Identification of a defoliation severity threshold for changing fruitset, bunch morphology and fruit composition in Pinot Noir

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Abstract

Background and Aims: Early defoliation has been proposed as a tool to reduce bunch susceptibility to fungal infections through a reduction in bunch compactness. This research aims to determine the desired level of defoliation producing looser bunches and to investigate the impact of the application of early defoliation on Pinot Noir vines under cool climate conditions.

Methods and Results: We applied leaf removal treatments at full flowering or EL-20 phenological stage on Pinot Noir in 2 consecutive years using five levels of defoliation: no leaves removed, and leaves removed from four, six, eight and ten basal nodes. The effects on fruitset, bunch morphology, fruit chemistry, yield and rot severity were recorded. We identified the defoliation of eight basal nodes as the threshold resulting in significantly lower fruitset. The removal of eight or ten leaves reduced bunch compactness but did not alter the proportion of rot. Finally, fruit composition at harvest of the treated vines was found to be improved compared with that of the control vines.

Conclusions: The defoliation of six to eight basal nodes at full flowering can regulate fruitset and bunch compactness in Pinot Noir under cool climate conditions. Application over 2 years showed no adverse effects on bud fruitfulness or vine performance the following year.

Significance of the Study: Early leaf removal can be an efficient tool to alter bunch architecture, yield and fruit composition without impacting vine health.

Keywords*: bunch compactness, bunch rot, cool climate, juice colour, leaf removal, yield, vine health,* Vitis vinifera

Introduction

Pinot Noir is an early ripening cold hardy red *Vitis vinifera* L. cultivar showing some tolerance for a temperature as low as −23°C and, thus, potential as an attractive choice for cool-climate vine growing areas characterised by cold winters (Reisch et al. 1993). For this reason, it is the most widely planted red cultivar in Michigan with 95 ha under cultivation, 8% of the total area dedicated to winegrapes (Michigan Department of Agriculture and Rural Development 2011). The tight bunch morphology of Pinot Noir, however, and local weather conditions, for example rain and high humidity, favourable to bunch rot infections, make growing this cultivar in Michigan a real challenge for grapegrowers (Sabbatini and Howell 2010). Consequently, early harvest is often required before the ripening grapes reach technological maturity, a balance between sugar concentration, titratable acidity and pH. More importantly, under favourable conditions for fungal infection, bunch rot rapidly spreads through a bunch causing significant yield reduction and compromising overall fruit composition.

In several scientific articles, early defoliation has been confirmed as an efficient tool to reduce bunch compactness, to reduce the spreading of bunch rot from infected to healthy berries and to improve fruit composition and control of crop load in other cultivars characterised by a large bunch size (Poni et al. 2006, 2008, Intrieri et al. 2008, Lohitnavy et al. 2010, Sabbatini and Howell 2010, Tardaguila et al. 2010, 2012). In a recent study on Pinot Noir vines, a cultivar with small and tight bunches, grown in Michigan, the effect of timing of defoliation on fruitset and bunch compactness was tested in 3 consecutive years (Sabbatini et al. 2010). Compared with post-flowering defoliation of six basal leaves, pre-flowering and flowering defoliation were more effective in reducing fruitset and bunch compactness. Even with the application of pre-flowering and flowering treatment, however, six leaves appeared insufficient to induce a source limitation stress that would trigger a significant reduction in fruitset every year. This study raised the question of what level of leaf removal would result in a significant decrease of both fruitset and bunch compactness. Moreover, at the beginning of the season, permanent structures (roots, trunk and canes) act as a source, supporting budburst and supplying growing shoots and undeveloped leaves with carbohydrate reserves stored over the winter (Williams 1996, Zapata et al. 2004). If the replenishment of the reserves in the previous growing season was affected by source limitation, insufficient reserves in the following spring would impact vine growth and fruit development. Howell et al. (1994) and Sabbatini and Howell (2010) demonstrated that Pinot Noir and Vignoles vines exposed to different leaf removal treatments in the previous year showed a reduction in berry size and an increase of shootless nodes on the defoliated positions. Although these authors did not find any observable impact upon bud fertility, other research on leaf removal in Sultana vines found that, 4 weeks after flowering, the number of bunches per shoot was greatly reduced in the following season, and the effect was more pronounced with more severe defoliation (May et al. 1969).

This work investigates the influence of early defoliation at multiple levels of severity. In this study, we hypothesised that leaves removed at full flowering would significantly reduce

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fruitset, would do so to a degree correlated to the number of leaves or leaf area and would result in a significant decrease in bunch compactness and consequently in harvest season bunch rot. We imposed the same level of defoliation at flowering on the same vines in 2 consecutive years. As a consequence, we were able to monitor vine performance under 2 years of repeated defoliation stress, measuring the growth parameters, assessing the fruit composition and analysing the yield components.

Material and methods

Plant material and experimental design

The research was carried out in a 6-year-old vineyard of *V. vinifera*, cv. Pinot Noir (clone 777 grafted onto C3309 rootstock) during 2011 and 2012. The vineyard was located at the Southwest Michigan Research and Extension Center (latitude 40°09′N, longitude 86°36′W, elevation 220 m) near Benton Harbor, Michigan. Vines were planted in a Spinks loamy fine soil (US Department of Agriculture, Soil Conservation Service 1957), with a spacing of 1.8 m between vines and 3.0 m between rows and trained to a vertical shoot positioning system. Vines were hand-pruned to three-node spurs during the winter, leaving approximately 60 buds per vine. No additional shoot or bunch thinning was performed before application of the treatments. Local cultural practices were followed with the pest management program based on scouting, experience and weather conditions. No sprays were applied during the flowering period to avoid physical damage to the inflorescence by the sprayer. After fruitset, a combination of fungicides and insecticides used as necessary for disease and pest control was rotated to prevent resistance during the summer (Wise et al. 2007). Pertinent weather data were recorded during the experiment by an automated weather station from the Michigan Automated Weather Network located 120 m from the experimental vineyard. Data retrieved from the station were daily precipitation, daily minimum, maximum and average temperature (Figure 1). Growing degree days (GDD) were calculated with the method using a base temperature of 10°C described by Baskerville and Emin (1969). No irrigation was used, and standard summer vineyard practices were applied. Shoots were trimmed with pruners on 25 July, day 206 of the calendar year, when they reached 30 cm above the highest pair of catch wires (2.1 m) and only in 2011.

