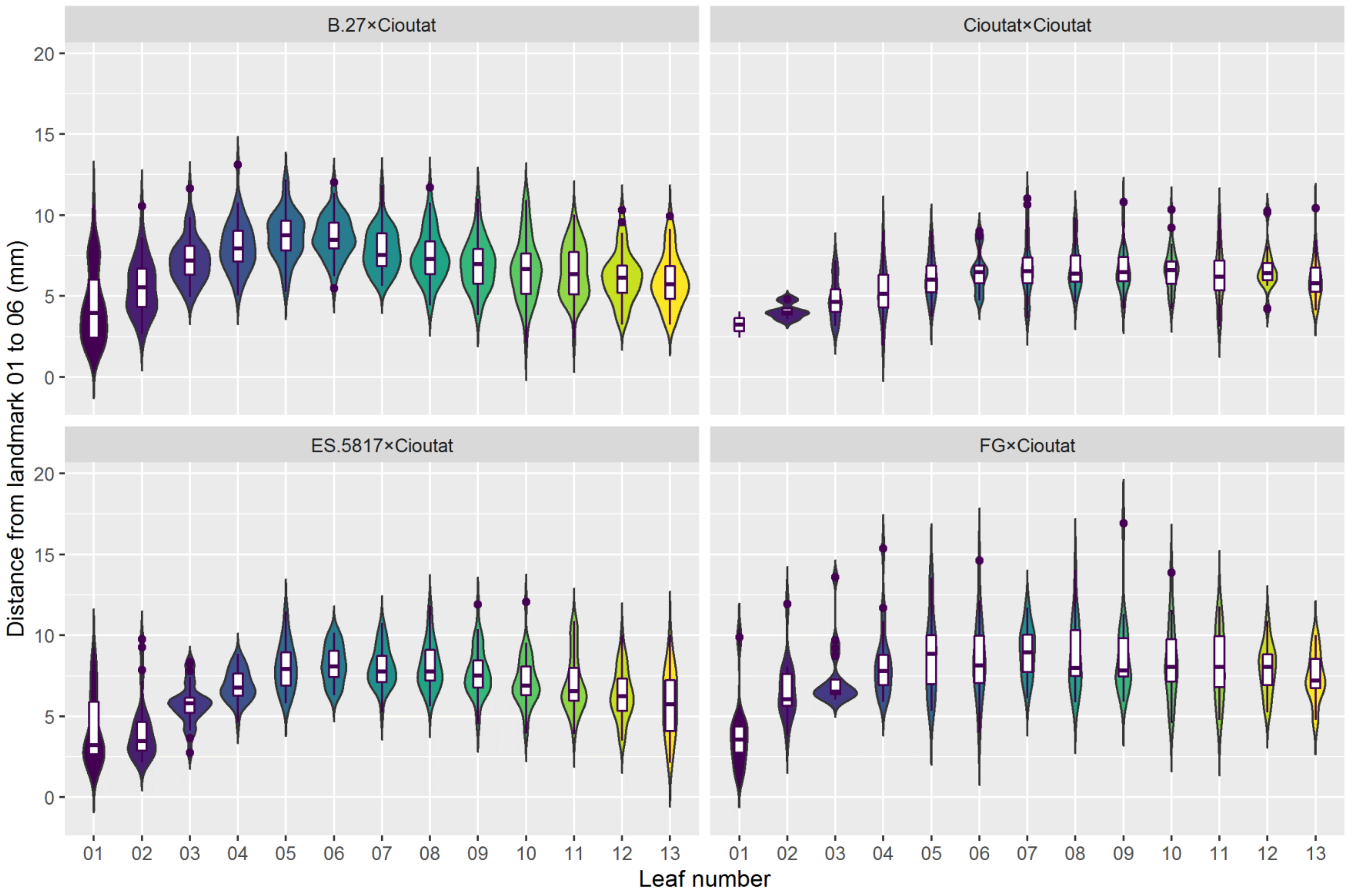

Figure A24. Length (mm) between landmark 01 and 06, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