The experiment was arranged in a randomised complete block design with one categorical factor, leaf removal (LR), with five levels of defoliation: no leaves removed (LR-0); leaves removed from four basal nodes (LR-4); leaves removed from six basal nodes (LR-6); leaves removed from eight basal nodes (LR-8); and leaves removed from ten basal nodes (LR-10). During the growing season, any lateral that eventually grew at the defoliated nodes was removed; at the time of the application of the treatment, shoots had an average of 15 leaves. Approximately 3 weeks before flowering, vines were organised into six blocks according to the number of inflorescences per vine, and each treatment was then randomly assigned to one vine per block. Additionally, a subsample of four shoots per vine was randomly chosen and tagged to make further measurements of shoot length, degree of fruitset, bunch parameters and fruit chemistry. Treatments were applied at full flowering (50% of cap fall), known as developmental stage EL-23 (Lorenz et al. 1995). In 2012, the described treatments were applied again at EL-23 on the same vines utilised the previous year. No additional shoot or bunch thinning was performed before treatment application. The timing of budburst, flowering, pea-size berries and harvest was also recorded (Table 1).

Estimation of leaf area

Shoot length was measured weekly on the tagged vines from 2 weeks before flowering up to 1 month after flowering. During the same period, a weekly sample of ten shoots was collected from guard vines (60 shoots total): shoot length was recorded, and leaf area (LA) was determined with a leaf area meter. A linear relationship between the LA per shoot (y) and shoot length (x): $y = 19.1x - 352.6$, $R^2 = 0.91$ in 2011; and $y = 17.51x - 87.52$, $R^2 = 0.82$ in 2012, was used for estimation of total LA. The calculated regression between shoot length and leaf area was then used to estimate the total leaf area (TLA) per shoot. Leaves removed using each defoliation level were collected in labeled, resealable poly zip bags, immediately stored in a portable cooler and transported to the campus laboratory. In the laboratory, TLA per shoot was determined with a leaf area meter (LI 3100; LI-COR Biosciences, Lincoln, NB, USA). After defoliation, leaf area removed per shoot was measured and subtracted from TLA to produce the retained leaf area (RLA) number.

Estimation of fruitset

Every basal bunch on each tagged shoot ($n = 120$) was photographed in the field at EL-20 (onset of flowering, with 30% flower cap fallen) and EL-31 (pea-size berries). Twenty bunches at EL-20 and 20 bunches at EL-31 were selected from the guard vines and photographed in the field against a dark background and then separately collected in resealable poly zip bags, stored in a portable cooler and transported to the laboratory. The actual number of florets and berries was

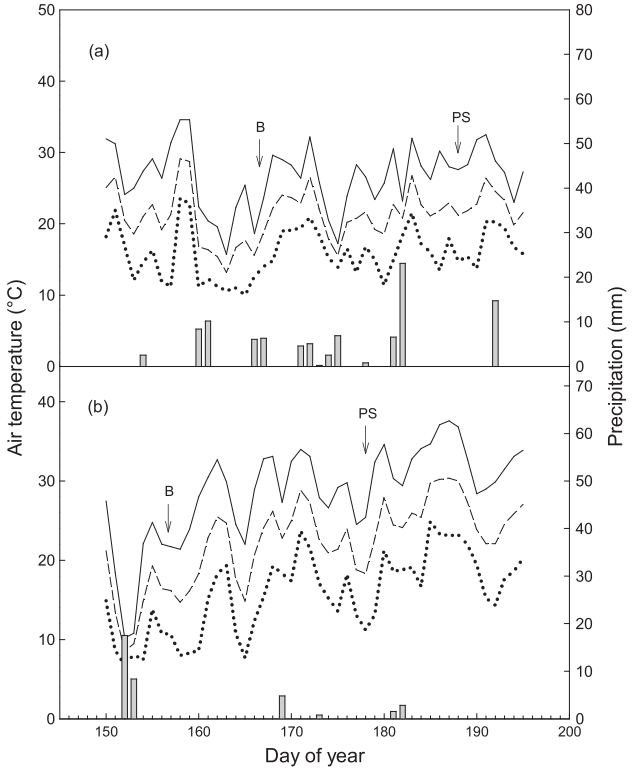

Figure 1. Daily precipitation (▯) and minimum (·····), maximum (----) and average air (- - -) temperature during flowering (B) and pea-size berry stage (PS) in (a) 2011 and (b) 2012 at Southwest Michigan Research and Extension Center.

destructively counted. The number of florets and berries visible in the photos was counted using Microsoft Office Paint (Windows XP; Microsoft, Redmond, WA, USA). Based on the methodology described by Poni et al. (2006), we observed a linear relationship between the actual number of florets (y) and the florets counted on the photographs (x) described by the equations: $y = 2.03x$, $R^2 = 0.86$ in 2011, and $y = 1.48x$, $R^2 = 0.95$ in 2012. Similarly, the relationship between the actual number of berries (a) and the berries counted in the pictures (b) was determined as $a = 1.50b$, $R^2 = 0.85$ in 2011 and $a = 1.38b$, $R^2 = 0.91$ in 2012. The equations were used to estimate the initial number of florets (F-20) and set berries (B-31) of each basal bunch per tagged shoot on the experimental vines. The proportion of fruitset was expressed in two ways: the proportion of fruitset at EL-31 calculated as the ratio between the B-31 and F-20; and the proportion of fruitset at EL-38 calculated as a ratio between F-20 and the number of berries at harvest (B-38).

Bunch parameters and morphology

At harvest, the basal bunches from tagged shoots were collected and weighed. Berries were separated from the rachis and counted, rachis and berries separately weighed, and the berries were returned to the sample poly bag and saved for subsequent chemical assessment. The bunch morphology was characterised by measuring the length of the rachis central axis (inner arm), the lateral wing or shoulder (outer arm) and the secondary branches (if they were longer than 5 mm). Rachis length was expressed as the sum of the three rachis components (Sabbatini and Howell 2010). Bunch compactness was calculated as the ratio between the B-38 and the rachis length and expressed as the compactness index (CI).