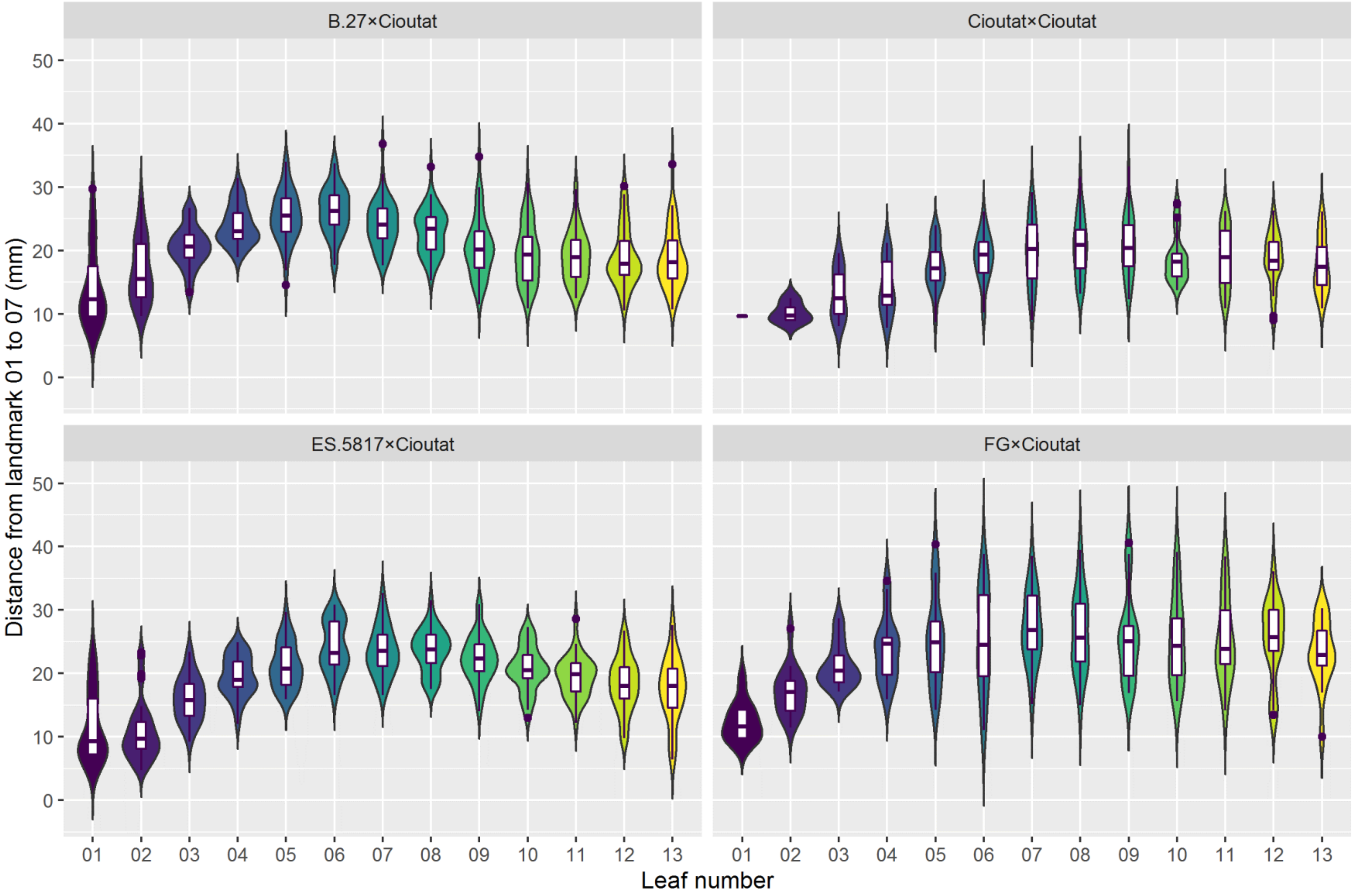

Figure A25. Length (mm) between landmark 01 and 07, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

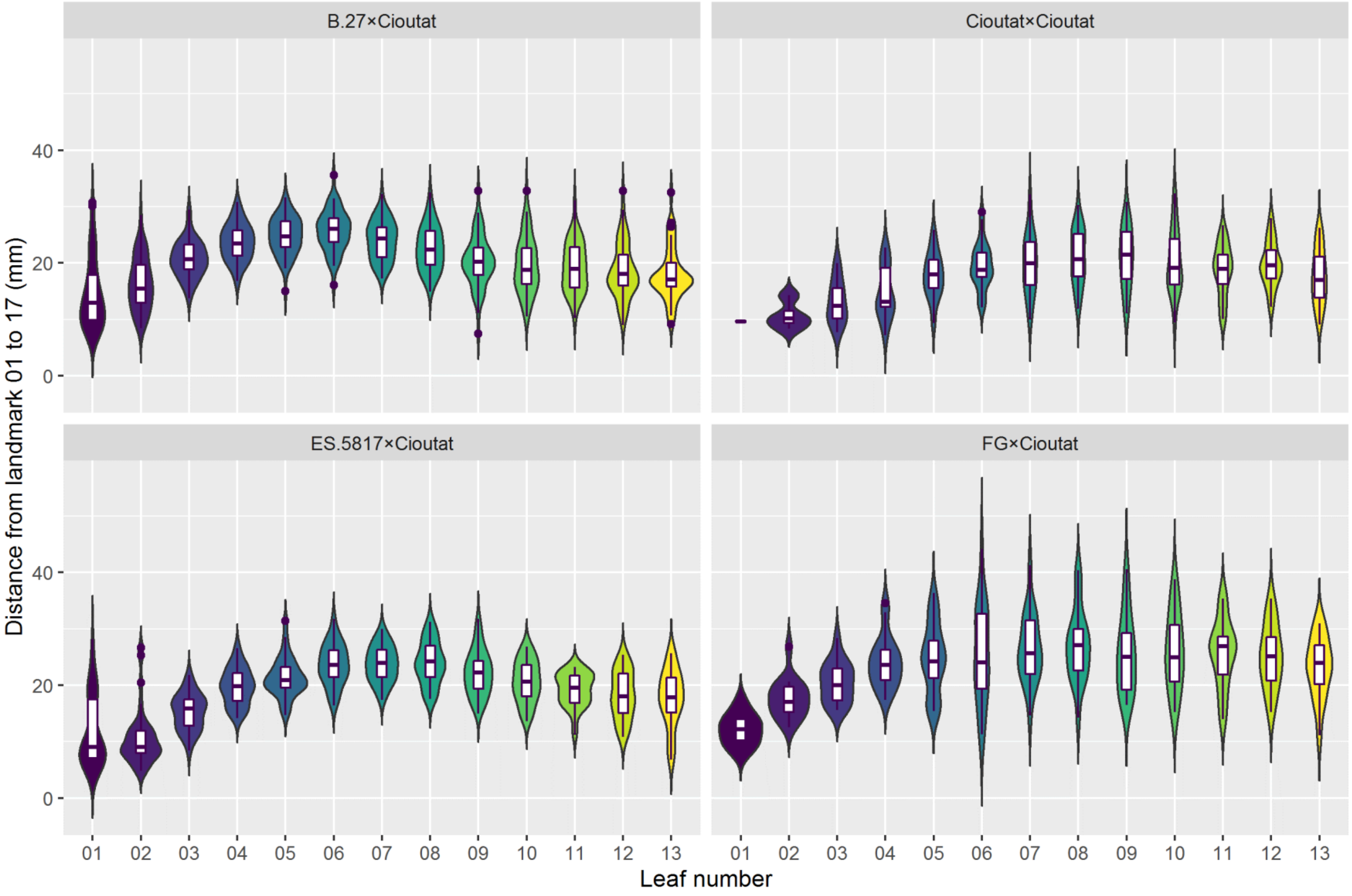

Figure A26. Length (mm) between landmark 01 and 17, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

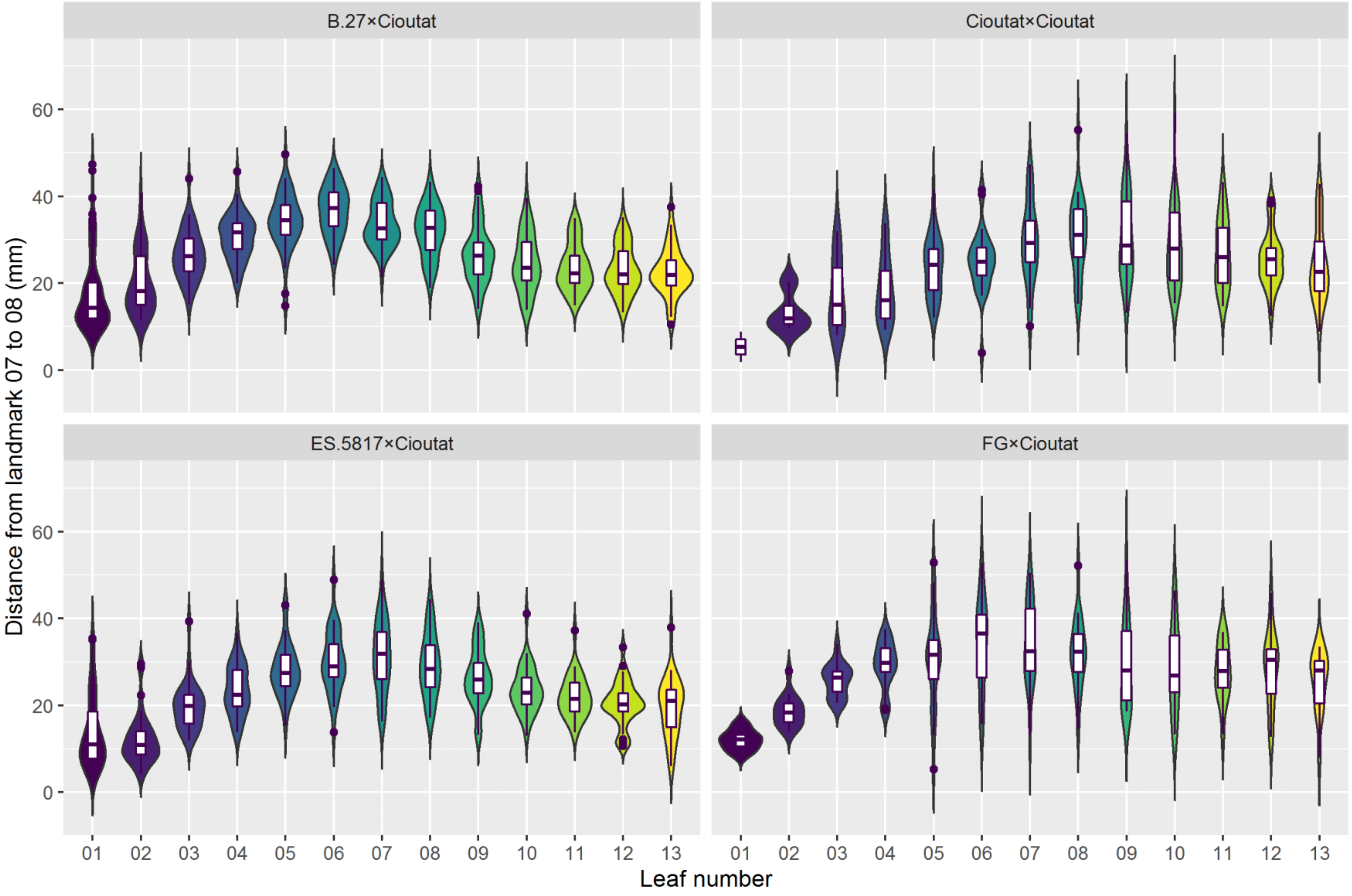

Figure A27. Length (mm) between landmark 07 and 08, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