Yield components, fruit chemistry and colour analysis

At harvest, yield per vine and number of bunches per vine were measured and recorded. Harvest bunch rot was determined as incidence (proportion of infected clusters per vine) and as severity (proportion of infected berries per bunch). Basic fruit chemistry and colour were determined as described by Iland et al. (2004). We extracted approximately 20 mL of juice from each bunch sample for analysis of both TSS (^{o}Brix) (Atago PAL-1 Refractometer; Kirkland, WA, USA) and pH (Thermo Scientific Orion 370 pH meter; Beverly, MA, USA). For determination of titratable acidity (TA), 10 mL of juice was titrated against a standardised 0.1 N NaOH solution in an automated titrator coupled to an auto-sampler and control unit (Titroline 96; Schott-Geräte, Mainz, Germany) and expressed as g/L of tartaric acid equivalents. Anthocyanins and phenolic substances were measured by the total phenol assay, using UV–VIS (Iland et al. 2004). One hundred berries stored at −30°C were partially thawed prior to grinding in a tissue homogeniser (Model PT 10/35; Brinkmann Instruments, Luzern, Switzerland) at a speed of four on the manufacturer's scale for about 1 min. Samples were ground while maintained in an ice bath to minimise oxidation, and the concentration of anthocyanins per gram of berry mass and the absorbance units of phenolic substances per gram of fresh berry were measured with a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan) (Iland et al. 2004).

Statistical analysis

Data were analysed using one-way ANOVA in PROC MIXED procedure, SAS 9.3 software (SAS Institute, Cary, NC, USA). Normality of the residuals was assessed by visual inspection of the normal probability plot and the Kolmogorov test. Whenever the distribution of the residuals was found to significantly diverge from the normal distribution, data were subjected to either logarithmic or square root transformation. Homogeneity of variances was checked using the side-by-side box plot and Levene's test (SAS 9.3 software). When the treatment effect was found to be significant at $P = 0.05$, all pair-wise comparisons among the treatments were conducted using the t-test. When the treatment effect was not statistically significant at $P = 0.05$, all pair-wise comparisons among the treatments were conducted using Tukey's HSD honest significant difference (HSD) test.

Results

Weather conditions

Compared with the local historical mean, 2011 had a lower heat accumulation (from 1 April to 31 October) with only 1467 GDD, while 2012 with 1635 GDD defines it as a year significantly above the average, marked by a period of unusual and rapid heat accumulation in March. Because of this, budburst occurred approximately 1 month earlier than normal and 40 calendar days earlier than in 2011. The 2012 season began early and had the longest time between budburst and harvest (160 days) when compared with that in 2011 (136 days). The date of flowering and pea-size was 1–2 weeks earlier as well as harvest that also occurred 2 weeks earlier in 2012. Total precipitation for the 2011 season matched the historical mean in Michigan (592 mm). In contrast, total precipitation for 2012 was only 458 mm, reduced because of rain events absent between June and July (Figure 1). Precipitation between flowering and pea-size was minimal and temperature ranged between 10 (minimum) and 30°C (maximum) with average temperature of about 25°C in both years.

Table 1. Timing of developmental stages in 2011 and 2012, expressed as a calendar date and day of the year, with growing degree days.

Developmental stage	2011			2012		
	Date	DOY	GDD	Date	DOY	GDD
Budburst	10 May	130	30	30 March	89	130
Flowering	15 June	166	377	6 June	157	449
Pea-size berry	7 July	188	631	27 June	178	703
Harvest	23 September	266	1476	6 September	249	1641

†Growing degree days were calculated using a base temperature of 10°C as described by Baskerville and Emin (1969). Heat accumulation in March 2012 was unusually high, so calculation for reported GDD included temperature values that were over the base temperature for March in 2011 and 2012. DOY, day of the year; GDD, growing degree days.

Defoliation impact on shoot growth and leaf area

In 2011, we removed 29, 43, 66 and 91% of the initial TLA (Table 2) from LR-4, LR-6, LR-8 and LR-10, respectively. The leaf area retained of all the defoliated treatments was therefore significantly different from that of LR-0 and separated from each other with the exception of LR-4 and LR-6. During the following 2 weeks, we observed an increase in shoot length and the development of new leaves: 1 month after defoliation, LR-0, LR-4, LR-6, LR-8 and LR-10 gained 113, 117, 174, 282 and 691% of the initial RLA, respectively. At this stage, LR-4 and LR-6 were not significantly different from that of the control, however, the removal of eight or more leaves represented the threshold earlier, which the growth response of the vines was not sufficient to compensate and fully recover the reduction in leaf area. Consequently, LR-8 and LR-10 showed a significant reduction in leaf area development, which resulted in a 39.3 and 65.7% decrease in RLA compared with that of the control vines 1 month after defoliation (Table 2).

At the beginning of season 2012, vines did not show any difference in shoot length and LA according to the leaf removal intensity, excluding an impact of the previous year leaf removal on the early shoot and leaf area development. When RLA was presented as a proportion of TLA, then LR-4, LR-6, LR-8 and LR-10 had, respectively, 61, 46, 20 and 17% of LR-0 (100%) on the day of defoliation (Table 2). During the month following defoliation, LR-0 gained 27% of RLA, while the increase in RLA was 47, 90, 131 and 221% for LR-4, LR-6, LR-8 and LR-10, respectively (Table 2). In contrast to the previous year, under these conditions of overall reduced vegetative development, the milder leaf reduction (LR-4 and LR-6) was severe enough to reduce the vine growth compensation response as measured 1 month after the application of the treatment.

Defoliation impact on fruitset

The reduction in inflorescence size year-to-year was striking with the number of florets at EL-23 in 2012 reduced by 59% from that of the previous year. Additionally, while in 2011, the initial number of florets was homogeneous among the treatments, and in 2012, we observed a reduction in LR-8 and LR-10, both significantly different from LR-4 and LR-6, which had the highest number of florets, suggesting that defoliation in the first year also impacted the number of florets in the second year (Table 3). We observed a distinct difference between the 2 years in the mean number of berries: in 2012, harvested bunches had 30.6% fewer berries than in 2011. This was, however, a smaller reduction than the one observed in the number of florets, but corresponded to an overall increase in fruitset (EL-38), 65% higher in 2012 (Table 3).