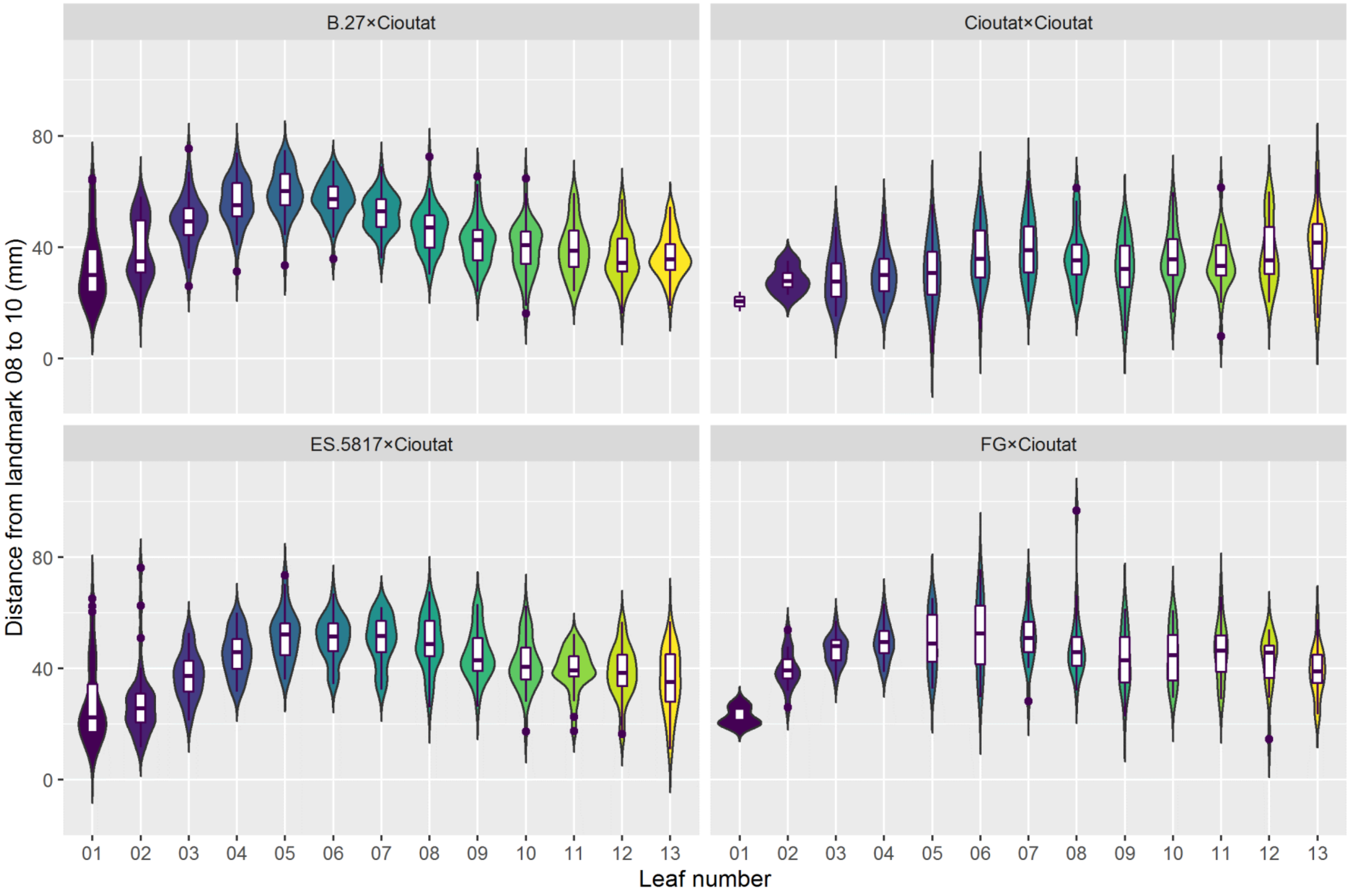

Figure A28. Length (mm) between landmark 08 and 10, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

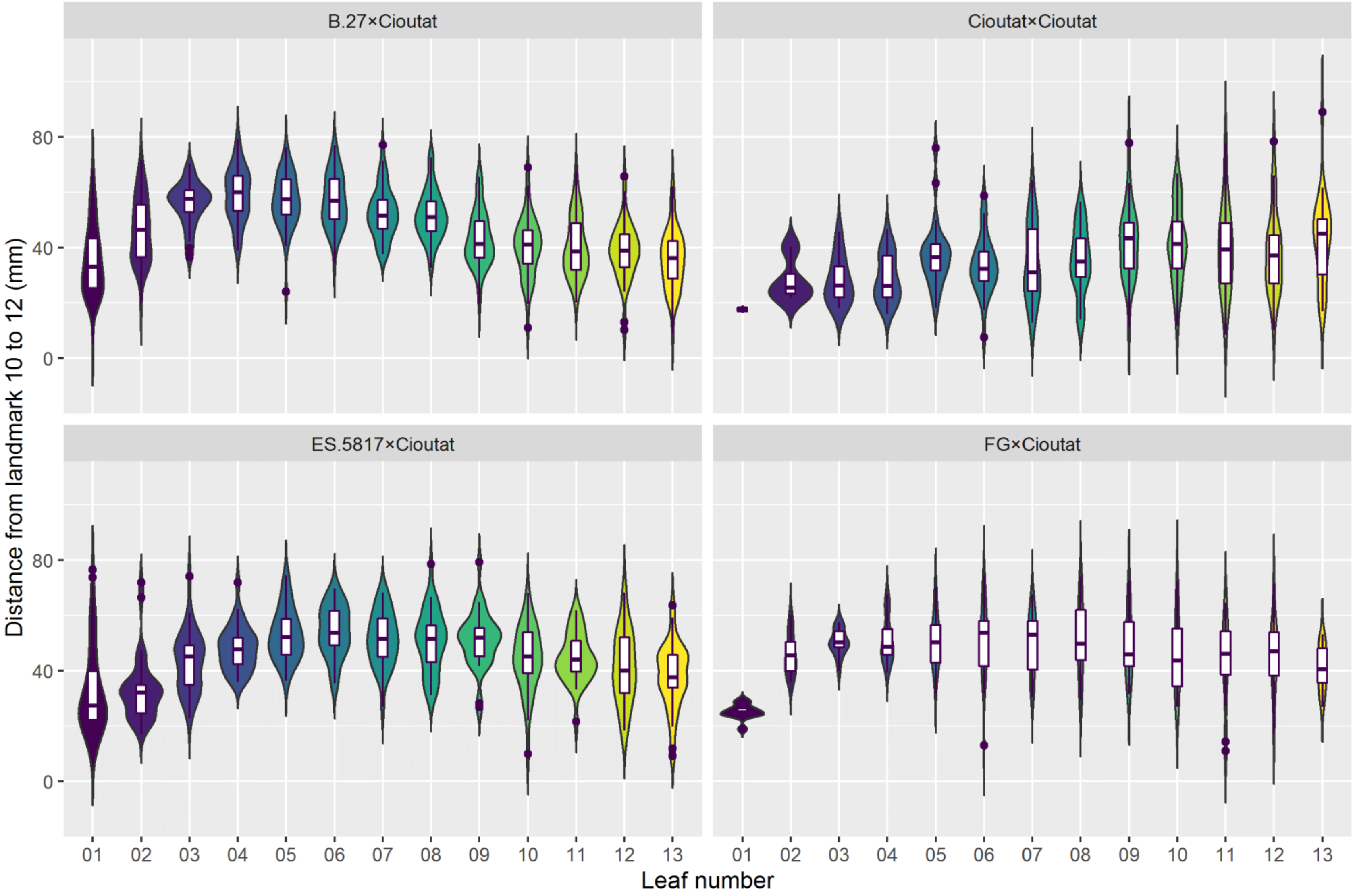

Figure A29. Length (mm) between landmark 10 and 12, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

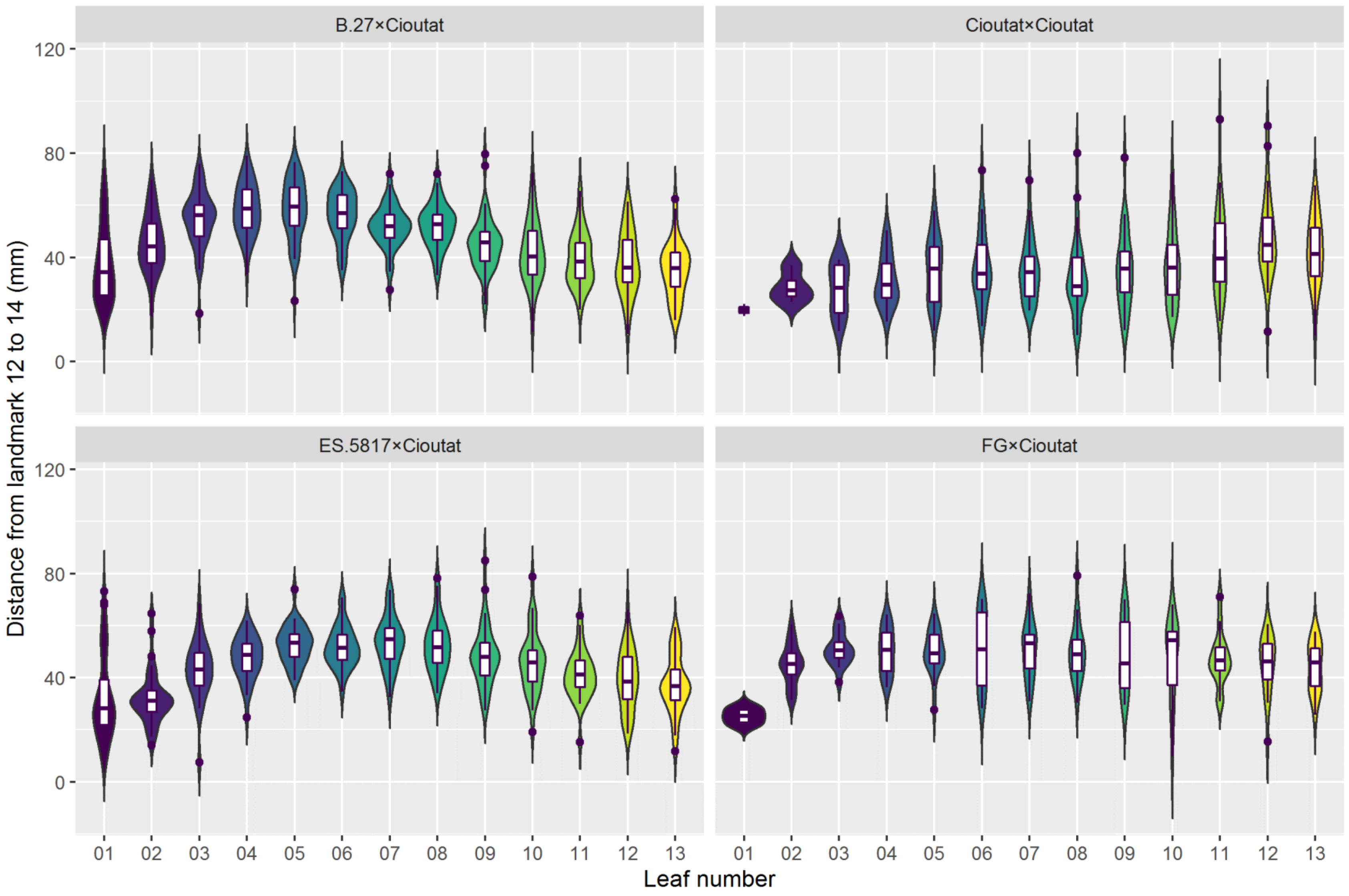

Figure A30. Length (mm) between landmark 12 and 14, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