The defoliation treatment was effective in both years in reducing the bunch size, with the LR-8 and LR-10 consistently showing a reduced number of berries when compared with that of the control LR-0. The removal of eight basal nodes appeared to be a stress threshold above which the vines were no longer able to effectively maintain a supply of resources to the reproductive organs of the vine. The first response to the defoliation in LR-8 was the reduction of fruitset in both years, as shown by the significantly lower FS-31 in 2011 and lower FS-38 in both years. This further resulted in fewer berries per bunch, that is 27 and 28% fewer berries than LR-0 at EL-31, and 51 and 35% at EL-38 in 2011 and 2012, respectively (Table 3).

The effect of the defoliation is, however, not limited to the actual stage of fruitset. Indeed, comparing the berry number at EL-31 with that at EL-38, we noticed additional berry reduction consistently increased with the severity of defoliation. The additional berry coulure (berry drop) occurred between the stages of pea-sized berry and harvest, and this led to a decrease in berry number from the LR-0 of 5, 3, 24 and 40% (2011) and of 1, 5, 13 and 21% (2012) in LR-4, LR-6, LR-8 and LR-10, respectively (Table 3).

Impact of early defoliation on bunch morphology and bunch rot severity

Early leaf removal had an impact upon bunch mass which was reduced in LR-10 by up to 65 and 62% in 2011 and 2012, respectively (Table 4). In 2011, the bunch mass was significantly affected with as little as four basal leaves removed, but in 2012, a significant difference from the un-defoliated LR-0 was achieved only with more severe defoliation (LR-8 and LR-10). Within the bunch mass components, while bunch size reduction was overall consistent with the decreased number of berries, the treatment effect on berry mass was reached only after 10 leaves had been removed in each of 2 consecutive years (Table 4). In the second year, 2012, there is a trend towards a reduction of berry mass with increasing leaf removal. The limitation of source availability during the early stages of bunch development did not significantly affect rachis length in 2011 but caused a reduction in 2012 of 36% in the LR-10 vines. The effect on rachis mass, however, was noticeable in both years and was induced by the removal of eight and ten leaves. The bunch compactness (indexed as CI, Table 4) was affected by the defoliation: in particular, we observed a dramatic 37 and 47%, respectively, reduction in bunch compactness of LR-8 and LR-10, in comparison with that of the control in 2011. A similar impact, at a lower magnitude, was assessed on the smaller sized-bunches in 2012. Although

Table 2. Total leaf area before treatment application in 2012, and retained leaf area per shoot immediately and 1 month after defoliation in 2011 and 2012.

Treatment†	2011			2012		
	TLA (cm^2)	RLA after defoliation (cm^2)	RLA 1 month after defoliation(cm^2)	TLA (cm^2)	RLA after defoliation(cm^2)	RLA 1 month after defoliation(cm^2)
LR-0	811n.s	811a	1730a	957 n.s.	957a	1220a
LR-4	809	576b	1252ab	928	584b	860b
LR-6	823	465b	1272ab	986	443c	842b
LR-8	814	275c	1050b	854	192d	444c
LR-10	694	75d	593c	955	159d	510c

Mean values were based on six replicates. Means within a column followed by the same letter are not significantly different by *t*-test ($P < 0.05$); n.s., not significant. †LR-0, no leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at flowering. RLA, retained leaf area; TLA, total leaf area.

Table 3. Effect of early defoliation on the number of florets and berries per bunch and proportion of fruitset at developmental stages EL-31 and 38 in 2011 and 2012.

Treatment†	Number of florets per bunch		Number of berries per bunch at EL-31		Number of berries per bunch at EL-38		EL-31 (%)‡		EL-38 (%)§	
Year	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
LR-0	425.4	185.2ab	102.8a	71.4a	116.5a	69.4a	25.8a	38.7	27.5a	37.7ab
LR-4	388.6	203.5a	98.3a	78.2a	92.6a	76.8a	26.0a	41.1	24.6a	40.0a
LR-6	441.8	201.0a	98.5a	62.8ab	95.2a	59.9ab	24.5ab	32.1	22.1a	30.0bc
LR-8	423.7	157.4b	74.7b	52.2bc	56.9b	45.1b	19.6b	33.8	14.0b	29.8c
LR-10	442.5	124.7c	73.5b	38.5c	44.5b	30.2c	19.0b	32.5	10.7b	25.8c
Level of significance	n.s.	**	*	*	**	**	*	n.s.	**	*
ANOVA										
Treatment	0.0018	<0.0001	<0.0001		<0.0001		0.0031		<0.0001	
Year	—	—	<0.0001		0.0002		<0.0001		<0.0001	
Treatment*year	—	—	0.5652		0.3176		0.2438		0.0053	

Means values were based on six replicates. Means within the column followed by the same letter are not significantly different by Tukey's honest significant difference test; ** $P < 0.05$; * $P < 0.1$; n.s., not significant. †LR-0, no leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at flowering. ‡Proportion of fruitset, based on berry number at EL-31 (Lorenz et al. 1995). §Proportion of fruitset, based on berry number at EL-38 (Lorenz et al. 1995).

the LR-8 or LR-10 treatments consistently reduced the CI, as well as the other components of bunch morphology, this did not alter the proportion of berries affected by rot when compared with that of the controls (Table 4). Even if means were not significant, all leaf removal treatments had a positive effect on rot reduction.

Impact of early defoliation on fruit composition and yield

The reduction of leaf area achieved with defoliation of up to six basal nodes did not significantly affect the final yield per vine (Table 5) but did affect the number of bunches per vine (Table 6). Early removal of eight and ten leaves, however, reduced yield per vine, respectively, by up to 33.7 and 55.6% in the first year and 51.3 and 70.8% in the second year of defoliation compared to that of the control. Such yield reduction in the LR-8 and LR-10 treatments corresponded to an increased level of TSS only in 2011, as well as to a reduced TA, with comparable leaf-to-fruit ratio (Table 7). We observed no beneficial effect on the concentration of juice anthocyanins at harvest, even though these treatments increased the exposure of the bunches to the sun for the full season (Table 5). In contrast, the concentration of phenolic substances of the treated vines showed a consistent positive response in which LR-8 and LR-10 developed considerably more phenolic substances than that of the control.