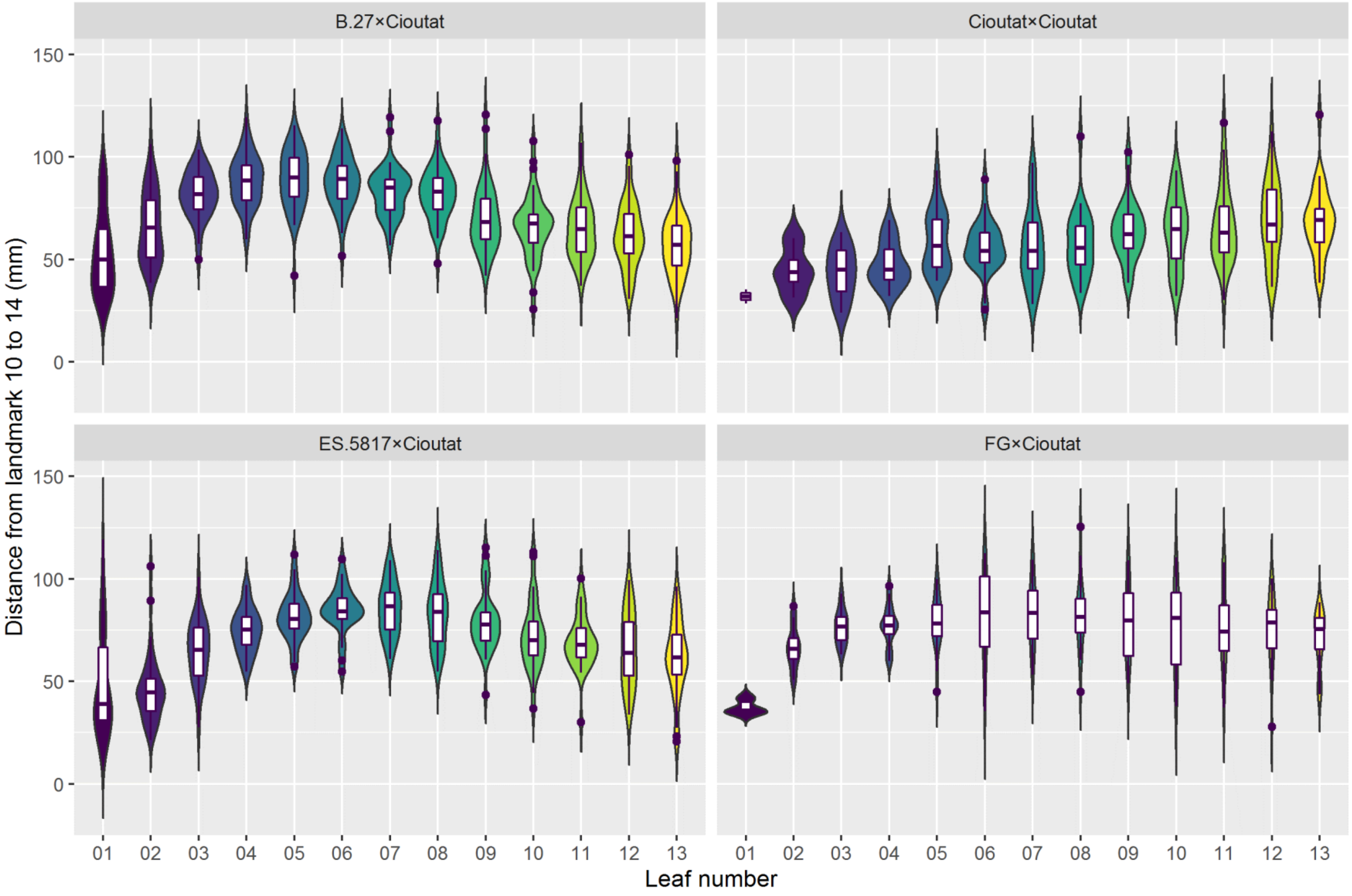

Figure A31. Length (mm) between landmark 10 and 14, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

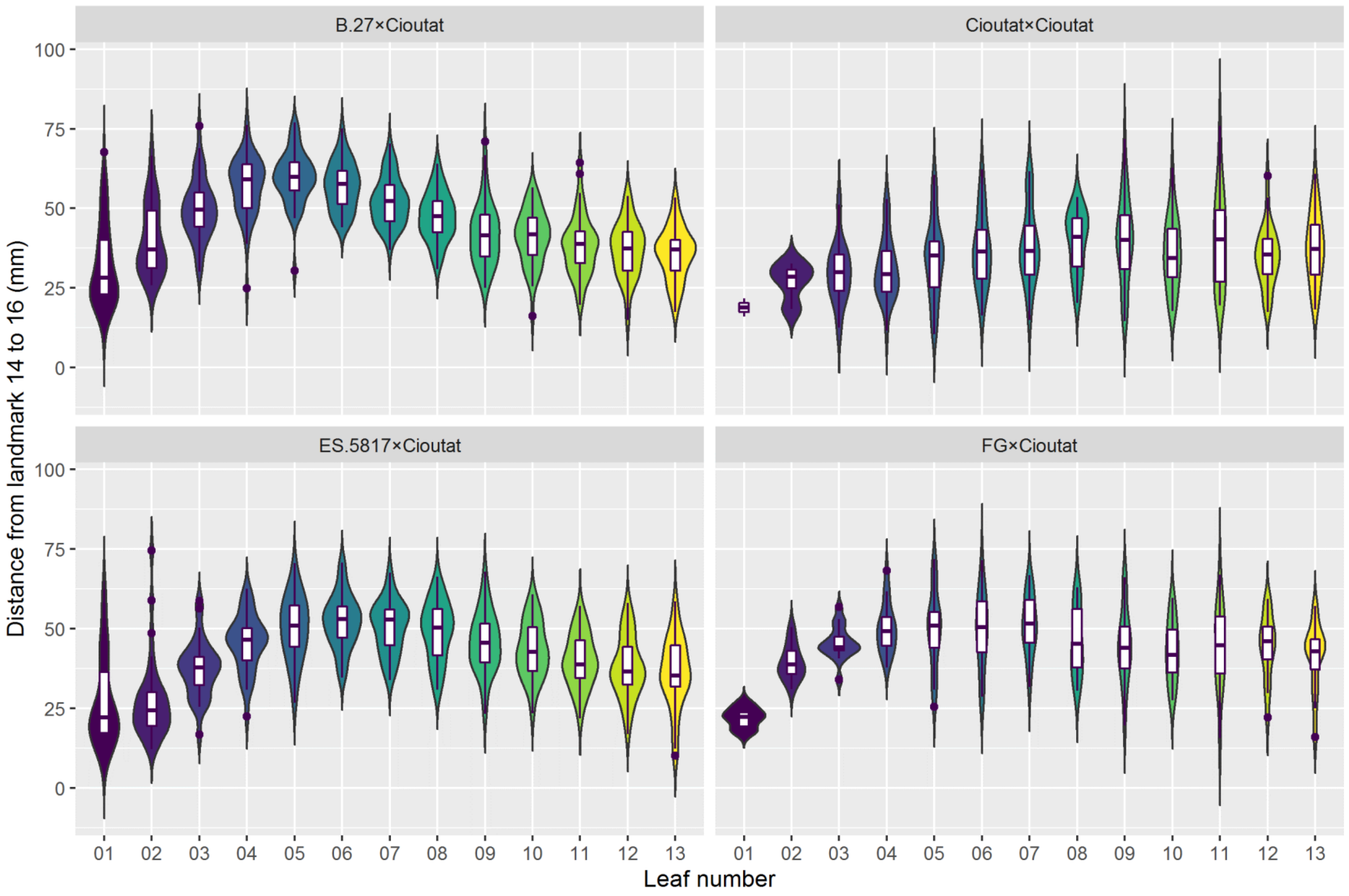

Figure A32. Length (mm) between landmark 14 and 16, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