Discussion

The development of a grape inflorescence depends on the presence of carbohydrates, which originate from reserves, leaves or the inflorescence itself (Morinaga et al. 2003, Vasconcelos et al. 2009, Vaillant-Gaveau et al. 2011). During flowering, the inflorescence generates a significant amount of new assimilated carbon for itself, but also a surplus that is distributed to the growing leaves (Lebon et al. 2008, Palliotti et al. 2010, Vaillant-Gaveau et al. 2011). Chlorophyll concentration decreases substantially, and in parallel, inflorescence photosynthesis declines and becomes negligible at fruitset (Lebon et al. 2005). From this stage forward, the

Table 4. Impact of early defoliation on components of bunch morphology and severity of bunch rot in 2011 and 2012.

Treatment†	Bunch mass (g)		Berry mass (g)		Rachis mass (g)		Rachis length (cm)		CI		Rot severity‡ (%)	
Year	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
LR-0	132.1a	79.5ab	1.08	1.12a	6.22a	2.46a	16.9	10.5ab	7.0a	6.7a	7.8a	25.6
LR-4	102.7b	88.1a	1.04	1.09a	4.92a	2.67a	14.5	12.1a	6.7a	6.8a	1.6b	12.9
LR-6	101.3b	66.6b	1.04	1.05ab	4.79a	2.14ab	16.7	10.8ab	6.1a	5.6ab	3.9ab	14.9
LR-8	60.1c	45.7c	1.01	0.98ab	3.05b	1.60bc	13.5	8.5bc	4.4b	5.8ab	4.5ab	10.2
LR-10	46.1d	29.8d	0.98	0.92b	2.40b	1.25c	13.0	6.7c	3.7b	4.7b	7.4ab	16.3
Level of significance	*	**	n.s.	*	**	**	n.s.	**	**	**	*	n.s.
ANOVA												
Treatment	<0.0001		0.0187		<0.0001		0.0002		<0.0001		0.0438	
Year	<0.0001		0.7489		<0.0001		<0.0001		0.2292		<0.0001	
Treatment*year	0.0367		0.6989		0.4023		0.1967		0.1204		0.1197	

Means values were based on six replicates. Means within the column followed by the same letter are not significantly different by Tukey's honest significant difference test; **, $P < 0.05$; *, $P < 0.1$; n.s., not significant. †LR-0, leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at bloom. ‡Rot severity was calculated as a proportion of affected berries per tagged bunch. CI, compactness index expressed as number of berries per rachis length.

inflorescence/bunch development relies mainly on leaf assimilates. The important role of supplying carbohydrates and other assimilates to the bunch is assigned to the leaves on the adjacent nodes below and above the bunch (Hale and Weaver 1962), with a major contribution of the leaves on the bunch side of the shoot (Motomura 1990). If the supply of carbohydrates for the bunches is restricted at flowering by defoliation, then poor fruitset and the abortion of fruitlets are inevitable (Coombe 1959, Candolfi-Vasconcelos and Koblet 1990). This outcome is consistent with what we observed in our study, where defoliated Pinot Noir vines subjected to the removal of eight or ten leaves showed a considerably reduced proportion of FS-31, FS-38 and of the number of berries per bunch at EL-38 compared with that of the control in 2011 and 2012. The reduction in berry count, however, was already evident at EL-31 in the first year alone. The amount of LA per shoot that was either removed or retained was of similar importance for the number of berries measured at EL-31 ($r = -0.81$, $r = 0.77$ in 2011 and $r = -0.60$ and $r = 0.66$ in 2012). Based on coefficient of determination (Figure 2), the amount of LA per shoot that was removed was negatively correlated with the number of berries at EL-38 for both years, and in 2011, it was $R^2 = 0.64$. There is, however, 36% of total variation of the number of berries per bunch at EL-38 that could be explained by removed LA in 2012. The existing difference between EL-31 and EL-38 in defoliated vines can be explained by the difference between the number of berries per bunch at phenological stages EL-31 and EL-38. Furthermore, comparing these two stages, control bunches showed little change in the number of berries from the pea-size berry stage until harvest. The difference between the number of berries per bunch at EL-31 and EL-38 is more obvious when presented as the proportional decrease between the two, and then it can be seen that the proportion of berries that dropped proportionally increased with defoliation severity in 2011 (Figure 3), which was similar to that reported by Candolfi-Vasconcelos and Koblet (1990). The linear correlation, however, between the removed LA and the proportional decrease in berry number from EL-31 to EL-38 was not that strong in 2012 (Figure 3). This is probably due to the occurrence of an extremely hot and dry period, which lasted for almost 2 months and coincided with berry development and ripening and which most affected the control vines with the largest LA. Based on Figure 3, the control had more or equal proportion of decrease in berry number as that for LR-4 and LR-6.

Table 6. Impact of early defoliation on number of bunches per vine in 2011 and 2012.

Treatment†	Number of bunches per vine	
Year	2011	2012
LR-0	91.7ab	86.3ab
LR-4	96.3ab	90.2ab
LR-6	107.0a	98.2a
LR-8	92.5ab	77.3ab
LR-10	81.5b	60.5b
Level of significance	**	**
ANOVA		
Treatment	0.0009	
Year	0.1067	
Treatment*year	0.7521	

Mean values were based on six replicates. Means within the column followed by the same letter are not significantly different by Tukey's honest significant difference test; $^{**}P < 0.05$; $^{*}P < 0.1$; n.s., not significant. †LR-0, no leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at flowering.

By 2012, the vines had been subjected to defoliation stress for two seasons, and post-fruitset, the proportion berry drop was more pronounced in 2011 than that in 2012. This particularly refers to LR-8 and LR-10, and an explanation for it could be found in the different inflorescence size produced in each year. In 2011, inflorescences contained more than double the number of florets found in 2012. Additionally, defoliation in 2011 decreased considerably the number of florets in LR-8 and LR-10 in 2012. To obtain balance between source availability and sink requirement, the vines proportionally set less berries if bunches contained more florets before fruitset and fruitset was higher in 2012 than it was in 2011 (May 2004).

Interestingly, we found that early defoliation did not impact mean berry mass in the first year of defoliation. Many authors reported a decrease in berry mass as a consequence of source limitation during the early stage of berry development (Poni et al. 2006, 2008, Intrieri et al. 2008, Lohitnavy et al. 2010,

Table 5. Impact of early defoliation on fruit composition and yield in 2011 and 2012.

Treatment†	TSS (°Brix)		pH		Titratable acidity (g/L)		Anthocyanins (mg/g)		Phenolic substances (au/g)		Yield per vine (kg)	
Year	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
LR-0	20.8c	21.6	3.46b	3.64d	6.09a	3.77a	0.34	0.45	0.95bc	1.11b	9.12a	5.95a
LR-4	22.0abc	20.7	3.44b	3.82c	5.80ab	3.63ab	0.30	0.38	0.87c	1.15ab	7.92ab	5.65a
LR-6	21.6bc	21.9	3.49b	3.93b	5.49b	3.61ab	0.29	0.39	0.86c	1.10b	9.70a	5.30a
LR-8	22.8ab	22.1	3.51b	4.09a	5.44b	3.29b	0.35	0.45	1.12ab	1.36a	6.05b	2.87b
LR-10	24.0a	22.2	3.69a	3.97b	4.95c	3.98a	0.37	0.48	1.23a	1.37a	4.05c	1.72b
Level of significance	*	n.s.	**	**	*	*	n.s.	n.s.	*	**	**	*
ANOVA												
Treatment	0.0043		<0.0001		0.0251		0.0369		<0.0001		<0.0001	
Year	0.0046		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Treatment*year	0.5452		<0.0001		0.0077		0.9998		0.6015		0.3674	

Means values were based on six replicates. Means within the column followed by the same letter are not significantly different by Tukey's honest significant difference test; **, $P < 0.05$; *, $P < 0.1$; n.s., not significant. †LR-0, no leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at flowering.

Table 7. Impact of early defoliation on leaf-to-fruit ratio per vine in 2011 and 2012.

Treatment†	Leaf-to-fruit ratio (cm^2/g)	
Year	2011	2012
LR-0	14.2	14.4ab
LR-4	14.9	13.0ab
LR-6	12.8	12.5b
LR-8	14.0	13.6ab
LR-10	13.5	19.9a
Level of significance	n.s.	**
ANOVA		
Treatment	0.2754	
Year	0.4882	
Treatment*year	0.197	

Mean values were based on six replicates. Means within the column followed by the same letter are not significantly different by Tukey's honest significant difference test; $^{**}P < 0.05$; $^{*}P < 0.1$; n.s., not significant. †LR-0, no leaves removed; LR-4, leaves removed from four basal nodes; LR-6, leaves removed from six basal nodes; LR-8, leaves removed from eight basal nodes; LR-10, leaves removed from ten basal nodes at flowering.

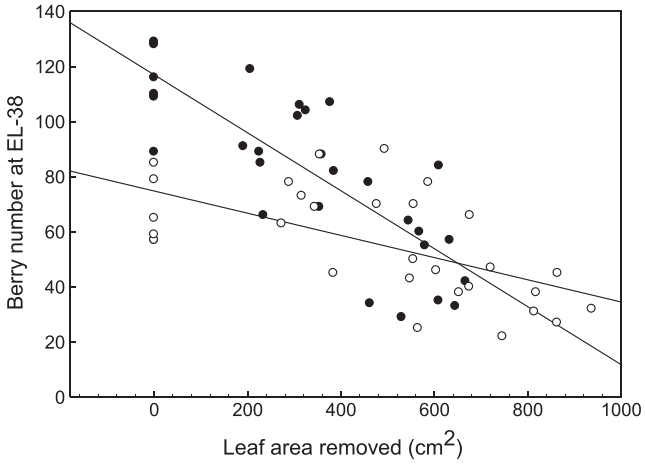

Figure 2. Linear regression between removed leaf area and berry number at development stage EL-38 (Lorenz et al. 1995). Regression is based on a sample of 30 vines for each year, 2011 (●) ($R^2 = 0.64$) and 2012 (○) ($R^2 = 0.36$).

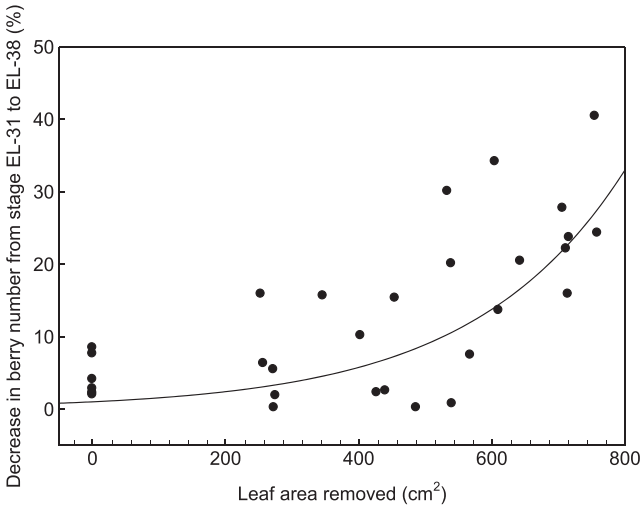

Figure 3. Linear correlation between removed leaf area and proportional decrease in berry number from developmental stage EL-31 to stage EL-38 (Lorenz et al. 1995). Correlation ($R^2 = 0.54$) is based on the sample of 30 vines and their mean values in 2011 and 2012.