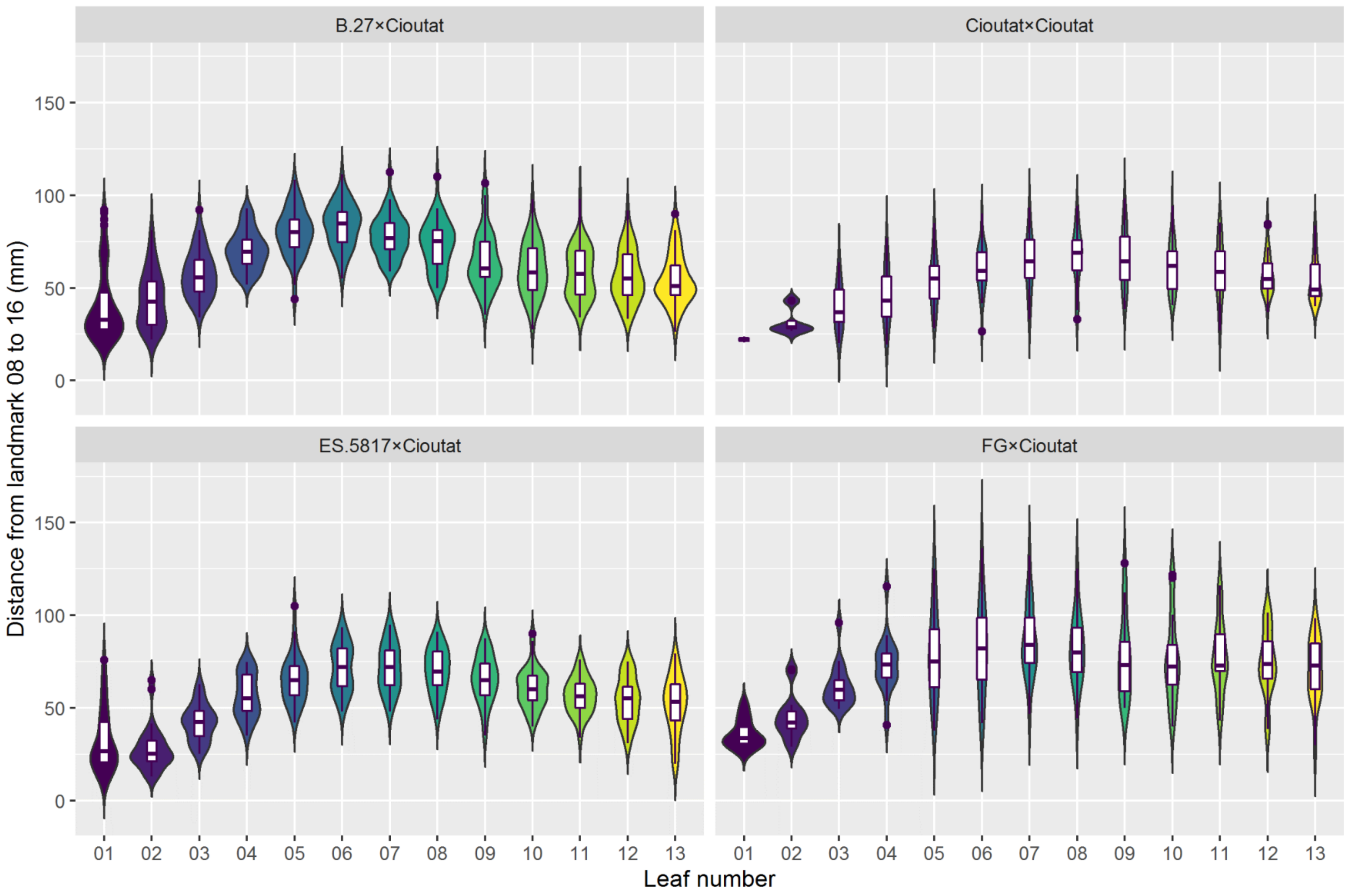

Figure A33. Length (mm) between landmark 08 and 16, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.

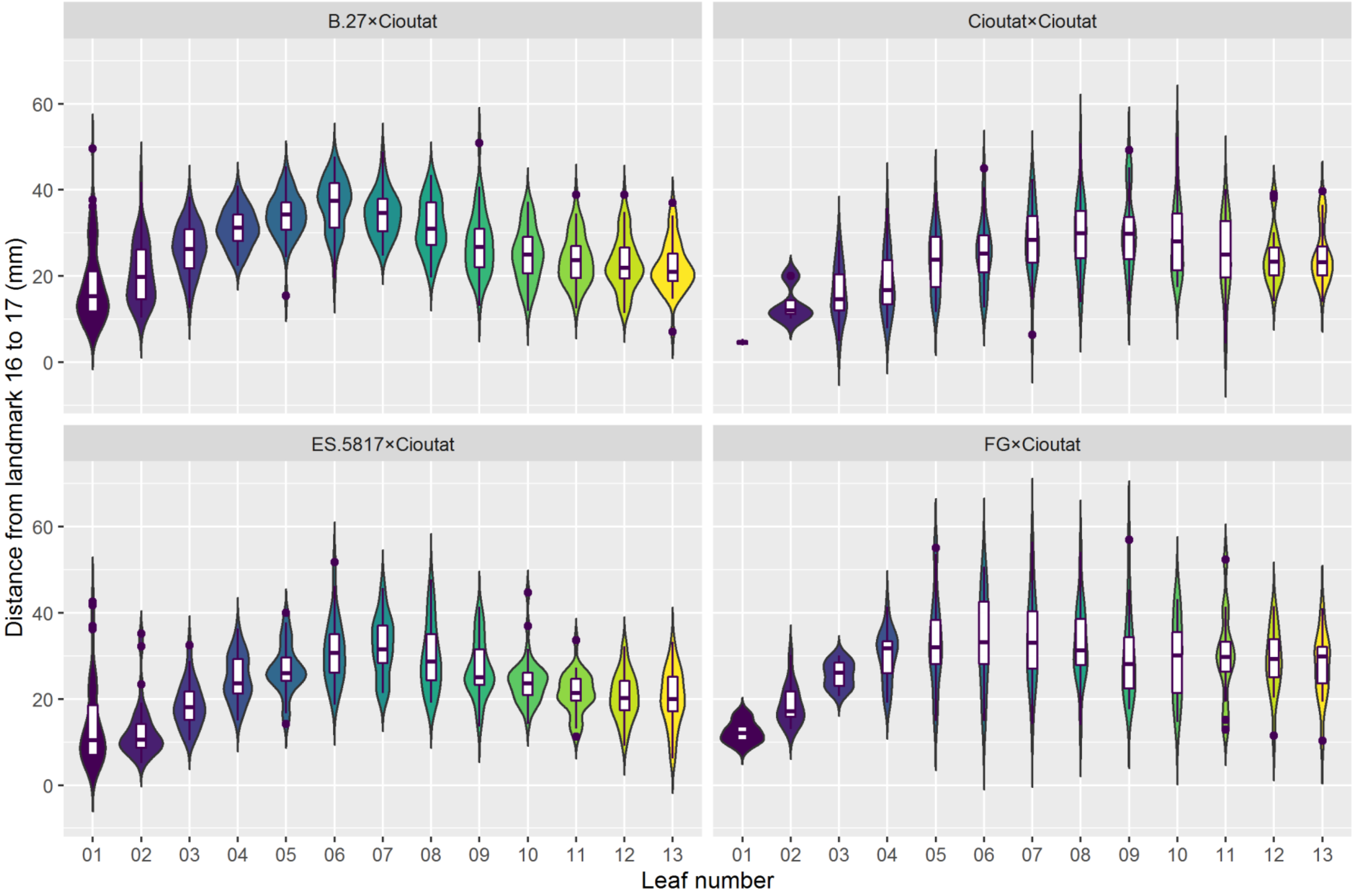

Figure A34. Length (mm) between landmark 16 and 17, across leaves 01 to 13 following cotyledon emergence (leaf 00) for three interspecific grapevine crosses with ‘Chasselas Cioutat’.