Tardaguila et al. 2010). Conversely, there are also reports that berry size may increase because of the compensation effect that promotes berries to reach full size (Poni and Bernizzoni 2010, Tardaguila et al. 2012). In the second year of defoliation, we found significant linear regression between RLA per shoot in 2012 and mean berry mass ($r = 0.67$). As for other yield components, mean berry mass was under the influence of the two-season defoliation, which induced a level of source limitation sufficient to reduce considerably berry size. Table 3 contains retained LA (%), from where removed LA (%) could be easily calculated. Unfortunately, none of the cited papers (Candolfi-Vasconcelos and Koblet 1990, Lee and Skinkis 2013) specify the proportion of LA removed. They reported that defoliation was performed only at flowering. In Lee and Skinkis (2013), five to six main leaves were removed, which corresponds to our treatment LR-6, while in Candolfi-Vasconcelos and Koblet (1990), all main leaves were removed, which corresponds to the LR-10 treatment. The influence of the two-season defoliation induced a level of source limitation sufficient to reduce berry mass considerably only in LR-10. Lee and Skinkis (2013) removed five to six leaves at flowering in 2 consecutive years on Pinot Noir vines at two locations and found no effect on berry mass, similar to that of the LR-6 treatment. This suggests that Pinot Noir vines may easily overcome the adverse influence of removing 56 to 43% of TLA (Table 3) at flowering on berry mass over multiple years. Removing more than 80% of TLA, however, presented a threshold to which vines responded by reducing berry mass; comparable results were reported by Candolfi-Vasconcelos and Koblet (1990).

One of the primary purposes of this research was to investigate the effect of leaf removal on bunch compactness. While many researchers report on bunch compactness using the ratio between berry number or berry mass and rachis length, little is known about the effect of defoliation stress on rachis development. Insensitivity of rachis length to the defoliation stress in 2011 could be because defoliation was applied at full flowering and thus failed to target the period of inflorescence primordium branching, which occurred from mid-August in the previous year and continued throughout budburst up to flowering in the current year (May 2000, Vasconcelos et al. 2009). This suggested that removing ten leaves in 2011 induced an effect on rachis length in 2012 (Table 4).

In the grape inflorescence, flowers are grouped in the dichasium, and they are attached together to the same base to form a branch (May 2004). In all likelihood, source limitation caused abortion of the flowers/berries that were weaker, and as consequence, the reduction of the number of berries per lateral branch decreased their mass. In the extreme case, the whole branch could dry and drop off, leading to fewer branches per bunch. Early defoliation of eight and ten leaves could consistently in both years caused a significant decline in the number of branches and therefore induced a reduction of the rachis mass compared with that of the control. About 65% of the variability in rachis mass can be associated with the degree of LA removed per shoot in 2012. Only 50% of the change in rachis length, however, can be associated with changing the proportion of LA removed per shoot in 2012.

Significant reduction of berry number in LR-8 and LR-10 resulted in a lower CI in 2011. Early defoliation in 2012, however, reduced CI only in LR-10, decreasing the berry number to a larger extent (56%) than shortening the rachis length (36%) compared with that of the control. Palliotti et al. (2012) reported that removal of 75–80% of the leaves in a canopy pre-flowering reduced the number of berries, bunch compactness [expressed as yield per rachis length or OIV rating(Organisation Internationale de la Vigne et du Vin 1983)] and, finally, botrytis rot in Ciliegiolo in 2 consecutive years. Two years of a six-leaf removal regimen reduced bunch compactness (bunch density) in Merlot, while bunch compactness in Cabernet Sauvignon was unaffected (Kotseridis et al. 2012). Although LR-8 and LR-10 reduced bunch compactness in 2011, none showed a reduction in the severity of bunch rot, likely because these treatments were advanced in ripening and more TSS was present in the grape juice making the berries more susceptible to infection (Hill et al. 1981). In 2012, only LR-10 showed a reduction in CI, but the effect on bunch rot severity was lacking again. According to Hed et al. (2015), the effectiveness of early leaf removal on bunch rot control is closely related to the number of berries per bunch. If the natural variability of bunch compactness is high, that is, more berries per bunch, the efficiency of early leaf removal is improved. The authors found that the best results were achieved when the control bunches of Chardonnay had over 100 berries, while in the year when that number was as low as 62, early leaf removal did not have an impact on the incidence or severity of Botrytis. In 2012, bunches from control vines of Pinot Noir had a reduced number of berries of about 40% when compared with that in 2011, meaning that their CI was low enough to cause no effect on the severity of bunch rot.

In 2011, all levels of defoliation significantly reduced bunch mass. This reduction, however, was due to a decreased number of berries per bunch only in LR-8 and LR-10, while mean berry mass showed no change. This resulted in the lower yield of LR-8 and LR-10 for 2011. In the following year, bunch mass was reduced in LR-8 and LR-10 by 42 and 62%, respectively, compared with that of the control. Clearly, fewer berries for both treatments and a smaller berry size in LR-10 contributed to this reduction. Additionally, early leaf removal caused fewer bunches per vine in LR-10. Therefore, defoliation of eight and ten leaves led to a vineyard yield of 5.3 and 3.2 t/ha, respectively, which is below an acceptable economic level for sustainable Pinot Noir production in Michigan. In contrast, two seasons of defoliation at the six nodes level resulted in slightly lighter bunches because of the decrease in berry mass but was not the limiting factor causing significant change in yield.

High TSS in the grape juice is primarily caused by low yield, meaning that the retained leaf area was sufficient to support bunch development and fruit ripening in the severely defoliated vines. Retained LA measured 1 month after defoliation in LR-10 was 66% less than that in the control. At that time, basal leaves in the control were at least 30 days old and, thus, less photosynthetically active. Similarly, bunch mass of LR-10 was also 62% less than that of the control. Therefore, the ratio between RLA per shoot and bunch mass was the same for LR-10 and the control. The leaves of LR-10, however, were younger and presumably more productive, which made a difference in the related fruit ripening stage (Table 7).

The increase in TSS with defoliation intensity cannot be explained solely by the leaf-to-fruit ratio in 2011, which appears to be unaffected by the early defoliation. The more likely cause of higher TSS accumulation in the severely defoliated vines (LR-8 and LR-10) could be linked to the age of the leaves present in the canopy during and after veraison when sink demand rises. Poni et al. (2008) stated that a progressive decline in leaf assimilation rate occurred after 50 days of age. Basal leaves on the control vines were approximately 70 days old during veraison. It is possible that younger leaves in LR-8 and LR-10 treatments could have contributed more efficiently to fruit ripening, increasing TSS. An increase in leaf assimilation of main and lateral leaves was also found on early defoliated Sangiovese vines during the period after veraison (Poni et al. 2006). Repeated defoliation in 2012, however, brought no change in TSS among the treatments. Moreover, significant difference in the leaf-to-fruit ratio was detected only between LR-6 and LR-10 (Table 7). Lee and Skinkis (2013) reported no difference in TSS after 2 consecutive years of defoliation of five to six basal leaves at flowering. In contrast to our results, Candolfi-Vasconcelos and Koblet (1990) measured a significant increase in TSS after two seasons of removing all main leaves 1 week after full flowering in Pinot Noir. Typically, values of pH in grape juice range from 3 to 3.5 and of TA from 5 to 10 g/L (Keller 2010) and are usually inversely related, which corresponds to our findings for 2011. The lower level of TA found in LR-6, LR-8 and LR-10 might be linked to an increased bunch exposure to sunlight and to intensive respiration of malate (Reynolds et al. 1986, Bergqvist et al. 2001). Both pH and TA of LR-10 were out of range, which suggested that the grapes were overripe. In the hot and dry season of 2012, however, all treated vines as well as the untreated control had a particularly low TA probably because of an enhanced degradation of malic acid influenced by temperature, which is known to be at high concentration and predominantly stored in Pinot Noir at maturity (Ruffner 1982a,b). The pH ranged from 3.64 to 4.09, and it was significantly higher in defoliated vines, which could be attributed to an intense potassium accumulation in berries caused by alteration of LA.

Over 2 years, none of the defoliation treatments improved the concentration of anthocyanins in the grape juice. The lack of the effect could be attributed to a detrimental influence of excessive bunch exposure and high temperature on anthocyanins biosynthesis (Bergqvist et al. 2001). There are reports, however, that five to six leaves removed at flowering in two different locations in Washington (Lee and Skinkis 2013) and six leaves removed at berry set and veraison in two different locations in Slovenia (Sternad Lemut et al. 2011) increased anthocyanins in Pinot Noir. Also, some other red cultivars (Merlot, Cabernet Sauvignon and Ciliegiolo) responded to early defoliation by increasing the concentration of anthocyanins in the grape juice (Kotseridis et al. 2012, Palliotti et al. 2012).

Differentiation of the uncommitted primordia into inflorescence primordia occurs between flowering until veraison determining the final inflorescence number on a vine for the following year (Keller 2010). During this time, cumulative radiation and heat experienced by the buds, as well as the availability of assimilates, play important roles promoting inflorescence formation and consequently increasing bud fruitfulness for the following season (May et al. 1969, Sommer et al. 2000). Thus, restricted availability of leaf assimilates decreases bud fruitfulness, which in our experiment was observed in the most severe defoliation treatment. Only leaf removal on the ten nodes in 2011 drastically reduced the number of inflorescences per vine. In previous studies, bud fertility, calculated as the number of bunches per number of

buds retained at pruning, was stimulated by 33% leaf reduction after budburst but reduced by the 66% defoliation after berry set (Hunter and Visser 1990). These authors referred to the severe defoliation as the cause of reduced nutrient availability for the initiation and differentiation of inflorescence primordia, but we found a weak linear correlation between the removed LA per shoot in 2011 and the number of inflorescences per vine in 2012 ($r = 0.41$; $P = 0.025$). Reduction of the inflorescence number per shoot was reported when leaves above the fifth node or all main leaves on the shoots were removed in the previous season (Candolfi-Vasconcelos and Koblet 1990). Removal of the main leaves, however, up to the second node above the distal bunch at pre-flowering did not change the number of bunches per shoot nor the number of bunches per vine in the Ciliegiolo cultivar (Palliotti et al. 2012).

During bud swelling and budburst, the branching of the inflorescence primordia resumes, and flower initials are formed (Pratt 1971). This is the period when the final number of florets per inflorescence is determined by the environmental conditions and the reserve status of the vine (Vasconcelos et al. 2009). It was also shown that higher temperature during the 2 weeks before budburst reduced the number of florets per inflorescence and the temperature influence on florets differentiation weakened as budburst advanced (Petrie and Clingeleffer 2005). From the middle of March 2012, the maximum air temperature fluctuated from 24 to 29°C for a week, which coincided to 2 weeks before budburst. This unusually high temperature in this growing region could be a reason for the generally lower number of florets per inflorescence in 2012 compared with that in 2011. Moreover, defoliation in 2011 showed a negative impact on the number of florets likely because of a reduction in the amount of stored reserves, which were of crucial importance for flower initiation and differentiation in early spring. Therefore, the LR-10 treatment showed the most significant decrease, reducing the floret number by 32% compared with that in LR-0. Similar results were reported when 75% defoliation of Chardonnay was applied earlier in the season in contrast to 12 weeks after flowering, which did not have any impact on the number of florets (Bennett et al. 2005). We found a significant linear correlation between floret numbers in 2012 and removed leaf area per shoot ($r = 0.55$, $P = 0.002$).

Conclusion

The aims of this experiment were to evaluate two particular causal relationships: the specific impact of early leaf removal on both grapevine fruitset and bunch compactness and the broader carry-over effect of 2 years application of early defoliation on growth characteristics, fruit composition and yield components of Pinot Noir grown in a cool climate. For the first relationship, the restricted supply of carbohydrates induced by defoliation of eight and ten leaves caused a consistent dampening effect on fruitset and number of berries per bunch; however, results on berry mass were mixed. The eight-leaf defoliation treatment represented a breaking point for CI reduction. Rot infection in 2011 was generally mild and that was a reason for the low effectiveness of reduced CI achieved by LR-8 and LR-10 in bunch-rot control. Because of a natural variability of bunch size over years, overall number of berries per bunch was notably lower in 2012 resulting in generally low CI (control included), so any further reduction of bunch compactness caused by defoliation was not successful in rot control. Although removal of more than eight leaves effectively reduced CI, this level of source limitation caused an economically unacceptable yield reduction especially in the second year. Unlike other defoliation studies on Pinot Noir, our experiment showed no beneficial effect of early defoliation on juice colour, although defoliation of ten leaves consistently increased the concentration of phenolic substances.

The potential long-term impact of defoliation on vine health would depend on the intensity of defoliation. Introducing the removal of eight or more leaves as a standard vineyard practice would definitely impair the ability of the vine to renew stored reserves. This would reflect on cold hardiness and probably decrease bud vitality and the survival rate of the vine during the winter period in the cool–cold climate viticultural region of the Great Lakes.

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